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SOME ASPECTS OF WHITE NOISE QUADRATIC FUNCTIONALS*

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Abstract

We are interested in **quadratic white noise functionals**. First we define the two subspaces $H_2^{(-2,1)}$ and $H_2^{(-2,2)}$ of the space of quadratic functionals of white noise. By introducing a random measure dz(ω), we consider a Hilbert space spanned by $\{\int f(u)\ dz(u)\}$. Then we can see the duality between the two spaces $H_2^{(-2,1)}$ and $H_2^{(-2,2)}$ In addition, when we consider the **Lévy Laplacian** on $H_2^{(-2,1)}$ we can obtain the adjoint of the Lévy Laplacian on $H_2^{(-2,2)}$

Keywords : quadratic white noise functional, Lévy Laplacian

Introduction

We are interested in quadratic functionals of white noise. Before discussing the quadratic functionals of white noise, we first recall the linear functional of white noise.

In the linear case, $(\dot{B})$ can be expressed as Wiener integral of the form

$$\dot{B}(f) = \int f(t)\,\dot{B}(t)\,dt, \quad f \in L^2(R^1)$$

The collection of such $\dot{B}(f)$ spans a Hilbert space H_1 under the $L^2(\Omega, P)$-topology. The above formula proves that the space H_1 is isomorphic to $L^2(R^1)$:

$$H_1 \cong L^2(R^1). \tag{1.1}$$

Extending the space $L^2(R^1)$ to Sobolev space K^{-1}, we obtain the space $H_1^{(-1)}$ of linear functional of white noise which is the corresponding extension of H_1.

Namely, we have a generalization of (1.1) :

$$H_1^{(-1)} \cong K^{-1}(R^1). \tag{1.2}$$

In reality, $H_1^{(-1)}$ is defined by (1.2). The space $H_1^{(-1)}$ is the space of linear functionals of white noise.

This isomorphism (1.2) determines the $H_1^{(-1)}$-norm denoted by $\| \ \|_{-1}$. More precisely, if $(\in H_1^{(-1)})$ corresponds to $f\ (\in K^{-1})$ by (1.2) then $\| \varphi \|_{-1} = \| f \|_{K^{-1}(R^1)}$.

It is not difficult to deal with linear functionals of $\dot{B}(t)$'s, but when we deal with nonlinear functionals of $\dot{B}(t)$'s, we need to renormalize them. We can see the idea of the renormalization by explaining for the quadratic forms of $\dot{B}(t)$'s.

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* Best Paper Award Winning Paper in Mathematics (2019)

2. Quadratic functionals of white noise (Ref.[4])

We now consider the quadratic functionals which is actually nonlinear functionals of $\dot{B}(t)$'s. We start by giving the definition of quadratic functionals.

2.1 Definition Let $Q(x) = Q(x(t))$, $t \in R^1$ be an $(L^2)^-$ *-functional and its S-transform be* $U(\xi)$. For any $\xi, \eta \in E$ and for any $\alpha, \beta \in R^1$, the function $U(\alpha\xi+\beta\eta)$ is a *homogeneous polynomial of degree 2 in* α *and* β, then $U(\xi)$ and hence $Q(x)$ is called a **quadratic functional or quadratic form**.

If the function $U(\alpha\xi+\beta\eta)$ is a *homogeneous polynomial of degree n in* α *and* β, then U and Q are called **entire homogeneous of degree n**.

Among others, the subspace $H_2^{(-2)}$ of $(L^2)^-$ consisting of *quadratic generalized white noise functionals* is particularly important.

Before we discuss the details of the analysis of quadratic forms of the $\dot{B}(t)$, we shall emphasize the significance of "quadratic".

Let $\{X_n\}$ be the independent identically distributed sequence such that each X_n being $N(0, 1)$ random variable. We can express the quadratic functional as

$$Q(X) = \sum_{1\le i,\, j \le n} a_{ij} X_i X_j = Q_1(X) + Q_2(X), \tag{2.1}$$

where

$$Q_1(X) = \sum_{i=1}^{n} a_{ii} X_i^2 \tag{2.2}$$

$$Q_2(X) = \sum_{1\le i,\, j \le n,\, i\neq j} a_{ij} X_i X_j, \quad a_{ij} = a_{ji}, \tag{2.3}$$

We consider the condition for convergence is quasi convergence as $n \to \infty$. Thus, we require the convergence for $\sum a_{ii}$ and square summability of coefficients a_{ij} and also, quasi-convergence of $Q_2(X)$ as $n \to \infty$.

We are now ready to discuss *the passage from discrete to continuous* (in Hida's words the passage from digital to analogue).

Thus we take X_j^n to be the variation of a Brownian motion with the idea of Lévy's construction of Brownian motion by interpolation. Namely, we set $X_j^n = \frac{\Delta_j^n B}{\sqrt{\Delta^n}}$, where $\{\Delta_j^n\}$ is the partition of [0, 1] with $\Delta_j^n = \left[\frac{j}{2^n}, \frac{j+1}{2^n}\right]$ i.e. $\bigcup_{j=1}^{n} \Delta_j^n = [0,1]$ and we can write Δ^n instead of Δ_j^n. Hence $\left|\Delta_j^n\right| = \left|\Delta^n\right| = \frac{1}{2^n}$.

Then ,

$$Q_2(X) = \sum_{i\neq j} a_{ij}^n \frac{\Delta_i^n B}{\sqrt{|\Delta^n|}} \frac{\Delta_j^n B}{\sqrt{|\Delta^n|}}$$

converges with the assumption

$$\sum \left(a_{ij}^n\right)^2 < \infty, \tag{2.4}$$

as $n \to \infty$.

The above inequality (2.4) guarantees the convergence of

$$\sum_{i\neq j} \frac{a_{ij}^n}{|\Delta^n|} \frac{\Delta_i^n B}{|\Delta^n|} \frac{\Delta_j^n B}{|\Delta^n|} |\Delta^n||\Delta^n|,$$

as letting $|\Delta^n| \to 0$ as $n \to \infty$. The sum tends to

$$Q_2(\dot{B}) = \int_0^1 \int_0^1 F(u, v)\, \dot{B}(u)\dot{B}(v)\, du\, dv \tag{2.5}$$

in H_2, where $\frac{a_{ij}^n}{\Delta^n}$ approximates $F(u, v) \in \widehat{L^2(R^2)}$ so that

As for the limit of $Q_1(X)$, we have to consider as follows. First we note that as $\Delta \to \{t\}$ the quantity $\left(\frac{\Delta B}{\Delta}\right)^2$ may be considered to tend to $\dot{B}(t)^2$, however it is not a generalized white noise functional. If we have the difference $\left(\frac{\Delta B}{\Delta}\right)^2 - \frac{1}{\Delta}$, then it converges to a generalized quadratic functional which is denoted by $:\dot{B}(t)^2:$.

We must modified $Q_1(X)$

$$Q_1(X) \to Q_1'(X) = \sum_i a_{ii}^n \left(\frac{\Delta_i^n B}{|\Delta^n|}\right)^2 - \frac{1}{|\Delta^n|}$$

which tends to

$$Q_1(\dot{B}) = \int_0^1 f(u) :\dot{B}(u)^2: du, \tag{2.6}$$

with $f \in L^2(R^1)$.

From the above modification, we can see the idea of renormalization. The result (2.6) is a Hida distribution while (2.5) defines an ordinary H_2-functional.

So far we observed some particular generalized functionals $\dot{B}(t)$ of degree 2, where we have seen the idea of the passage from discrete to continuous. The limit of $Q(X)$, is in fact, ***normal functional*** in terms of P. Lévy,

$$\int f(u) :\dot{B}(u)^2: du + \int\int F(u, v) :\dot{B}(u)\dot{B}(v): du\, dv. \tag{2.7}$$

We just mentioned above that equation (2.7) is the normal functionals in $\dot{B}(t)$. Thus, we should now give the general expression of *Lévy's normal functional* in terms of *S*-transform which is of the form

$$\int f(u)\,\xi(u)^2\,du + \int\int F(u,v)\,\xi(u)\,\xi(v)du\,dv, \qquad (2.8)$$

where $f \in L^1(R)$, $F \in L^2(R^2)$ and $\xi \in E$.

We now pause to explain the importance of normal functionals. Most significant reason is in the analytic property.

We may say that the first term of (2.8) has the Fréchet derivative and the kernel function has singularity only on the diagonal. We may therefore call it the singular part of the normal functional, while the second term is second Fréchet differentiable with kernel function $F(u, v)$. The following examples are given to see the singular and regular parts.

2.2 Example Let

$$U(\xi) = \int f(u)\,\xi(u)^2\,du.$$

Taking its variation

$$\delta U(\xi) = 2\int f(u)\,\xi(u)\,\delta\,\xi(u)\,du$$

$$U'(\xi, t) = 2\,f(t)\,\xi(t) = 2\int f(u)\,\xi(u)\,\delta_t(u)\,du$$

$$\delta U'(\xi, t) = 2\int f(u)\,\delta\xi(u)\,\delta_t(u)\,du$$

$$U''(\xi, t) = 2\,f(t)\,\frac{1}{dt}$$

Thus we can see that second *Fréchet derivative* does not exist. It is the singular part of the normal functional (2.8).

2.3 Example Let

$$U(\xi) = \int_{R^2} F(u,v)\xi(u)\xi(v)du\,dv$$

with smooth symmetric kernel $F(u, v)$.

The variation of U is

$$\delta U = 2\int F(t,u)\,\xi(u)\,\delta\,\xi(u)\,du,$$

thus

$$U'(\xi) = 2\int F(t,u)\,\xi(u)\,du$$

$$U''(\xi) = 2\,F(t,t)\;\xi(t).$$

We now see that it is second Fréchet differentiable and so it is the regular part of the normal functional (2.8).

Some other characteristics of the quadratic functional can be seen through the representation by using the *T* or *S* transform.

For an ordinary functionals, we are given a representation in terms of symmetric $L^2(R^2)$ function. We can then appeal to Mercer's theorem to develop the original quadratic random functional decomposed into the sum of countably many independent random variables with χ^2 – distribution.

A plausibility of renormalization can be seen from the Quadratic forms of white noise functionals. Reductionism, which is Hida's favorite idea, suggests us to start the analysis with the algebra of polynomials in $\dot{B}(t)$'s.

Except linear functionals, those polynomials need renormalization in order to be generalized functionals of $\dot{B}(t)$'s. Quadratic form that is the limit of $Q_1(x)$ shows why and how the renormalization is necessary to be a generalized quadratic functional. We emphasize that such a generalized functional plays significant role also in the applications in physics.

When Laplacian is discussed, normal quadratic forms illustrate how it works from the view point of "harmonic analysis", where infinite dimensional rotations are acting.

Remark. The harmonic property can be discussed after the Lévy Laplacian Δ_L is defined. Both Q_1 and Q_2 have enough analytic properties, in particular they are in the domain of Δ_L. We see, as mentioned before, that Q_2 is always harmonic, while Q_1 may have non-zero trace.

3. Space of quadratic functionals of white noise (Ref. [4])

Let $K^{-3/2}(R^2)$ be the dual space of Sobolev space $K^{3/2}(R^2)$. There is an isomorphism

$$H_2^{(-2)} \cong \widehat{K^{-3/2}(R^2)} \tag{3.1}$$

as an extension of the known isomorphism

$$H_2 \cong \widehat{L^2(R^2)}. \tag{3.2}$$

We now have the Gel' fand triple

$$\mathrm{H}_2^{(2)} \text{ Ì } \mathrm{H}_2 \text{ Ì } \mathrm{H}_2^{(-2)}, \tag{3.3}$$

where $\mathrm{H}_2^{(2)}$ is the dual space of $\mathrm{H}_2^{(-2)}$, defined in the usual manner based on the scalar product in H_2.

According to the isomorphism (3.1), for $\varphi \in \mathrm{H}_2^{(-2)}$ there is a function $F(\mathrm{u}, \mathrm{v})$ in the space $\widehat{K^{-3/2}(R^2)}$ to have the representation

$$\varphi(\dot{B}) = \int F(\mathrm{u}, \mathrm{v}) \, : \dot{B}(\mathrm{u})\dot{B}(\mathrm{v}) : \mathrm{du}\, \mathrm{dv}. \tag{3.4}$$

where the notation **: - :** may be considered as the Wick product, i.e. renormalized product.

In the above we have obtained the Gel'fand triple

$$H_2^{(2)} \grave{I}\ H_2 \grave{I}\ H_2^{(-2)}, \tag{3.5}$$

where

$$H_2^{(2)} = \{\int\int_{I^2} F(u,v) : \dot{B}(u)\dot{B}(v) : du\ dv\}, F \in \widehat{K^{3/2}(I^2)} \tag{3.6}$$

$$H_2 = \{\int\int_{I^2} F(u,v) : \dot{B}(u)\dot{B}(v) : du\ dv\}, F \in \widehat{K^{2}(I^2)} \tag{3.7}$$

and the quadratic Hida distribution space

$$H_2^{(-2)} = \{\int\int_{t^2} F(u,v) : \dot{B}(u)\dot{B}(v) : du\ dv\}, F \in \widehat{K^{3/2}(I^2)}, \tag{3.8}$$

where $\hat{\cdot}$ means symmetric.

It can be seen that the space $H_2^{(-2)}$ is the space of quadratic functionals of $\dot{B}(t)$, $t \in I = [0, 1]$. We define the new subspace of $H_2^{(-2)}$:

$$H_2^{(-2,1)} = \{\int_I f(u) : \dot{B}(u)^2 : du, f \in L^2(I)\} \tag{3.9}$$

The function f is viewed as $f(\frac{u+v}{2})\,\delta(u-v) \equiv f(u)$.

$$(\int_I f(u) : \dot{B}(u)^2 : du,\ \int_I g(u) : \dot{B}(u)^2 : du) = (f, g)_{K^{-3/2}} \tag{3.10}$$

The null space of $H_2^{(-2,\,1)}$ is $\{0\}$. Hence the Hilbert space $H_2^{(-2,\,1)}$ is defined as a subspace of $H_2^{(-2)}$. We now introduce a new vector space in a formal expression

$$H_2^{(-2,\,2)} = \{\int_I g(u) : \dot{B}(u)^2 : du^2, g \in L^2(I)\}. \tag{3.11}$$

Here we give the interpretation of the elements in $H_2^{(-2,\,2)}$ as follows.

$$\left|\Delta_{2k}^{(n+1)}\right| = \left|\Delta_{2k+1}^{(n+1)}\right| = 2^{-(n+1)}\ \left(= \Delta^{(n+1)}\right),$$

We consider the following conditional expectation.

$$E\left(: \left(\frac{\Delta_{2k}^{(n+1)} B}{\sqrt{\Delta^{(n+1)}}}\right)^2 : + : \left(\frac{\Delta_{2k+1}^{(n+1)} B}{\sqrt{\Delta^{(n+1)}}}\right)^2 : \middle| : \left(\frac{\Delta_{k}^{(n)} B}{\sqrt{\Delta^{(n)}}}\right)^2 : \right) = : \left(\frac{\Delta_{k}^{(n)} B}{\sqrt{\Delta^{(n)}}}\right)^2 :$$

Then we can prove that

$$E\left[\sum_k \left(: \left(\frac{\Delta_{2k}^{(n+1)} B}{\sqrt{\Delta^{(n+1)}}}\right)^2 : + : \left(\frac{\Delta_{2k+1}^{(n+1)} B}{\sqrt{\Delta^{(n+1)}}}\right)^2 : \middle| : \frac{\left(\Delta_{k}^{(n)} B\right)^2}{\Delta^{(n)}} :, 0 \le k \le 2^n \right) \right]$$

$$= \sum_k E\left[\left(:\left(\frac{\Delta_{2k}^{(n+1)}B}{\sqrt{\Delta^{(n+1)}}}\right)^2 : + :\left(\frac{\Delta_{2k+1}^{(n+1)}B}{\sqrt{\Delta^{(n+1)}}}\right)^2 : \right| : \frac{\left(\Delta_k^{(n)}B\right)^2}{\Delta^{(n)}} : \right)\Bigg]$$

$$= \sum_k :\left(\frac{\Delta_k^{(n)}B}{\sqrt{\Delta^{(n)}}}\right)^2 :$$

Hence

$$E\left[\sum_k \; : \frac{\left(\Delta_k^{(n+1)}B\right)^2}{\Delta^{(n+1)}} : \Delta^{(n+1)} \;\middle|\; :\left(\Delta_k^{(n)}B\right)^2 :, 0 \le k \le 2^n - 1\right]$$

$$= \sum_k :\left(\frac{\Delta_k^{(n)}B}{\Delta^{(n)}}\right)^2 : \Delta^{(n)}$$

Thus, for the quadratic form $\sum_j :\left(\frac{\Delta_j^{(n)}B}{\Delta^{(n)}}\right)^2 : \Delta^{(n)}$, we obtain its average

$$\frac{1}{2^n}\sum_j :\left(\frac{\Delta_j^{(n)}B}{\Delta^{(n)}}\right)^2 : \Delta^{(n)} = \sum_j :\left(\frac{\Delta_j^{(n)}B}{\Delta^{(n)}}\right)^2 : \left(\Delta^{(n)}\right)^2 \qquad (3.12)$$

is consistent in n by the projection which is realized by the conditional expectation $E(\cdot|B_n)$ where B_n is generated by $:\left(\Delta_j^{(n)}B\right)^2 :$, $0 \le j \le 2n-1$. Thus $\int_I : \dot{B}(t)^2 : (dt)^2$ is its limit.

The topology is to be introduced below so that the space $H_2^{(-2,\,2)}$ has meaning. Let us set

$$dz(u) = \tfrac{1}{\sqrt{2}} : \dot{B}(u)^2 : (du)^{\frac{3}{2}} \qquad (3.13)$$

Thus, we have a system of independent (hence orthogonal) infinitesimal random variables and $E\,(: \dot{B}(u)^2 :^2) = \frac{2}{(du)^2}$.

Thus, $dz(u)$ is a random measure with $E(\,|\,dz(u)\,|^2\,) = du$ and we can define a Hilbert space L spanned by $\left\{ \int f(u)dz(u) \right\}$.

The bilinear form $\ll\cdot,\,\cdot\gg$ which connects the two spaces $H_2^{(-2,\,1)}$ and $H_2^{(-2,\,2)}$ is given by the Hilbertian norm $\langle\cdot\,,\,\cdot\rangle$ with respect to the random measure $dz(u)$ as in the following manner.

For the two functionals $\varphi \in \mathrm{H}_2^{(-2,\,1)}$ and in $\mathrm{H}_2^{(-2,\,2)}$ such that

$$\varphi = \int f(\mathrm{u}) : \dot{B}(u)^2 : \mathrm{du}$$

and

$$\psi = \int g(\mathrm{u}) : \dot{B}(u)^2 : (\mathrm{du})^2,$$

$$\ll \varphi, \psi \gg = \langle \int f(\mathrm{u}) : \dot{B}(u)^2 : \mathrm{du},\ \int g(\mathrm{u}) : \dot{B}(u)^2 : (\mathrm{du})^2 \rangle_{\mu}$$

$$= \langle \int f(\mathrm{u}) : \dot{B}(u)^2 : (\mathrm{du})^{\frac{3}{2}},\ \int g(\mathrm{u}) : \dot{B}(u)^2 : (\mathrm{du})^{\frac{3}{2}} \rangle_{\mu}$$

$$= E[(\int f(\mathrm{u}) : \dot{B}(u)^2 : (\mathrm{du})^{\frac{3}{2}})(\int g(\mathrm{u}) : \dot{B}(u)^2 : (\mathrm{du})^{\frac{3}{2}})]$$

$$= \int f(\mathrm{u}) g(\mathrm{u})\, \mathrm{du}$$

since $E\,(: \dot{B}(u)^2 :^2) = \frac{2}{(\mathrm{du})^2}$. We denote $\ll \varphi, \psi \gg$ by $f(\varphi)$. Thus f_{ψ} is a continuous linear functional defined on $\mathrm{H}_2^{(-2,\,1)}$.

3.1 Theorem $\mathrm{H}_2^{(-2,\,1)}$ and $\mathrm{H}_2^{(-2,\,2)}$ are the dual pair with respect to random measure d$z(u)$.

4 The adjoint of the Lévy Laplacian Δ_L

An infinite dimensional Lévy Laplacian (Ref.[1], [3])

$$\lim_{\mathrm{n}\to\infty} \frac{1}{\mathrm{n}} \sum_{1}^{\infty} \frac{\partial^2}{\partial \xi_{\mathrm{i}}^2}$$

is rephrased in terms of the ∂t (H – H Kuo)

$$\Delta_L = \int \partial_{\mathrm{t}}^2 (\mathrm{dt})^2 \,. \tag{4.1}$$

We define the operator

$$\Delta_L^* = \int (\partial_{\mathrm{t}}^*)^2 (\mathrm{dt})^2 \tag{4.2}$$

in a formal expression. Any member of $\mathrm{H}_2^{(-2,\,1)}$ is in the domain of Δ_L.

4.1 Theorem By the above relation with the choice of the dual pair in the previous section, Δ_L^* can be understood as the adjoint of the Lévy Laplacian Δ_L.

Proof. Since any member of $\mathrm{H}_2^{(-2,\,1)}$ is in the domain of Δ_L, we take

$$\varphi = \int f(u) : \dot{B}(u) :^2 \mathrm{d}u \in \mathrm{H}_2^{(-2,\,1)}.$$

Take the *S*-transform we obtain the *U*-functional, we have

$$U(\xi) = \int f(u)\, \xi(u)^2 \mathrm{d}u.$$

In Example 2.2, we have obtained

$$U''(\xi, t) = 2 f(t) \frac{1}{\mathrm{d}t}.$$

Taking back S^{-1}-transform

$$\partial_{\mathrm{t}}^2 \varphi = 2 f(t) \frac{1}{\mathrm{d}t}$$

$$\Delta_L \varphi = \int \partial_{\mathrm{t}}^2 \varphi\, (\mathrm{d}t)^2$$

$$= \int 2 f(t)\, \mathrm{d}t$$

$$= \text{constant}$$

On the other hand we take

$$\psi = \int g(u) : \dot{B}(u)^2 : (\mathrm{d}u)^2 \ \in \mathrm{H}_2^{(-2,\,2)} \tag{4.3}$$

then

$$\Delta_L^* = \int_I (\partial_{\mathrm{t}}^*)^2 (\mathrm{d}t)^2 \int g(u) : \dot{B}(u)^2 : (\mathrm{d}u)^2 \tag{4.4}$$

Therefore

$$\langle \Delta_L \varphi, \psi \rangle = \langle 2 \int f(u)\, \mathrm{d}u\, ,\, \int g(u) : \dot{B}(u)^2 : (\mathrm{d}u)^2 \rangle_\mu$$

$$= E[\, 2 \int f(u)\, \mathrm{d}u \int g(u) : \dot{B}(u)^2 : (\mathrm{d}u)^2\,]$$

$$= 2 \int f(u)\, \mathrm{d}u \int g(u) \frac{1}{\mathrm{d}u}\, (\mathrm{d}u)^2$$

$$= 2 \int f(u)\, \mathrm{d}u \int g(u)\, \mathrm{d}u \tag{4.5}$$

On the other hand

$$\langle \varphi,\ \Delta_L^* \psi \rangle = \langle \int f(u) : \dot{B}(u)^2 : \mathrm{d}u\, ,\ \Delta_L^* \int g(u) : \dot{B}(u)^2 : (\mathrm{d}u)^2 \rangle_\mu$$

$$= E[\int f(u) : \dot{B}(u)^2 : \mathrm{d}u \int (\partial_{\mathrm{u}}^*)^2 \left(\mathrm{d}u\right)^2 \int g(u) : \dot{B}(u)^2 : (\mathrm{d}u)^2\,]$$

$$= E[\int f(u) : \dot{B}(u)^2 : \mathrm{d}u \int : \dot{B}(u)^2 : \left(\mathrm{d}u\right)^2 \int g(u) : \dot{B}(u)^2 : (\mathrm{d}u)^2\,]$$

$$= \int f(u)\, \mathrm{d}u \frac{1}{\mathrm{d}u} \int g(u)\ \frac{2}{(\mathrm{d}u)^2}\ (\mathrm{d}u)^4$$

$$= 2 \int f(u)\, \mathrm{d}u \int g(u)\, \mathrm{d}u \qquad (4.6)$$

The equations (4.5) and (4.6) gives

$\langle \Delta_L \varphi, \psi \rangle = \langle \varphi, \Delta_L^* \psi \rangle$.

Thus, the assertion is proved.

Conclusion

In this paper we have constructed the dual pair which are the subspaces $\mathrm{H}_2^{(-2,\,1)}$ and $\mathrm{H}_2^{(-2,\,2)}$ of the space H_2 of quadratic white noise functionals. Consequently the adjoint of Lévy Laplacian can also be constructed.

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VERTEX-TO-VERTEX MEDIAN AND VERTEX-TO-EDGE MEDIAN OF A DOUBLE LOLLIPOP

Theint Pa Pa Lin[1], Cho Kyi Than[2]

Abstract

In this paper, we describe some definitions and results on the vertex-to-vertex medians and the vertex-to-edge medians of general graphs and some particular graphs. Then we introduce the definition of a double lollipop which is a particular type of a bicyclic graph and investigate the structures of the vertex-to-vertex median and the vertex-to-edge median of a double lollipop.

Keywords: vertex-to-vertex median, vertex-to-edge median, bicyclic graph, double lollipop.

1. Some Graph Theoretic Terms and Notations

We first introduce some graph theoretic terms and notations which are used in this paper.

A ***graph*** $G=(V(G),E(G))$ consists of a nonempty finite set $V(G)$ of ***vertices*** and a finite set $E(G)$ of ***edges*** where $E(G)$ is disjoint from $V(G)$ and each edge of $E(G)$ corresponds to an unordered pair of (not necessarily distinct) vertices of $V(G)$. If an edge $e\in E(G)$ corresponds to an unordered pair $\{u, v\}$ of two vertices in $V(G)$, we write $e=uv$ or $e=vu$; and we say that e ***joins*** u and v; and we also say that u and v are ***adjacent***; e is ***incident*** with u and v; and the vertices u and v are called the ***ends*** of e. An edge with identical ends is called a ***loop*** and an edge with distinct ends is a ***link***. If two edges e and f join the same pair of vertices, then e and f are called ***parallel edges***. A graph is said to be ***simple*** if it contains no loops and no parallel edges. Throughout this paper we will consider only simple graphs. A simple graph in which each pair of distinct vertices is joined by an edge is called a ***complete graph***. A complete graph on n vertices is denoted by K_n. A ***walk*** in a graph G is a finite sequence $W=v_0e_1v_1e_2v_2\cdots e_kv_k$ whose terms are alternately vertices and edges, such that, for $1\le i\le k$, the ends of e_i are v_{i-1} and v_i.The vertices v_0 and v_k are called the ***origin*** and ***terminus*** of W respectively, and $v_1,v_2,\cdots,v_{k-1}$, its ***internal vertices***. If it does not lead to confusion, we will simply denote the walk W by the sequence $v_0v_1v_2\cdots v_k$ of its vertices. The ***length*** of a walk is the number of edges appearing in it, and so the walk given above has length k. If the edges $e_1,e_2,\cdots,e_k$ of a walk W are distinct, W is called a ***trail***. If, in addition, $v_0,v_1,v_2,\cdots,v_k$ are distinct, W is called a ***path***. A walk (respectively a path) with origin u and terminus v is called a (u, v)-***walk*** (respectively a (u, v)-***path***). A graph G is called ***connected*** if for any two vertices u and v in G there is a (u, v)-path, otherwise G is ***disconnected.*** A ***subgraph*** of a graph $G=(V(G),E(G))$ is a graph $H=(V(H),E(H))$ with $V(H)\subseteq V(G)$ and $E(H)\subseteq E(G)$ and it is written by $H\subseteq G$. If $H\subseteq G$ but $H\neq G$, we write $H\subset G$ and it is called a ***proper subgraph*** of G. Suppose that V' is a nonempty subset of $V(G)$. The subgraph of G whose vertex set is V' and whose edge set is the set of those edges of G that have both ends in V' is called the subgraph of G ***induced*** by V' and is denoted by $G[V']$; and we say that $G[V']$ is an ***induced subgraph*** of G. A maximal connected subgraph of G is called a

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component of *G*. A walk $v_0 e_1 v_1 e_2 \cdots e_k v_k e_{k+1} v_0$ of a graph *G* is a ***cycle*** if $k \geq 2$ and all the vertices $v_0, v_1, v_2, \cdots, v_k$ are distinct. A connected graph without a cycle is called a ***tree***. A vertex *v* of a graph *G* is a ***cut vertex*** if $G - v$ has more components than *G* where $G - v$ means the graph obtained from *G* by deleting the vertex *v* and all edges incident with *v*. A connected graph without a cut vertex is called a ***block***. A ***block of a graph*** *G* is a subgraph of *G* which is a block and is maximal with respect to this property. An edge *e* of a graph *G* which is a ***cut edge*** if $G - e$ has more components than *G* where $G - e$ means the graph obtained from *G* by deleting the edge *e*.

2. The Vertex-to-Vertex Median of a Graph

In this section we give the definition of the vertex-to-vertex median of a graph and state known results on it.

2.1 Definitions. Let *G* be a connected graph with the vertex set $V(G)$ and the edge set $E(G)$. If *u* and *v* are two vertices in *G*, the ***vertex-to-vertex distance*** between *u* and *v* is denoted by $d(u,v)$ and defined as the length of a shortest path joining them. The ***vertex-to-vertex distance sum*** $s(v)$ of a vertex *v* of *G* is

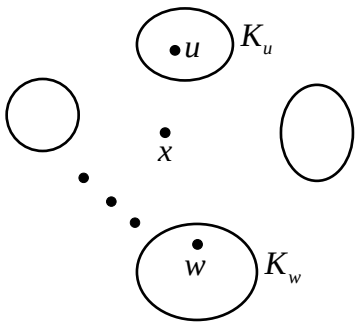
$$s(v) = \sum_{u \in V(G)} d(v, u).$$

The subgraph of G induced by the set of all vertices of G with minimum vertex-to-vertex distance sum is called the ***vertex-to-vertex median*** of G and is denoted by $M(G)$.

An interesting result on the structure of the vertex-to-vertex median of a connected graph is given below.

2.2 Theorem. If *G* is a connected graph, all vertices of the vertex-to-vertex median *M*(*G*) of *G* lie in the same block of *G*.

Proof. To prove the theorem by contradiction, suppose that there exist two vertices *u* and *w* of *M*(*G*) lying in distinct blocks of *G*. This implies that there exists a cut vertex *x* of *G* such that *u* and *w* lie in distinct components of $G - x$. Let K_u and K_w be the components of $G - x$ containing the vertex *u* and *w* respectively.



Suppose that

(1) $\quad |V(K_u)| < \frac{1}{2}|V(G)|.$

It is obvious that

$d(x, v) \leq d(x, u) + d(u, v)$ for each vertex v in K_u,

that is,

(2) $d(x, v) \leq d(u, v) + d(x, u)$

and

$d(u, v) = d(u, x) + d(x, v)$ for every vertex v not in K_u,

that is,

(3) $d(x, v) = d(u, v) - d(x, u)$.

Therefore

$$s(x) = \sum_{v \in V(G)} d(x, v)$$

$$= \sum_{v \in V(K_u)} d(x, v) + \sum_{v \notin V(K_u)} d(x, v)$$

and by using (2) and (3),

$$s(x) \leq \sum_{v \in V(K_u)} (d(u, v) + d(x, u)) + \sum_{v \notin V(K_u)} (d(u, v) - d(x, u))$$

$$= \sum_{v \in V(G)} d(u, v) + d(x, u)\left[|V(K_u)| - (|V(G)| - |V(K_u)|)\right]$$

(4) $$= s(u) + d(x, u)\left[2|V(K_u)| - |V(G)|\right].$$

From (1) and (4), we obtain

$s(x) < s(u)$

and this is a contradiction to the fact that u is a vertex in $M(G)$. Therefore our assumption (1) is false and hence

(5) $|V(K_u)| \geq \frac{1}{2}|V(G)|$.

Similarly,

(6) $|V(K_w)| \geq \frac{1}{2}|V(G)|$.

By (5) and (6),

$|V(K_u)| + |V(K_w)| \geq |V(G)|$

and this is impossible since

$V(K_u) \cup V(K_w) \cup \{x\} \subseteq V(G)$.

Thus there do not exist two vertices *u* and *w* of *M*(*G*) lying in distinct blocks of *G*, and this means that all vertices of *M*(*G*) lie in a block of *G*.

2.3 Corollary. If *T* is a tree, then the vertex set of the vertex-to-vertex median *M*(*T*) of *T* consists of one vertex or two adjacent vertices.

Proof. The corollary easily follows from Theorem 2.2 and the fact that each block of a tree *T* is an edge.

2.4 Definitions. A connected graph containing exactly one cycle is called a ***unicyclic graph***. A connected graph containing exactly two cycles is called a ***bicyclic graph***.

2.5 Corollary. Let *G* be a unicyclic graph containing a cycle *C*. Then the vertex set of the vertex-to-vertex median *M*(*G*) of *G* consists of one vertex or two adjacent vertices or some vertices of the cycle *C*.

Proof. The corollary easily follows from Theorem 2.2 and the fact that a block of the unicyclic graph *G* is either an edge (not in *C*) or the cycle *C*.

2.6 Corollary. Let *G* be a bicyclic graph containing two cycles C_1 and C_2. Then the vertex set of the vertex-to-vertex median *M*(*G*) of *G* consists of one vertex or two adjacent vertices or some vertices of C_1 or some vertices of C_2.

Proof. The corollary easily follows from Theorem 2.2 and the fact that a block of the bicyclic graph *G* is an edge (not in C_1 or C_2) or the cycle C_1 or the cycle C_2.

3. The Vertex-to-Edge Median of a Graph

In this section we describe the concept of the vertex-to-edge median of a graph introduced by Santhakumaran.

3.1 Definitions. Let *G* be a connected graph with the vertex set $V(G)$ and the edge set $E(G)$. If *v* is a vertex and $f = xy$ is an edge of *G*, the ***vertex-to-edge distance*** between *v* and *f* is denoted by $d(v, f)$ and defined as

$$d(v, f) = \min\{d(v, x), d(v, y)\}.$$

The ***vertex-to-edge distance sum*** $s_1(v)$ of a vertex *v* in *G* is

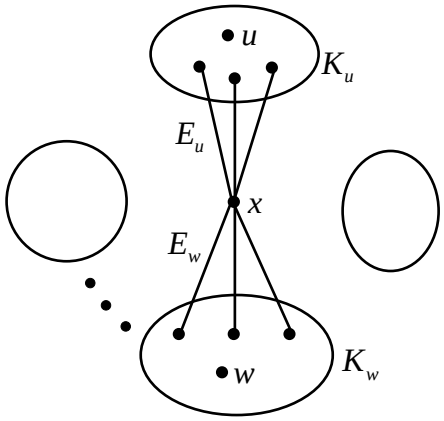
$$s_1(v) = \sum_{e \in E(G)} d(v, e).$$

The subgraph of *G* induced by the set of all vertices of *G* with minimum vertex-to-edge distance sum is called the ***vertex-to-edge median*** of *G* and is denoted by $M_1(G)$.

The next theorem on the structure of the vertex-to-edge median of a connected graph is a counter path of Theorem 2.2.

3.2 Theorem. If *G* is a connected graph, all vertices of the vertex-to-edge median $M_1(G)$ of *G* lie in the same block of *G*.

Proof. To prove the theorem by contradiction, suppose that there exist two vertices *u* and *w* of $M_1(G)$ lying in distinct blocks of *G*. This implies that there exists a cut vertex *x* of *G* such that *u* and *w* lie in distinct components of $G-x$.



Let K_u and K_w be the components of $G-x$ containing the vertex *u* and *w* respectively.

Let the edge sets E_u and E_w be defined by

$$E_u = \{\, xy : \; y \in V(K_u)\},$$

$$E_w = \{\, xy : \; y \in V(K_w)\}.$$

We also define the edge sets E'_u and E'_w as follows:

$$E'_u = E_u \bigcup E(K_u),$$

$$E'_w = E_w \bigcup E(K_w).$$

Suppose that

(1) $$|E'_u| \le \frac{1}{2}|E(G)|.$$

It is easy to see that

(2) $d(x,e) \le d(x,u) + d(u,e)$ for each edge *e* in $E(K_u)$,

(3) $d(x,e) < d(x,u) + d(u,e)$ since $d(x,e) = 0$,

and

$d(u,e) = d(u,x) + d(x,e)$ for each edge *e* in $E(G) \setminus (E(K_u) \bigcup E_u) = E(G) \setminus E'_u$,

that is,

(4) $$d(x,e) = d(u,e) - d(x,u).$$

Now

$$s_1(x) = \sum_{e \in E(G)} d(x,e)$$

$$= \sum_{e \in E'_u} d(x,e) + \sum_{e \in E(G) \setminus E'_u} d(x,e)$$

and by using (2), (3) and (4), we get

$$s_1(x) < \sum_{e \in E'_u} \left[d(x,u) + d(u,e) \right] + \sum_{e \in E(G) \setminus E'_u} \left[d(u,e) - d(x,u) \right]$$

$$= \sum_{e \in E(G)} d(u,e) + d(x,u) \left[\left| E'_u \right| - \left| E(G) \setminus E'_u \right| \right]$$

$$= s_1(u) + d(x,u) \left[2\left| E'_u \right| - \left| E(G) \right| \right]$$

$$\leq s_1(u)$$

by virtue of our assumption (1) and we have a contradiction to the fact that *u* is a vertex of $M_1(G)$. Therefore our assumption (1) must be false and we must have

$$(5) \qquad \left| E'_u \right| > \frac{1}{2} \left| E(G) \right|.$$

Similarly, we have

$$(6) \qquad \left| E'_w \right| > \frac{1}{2} \left| E(G) \right|$$

and by combining (5) and (6), we obtain

$$\left| E'_u \right| + \left| E'_w \right| > \left| E(G) \right|.$$

This is impossible since

$$E'_u \bigcap E'_w = \varnothing \text{ and } E'_u \bigcup E'_w \subseteq E(G).$$

So the vertices *u* and *w* cannot lie in distinct blocks of *G*. This means that all vertices of $M_1(G)$ lie in the same block of *G* and the theorem is proved.

3.3 Corollary. If *T* is a tree, then the vertex set of the vertex-to-edge median $M_1(T)$ of *T* consists of one vertex or two adjacent vertices.

Proof. The corollary easily follows from Theorem 3.2 and the fact that each block of a tree *T* is an edge.

3.4 Corollary. Let *G* be a unicyclic graph containing a cycle *C*. Then the vertex set of the vertex-to-edge median $M_1(G)$ of *G* consists of one vertex or two adjacent vertices or some vertices of the cycle *C*.

Proof. The corollary easily follows from Theorem 3.2 and the fact that a block of the unicyclic graph *G* is either an edge (not in *C*) or the cycle *C* in *G*.

3.5 Corollary. If *G* is a bicyclic graph containing exactly two cycles C_1 and C_2, then the vertex set of the vertex-to-edge median $M_1(G)$ of *G* consists of one vertex or two adjacent vertices or some vertices of C_1 or some vertices of C_2.

Proof. The corollary easily follows from Theorem 3.2 and the fact that a block of the bicyclic graph *G* is an edge (not in C_1 or C_2) or the cycle C_1 or the cycle C_2.

4. Vertex-to-Vertex Median and Vertex-to-Edge Median of a Double Lollipop

In this section, we investigate the structures of the vertex-to-vertex median and the vertex-to-edge median of a double lollipop which is a particular type of a bicyclic graph.

4.1 Definition. Let *G* be a connected graph with the vertex set $V(G)=\{x_1, x_2, x_3, \ldots, x_{m_1}, z_1, z_2, z_3, \ldots, z_n, y_1, y_2, y_3, ..., y_{m_2}\}$ and the edge set $E(G)=\{x_1x_2, x_2x_3, x_3x_4, \ldots, x_{m_1}x_1, x_1z_1, z_1z_2, \ldots, z_{n-1}z_n, z_ny_1, y_1y_2, y_2y_3, \ldots, y_{m_2}y_1\}$, where m_1, m_2 and *n* are positive integers with $m_1 \geq 3$, $m_2 \geq 3$ and $n \geq 1$, which consists of two cycles $C_1 = x_1\, x_2 \ldots x_{m_1}\, x_1$, $C_2 = y_1\, y_2 \ldots y_{m_2}\, y_1$ and a path $P = x_1\, z_1\, z_2 \ldots z_n\, y_1$. Then *G* is called a ***double lollipop***, see Fig. 1.

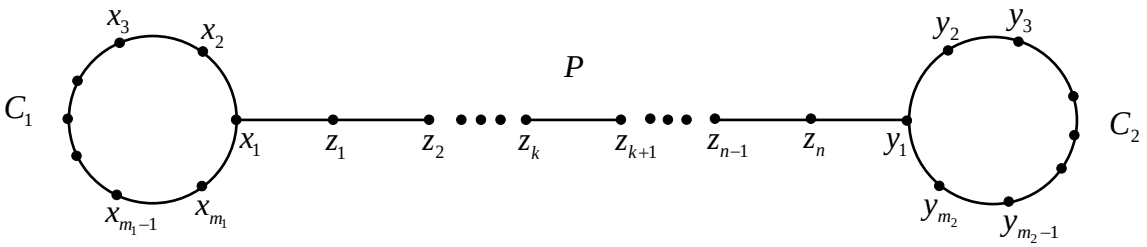


Figure 1

In investigating the structure of the vertex-to-vertex median and the vertex-to-edge median of a double lollipop, the following two propositions and two lemmas are useful.

4.2 Proposition. Let *G* be a connected graph, xy be a cut edge of *G*, and G_x and G_y be the components of $G - xy$ containing the vertex *x* and *y* respectively. Then

(1) $s(x) < s(y) \Leftrightarrow |V(G_x)| > |V(G_y)|$,

(2) $s(x) = s(y) \Leftrightarrow |V(G_x)| = |V(G_y)|$.

Proof. We observe that

$d(x, v) = d(y, v) - 1$ for each $v \in V(G_x)$

and

$d(x, v) = d(y, v) + 1$ for each $v \in V(G_y)$.

Therefore

$$s(x) = \sum_{v \in V(G)} d(x, v)$$

$$= \sum_{v \in V(G_x)} d(x, v) + \sum_{v \in V(G_y)} d(x, v)$$

$$= \sum_{v \in V(G_x)} (d(y, v) - 1) + \sum_{v \in V(G_y)} (d(y, v) + 1)$$

$$= \sum_{v \in V(G_x)} d(y, v) - |V(G_x)| + \sum_{v \in V(G_y)} d(y, v) + |V(G_y)|$$

$$= \sum_{v \in V(G)} d(y, v) + |V(G_y)| - |V(G_x)|.$$

So

(3) $\quad s(x) = s(y) + |V(G_y)| - |V(G_x)|.$

Now it immediately follows from Equation (3) that

$$s(x) < s(y) \Leftrightarrow |V(G_x)| > |V(G_y)|$$

and

$$s(x) = s(y) \Leftrightarrow |V(G_x)| = |V(G_y)|.$$

4.3 Proposition. Let G be a connected graph, $e^* = xy$ be a cut edge of G, and G_x and G_y be the components of $G - xy$ containing the vertex x and y respectively. Then

(1) $\quad s_1(x) < s_1(y) \Leftrightarrow |E(G_x)| > |E(G_y)|,$

(2) $\quad s_1(x) = s_1(y) \Leftrightarrow |E(G_x)| = |E(G_y)|.$

Proof. We obverse that

$$d(x, e) = d(y, e) - 1 \text{ for each } e \in E(G_x),$$

$$d(x, e) = d(y, e) + 1 \text{ for each } e \in E(G_y)$$

and

$$d(x, e^*) = 0 = d(y, e^*).$$

Therefore

$$s_1(x) = \sum_{e \in E(G)} d(x, e)$$

$$= d(x, e^*) + \sum_{e \in E(G_x)} d(x, e) + \sum_{e \in E(G_y)} d(x, e)$$

$$= d(y, e^*) + \sum_{e \in E(G_x)} (d(y, e) - 1) + \sum_{e \in E(G_y)} (d(y, e) + 1)$$

$$= d(y,e^*) + \sum_{e \in E(G_x)} d(y,e) - |E(G_x)| + \sum_{e \in E(G_y)} d(y,e) + |E(G_y)|.$$

So

(3) $s_1(x) = s_1(y) - |E(G_x)| + |E(G_y)|.$

Now it immediately follows from Equation (3) that

$$s_1(x) < s_1(y) \Leftrightarrow |E(G_x)| > |E(G_y)|$$

and

$$s_1(x) = s_1(y) \Leftrightarrow |E(G_x)| = |E(G_y)|.$$

4.4 Lemma. Let G be a double lollipop given in Definition 4.1. For any i with $2 \le i \le m_1$

(1) $s(x_i) > s(x_1)$,

(2) $s_1(x_i) > s_1(x_1)$.

Proof. (1) It is not difficult to see that

$$\sum_{j=1}^{m_1} d(x_i, x_j) = \sum_{j=1}^{m_1} d(x_1, x_j)$$

and that

$$d(x_i, v) > d(x_1, v)$$

for any vertex $v \in V(G) \setminus V(C_1)$.

It follows from the above equation and inequality that

$$s(x_i) > s(x_1).$$

(2) It is clear that

$$\sum_{e \in E(C_1)} d(x_i, e) = \sum_{e \in E(C_1)} d(x_1, e)$$

and that

$$d(x_i, e) > d(x_1, e)$$

for any edge $e \in E(G) \setminus \mathrm{E}(C_1)$.

It follows from the above equation and inequality that

$$s_1(x_i) > s_1(x_1).$$

Similarly we can prove the following lemma.

4.5 Lemma. Let G be a double lollipop given in Definition 4.1. For any i with $2 \le i \le m_2$

(1) $s(y_i) > s(y_1)$,

(2) $s_1(y_i) > s_1(y_1)$.

By using the above propositions and lemmas, we can now derive some results on the vertex-to-vertex median and the vertex-to-edge median of a double lollipop.

4.6 Theorem. Let G be a double lollipop given in Definition 4.1. Suppose that $m_1 > m_2 + n$. Then $V(M(G)) = V(M_1(G)) = \{x_1\}$.

Proof. Since $m_1 > m_2 + n$, it follows from Propositions 4.2 and 4.3 that

$$s(x_1) < s(z_1) < s(z_2) < \cdots < s(z_n) < s(y_1),$$

$$s_1(x_1) < s_1(z_1) < s_1(z_2) < \cdots < s_1(z_n) < s_1(y_1).$$

From these inequalities, Lemma 4.4 and Lemma 4.5, it follows that

$$V(M(G)) = V(M_1(G)) = \{x_1\}.$$

Similarly we can prove the next theorem.

4.7 Theorem. Let G be a double lollipop given in Definition 4.1. Suppose that $m_2 > m_1 + n$. Then $V(M(G)) = V(M_1(G)) = \{y_1\}$.

4.8 Theorem. Let G be a double lollipop given in Definition 4.1. Suppose that $m_1 = m_2 + n$. Then $V(M(G)) = V(M_1(G)) = \{x_1, z_1\}$.

Proof. Since $m_1 = m_2 + n$, it follows from Propositions 4.2 and 4.3 that

$$s(x_1) = s(z_1) < s(z_2) < \cdots < s(z_n) < s(y_1),$$

$$s_1(x_1) = s_1(z_1) < s_1(z_2) < \cdots < s_1(z_n) < s_1(y_1).$$

From these inequalities, Lemma 4.4 and Lemma 4.5, it follows that

$$V(M(G)) = V(M_1(G)) = \{x_1, z_1\}.$$

Similarly we can prove the following theorem.

4.9 Theorem. Let G be a double lollipop given in Definition 4.1. Suppose that $m_2 = m_1 + n$. Then $V(M(G)) = V(M_1(G)) = \{z_n, y_1\}$.

4.10 Theorem. Let G be a double lollipop given in Definition 4.1. Let $m_1 < m_2 + n$, $m_2 < m_1 + n$ and $m_1 + m_2 + n$ is even. Then

$$V(M(G)) = V(M_1(G)) = \{z_k, z_{k+1}\}$$

where $k = \dfrac{m_1 + m_2 + n}{2} - m_1.$

Proof. By our choice of *k* we have

$$\left|V(G_{z_k})\right| = \left|V(G_{z_{k+1}})\right| = \frac{m_1 + m_2 + n}{2},$$

$$\left|E(G_{z_k})\right| = \left|E(G_{z_{k+1}})\right| = \frac{m_1 + m_2 + n}{2}.$$

Therefore by Propositions 4.2 and 4.3, we obtain

$$s(x_1) > s(z_1) > s(z_2) > \cdots > s(z_k) = s(z_{k+1}) < s(z_{k+2}) < \cdots < s(z_n) < s(y_1)$$

and

$$s_1(x_1) > s_1(z_1) > s_1(z_2) > \cdots > s_1(z_k) = s_1(z_{k+1}) < s_1(z_{k+2}) < \cdots < s_1(z_n) < s_1(y_1).$$

From these relations, Lemma 4.4 and Lemma 4.5, it follows that

$$V(M(G)) = V(M_1(G)) = \{z_k, z_{k+1}\}.$$

4.11 Theorem. Let *G* be a double lollipop given in Definition 4.1. Suppose that $m_1 < m_2 + n$, $m_2 < m_1 + n$ and $m_1 + m_2 + n$ is odd. Then

$$V(M(G)) = V(M_1(G)) = \{z_k\}$$

where $k = \dfrac{m_1 + m_2 + n + 1}{2} - m_1.$

Proof. From our choice of *k* and Propositions 4.2 and 4.3, it follows that

$$s(x_1) > s(z_1) > s(z_2) > \cdots > s(z_{k-1}) > s(z_k) < s(z_{k+1}) < s(z_{k+2}) < \cdots < s(z_n) < s(y_1)$$

and

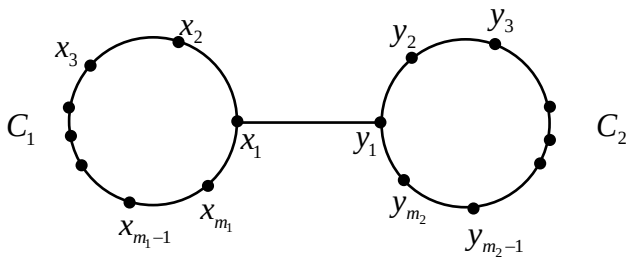
$$s_1(x_1) > s_1(z_1) > s_1(z_2) > \cdots > s_1(z_{k-1}) > s_1(z_k) < s_1(z_{k+1}) < s_1(z_{k+2}) < \cdots < s_1(z_n) < s_1(y_1).$$

From these relations, Lemma 4.4 and Lemma 4.5, imply that

$$V(M(G)) = V(M_1(G)) = \{z_k\}.$$

This completes the investigation of the structures of the vertex-to-vertex medians and vertex-to-edge medians of double lollipops.

4.12 Remark. Our definition of a double lollipop does not include the following graph.



However, by using similar arguments we have applied above we can prove the following.

(1) If $m_1 > m_2$, then

$$V(M(G)) = V(M_1(G)) = \{x_1\}.$$

(2) If $m_2 > m_1$, then

$$V(M(G)) = V(M_1(G)) = \{y_1\}.$$

(3) If $m_1 = m_2$, then

$$V(M(G)) = V(M_1(G)) = \{x_1, y_1\}.$$

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A LEARNER AIDS SYSTEM FOR IMPROVING THE ACCESS OF ONLINE LEARNING*

Htway Htway Khaing[1], Nem Khan Dim[2], Soe Mya Mya Aye[3]

Abstract

An increasing number of institutions are incorporating online courses and degrees into their curriculum. Online learning offers benefits which traditional learning does not, including flexibility, accessibility, and increased interaction with instructors and fellow students. Successful learning requires students to be motivated to achieve the desired learning goals. But most of the online learning environment lack of the interaction, adaptation and communication of learners with lectures contents. This paper introduces interactive aids and accessibility features to provide students with learning aids that will increases their motivation in the learning process. Learning aids and accessibility features implemented in the paper are helpful for both ordinary students and especially for students with visual impairments.

Keywords: Learning aids system, blind learning system, Accessibility, Online Learning

Introduction

Technology has become the key to a new world of education. The rapid improvement of computer and Internet technologies has dramatically increased the ways of teaching and learning. People learn in different ways. It is important to be aware of the differences between learners. New delivery mechanisms are required, including online, open and distance learning. These issues can be partially resolved by providing student-centered, self-paced, highly interactive teaching materials and introducing automatic and asynchronous teaching methods. Online learning environments have been used by a much wider variety of students. Each student may have different backgrounds, learning styles, individual preferences, and knowledge levels and disabilities.

In this paper, the main focus lies on the assumption that the learner aids system must be flexible to be suitable for both ordinary students and especially for students with disabilities. Therefore, the learner aid in this paper was designed to be assessable to both ordinary and to student with visual impairment. Students with visual impairments began joining online learning, with assistance from trained special education teachers, and today, most visually impaired students attend regular school systems where they learn in classrooms with sighted peers. The improvement of human-computer interaction techniques in the online learning systems can improve the effectiveness of the learning and solve some pedagogical, psychological problems of online learning concerning the issues of user-teacher feedback, learning material presentation for both ordinary students and especially for students with visual impairments.

Evolution of online Education

The evolution of distance education beginning with correspondence and the use of parcel post, to radio, then to television, and finally to online education. As developments in educational technology continue to advance, the ways in which deliver and receive knowledge in both the

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traditional and online classrooms will further evolve. Correspondence education relies on the self-paced learning of the student as it does not include any face-to-face interaction at all—an instructor-centered, and not student-centered, one-way communication. Distance education is defined as a method of teaching where the student and teacher are physically separated. Unlike correspondence education, today's distance education takes advantage of ever-improving, fast Internet technology. Typically, the instruction is delivered instantaneously via live chat in the virtual classroom. Some models may incorporate emails and live chats as well as audio or video recordings.

Online education is defined as a form of distance education that uses computers and the Internet as the delivery mechanism for the course content delivered online. Over time computer processor speed vastly improved and web browsers became more user-friendly, making online education more widely available. Day by day more and more online educational institutes are emerging and online education degrees are becoming increasingly popular as these institutes are providing affordable higher education with advantages of flexibility and easy accessibility among many. However, the biggest impact for online education students was lack of the interaction, adaptation and communication of learners with lectures contents and the improvement in Internet connection speeds.

Online Learning

Online Learning takes place via the Web and may include text, graphics, animation, audio, video, discussion boards, e-mail, and testing. Online learning is typically "on demand" and self-directed but may include synchronous chat, web based teleconferencing (audio graphics), or similar technology.

Online learning can be divided into three classes:

- Contact learning supported by the net
- Multiform learning in the net
- Self-studying in the net

In the first class some parts of a course can be in the net for example the delivery of learning material and the lectures are given as contact learning. The second class is multiform learning which means using multiple options in learning for example: forum discussions, help from tutors and learning objects (e-books, videos, et cetera). The third class means that the learner studies alone in the net or in a virtual learning environment without outside help.

Learner Aids Technology of Online Learning

Online learning offers benefits which traditional learning does not, including flexibility, accessibility, and increased interaction with instructors and fellow students. Interaction methods and tools provide additional possibilities for the learner to deepen their understanding of the content, such as: tests in questions and answers format, simulators, and interactive objects (e.g., images and shapes require actions and reactions). In order to clarify the ideas contained within the learning content, many different aids such as images, animations, charts, graphs, videos, texts, and many other means are used for this purpose. A well-recognized classification of interactions in online teaching and learning are (1) learner-instructor, (2) learner-learner, and (3) learner-content interaction.

Learner-instructor interactions establish an environment that encourages learners to understand the content better. This type of interaction is "regarded as essential by many educators and highly desirable by many learners". Learner-learner interactions take place "between one learner and other learners, alone or in group settings, with or without the real-time presence of an instructor". Many studies show that this type of interaction is a valuable experience and learning resource. Empirical evidence shows that students actually desire learner-learner interactions, regardless of the delivery method. Learner content interaction is defined as "the process of intellectually interacting with content that results in changes in the learner's understanding, the learner's perspective, or the cognitive structures of the learner's mind". Different contents may require different interaction patterns, and, thus, it is difficult to have a generalized discussion about such interaction.

For student who do not use GUI, for accessing the web cannot get an overview of the structure of a text with one quick glance at the screen. Thus these users can be "lost in hyperspace" very quickly. Producing a document overview is one of the main issues to be considered in an application for surfing the Web which has a vocal interface.

Accessible and Usable Technologies

Accessibility and usability are two related but distinct concepts. Accessibility allows users access to system functionality. For users with disabilities, accessibility is treated as a technical construct that allows assistive technologies, such as screen-readers, the necessary access to interface elements. Usability refers to how well a system conforms to users' conceptualization of performing a task using it. It is a cognitive construct that depends on the task the user performs. A system that is not accessible is not usable; however, an accessible system does not guarantee usability. Accessibility problems prevent access to system features and functionality. Usability problems prevent the use of these features and functionality to meet objectives. Therefore, systems accessibility and usability are key to deriving the utility of a system.

The most used tool for accessing information on the internet and the best solution for distributing educational material for online learning is Web. A Learner aid system which has some fully accessible tools for learning may be unsatisfactory for visually disabled users if the learning methodology was designed for sighted users. Thus, the enabling technologies are very important but not enough. Similarly, a well-designed learning contents methodology, if not supported by a set of accessible tools, is not enough to allow the disabled students to learn on the net. In an educational context designing the content interaction is extremely important in order to reach a learning goal.

Online-learning environments should be usable by anyone. For this reason, it is important to also verify the accessibility and usability of e-learning collaborative tools for people with special needs. Accessibility and usability should always be considered during the design of a user interface allowing universal access to anyone. Accessibility permits users to reach on-line application content, while usability provides simple, efficient and satisfying navigation and interaction. Web content more accessible and usable for people with disabilities are organized into four principles: clear perception of content information (content perceivable), complete interaction with an interface in its functions (interface elements operable), comprehension of

meaning (content understandable), and maximizing the interface's compatibility with new assistive technologies and devices (content robustness).

Learner Aid System

The Learner aid system for online learning (LASOL) is designed an e-learning architecture based on the accessible and usable technology. It starts with an overview of the different ways disabled people work with computers and assistive technology. Access to information and communication for people with disabilities through modern technology is acknowledged as an important requirement for social inclusion.

Design Process - How online Learning aid works

At the heart of any online education is a website or portal through which students can submit their application and apply for the course they are interested in. Each time a new application is submitted to the system, administrator gets notified. An admission board (AB) will review the application of the student and will contact student in case they need any further information from them. Once the application of the student is approved they are ready to start with their respective classes.

As the learning aid system was developed for both sighted and blind students, system options will be provided to students so that they can opt for Normal mode and Assistive mode. In assistive mode, speech output will be activate for learners with visual impairments. The system model is shown in Figure 1.

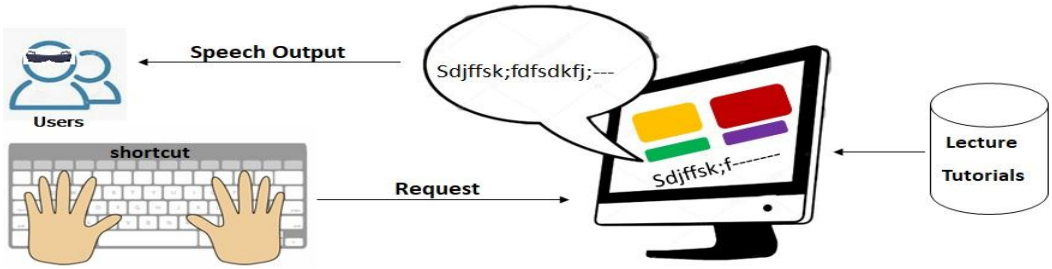

Figure 1 A Model for Learner Aid System in Online Learning

When students need to find the correct answer for a given question, they will be directed on the answer files where the paragraph with answers is highlighted. For students with visual impairments, because visual information and visual clues are not accessible to them, speech feedback will be used to read out the designated paragraph for them. To access the system functions such as browsing menus or opening a web file, shortcut keys will be used for them

Keyboard Shortcuts and Speech Outputs

An analysis of the systems currently in use indicates that each system has its own predefined shortcuts, which does not allow for efficient use and requires time to learn the various shortcuts. Keyboard shortcuts should be standardized, so the following seven basic shortcuts are recommended for navigating act in (LASOL):

1. Home page – CTRL + ALT +H(ome)
2. Content search – CTRL + ALT + S(earch)
3. Top of page – CTRL + ALT + T(op)

4. Bottom of page – CTRL +ALT+ D(own)

5. Go to menu column – CTRL + ALT + R(ead) selected file/page

6. Go to content column – CTRL + ALT + P(ause) reading

The CTRL+ALT combination was defined in order to avoid overlap with existing shortcuts employed by operating systems and application solutions (MS Office, Inter-net browsers and more).

For the accessibility output, speech output is provided powered by the Text-to-Speech (TTS) library of C# in Microsoft .Net Framework. In current system, the TTS engine is used for English language as all lectures in tutorials in current system are in English.

Implementation

To develop the learning aid system, PHP (Hypertext Processor) scripting language was used for the server side. For the front end designing, HTML, CSS and Java Script languages were used. For the backend database system, MySQL database management system was used.

Each student is given a login detail which they use to access their classes and this act as a common platform where instructor can interact with their students. This system also feature a notification area that is visible to the student each time they log in as shown in figure 2.

Figure 2 Login Feature

During the learning process, students will be able to learn online courses, answer tutorials and questions, check their answers and get feedback or the right answers from online learning aid system as shown in figure 3.

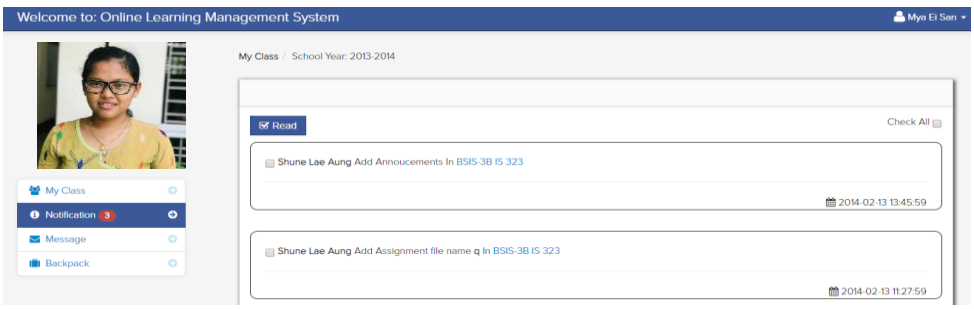

Figure 3 Student Profile

Also contain download section from where students can download lecture by Pdf format or paper format or presentation format, audio or video lectures from the instructor are shown in figure 4.

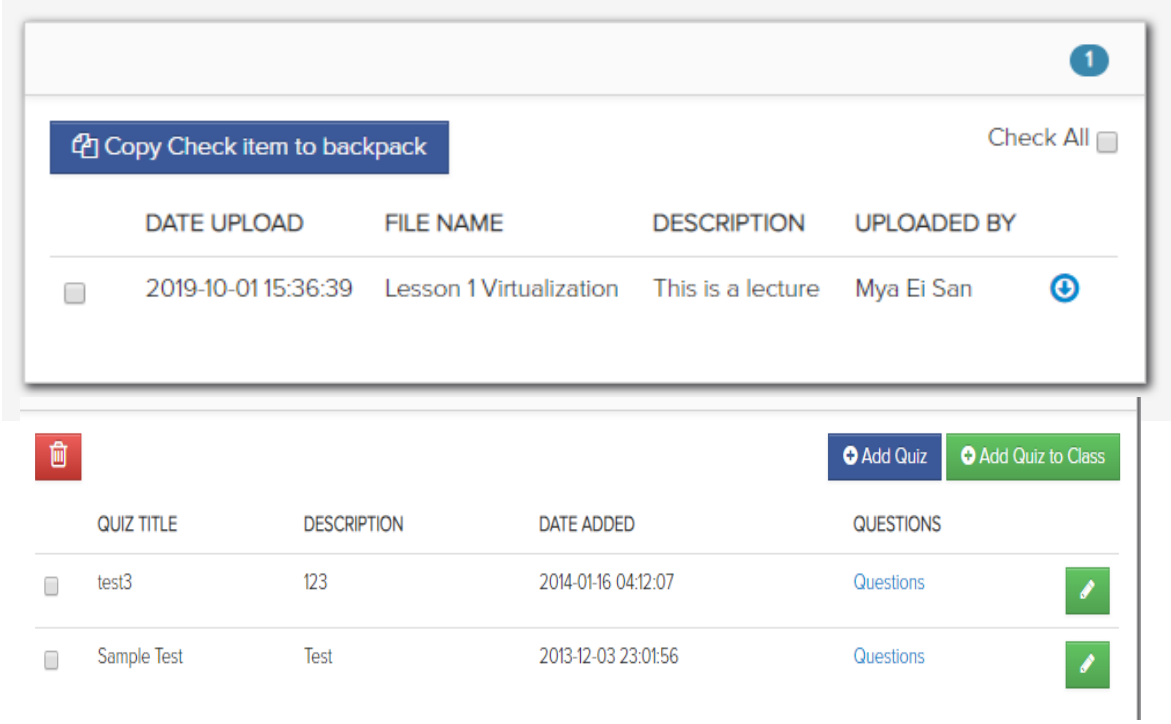

Figure 4 Learning Feature

Online classes will have chapter wise exercises and assignment as shown in Figure 5. Online students can know immediately their exercises marks and also can review the lecture which need to understand. Online student can learn their lecture by audio feature.

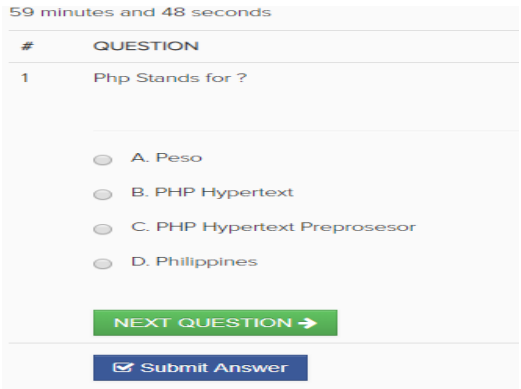

Figure 5 Exercise Feature

Result and Discussion

This paper has presented some studies about the accessibility of the e-learning and some tools to give the Internet a voice. These vocal tools have proved to be effective in supporting blind and visually impaired people in using Internet information and communication services especially for educational purposes. Our conclusions suggest that when designing technology for

people with disabilities it is necessary not simply to respond to their disadvantages but to take a more comprehensive view of their communicative and social needs as well as considering their overall capacities and knowledge. While interaction, in all its varied formats, is perceived as an effective means for learning, students tend to vary in their preferences about additional interaction in their online courses. Such variations tend to be related to individual personalities or learning style differences. Further research is needed to determine the relationships between learner preferences related to online interactions and individual differences. Rapid growth of the Internet is leading many educational institutions to offer a large variety of online courses/programs in wide range of fields. As online educational system is becoming increasingly popular and legitimate in society and corporate world, there is now a need to examine its benefits and drawbacks as well as its functioning driving the justification and design behind its foundation.

Conclusion

Online learning involves the use of digital tools for teaching and learning. It makes use of technological tools to enable learners study anytime and anywhere. It involves the training, delivery of knowledge and motivates students to interact with each other, as well as exchange and respect different point of views. It eases communication and improves the relationships that sustain learning. These technologies have to be accessible in order to enable people with disabilities to take part in education and the live-long learning.

Acknowledgement

The author is deeply indebted to Dr Nwe Nwe Win (Retired professor, Department of Computer Studies, University of Yangon) and Dr Pho Kaung (Rector, University of Yangon) for their helpful suggestion and valuable discussion. Thanks are due to Dr Soe Mya Mya Aye (Professor and Head, Department of Computer Studies, University of Yangon) for her permission to carry out this paper.

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PREVENTIVE MECHANISM FOR POTENTIAL SECURITY THREATS AND ATTACKS ON VIRTUAL CLOUD LDAP SERVER

Su Su Win[1], Mie Mie Su Thwin[2]

Abstract

Today, universal cloud computing and digital world cannot exist without virtualization architecture. They are basic tools to support varying demands without sluggish, expensive physical reconfiguration and software stack investment. On the other hand, Information and data are live source of today business in an organization. These data are created more and more every day. Data can be saved, but the weakest link in the cloud security is end point. Users can move on and data can be lost. This can bring more exposure to security threats, no reliable and safety. So, cloud security is becoming an important research topic. To demonstrate the weakness and vulnerabilities of LDAP (Lightweight Directory Access Protocol) server on virtual cloud environment, paper is developed by allowing Kerberos Single-Sign-On with LDAP directory service environment with client/server model in order to prevent third party password sniffing, eavesdropping and stealing password from LDAP database.

Keywords: LDAP Server, Single Sign On, Kerberos Authentication, Ticket, Password Sniffing

Introduction

LDAP server is frequently used by medium-to-large organizations and the scope is ranging from small servers for workgroups to large organizational and public servers. When the enterprise has own LDAP server, this organization can use this service to look up contact of users' information, user management and controlling authentication safely. E-mail register controlling mechanism can also be done by centralized up to date administration. LDAP servers are adaptable and able to replicate data both pushing or pulling methods. LDAP directory server that stores users' information data by means of hierarchically.

One of the techniques to partition the directory is to use LDAP reference model, which enable users to refer LDAP requests to a different server. The main concept of LDAP is the information model, which deals with the kind of information saved in directories and the structuring of information. The information shape model revolves around an entry, which is a collection of attributes with type and value. Entries are organized in a tree-like structure called the directory information tree (DIT). The entries are created around real world concepts, organization, people and objects. Attribute types are link with syntax defining allowed information. A single attribute can enclose multiple values within it. The distinguished names of the configuration in LDAP are read from bottom to top. The other left part is called the relative distinguished name and the right part is the base distinguished name.

The proposed system performed empirical analysis with LDAP server password sniffing attack with Kali Linux platform by using python and Wireshark tool. LDAP server password sniffing procedure can gather client's password information by querying the LDAP host server. LDAP is a kind of single sign on Client/Server model and when the information travel across the network and internet, Unsigned and malicious network traffic is susceptible to man-in-the-middle attacks. In such attacks, an intruder captures packets between the server and the client device, modifies them, and then forwards them to the client device. Where LDAP servers are concerned, an attacker could cause a client device to make decisions that are based on false records from the

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LDAP directory. To lower the risk of such an intrusion attack in an organization's network, some kind of potential attacks that happen on LDAP server such as LDAP injection and LDAP enumeration attacks can be found on recent research topic.

Background Theory

Kerberos is a kind of network protocol in client/server environment and uses secret key cryptography when clients want to communicate to the server. It is man in middle server. Whenever client want to use the server services, Kerberos requests an encrypted ticket by use of authenticated server. Kerberos, the name of the protocol come from three-headed dog for security guarded at the gates of Hades in Greek mythology. Kerberos was begun and developed by the name of project Athena- it is a joint project linking between the Massachusetts Institute of Technology (MIT), Digital Equipment Corporation and IBM that ran between 1983 and 1991.

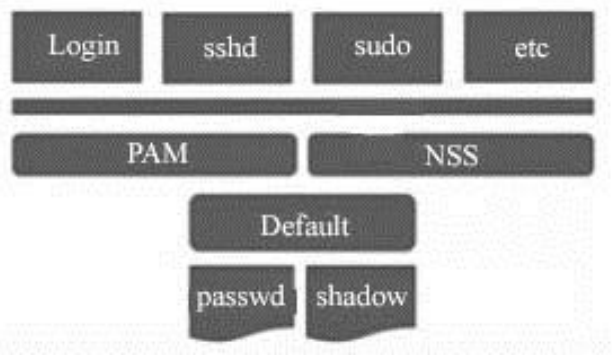


Figure 1 Linux Password Storage system in PC

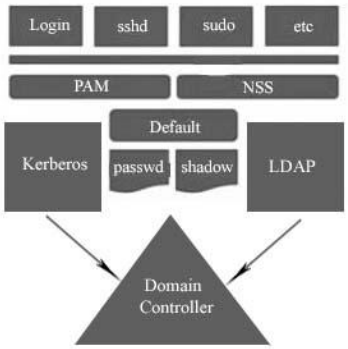


Figure 2 Integrated Design with Kerberos

An LDAP authentication server utilizes a Kerberos ticket to give permission server access and build a session key depend on the requested person's password. Then, the ticket-granting ticket (TGT) is connected to the ticket-granting server (TGS), in order to use the similar authentication server process. The requester (client) receives an encoded TGS key with a time stamp and service ticket, after that returned to the requester and decoded. The client sends the TGS this information and forwards the encoded key to the server to get the required service. When all events are handled accurately, the server takes the ticket and completes the requested user service, which have to verify the timestamp, decode the key and communicate the distribution center to get session keys. This session key is sent to the client requester, which decodes the ticket. As soon as timestamp and keys are valid and acceptable, client-server communication continues to establish. The TGS ticket means time stamped to permit concurrent and parallel client requests during the allow time frame.

Related Works

Kerberos was introduced at the Massachusetts Institute of Technology (MIT) to defend network services support by Project Athena. Versions 1–3 used only internally at MIT. Although Steve Miller and Clifford Neuman are the ordinary designers of Kerberos 4, many members and followers of Project Athena supplied to the design and implementation of Kerberos.

Kerberos 4 was available and published in the late 1980s. Even if it was pointed mainly for Project Athena, growing of it to be used in recent computer networks. Version 5 was invited by John Kohl and Clifford Neuman. It performed as RFC 1510 in 1993 (made obsolete by RFC 4120 in 2005).

To overcoming the limits and security problems troubleshooting of version 4, discuss in that was published in 1988 to support and know the fundamental motives for why Kerberos 4. This contribution is quite related to Kerberos 5. But, the basic principal ideas of the protocol have remained the same.

Problem Statement

Users login and password are recorded as centralized manner in Kerberos architecture, that protects clients from storing passwords on their related machines. Network security authentication protocol weaknesses due to unencrypted data transfer on network facilities of services can be reduced with the help of Kerberos. These are some issue that happen in proposed virtual environment system.

- Sniffing password from LDAP server
- Stealing password from LDAP database

Implementation and Contribution

In this experiment, the system tested with VMware type-1 hypervisor implementation.

As first step of proposed system design, a virtual environment was created for type-1and the networks were configured on the host server machine, with one network allowing access to the Internet and an internal one with the IP address range shown in table (1) and figure (4) as IP address domains. For IP address allocation, 10.0.0.0/24 is used to communicate between the virtual machines. Then, the Management VM was created with Linux Open source software through a desktop environment installed, before the network interfaces were configured and SSH (Secure Socket Shell) access was enabled for remote access.

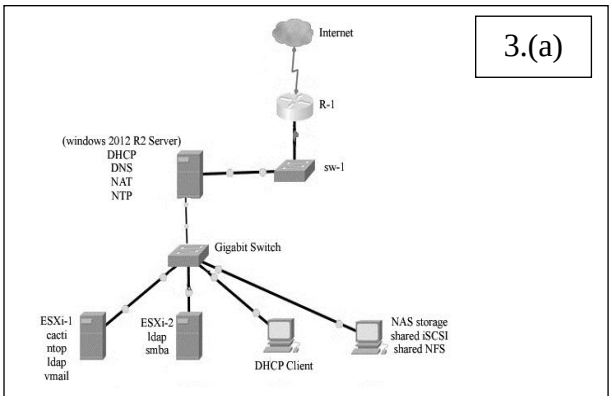


Figure 3(a) Virtualization with LDAP Server Architecture

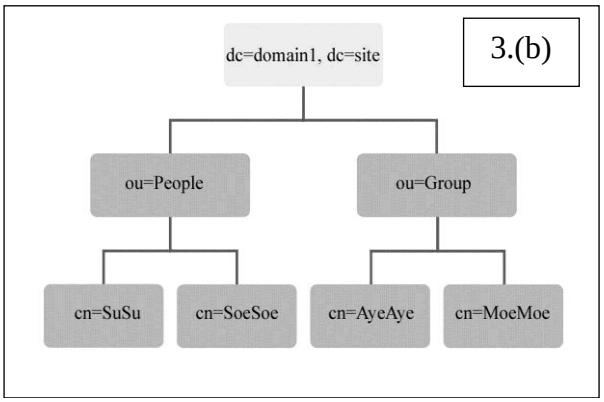
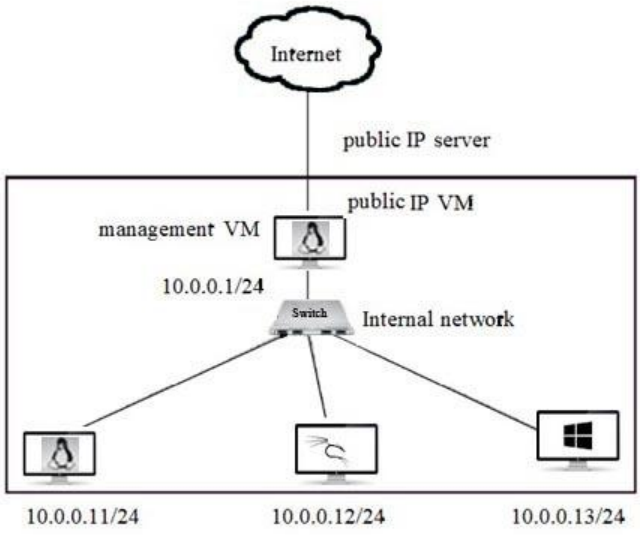


Figure 3(b) LDAP Tree Structure

Table1 IP Addressing scheme of Tested Virtualization Environment

No	FQDN	IP Address	System	Installations
1	vcenter.domain1.site	172.16.10.1	Windows 2012 R2	DNS, DHCP, NAT, NTP and VMware vCenter Server
2	esx1.domain1.site	172.16.10.11	VMware ESX	ESXi 5.0
3	esx2.domain1.site	172.16.10.12	VMware ESX	ESXi 5.0
4	nas1.domain1.site	172.16.10.21	NAS	Openfiler
5	research.domain1.site	DHCP	Windows XP (Management PC)	VMware vShpere Client

The next step is the creation of the basic VM template which was used to create a total of three VMs for the internal network by installing their respective servers one by one.



LDAP (Linux) | Hacker (Kali Linux) | Wireshark (Monitoring)

Figure 4 Tested Procedure for Sniffing Traffic

To implement a Kerberos security system with a proposed system, users have to pass network through three layers before they can access network services from the server. Firstly, Authentication to the Boundary Router and describes the operations to follow in the authentication process. A remote distance user who successfully initiates a PPP (Peer to Peer) session to the communicate the intended site is prompted by the router in order to register with login and password. Although in this phase the user is inside the firewall, to gain access to the network services, it still must authenticate to the Key Distribution Centre (KDC).

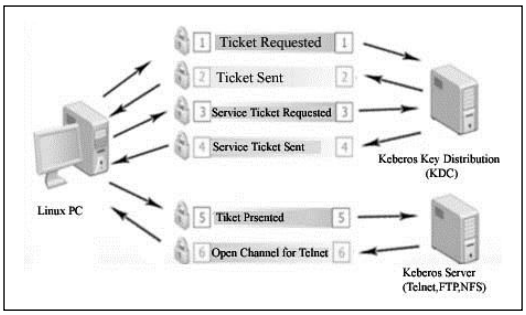


Figure (5-a)

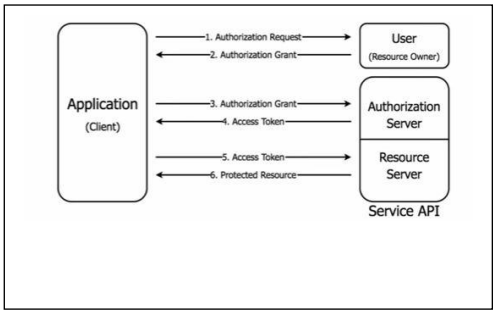


Figure (5-b)

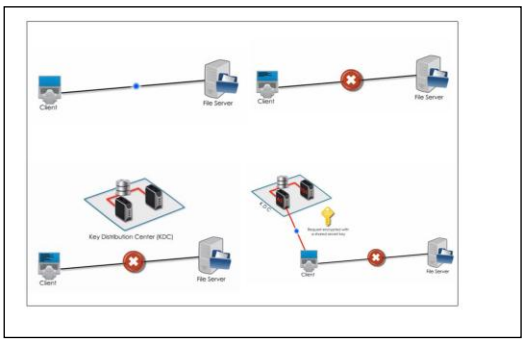


Figure (5-c)

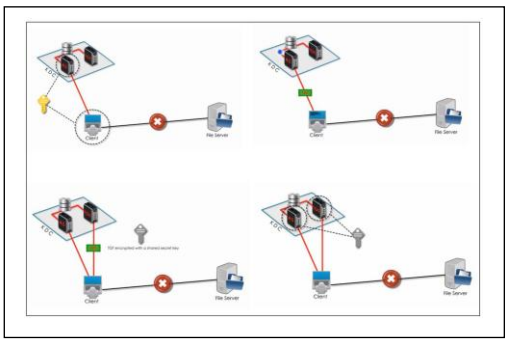


Figure (5-d)

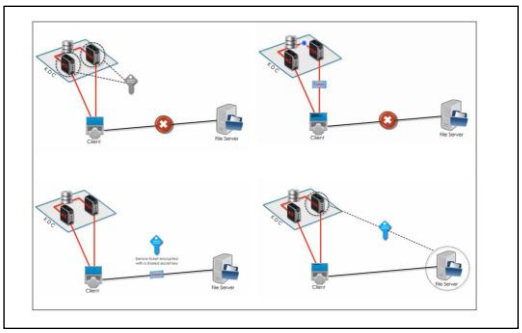


Figure (5-e)

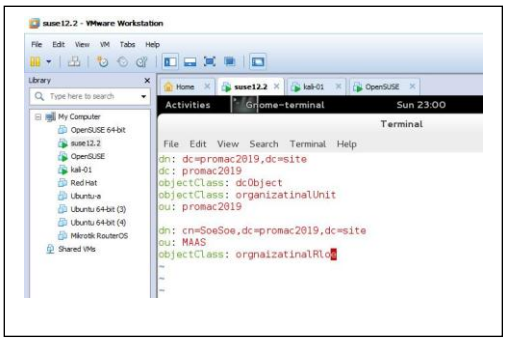


Figure (5-f)

Figure 5 (a-f): Step by Step Working with Kerberos function in Tested Area

After that, ticket TGT issued by the KDC is stored on the router and is not useful for additional authentication unless the user physically logs on the router. Therefore, the next step is Obtaining a TGT from a KDC. it prompts the user for the password to decrypt the ticket if it is successfully, the user has a TGT and can communicate securely with the KDC.

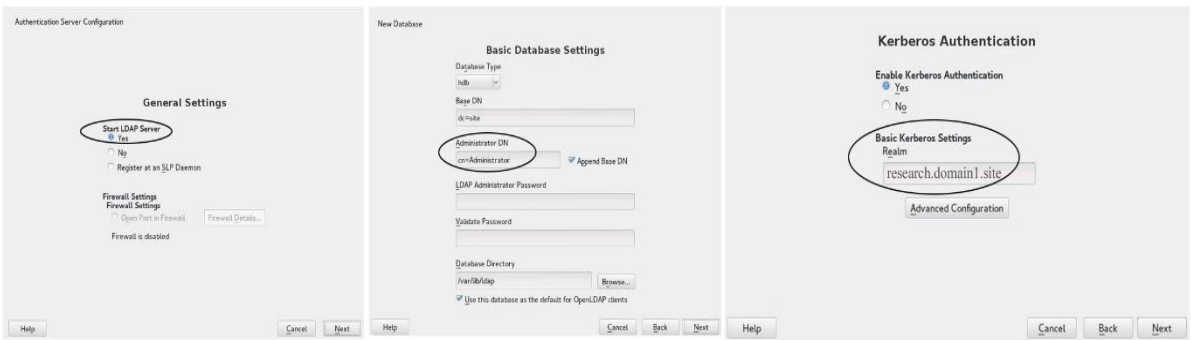


Figure 6 LDAP Server with Kerberos Authentication

In the following figure-7, that illustrate password sniffing for a LDAP server via Wireshark tool.

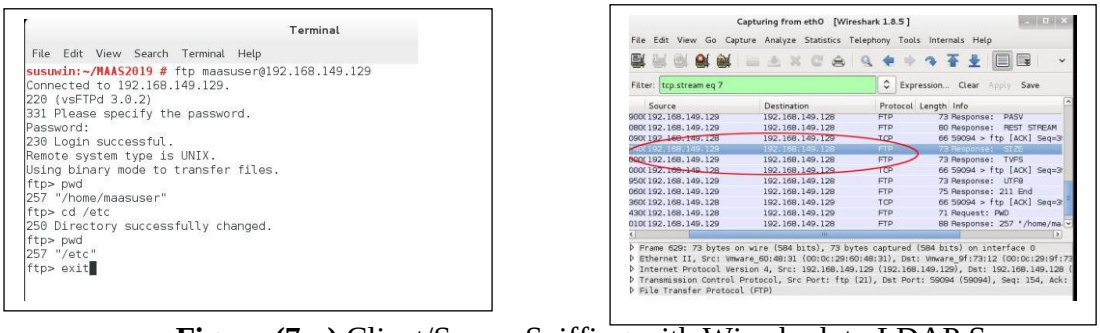


Figure (7-a) Client/Server Sniffing with Wireshark to LDAP Server

Figure (7-b) Plain Text Showing Password that Sniff in Wireshark Tool

In Figure-8, that demonstrate the configuration file of LDAP server for user input.

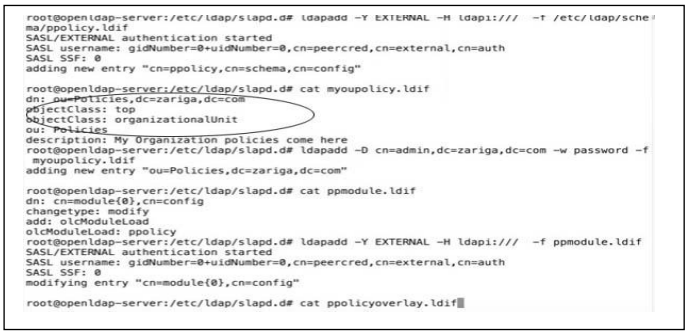


Figure 8 Creating Password Policy in LDAP Server

After the configuration of (ldif.conf) file, this is the output home page of LDAP server in GUI view.

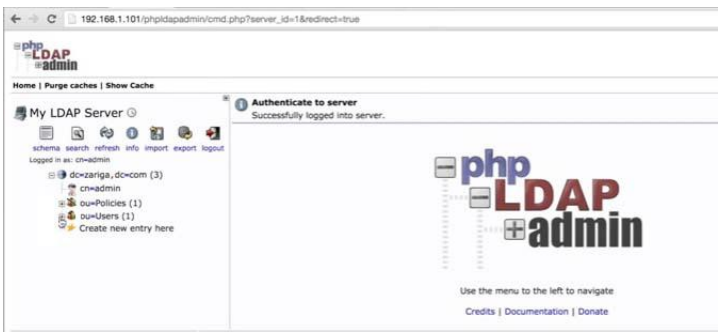


Figure 9 Home Page of LDAP after Configuration

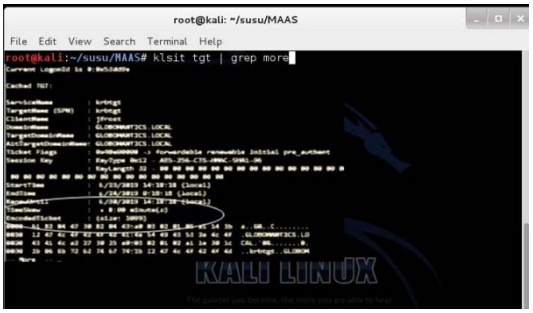


Figure 10 Output Command of Checking Kerberos Ticket

Finding Method and Discussion

The submitted proposal architecture supports LDAP server including both server-side and client-side components. The LDAP queries are known as LDAP exploration filters, which are constructed using prefix symbolization. Below is an sample case of an LDAP search filter: find ("(&(canonical name=" + client username +")(client password=" + pass +"))")

This prefix attachment filter and sorting notation established the query to find an LDAP client with the specified username and password. If the user-controlled values are prefixed to the LDAP search filter without any proof or decleration, a username and password value of '*' alters the intended sense of meaning. The method to prevent code injection techniques include defensive software design with programming, complex input validation, dynamic and live checks with static source code analysis. The job of mitigating LDAP injections must involve similar techniques.

Understanding LDAP database password stealing is little difficult from traffic sniffing other things like SNMP traffic, DNS traffic and NetBIOS information. because the attacker must have good knowledge of Active directory configuration about LDAP server configuration. The system used Wireshark and Kali Linux when it is tested.

Benefits and Limitations

- In Kerberos design architecture, user login information and their credentials are kept in the main centralized server. So, it is a difficult job to transfer all login credentials from local machines #/etc/passwd and #/etc/shadow files to the central server.
- If some hacker gets privilege to control the central server, the entire organization infrastructure will be under control of threat.
- The functions can be protected using Kerberos must have Kerberos efficiently built into the system.
- It is no standards for the authentication management of the Kerberos protocol. This kind of event is different from server implementations.
- The proposed system Kerberos necessitates constant availability of the KDC. When the Kerberos server is not working, the system will have weak point and vulnerable to the single point of failure problem. This can be mitigated by replacing of multiple Kerberos servers.

Conclusion

The system is tested in secure log in that focus on cryptographic protocol intended to achieve authentication over the secure network. The design objective is not only susceptible to password guessing attacks but deploy Kerberos protocol with Linux open SUSE. The proposed system presents a general overview of Kerberos network authentication protocol. Another part of the proposed system is focusing on the Kerberos' successful authentication in the of client/server architecture integrated with LDAP server to prevent password sniffing is demonstrated with Wireshark network traffic sniffing tool. Finally, the system gave the idea of its benefits and limitations.

Acknowledgement

Firstly, I would also like to acknowledge Dr Than Naing Soe, Head of University of Computer Studies, Myint Kyi Nar, Ministry of Education for allow me to submit this paper.

Secondly, I am extremely grateful to my supervisor Dr Mie Mie Su Thwin, Professor and Head of Cyber Security Research Lab, University of Computer Studies, Yangon for her invaluable guidance, supervision, patience, encouragement during the period of this paper.

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ASSISTIVE INTERFACE FOR PEOPLE WITH VISUAL IMPAIRMENTS

Shune Lae Aung[1], Nem Khan Dim[2], Htway Htway Khaing[3], Soe Mya Mya Aye[4]

Abstract

Although technology is rapidly developing, mobile applications are difficult to use for visually impaired people. The potential of technology to give invaluable support for people with visual impairments remains unexplored especially accessing the advanced functions of a smart phone is not a trivial task for them. Currently, visually impaired users rely on screen readers and voice commands to discover and execute functions on smartphones. Despite its common use, screen readers are not convenient in public places because of continuous read out functions. Also, voice commands are difficult for a system to recognize in noisy environments. Therefore, visually impaired people are unable to fully access advanced smartphone functions until now. In this paper, to increase the accessible rate, marking menu is implemented as an assistive interface for visually impaired people.

Keywords: Visual Impairments, Assistive Interface, Marking Menus

Introduction

According to the statistics, the population of blind people is increasing around the world. This population encounter difficulties for safe and independent mobility. Furthermore, they face more problems related to communication and access to information. Nowadays, there are a variety of assistive technologies helping to reduce many of these barriers. The use of technology for tasks such as reading, writing, communicating, navigating, and searching for information would enable blind people to perform a wide range of activities independently.

However, disabled population continue to face barriers in many situations. Specifically, modern devices such as smartphones that require visual clues presents challenges for people with visual impairments to access the advanced functions in it. Because of these challenges, persons with visual impairments can't get fully access and difficult to use touch screen.

In this research, a survey was conducted with thirty participants at Kyi-Myin-Daing and Kha-Wae-Chan Blind Schools. They all used smart phones for call. Thirteen people used for social media (facebook, viber, messenger, youtube etc) and fourteen people used map application to navigate their way. They give comments and several feedbacks on using smart phone. Motivated by user comments and available technologies, we developed marking menus that can provide eye-free access. This assistive interface will provide users to access smartphones' functionality easily. After developing the system, we conducted a preliminary study with users.

The following sections will present the literature reviews, usability studies, discussion of design guidelines drawn from user feedback during the user studies, and conclusion.

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Assistive Interfaces for People with Visual Impairments

There may be several different ways of using the device to perform an interaction task. An interaction technique is a way of using a physical device to perform an interaction task. Assistive interfaces for people with visual impairments based on two main aspects: (1) inputs, and (2) outputs.

Inputs

- **Hands**

Hand gestures on surface screens are a way of common today interaction. Surface gestures were used as input in tactile graphs, media player and other touchscreen devices. Especially modern devices nowadays mostly rely on hand gestures performed on touchscreens. The use of hand gestures for eyes-free input on a mobile touch screen was demonstrated by some researchers (Kane et al. 2008).

- **Body movements**

Hand position, foot position, head position, and even the direction of gaze of the eyes are also usable as computer inputs. Another way of interaction is motion gestures where users interact with a device, in 3D space by translating or rotating the device, or by moving the body parts without holding any devices. Bauer et al. (2013) implemented motion gestures for menu selections on large wall-mounted displays.

- **Voice**

Another natural way of interaction is speech that can be used with unrecognized speech discrete word recognition, or continuous speech recognition. Even if the computer could recognize all the user's words in continuous speech, the problem of understanding natural language is a significant and unsolved one.

- **Braille**

Braille inputs are where users perform inputs to systems using braille tactile buttons. Braille was the primary assistive input technique for blind people before (Caleb et al. 2012).

Outputs

Output modalities are classified into four categories.

- **Braille displays**

Braille displays are where system responds are displayed through tactile braille.

- **Sound**

Sound outputs are where the system responds to users through non-verbal sounds such as beeps.

- **Speech**

Speech outputs are where users receive system responses through verbal speech Vibration outputs are where users receive system response through vibrations which encode information in different patterns.

- **Vibration**

Vibration is a non-intrusive eyes-free output. They are used to convey a variety of information to users non-visually. Various information can be encoded in different temporal and spatial patterns of vibration.

Current technologies and interfaces are limitedly accessible and less engaging to visually impaired users. This is due to the need of better understanding of users, which will guide to designing and developing more efficient and engaging technologies for them. In another word, not a single existing interface can work perfect for this group of users in all situations. There is a pressing need of supplement or alternative interaction technique to make technologies more accessible to them. This research will propose new interaction technique for visually impaired users which are based on close studies and understanding users.

Survey Development

Preliminary Interview

We developed the survey to explore the current use of mobile devices and apps for people with visual impairments, as well as their perceptions of the apps. The survey consisted of information (such as age, gender, education and occupation), use of mobile devices and apps in general (device type, years of using a mobile device, the number of apps downloaded in a month, apps in use, frequently used apps, and usability and accessibility of apps), and use of apps specifically designed for people who are visually impaired (usability, accessibility). Based on their feedback, survey items were revised for clarity and accessibility. The survey took no more than fifteen minutes to complete. The participants' responses to survey items were saved and analyzed by Microsoft spreadsheet.

Formative Interview

In order to identify usability issues with mobile devices and touch screens, we conducted formative interviews with thirty blind mobile device users from Kyi-Myin-Daing and Kha-Wae-Chan Blind School. Studies have been conducted with persons with visual impairments for smart phone usage. Questionnaires were used to examine how smart phones play a role in their daily lives and how they can get benefits or assist from them and how they face difficulties in using smartphones. The participants strongly agreed that they need smart phone to assist them in their daily lives. They all use smartphones' technology to contact people, send message and use entertainments. The usage of smart phone for people with visual impairments is shown in Figure1.

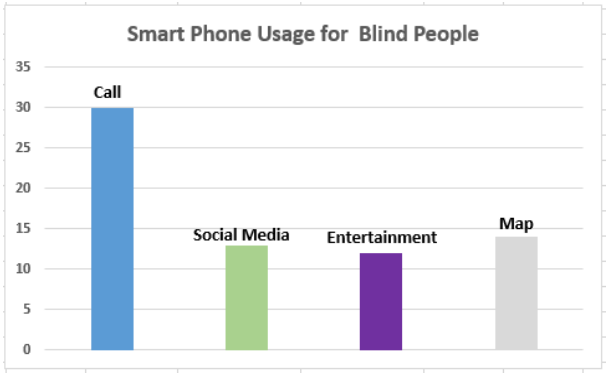

Figure 1 Survey for Smart Phone's Usability for Blind People

But, they are less accessible than enabled people. They have problems to use smartphones in public places because of screen reading functions and also the reader doesn't also recognize in public places. So, they can't use smartphone effectively and efficiently as shown in Figure 2.

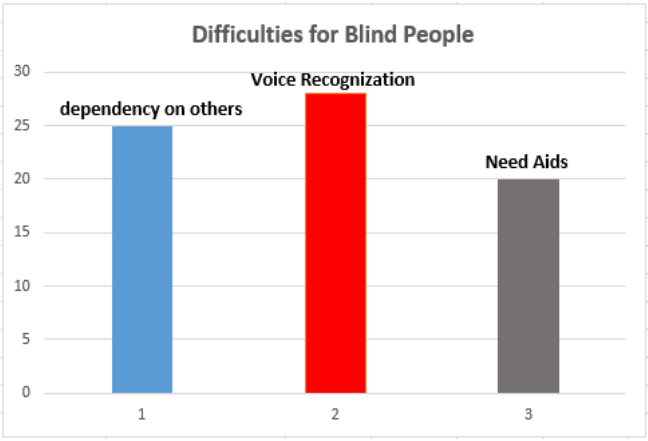

Figure 2 Survey for Difficulties for Smart Phone's for Blind People

Based on the user comments and qualitative analyses, we will provide design implications and guidelines for development of assistive interface for people with visual impairments.

Menu System

A menu is a list of options or commands presented to the user of a computer or mobile phone or communication system. A user chooses an option from a menu by using an input device. Some of the input devices used in the menu interfaces are touchscreens, keyboards, mice, remote controls, and microphones. In a voice-activated system, such as interactive voice response, a microphone sends a recording of the user's voice to a speech recognition system, which translates it to a command. Menus are used extensively in human computer interfaces. They provide critical information on what commands are available and a way to invoke commands.

Marking Menu Method

A marking menu is an interaction technique that allows a user to select from a menu of items. It supports to make interactions more efficient and easier to learn. There are two basic ways (or modes) in which a selection can be performed:

Menu mode In this mode a user makes a selection by displaying a menu. A user enters this mode by pressing the pen against the display. A menu of items is then displayed centered around the pen tip. A user can select a menu item by moving the pen tip into the sector of the desired item. The selected item is highlighted and the selection is confirmed when the pen is lifted from the display. (See Figure 2.1)

Mark mode In this mode, a user makes a selection by drawing a mark. A user enters this mode by pressing the pen against the display and immediately moving in the direction of the desired menu item. Rather than displaying a menu, the system draws an ink-trail following the pen tip. When the pen is lifted, the item that corresponds to the direction of movement is selected. (See Figure 3)

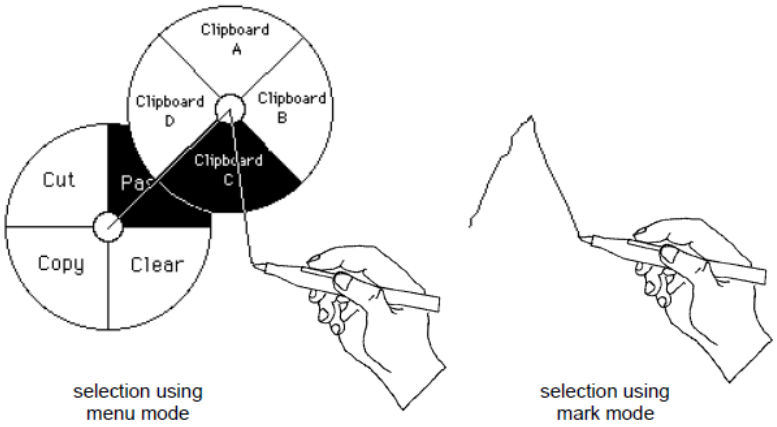

Figure 3 The two basic ways of selecting from a marking menu.

Advantages of Marking Menu

Marking menu support advantages over the traditional menus. With these types of menus, selection is performed by popping up the menu and selecting items by pointing with the mouse. Menu items can also be selected by pressing an accelerator key associated with a menu item. There are several specific advantages marking menus have over these traditional menus:

Keyboard less acceleration

Marking menus allow menu selection acceleration without a keyboard. With traditional linear menus, key presses must be used to accelerate selection. Marking menus provide a method of accelerating menu selections when no keyboard is available. With marking menu, the selection of all items can be accelerated by the user marking a mark.

Ease of drawing

Marking menus use a very simple set of marks consisting of straight and zig-zag marks. Marks are easy and fast to draw and are therefore suitable for accelerated performance. Ease of drawing is especially important when drawing precision is hampered by imperfect pen/display technology.

Eyes-free selection

Selection by a distinct physical movement with a marking menu lends itself to "eyes-free" selection. For example, we can draw the directions of a desired items without looking. Eyes-free selection is useful in situations where a user's visual attention must be on something other than the selection process. An eyes-free selection technique is also extremely valuable to the visually impaired.

Marking Menu Design

In our research, the following making menu was implemented on android OS by understanding the analysis of the user survey as shown in Figure 4. We will conduct the experiment on later.

Figure 4 Marking Menu Layout based on User Survey

Results and Discussion

Thirty participants from Kyi-Myin-Daing and Kha-Wae-Chan Blind School are included to complete the survey. According to the survey results, our findings are that all participants are currently using smart phone. They all use smartphones' technology to contact people and use social media and entertainment. But they have problems to use smartphones in public places because of continuous screen reading functions. We have to provide making menu as an effective input technique to carry out their desire work. Our major finding that voice recognition is needed to use smartphone more effectively and efficiently. But this system can't work exactly in public places because of the noise.

Therefore, we developed a making menu as an effective input based on user centered approach to get fully access and make their desire thing effectively and efficiently. After the system was implemented, we conducted a preliminary study to investigate the usability of our system with five blind-folded users. The purpose of the study was to validate the performance of our proposed system in terms of errors and task completion time. Results from the preliminary study indicates that marking menus were desirable eyes-free menus on smartphones with error rates less than 4% and approximate task completion time of 077 second.

Conclusions

The number of all ages with visual impairments has been increasing throughout the world. They face several challenges in their daily lives. Nowadays, mobile devices are widely used by people with disabilities. They cannot benefit from these devices e.g. current smart phone with rich functions. To satisfy their lives and productive livings assistive interfaces or technologies are needed to provide people with visual impairments (blind or low vision).So, assistive interfaces are need to provide their satisfactory and productive livings. They complained that screen reading function is not convenient in public places. Therefore, we proposed marking menus as effective input methods for blind people. Our finding confirms that marking menus are promising assistive interface for people with visual impairments.

Acknowledgement

The authors would like to thank Professor Dr. Phoe Kaung, Rector, University of Yangon for granting and supporting to do this research. We would like to thank to professor Dr. Soe Mya Mya Aye, Head of Department of Computer Studies, University of Yangon for giving us a chance to do this research and for encouraging us to complete in time. Also, we would like to thank all staff members and our colleagues at the Department of Computer Studies for their continuous support. We also would like to express our special gratitude to the principles, staffs and students of Kya Myin Daing and Kha Wae Chan Blind school for their active participation in this research.

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PERSONALIZED RECOMMENDATION SYSTEM IN MOBILE COMMERCE BY USING COLLABORATIVE FILTERING METHOD

Tin Nilar Win[1] and Wint Pa Pa Kyaw[2]

Abstract

Mobile commerce (m-commerce) is the delivery of electronic commerce capabilities directly into the consumer's hand, anywhere and anytime via wireless technology. Mobile phones are becoming a primary platform for accessing information and when coupled with recommendation systems technologies they can become key tools for mobile users both for leisure and business applications. Also, the huge amount of data in mobile business processes and physical limitations have increased the importance of personalization process. The users are flooded with so much of choices that it is hard for them to find appropriate and suitable items in m-commerce. Recommendation system can aid users in discovering information or items in a personalized manner. A mobile-based tourism recommendation system can help customers in travel planning because it may be so complicated and confusing to process a lot of information on the travel sites. Collaborative filtering method compares the user's past ratings with those of other users (neighbors) to find users with similar preferences. Highly rated items by these neighbors will be recommended. In this research, the system suggests personalized travel locations to users based on their rating profiles and interests by using collaborative filtering method.

Keywords: M-commerce, Recommendation System, Collaborative Filtering

Introduction

Increasing amounts of information on traveling are available on the web. As is the case for many other domains, the web is becoming the most important information source for planning a holiday. Specialized web sites, such as Expedia or Sky Scanner, exist for finding the best deals, flight tickets or travel packages. Others, such as Wiki Voyage or Frommers, are specialized in providing information and travel advice on different destinations. Reviews and evaluations of hotels, restaurants, and attractions can be read on websites such as Trip Advisor. Although these services are all valuable information sources, they typically give no personal advice which holiday destination to choose.

Now-a-days recommendation system is becoming very popular and people are getting attracted to it, as it is assisting them in discovering interesting items over huge amount of information. Recommendation systems are used in digital libraries, electronic stores, travel tours, restaurants, hospitals and in general can be useful in any decision-making process to provide predictions of appropriate items to specific users. During a commercial interaction, recommendation systems have advantages for both customers and merchants.

In a business interaction through the online shopping, recommendation systems can help customers to find their favorite items among an overwhelming number of items in an electronic department store. Therefore, recommendation systems can facilitate and accelerate shopping for users. Merchants proffer their products and hereby they can increase their sales and customers satisfaction by offering the new and preferable items. Similarly, in a digital library, recommendation systems can manage information overload by helping users to choose appropriate information items from a large set of alternatives.

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In the tourism field, travel recommendation systems aim to match the characteristics of tourism and leisure resources or attractions with the user needs. The travel companies have to aware of these preferences from different tourists and serve more attractive packages to get more business and profit. Therefore, the demand for intelligent tour services, from both travellers and tour companies, is expected to increase dramatically. Since recommendation systems have been successfully applied to enhance the quality of service for customers in a number of fields, it is natural direction to develop recommendation systems for personalized travel package recommendation.

Personalization Technology and Mobile Commerce

Personalization issues

A particular e-commerce and mobile commerce (m-commerce) technology, namely personalization, focuses on making a site more receptive to the unique and individual needs of each user. Understanding user needs requires understanding how users view the data available and how they actually use the e-commerce or m-commerce site.

Personalization is the provision to the individual of tailored services, products, information or information relating to service or products. It is a huge area, also covering recommendation systems, customization, and adaptive Web sites. Undoubtedly, delivering personalized information on the Web is a critical factor concerning the effectiveness of a Web site: the organization knows how to treat each visitor on an individual basis and emulate a traditional face-to-face transaction. Thus, has the ability to treat people based on their personal qualities and prior history with its site and furthermore has the ability to customize its resources to better match the needs of each user.

Mobile commerce and its features

The simplest way to describe m-commerce would be the buying and selling of products or the conduct of commercial transactions and activities through telecommunication and other mobile devices that run or operate on wireless network technologies. M-commerce is an upgraded version of e-commerce. In fact, m-commerce has been defined as the conduct of e-commerce activities using mobile devices. If the use of wireless telecommunication networks is included in the transactions of business, then it is highly likely to fall under m-commerce.

Portability and Flexibility are major advantages of m-commerce over e-commerce. While internet connectivity is required in e-commerce, that is not the case in m-commerce since these devices come with their own connection to the internet using telecommunication networks. Users can literally conduct commercial activities anywhere, even in places with no electricity because the mobile devices are also smaller and more portable.

The essence of m-commerce revolves around the idea of reaching customers, suppliers, and employees regardless of where they are located. It provides users the ability to access the Internet from anywhere at any time, the capability to pinpoint an individual mobile terminal user's location, the functionality to access information at the point of need, and a need-based data/information update capability. M-commerce has features not available to traditional e- commerce. These are user mobility, device portability and wireless connectivity.

User mobility means that the user can access a mobile information system in different locations. For example, a traveler landed at Yangon International airport, can use a mobile phone to access Trip advisor recommender system, for deciding where to book a room in Yangon. A good recommender system should be designed in such a way that this user mobility is supported and exploited by using the knowledge about his current context i.e., that his current position is Vienna.

Device portability refers to the fact that the device used to access the information system is mobile. For instance, a user may access the hotel recommender system with a laptop or a smart phone, or a PDA (Personal Digital Assistant). Device portability is the dimension because of the impact of the physical characteristics of the mobile devices, i.e., limited screen size, limited computation power and data storage, on the human/computer interaction.

Wireless connectivity refers to the fact that the device used to access the recommender system is networked by means of a wireless technology such as Wi-Fi. Some components of the recommender system use the network to access that are not residing on the device, and without the need of any wire. Actually, this may be the most important technological development, which really caused the mobile revolution, but it has not been extensively exploited in the recommender system literature.

Anatomy of Recommendation System

Recommendation systems are made to help users in their search for a fitting product from an overwhelming array of options. Recommendation systems can nowadays be found in a broad range of applications and are very common in e-business solutions. Every recommendation system needs to at least consist of two base elements: the user profile and the information filtering technique.

The user profile is needed for the system to represent the user's information and preferences. Without a user profile, it becomes impossible to generate personalized recommendations. Based on this user profile, the recommendation will need a certain matching (filtering) approach to match users with items. Figure 1 shows the process of recommendation system.

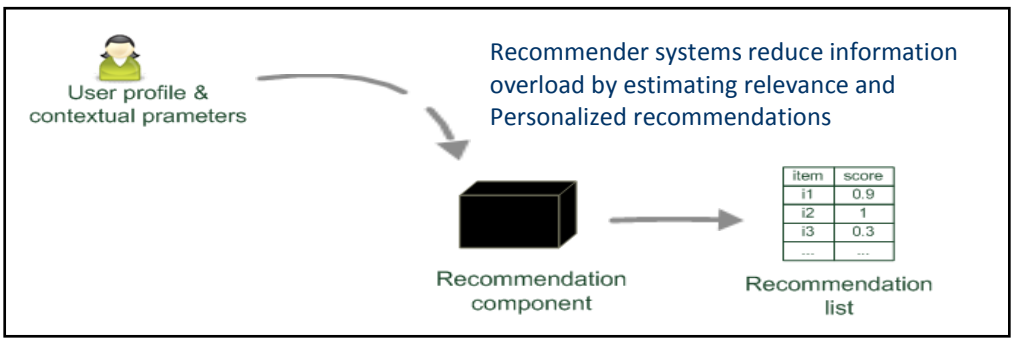

Figure 1 Paradigm of Recommendation System

User profiles

A user profile includes all personal information needed of a user to help with making recommendations. For constructing and maintaining user profiles, there are many different ways to represent the user's preferences. Two of the most successfully used techniques are to save a user-item matrix with ratings a user made in the past combined with the use of a feature vector, representing the affinity of a user to predefined features. For example, in a travel recommendation, feature can represent hobby. The user profile would then represent what are user hobbies. The user can also be asked for explicit input. In such case the users can state their preferences by answering questions or indicating their interests from a list.

Recommendation systems always try to improve their user's profiles and adapt to changing user's preferences over time. So, user feedback is another important aspect of any system. Explicit feedback can be attained by asking the user to rate items or ask his opinion (like/dislike) on a recommendation. Explicit feedback is the most accurate information but ask the user for to make an effort for the system. The more user-friendly approach is to collect implicit feedback from a user's behaviour and (natural) interactions with the system. Processing this information can also give insight to the user's preferences.

Filtering approaches

With the user profile and a database of items available, the final step to make a recommendation is to match users with suitable items. The filtering method determines how these are found. This work categorizes recommendation systems by their filtering approach and distinguishes between four different ones.

(1) In Content-Based Filtering, where the system makes use of the user's profile to recommend items that exhibit similar characteristics to what he has liked in the past.

(2) In Collaborative Filtering, the recommendation compares the user's past ratings with those of other users to find users with similar taste. Highly rated items by these neighbors will be recommended.

(3) Knowledge-Based recommendations make use of domain specific information to match user interests with items.

(4) Hybrid systems represent any system that combines two or more of the above approaches to a more complex whole.

Rating Estimations

An important element in recommendation systems is the user-item ratings. Ratings in recommendation systems represent how pleasing or useful a certain item is to a user. When a user has experienced the product, he can give it an explicit rating. But for most products, such rating is not known. Most recommendation approaches reduce the problem of making a recommendation to estimating ratings for items a user hasn't rated yet. Given these estimations, the system can then recommend the highest scoring items to the user.

Explicit ratings

Asking for explicit item ratings is probably the most precise one among the existing alternatives for gathering users' opinions. In most cases, five-point or seven-point Likert response scales ranging from "Strongly dislike" to "Strongly like" are used; they are then internally transformed to numeric values so the previously mentioned similarity measures can be applied. Some aspects of the usage of different rating scales, such as how the users' rating behavior changes when different scales must be used and how the quality of recommendation changes when the granularity is increased.

Explicit ratings require additional efforts from the users of the recommendation system and users might not be willing to provide such ratings as long as the value cannot be easily seen. Thus, the number of available ratings could be too small, which in turn results in poor recommendation quality. Figure 2 shows the five-point interval ratings scale.

Figure 2 Example of 5-point interval ratings

Implicit ratings

Implicit ratings are typically collected by the web shop or application in which the recommendation system is embedded. When a customer buys an item, for instance, many recommendation systems interpret this behavior as a positive rating. The system could also monitor the user's browsing behavior. If the user retrieves a page with detailed item information and remains at this page for a longer period of time, for example, a recommendation could interpret this behavior as a positive orientation toward the item.

Although implicit ratings can be collected constantly and do not require additional efforts from the side of the user, one cannot be sure whether the user behavior is correctly interpreted. Still, if a sufficient number of ratings is available, these particular cases will be factored out by the high number of cases in which the interpretation of the behavior was right. In some domains (such as personalized online radio stations) collecting the implicit feedback can even result in more accurate user models than can be done with explicit ratings.

Collaborative Filtering

The major purpose of collaborative filtering approaches is to exploit information about the past behavior or the opinions of an existing user community for predicting which items the current user of the system will most probably like or be interested in. These types of systems are in widespread industrial use today, in particular as a tool in online retail sites to customize the content to the needs of a particular customer and to thereby promote additional items and increase sales.

From a research viewpoint, these types of systems have been explored for many years, and their advantages, their performance, and their limitations are nowadays well understood. Years ago, many types of algorithms and techniques have been proposed and successfully evaluated on real-world and artificial test data.

A matrix of given user–item ratings is taken as the only input and typically produced the following types of output in pure collaborative approaches. These are (a) a numerical prediction indicating to what degree the current user will like or dislike a certain item and (b) a list of *n* recommended items. Such a *top-N* list should, of course, not include items that the current user has already bought.

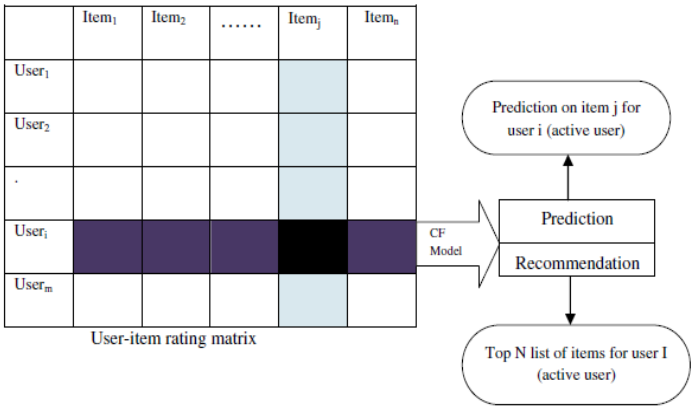

Figure 3 Collaborative Filtering Process

User-based collaborative filtering

User–based collaborative filtering is a straightforward algorithmic interpretation of the core premise of collaborative filtering: find other users whose past rating behavior is similar to that of the current user and use their ratings on other items to predict what the current user will like. In a travel recommendation system, to predict Mary's preference for an item she has not rated, user–based collaborative filtering looks for other users who have high agreement with Mary on the items they have both rated. These users' ratings for the item in question are then weighted by their level of agreement with Mary's ratings to predict Mary's preference.

Besides the rating matrix R, a user–based collaborative filtering system requires a similarity functions: U×U → R computing the similarity between two users and a method for using similarities and ratings to generate predictions. The main idea of user-based collaborative filtering is that given a ratings database and the ID of the current (active) user as an input, identify other users referred to as peer users or nearest neighbors that had similar preferences to those of the active user in the past. Then, in travel recommendation, for every tour package *p* that the active user has not yet seen, a prediction is computed based on the ratings for *p* made by the peer users. The underlying assumptions of such methods are that (a) if users had similar tastes in the past, they will have similar tastes in the future and (b) user preferences remain stable and consistent over time.

With respect to the determination of the set of similar users, one common measure used in recommendation systems is Pearson's correlation coefficient. The similarity *sim* (*a*, *b*) of users *a* and *b*, given the rating matrix *R*, is defined in the following formula. The symbol $\bar{r_a}$ corresponds to the average rating of user *a*.

$$sim(a,b) = \frac{\sum_{p\in P}(r_{a,p} - \overline{r_a})(r_{b,p} - \overline{r_b})}{\sqrt{\sum_{p\in P}(r_{a,p} - \overline{r_a})^2}\sqrt{\sum_{p\in P}(r_{b,p} - \overline{r_b})^2}}$$

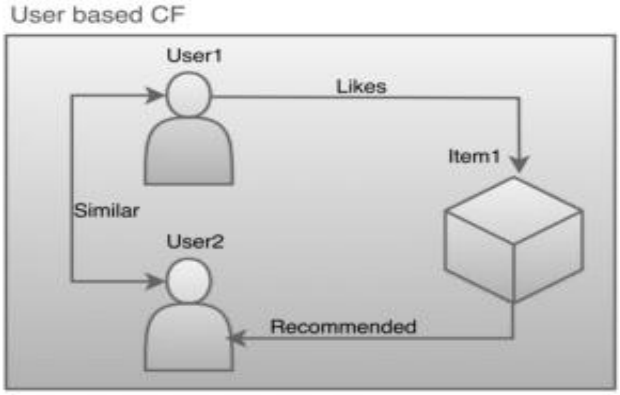

Figure 4 User-based Collaborative Filtering Process

Results and Discussion

User-based collaborative filtering recommendation based on nearest-neighbors enjoy a huge amount of popularity, due to its simplicity, efficiency, and ability to produce accurate and personalized recommendations. Table 1 shows a database of ratings of the current user, Mary, and some other users. Mary has, for instance, rated "Package1" with a "5" on a 1-to-5 scale, which means that she strongly liked this item. The task of a recommendation system in this simple example is to determine whether Mary will like or dislike "Package5", which Mary has not yet rated or seen.

In this sample, $U = \{u_1, \ldots, u_n\}$ to denote the set of users, $P = \{p_1, \ldots, p_m\}$ for the set of tour packages (items), and R as an $n \times m$ matrix of ratings $r_{i,j}$, with $i \in 1 \ldots n$, $j \in 1 \ldots m$. A numerical scale from 1 (strongly dislike) to 5 (strongly like) can be defined as the possible rating values. If an item j has not been rated by a certain user, the corresponding matrix entry $r_{i,j}$ remains empty.

Table 1 Sample Ratings Database for Collaborative Recommendation

	Package1	Package2	Package3	Package4	Package5
Mary	5	3	4	4	?
User1	3	1	2	3	3
User2	4	3	4	3	5
User3	3	3	1	5	4
User4	1	5	5	2	1

By substituting the rating value from Table 1 in Pearson's correlation coefficient formula, the similarity of Mary to User1 is thus as follows: ($\overline{r_{Mary}} = \overline{r_a}$=4, ($\overline{r_{User1}} = \overline{r_b}$=2.4:

$$\frac{(5-\overline{r_a})*(3-\overline{r_b}) + (3-\overline{r_a})*(1-\overline{r_b}) + \cdots + (4-\overline{r_a})*(3-\overline{r_b}))}{\sqrt{(5-\overline{r_a})^2 + (3-\overline{r_a})^2 + \cdots}\sqrt{(3-\overline{r_b})^2 + (1-\overline{r_b})^2 + \cdots}} = 0.85$$

The Pearson correlation coefficient takes values from +1 (strong positive correlation) to −1 (strong negative correlation). The results 0.70, 0.00, and −0.79 are the similarities to the other users, *User2* to *User4* respectively.

Based on these calculations, *User1* and *User2* were somehow similar to *Mary* in their rating behavior in the past. The Pearson measure regards the fact that users are different with respect to how they interpret the rating scale. Some users tend to give only high ratings, whereas others will never give a 5 to any package. The Pearson coefficient factors these averages out in the calculation to make users comparable – that is, although the absolute values of the ratings of *Mary* and *User1* are completely different, a rather clear linear correlation of the ratings and thus similarity of the users is detected.

This fact can also be seen in the visual representation in Figure 5, which both illustrates the similarity between *Mary* and *User1* and the differences in the ratings of *Mary* and *User4*. To make a prediction for *Package5*, which of the neighbors' ratings shall be taken into account and how strongly shall be valued their opinions. In this example, an obvious choice would be to take *User1* and *User2* as peer users to predict Mary's rating.

A possible formula for computing a prediction for the rating of user *a* for package *p* that also factors the relative *proximity* of the nearest neighbors *N* and *a*'s average rating $\overline{r_a}$ is the following:

$$pred(a, p) = \overline{r_a} + \frac{\sum_{b \in N} sim(a, b) * (r_{b,p} - \overline{r_b})}{\sum_{b \in N} sim(a, b)}$$

In the sample, the prediction for Mary's rating for *Package5* based on the ratings of near neighbors *User1* and *User2* will be:

$4 + 1/(0.85 + 0.7) * (0.85 * (3 - 2.4) + 0.70 * (5 - 3.8)) = 4.87$

Given these calculation schemes, rating predictions for Mary can be computed for all items she has not yet seen and include the ones with the highest prediction values in the recommendation list. It will most probably be a good choice to include *Package5* in such a list.

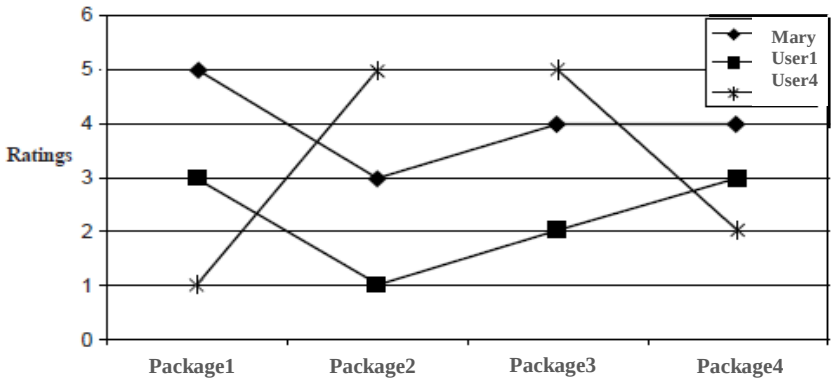

Figure 5 Comparing Mary with two other users

Conclusion

Recommendation systems open new opportunities of retrieving personalized information on the Internet. Recommendation techniques have coped with the information overload problem and have proven their usefulness as a tool in many classical domains such as movies, books, and music. A variety of approaches have been used to perform recommendations in these domains, including content-based, collaborative, and knowledge-based.

This paper proposes a recommendation system that offers personalized recommendations for travel destinations to individuals. It can help overcome information overload problem by exposing users to interesting, novel, surprising and relevant items based on preferences users have expressed either explicitly or implicitly. It can introduce users to new items that have not been known or have not been retrieved. So recommendations can help users in meeting their information needs.

On the whole, it is mainly an intelligent application, created to support users by personalized recommendations in search process and their decision-making while interacting with large information spaces. These recommendations are based on the users' rating profile, personal interests, and specific demands for their travel destination by using user-based collaborative filtering approach. Recommendation system automates some of these strategies with the goal of providing affordable, personal, and high-quality recommendations.

Acknowledgement

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AN ANALYSIS OF NETWORK MODELS IN PROJECT MANAGEMENT

Kyaw Moe Min*

Abstract

In the analysis of network models, **P**rogram **E**valuation and **R**eview **T**echnique (PERT) and **C**ritical **P**ath **M**ethod (CPM) are the methods which have been widely applied to industrial project planning and control in practice. PERT which is a large scale model, analyzes the project using a standard *forward-backward* analysis method. **PERT** and **CPM** are available to assist the project manager in carrying out these responsibilities. These techniques make heavy use of *networks* to help plan and display the coordination of all the activities. This paper illustrates how the application of ***network*** or ***precedence*** diagram can be used in project management. In this study, all the project activities will be able to construct Gantt chart using Excel spreadsheet and MS Project.

Keywords: Project management, Gantt chart, Network models, PERT, CPM

Introduction

Project management differs from management of more traditional activities mainly because of its limited time framework and the unique set of activities involved, which gives rise to a host of unique problems. The core technique available to Project Managers for planning and controlling their projects is **Network Analysis**.

Network analysis is one of the most wide spread methods in the planning and controlling of the project. Sources of complex projects can be managed more effectively by analyzing with networks that are modal illustrations of a series of events and activities. Network is a technique used for planning and scheduling of large projects in the fields of construction, maintenance, fabrication, purchasing, computer system instantiation, research and development planning etc.

Network Model

Networks model the interrelated flows of work that must be accomplished to complete a project. The two best-known techniques for network analysis are Program Evaluation and review Technique (PERT) and Critical Path Method (CPM).

Network analysis is a technique of planning, scheduling and controlling of a large and complex project comprising various activities. Network techniques provide a rational approach to the planning and controlling of construction works.

PERT/CPM are two of the most widely used techniques for ***planning and coordinating large-scale projects***. PERT and CPM are the two network-based project management techniques, which exhibit the flow and sequence of the activities and events. Although PERT and CPM were developed independently, Figure 1 is shown the relationship between PERT and CPM.

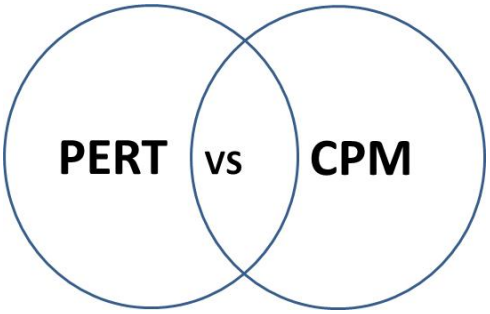


Figure 1 Relationship between PERT and CPM

For practical purposes, the two techniques now are the same; the comments and procedures described will apply to CPM analysis as well as to PERT analysis of projects. PERT and CPM are the two most widely applied techniques shown in Figure 2.

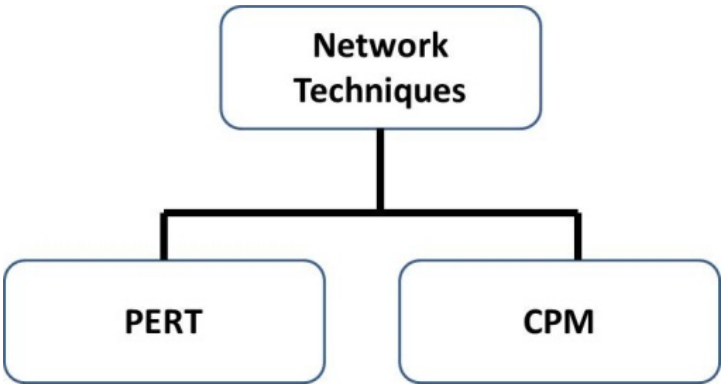


Figure 2 The two most widely applied techniques

In CPM method, fixed and defined processing times are accepted. **Critical path** is a path that has the ***longest total activity*** period that supplies project to be completed in the shortest time between starting and ending point on the project network. PERT method is established on probability estimation of operation times and completion duration of the project. The most important difference between CPM and PERT is estimation of operation time. PERT was designed for projects in which durations are unclear such as research and development projects. CPM and PERT are calculated with the same method.

Building the Network Diagram

One of the main features of PERT and related techniques is their use of a network or precedence diagram to depict major project activities and their sequential relationships. There are two slightly different conventions for constructing these network diagrams. Under one convention, the *arrows* designate activities; under the other convention, the *nodes* designate activities.

These conventions are referred to as activity-on-arrow (AOA) and activity-on-node (AON). Activities consume resources and/or *time*. The nodes in the AOA approach represent the activities' starting and finishing points, which are called *events*. Events are points in time. Unlike activities, they consume neither resources nor time. The *nodes* in an AON diagram represent *activities*. This gives an *activity-on-arrow* (AOA) network, usually associated with PERT. The

activity-on-node (AON) is often associated with CPM. This describes a small project represented by the activities and precedence shown in Table 1.

Table 1 A sample set of Project Activities and Precedence

Activity No	Activity Name	Predecessor
1	Locate facilities	-
2	Order furniture	1
3	Interview	-
4	Hire and train	3
5	Remodel	1
6	Furniture setup	2
7	Move in	5,4

The AOA network is generally more difficult to draw, but depicts the technical relationships of the activities quite well. Beginning the same way, it creates a Start node from which flow all activities that have no predecessors, in this case 1. The completion of these activities results in Figure 3. Because it is easiest to draw, it starts with the AON network. Because activity 1 has no predecessor, it follows the Start node. They are connected to the starting node with arrows as in Figure 4. A simple project network diagram which is PERT and CPM with AOA and AON is shown in the following figure 3 and figure 4.

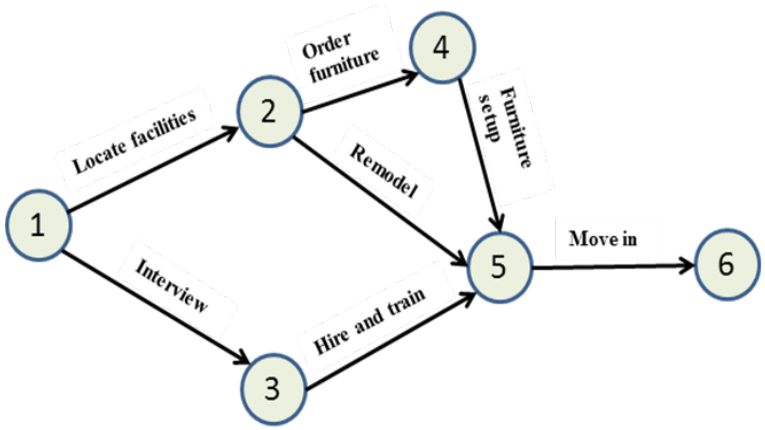


Figure 3 Activity-on-arrow (AOA) diagram

In the AOA diagram, the arrows represent activities and they show the sequence in which certain activities must be performed (e.g., Local facilities precedes Order furniture, Interview precedes Hire and train) from Table 1. Activities in AOA networks can be referred to in either of two ways. One is by their end points (e.g., activity 2-4) and the other is by a letter assigned to an arrow (e.g., activity *c*).

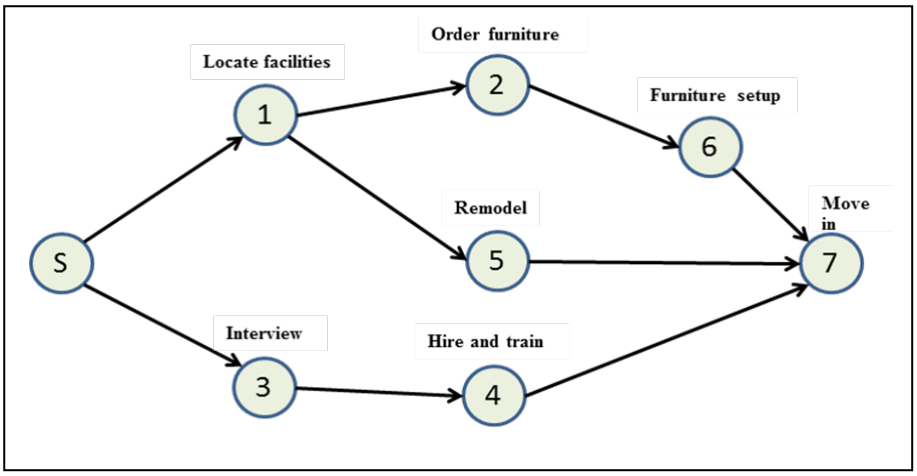


Figure 4 Activity-on-node (AON) Diagram

In the AON diagram, the arrows show only the sequence in which certain activities must be performed while the nodes represent the activities. Activities in AON networks are referred to by a letter (or number) assigned to a node (e.g. Local facilities assigned to number 1, Order furniture assigned to number 2, and so on.) from Table 1. The AON diagram has a starting node, S, which is actually not an activity but is added in order to have a single starting node.

Finding the Critical Path and Critical Time

A sample problem for finding the critical path and critical time is given in Table 2. Firstly, a project table will be created by using the given data in MS Excel sheet.

Table 2 A sample problem for finding the critical path and critical time

Activity	**Predecessor**	**Duration(**days)
A	-	5
B	-	4
C	A	3
D	A	4
E	A	6
F	B, C	4
G	D	5
H	D, E	6
I	F	6
J	G, H	4

To find the critical path and critical time using the data from Table 2, it can start drawing the associated **AON network** as in Figure 5. The activity names and durations are shown in the appropriate node.

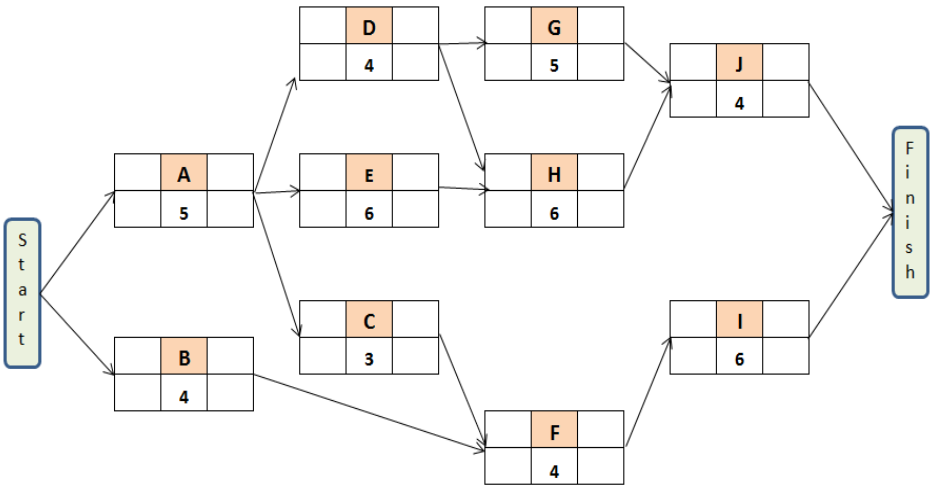


Figure 5 A complete network for Table 2

After using the data from Table 2, information contents can be added to the nodes in the network. Just above each node it is common practice to show what is called the earliest start time (ES) and earliest finish time (EF) for associated activity. Just below each node is shown the latest start time (LS) and latest finish time (LF) for the activity. The information contents in an AON node can be shown in Figure 6. Activity name is J and expected duration is 4 days. Earliest start is 17 days and earliest finish is 21 days. Then Latest start is 17 and latest finish is 21 days.

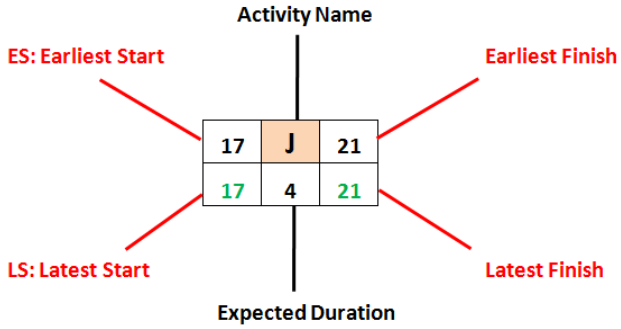


Figure 6 Information contents in an AON node

All activities and thus all paths must be completed to finish the project. The shortest time for completion of the network is equal to the ***longest path*** through the network, in this case A-E-H-J. If any activity on the A-E-H-J path is even slightly delayed, the project will be delayed and that identifies A-E-H-J as the critical path and 21 days as the critical time. In Figure 8, the critical path is depicted by thick lines – a common practice with PERT/CPM networks. It can be found that the critical path is A→E→H→J.

The ES and EF begin for each activity quite easily at the start node and move from left to right through the network, calculate from node to node. This is called a ***forward pass*** (left to right pass) and makes it simple to find the critical path and time for PERT/CPM networks as shown in Figure 7.

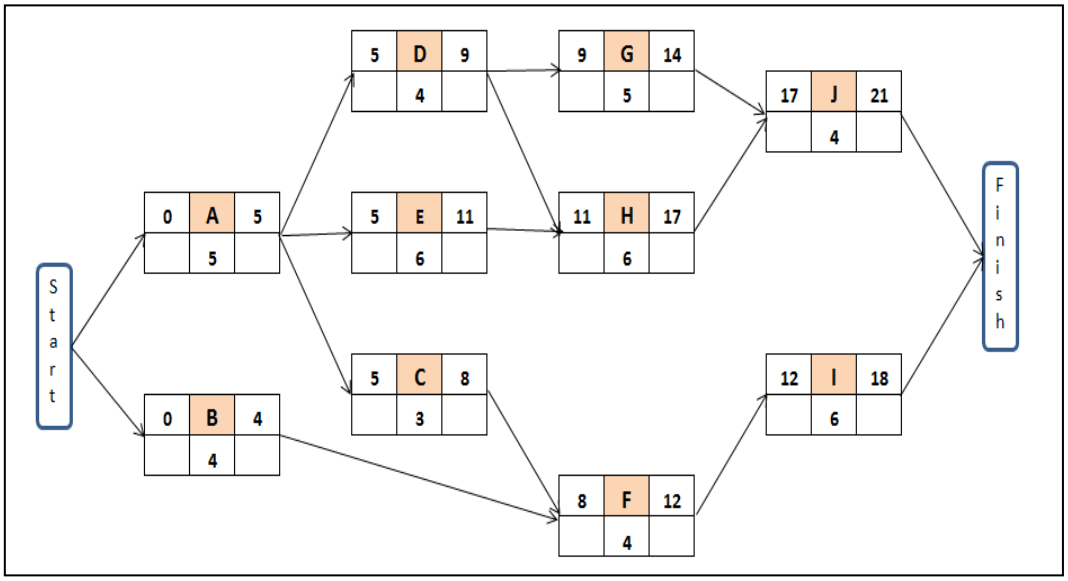


Figure 7 Forward pass calculation for the critical path and time

In a similar fashion, this can perform a **backward pass** (right-to-left pass) to calculate the LS and LF values for each activity. The thin arrows are represented for a backward pass of each activity. The thickest arrow- lines are represented for a critical path of all activities. After completing the backward pass calculation, the critical path can be easily determined in Figure 8. This can be easily seen that the critical time is 21 days.

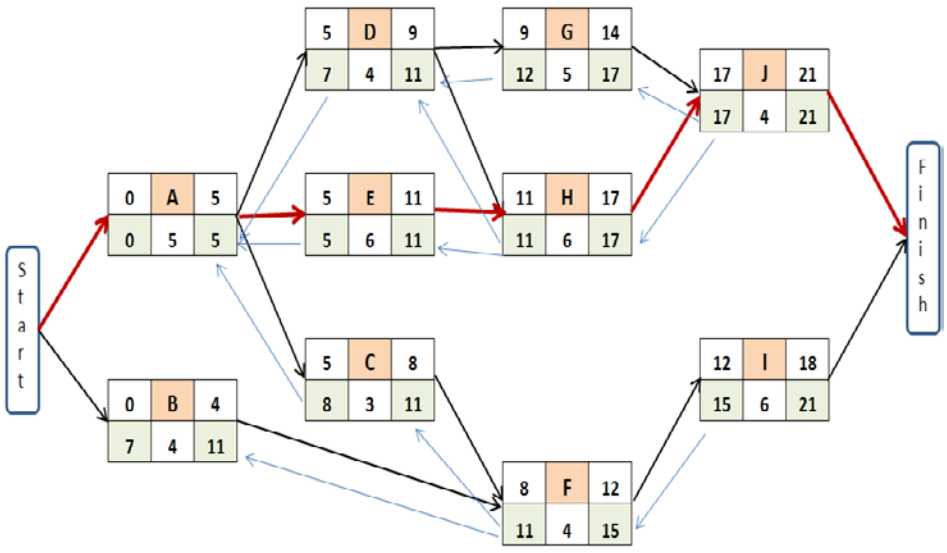


Figure 8 Backward pass calculation for the critical path and time

Calculating Activity Slack

The allowable slippage for any path is called **slack,** and it reflects the difference between the length of a given path and the length of the critical path. ***The critical path, then, has zero slack time.*** The amount of time a non-critical task can be delayed without delaying the project is called slack or float. The slack for any activity is easily calculated as LS-ES = LF-EF = slack. By using the data from Table 2, this will be calculated firstly earliest finish (EF) and latest finish

(LF). The result of them is shown in Table 3-1. After resulting EF and LF, it will be calculating slack time and critical path (CP). Then it can be seen zero slack time and 'YES' in CP column in Table 3-2.

Table 3-1 Calculating earliest finish and latest finish

Activity	Duration (Days)	Preceding Activity	ES	EF =ES+ Duration	LS= LF Duration	LF
A	5	-	0	5	0	5
B	4	-	0	4	4	8
C	3	**A**	5	8	8	11
D	4	**A**	5	9	7	11
E	6	**A**	5	11	5	11
F	4	**B,C**	8	12	11	15
G	5	**D**	9	14	12	17
H	6	**D,E**	11	17	11	17
I	6	**F**	12	18	15	21
J	4	**G,H**	17	21	17	21

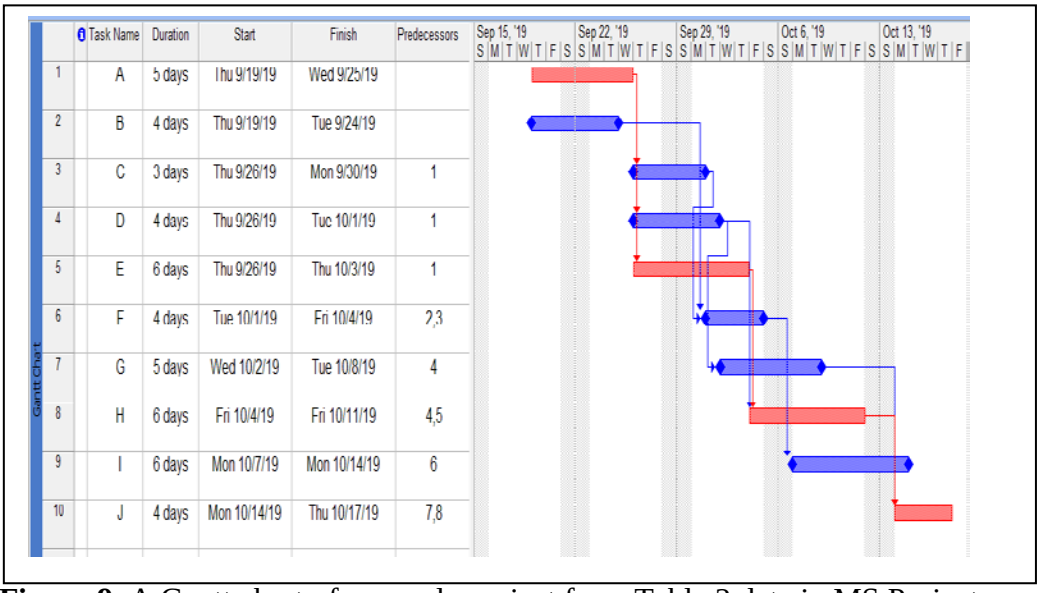
Table 3-2 Calculating activity slack for the critical path

Activity	Duration (Days)	Preceding Activity	ES	EF = ES + Duration	LS= LF - Duration	LF	Slack = LF-EF	CP
A	5	-	0	5	0	5	**0**	YES
B	4	-	0	4	4	8	4	NO
C	3	**A**	5	8	8	11	3	NO
D	4	**A**	5	9	7	11	2	NO
E	6	**A**	5	11	5	11	**0**	YES
F	4	**B,C**	8	12	11	15	3	NO
G	5	**D**	9	14	12	17	3	NO
H	6	**D,E**	11	17	11	17	**0**	YES
I	6	**F**	12	18	15	21	3	NO
J	4	**G,H**	17	21	17	21	**0**	YES

By calculating activity slack in MS E**xcel**, the critical path has zero slack time shown in Table 3-2. In this case, the project can be identified **A-E-H-J** as the critical path.

Furthermore, once the project plan data are entered, **MS Project** will automatically draw an AON PERT/CPM network and Gantt chart as shown in Figure 8 and Figure 9. A Gantt chart displays project activities as bars measured against a horizontal time scale. It is the most popular way of exhibiting sets of related activities in the form of schedules.

Figure 9 shows a Gantt chart of the sample project for given data from Table 2. Any activity that has no predecessors starts at the beginning of Day 1 and extends to its duration. Any activity with predecessors begins when its latest predecessor has been completed. Figure 9 shows a PERT/CPM Network Diagram of the sample project for given data from Table 2.



	Task Name	Duration	Start	Finish	Predecessors
1	A	5 days	Thu 9/19/19	Wed 9/25/19	
2	B	4 days	Thu 9/19/19	Tue 9/24/19	
3	C	3 days	Thu 9/26/19	Mon 9/30/19	1
4	D	4 days	Thu 9/26/19	Tue 10/1/19	1
5	E	6 days	Thu 9/26/19	Thu 10/3/19	1
6	F	4 days	Tue 10/1/19	Fri 10/4/19	2,3
7	G	5 days	Wed 10/2/19	Tue 10/8/19	4
8	H	6 days	Fri 10/4/19	Fri 10/11/19	4,5
9	I	6 days	Mon 10/7/19	Mon 10/14/19	6
10	J	4 days	Mon 10/14/19	Thu 10/17/19	7,8

Figure 9 A Gantt chart of a sample project from Table 2 data in MS Project

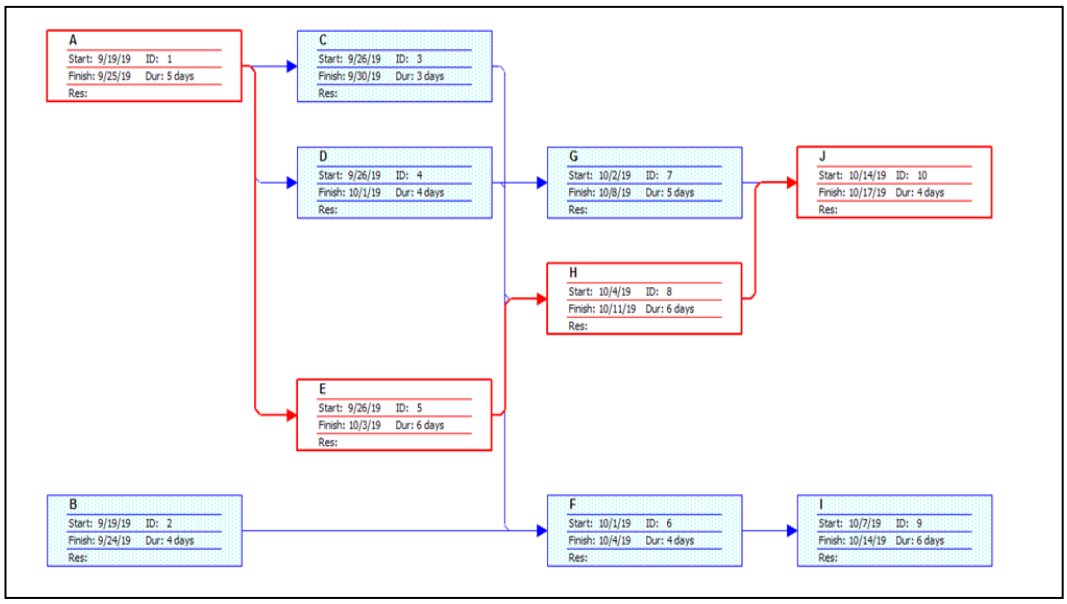


Figure 10 A PERT/CPM network from Table 2 data in MS Project

In order to Figure 9 and Figure 10, the result can be found that the critical path is **A-E-H-J.** Furthermore, Figure 11 shows a Gantt chart of Milestone and Critical in MS Project.

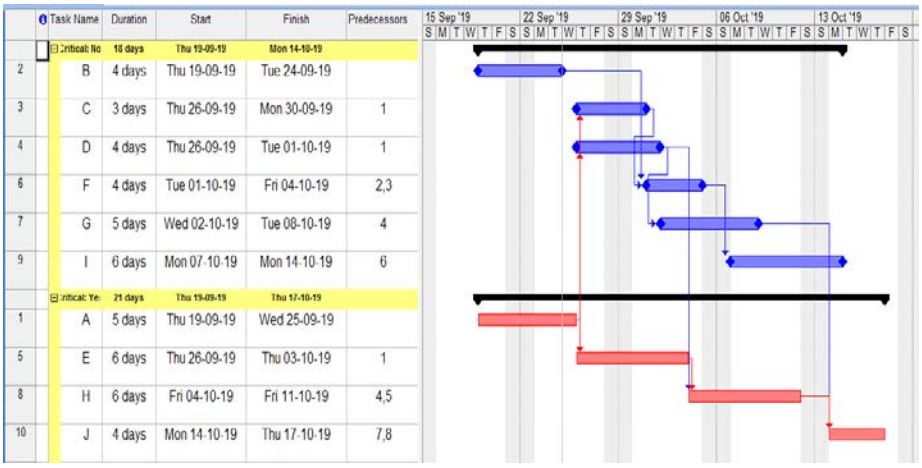


Figure 11 A Gantt chart of Milestone and Critical in MS Project

Difference between PERT and CPM

PERT is appropriate for the projects where the time needed to complete different activities are not known. **The Critical Path Method (CPM)** is appropriate for the projects which are recurring in nature. The primary difference between PERT and CPM is shown in Table 4.

Table 4 Differences between PERT and CPM

CPM	PERT
• CPM is **activity** oriented	• PERT is **event** oriented
• Single time estimates are used for the various activities i.e. the time estimates are **deterministic**.	• The time estimates for activities are **probabilistic**.
• Appropriate for Reasonable time estimate	• Appropriate for High precision time estimate
• Model under certainty	• Model under uncertainty
• Project management that manages well defined activities of a project	• Technique, used to manage uncertain activities of a project
• A method to control cost and time	• A technique of planning and control of time
• Suitable for Non-research projects like civil construction, ship building etc.	• Suitable for Research and Development Project

Conclusion

The two scheduling methods use a common approach for designing the network and for ascertaining its critical path. They are used in the successful completion of a project and hence used in conjunction with each other. Nevertheless, PERT is focuses on time and CPM is focuses on the time-cost trade-off. PERT and CPM are available to assist the **project manager** in carrying out these responsibilities. These techniques make heavy use of *networks* to help plan

and display the coordination of all the activities. These techniques are widely used for *planning and coordinating large-scale projects*. By using PERT or CPM, managers are able to obtain (1) a graphical display of project activities (2) an estimate of how long the project will take (3) an indication of which activities are the most critical to timely project completion (4) an indication of how long any activity can be delayed without delaying the project.

This paper illustrates how the application of network or precedence diagram can be used in project management. All the project activities are constructed project network and Gantt chart for a sample project using MS Excel spreadsheet and MS Project.

The difference between these two project management tools PERT and CPM is getting blurred as the techniques are merged with the passage of time. That is why, in most projects, they are being used as a single project. The primary distinguishes PERT from CPM is that the former gives the extreme importance of time, i.e. if the time is minimized, consequently the cost will also be reduced. It is illustrated the critical path and time for PERT/CPM by performing forward pass and backward pass. Furthermore, it is found easily the length of the critical path by calculating the slack time for any activity.

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DATA ANALYSIS FOR DECISION SUPPORT ON STUDENT INTAKE RESULT MANAGEMENT

Yi Mon Win*

Abstract

Data analysis for Decision Support Systems (DSS) technologies fixed to provide decision support in the higher education environments, by producing and showing their information which are helpful in taking the decision regarding admission management in universities. In this paper presented the implementation of a system to provide decisions in the student intake management allowed for real-time management by using the statistical data analysis methods such as hypothesis testing and classification methods. This paper is finding the total data of students who are missing their graduation that passing through start and end of the university year by using the decision support system of student intake management system. Decision makers of University can apply the DSS components for their important decision such as student registration, classroom management, facility management, extra curriculum, teacher, scholarship, hostel facility, transportation. This paper is created in order to query useful information from general data support in student databases to classify decision-making process by the information systems of any university.

Keywords: Decision support system, student registration, data analysis

Introduction

Universities need to have extensive analysis capabilities of student achievement levels in order to make appropriate academic decisions. Academic decisions will move to changes in academic performance, necessitating periodic assessment for the determination of the effect of changes. The study of decision-making process in university, effective resource management, personnel administration, automation of student registration, graduation and dismiss have always been of great interest to educationalists.

Information is considered to be an important asset for any academic institution. DSS are the most efficient tool to deal with any kind of situation, where the decisions are required to be taken efficiently. In higher education environments, DSS are well suited technologies to provide decision support by generating and presenting the relevant information and the knowledge towards quality improvement of education processes. In this research the author present the conceptual framework that can provide the required decision support especially while planning for taking the decisions in universities management. This includes a brief discussion of the DSS model analysis for higher education systems.

In this analyze the student data of university by using the system of student intake result management. This system can search, view and calculation the total data of students passing through start to end of their university year for example start 2012-2013 intakes to end 2015-2016 end of the university year. Decision-making needs to accurate data in timely. Sometime information does not get to decision makers in a useful form. Universities need to have extensive analysis capabilities of student registration that is to make appropriate academic decisions. Conversely, certain academic decisions will lead to changes in academic performance, necessitating periodic assessment for determining the effect of changes

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The specific objectives include;

1. To store the students registration data of university.
2. To create the university students database.
3. To check the student data for admin user timely.
4. To create system for easy to use friendly user interface.
5. To decide the specific decision for decision maker timely.

Decision-Analytic Decision Support Systems

An emergent class of DSSs known as decision analytic DSSs applies the principles of decision theory, probability theory, and decision analysis to their decision models. Decision theory is an axiomatic theory of decision making that is built on a small set of axioms of rational decision making. It expresses uncertainty in terms of probabilities and preferences in terms of utilities. These are combined using the operation of mathematical expectation. The attractiveness of probability theory, as formalism for handling uncertainty in DSSs, lies in its soundness and its guarantees concerning long-term performance. Probability theory is often viewed as the gold standard for rationality in reasoning under uncertainty. Decision analysis is the art and science of applying decision theory to real-world problems. There are two applied model:

- Systems with static domain models. In this class of systems, a probabilistic domain is represented by a large network encoding the domain's structure and its numerical parameters. The network comprising the domain model is normally built by decision analysts and domain experts. An example might be a medical diagnostic system covering a certain class of disorders. Queries in such a system are answered by assigning values to those nodes of the network that constitute the observations for a particular case and propagating the impact of the observation through the network in order to find the probability distribution of some selected nodes of interest.
- Systems with customized decision models. The main idea behind this approach is automatic generation of a graphical decision model on a per-case basis in an interactive effort between the DSS and the decision maker. The DSS has domain expertise in a certain area and plays the role of a decision analyst. During this interaction, the program creates a customized influence diagram, which is later used for generating advice. The main motivation for this approach is the premise that every decision is unique and needs to be looked at individually; an influence diagram needs to be tailored to individual needs.

In this research, using the data analytical tools as statistical data analysis. Finding structure in data and making predictions are the most important steps in Data Science. Here, statistical methods are essential since these can able to handle many different analytical tasks. Important statistical data analysis methods are the following.

a). Hypothesis testing is one of the pillars of statistical analysis. Questions arising in data driven problems can often be translated to hypotheses. Also, hypotheses are the natural links between underlying theory and statistics. Since statistical hypotheses are related to statistical tests, questions and theory can be tested for the available data. Multiple usages of the same data in different tests often lead to the necessity to correct significance levels. In applied statistics, correct multiple testing is one of the most important problems.

b) Classification methods are basic for finding and predicting subpopulations from data. In the so-called unsupervised case, such subpopulations are to be found from a data set without a priori knowledge of any cases of such subpopulations. This is often called clustering.

This paper studied the data analysis of student intake result management in Dagon University by using the student intake management system (SIRM) that framework made up the schematic view of DSS for the university shown in figure (1).

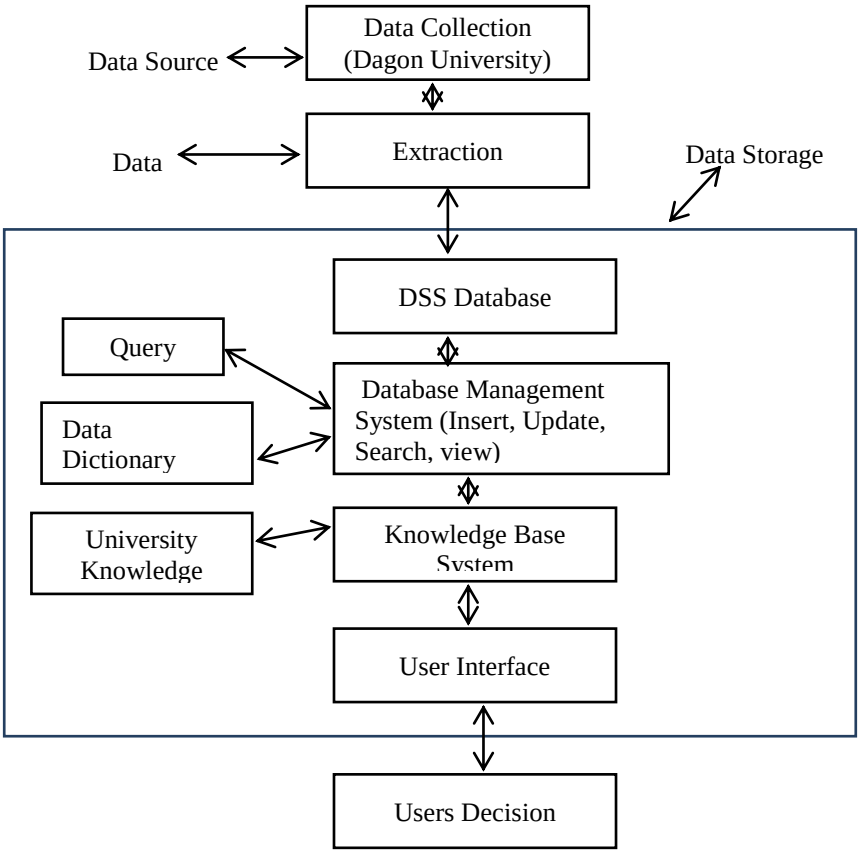

Figure 1 DSS Schematic View for Universities.

Result and Discussion of Proposed System

Student intake management system general analyses the following types of result:

1. Decision maker can analyze the total registered students to university.
2. Can analyze the students of undergraduate and postgraduate in university.
3. Can check the registration and graduate student by department.
4. Admin user can check resign, transferred, pass away student in university.
5. Can analyze the detail departmental student with undergraduate or postgraduate students by yearly.

In this work, the different ways in which student intake data can be analyzed and presented for academic decision-making are investigated. A software package called the Student Intake Result Management (SIRM) is designed and developed for this purpose. The software system makes extensive use of graphical displays for presenting the results. The SIRM has been developed for student intake result management system by using Microsoft C#.Net (2015) and

SQL server (2012) for database. The result data of program has been analyzed the data analysis method with Power Bi software.

The problem domain of this system is searching the total data of students through first year to final year students within their graduated year who are missing by resigned, transferred and fail, pass away student. Decision maker can search total student data of pass students, fail students, resigned student, pass away students, transferred students and graduated students within their university year. The SIRM system can support user can check the detail data (name, student ID, father name, address, etc.) of total students by their specialization. This system has two sites, admin site and students' site. In student site, student can register who want to enroll the university. In admin site of this system, admin staff can enter user name and password correctly for student data security. Authority person or admin user can view, update, search and calculate by data of intake and academic year. The main effective of this system is finding the student data that missing students of register to first year to final year of their graduated year. Admin user can view who are missing student that is transfer student, resign student, pass away etc. Student can fill the register form only data input part. Firstly, student have to register to the university with register form, students clicks the submit button after filled the register form. Total register student's data of Dagon University in 2012-2013 are shown in figure (2) for arts students and figure (3) for science students by their specialization.

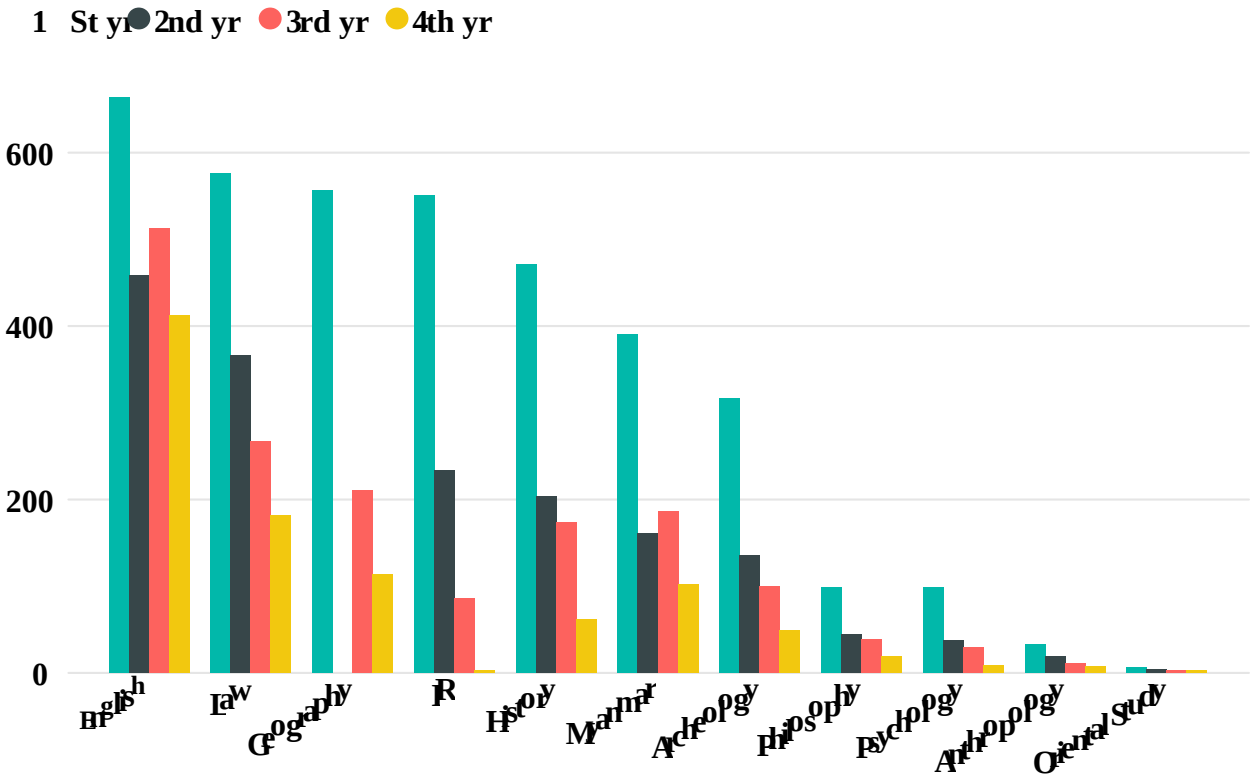

Figure 2 Total Register of Arts Students in Dagon University

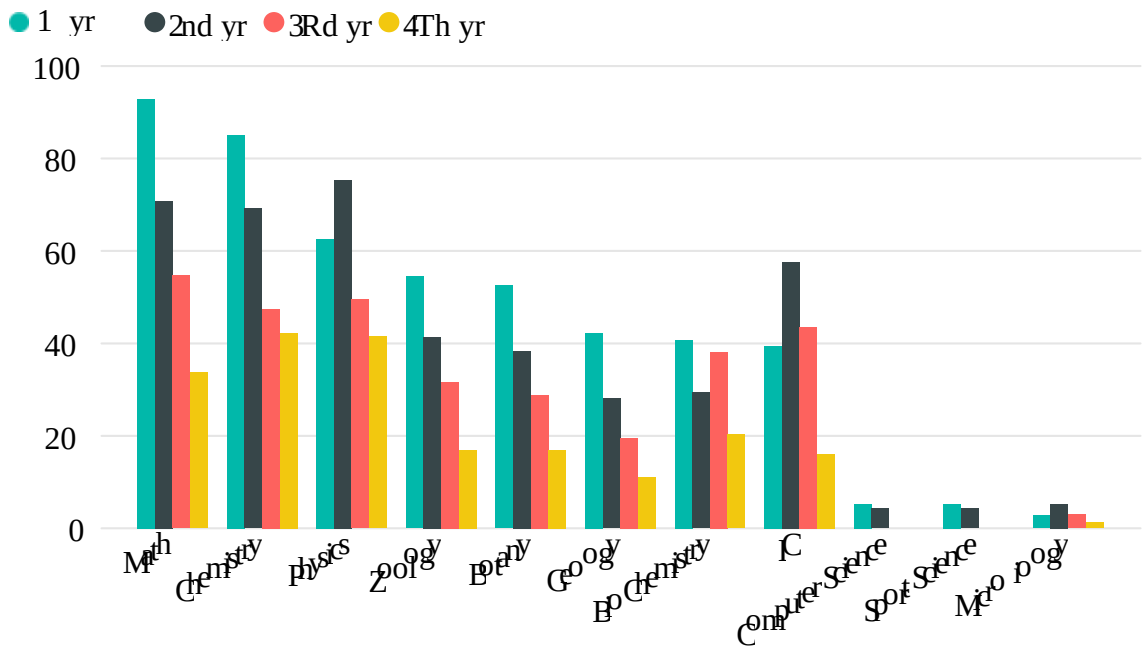

Figure 3 Total Register of Science Students in Dagon University

The SIRM system examines the 2012-2013, 2013-2014 and 2014-2015 intakes student of Dagon University. As the statistical information, intake students of 2012-2013 are received their concerning degree at 2015-2016 academic year, 2013-2014 students are got their concerning degree at 2016-2017 and 2014-2015 students are accepted their degree at 2017-2018 shown in figure (5), (6) and (7).

As the program result is show in Table 1 for 2012-2013 intake, Table 2 for 2013-2014 intake and Table (3) for 2014-15 intake data of Dagon University. In these tables show the total students, examinee, pass the exam students, fail students, honours/ qualify students and missing students.

Table 1 2012-2013 Intake Student Condition of Pass/Fail and Missing Condition

Year	Registered Student	Examinee Student	Pass Student	Fail Student	Honours/ Qualify	Missing Student	Missing Percent(%)
2012-13	7700	6794	5736	1058		906	11.76
2013-14	6153	5344	4687	657		809	13.14
2014-15	4796	4472	3880	592	267	324	6.75
2015-16	4154	3943	3470	450	232	211	5.07
				Total	Missing	2250	36.75

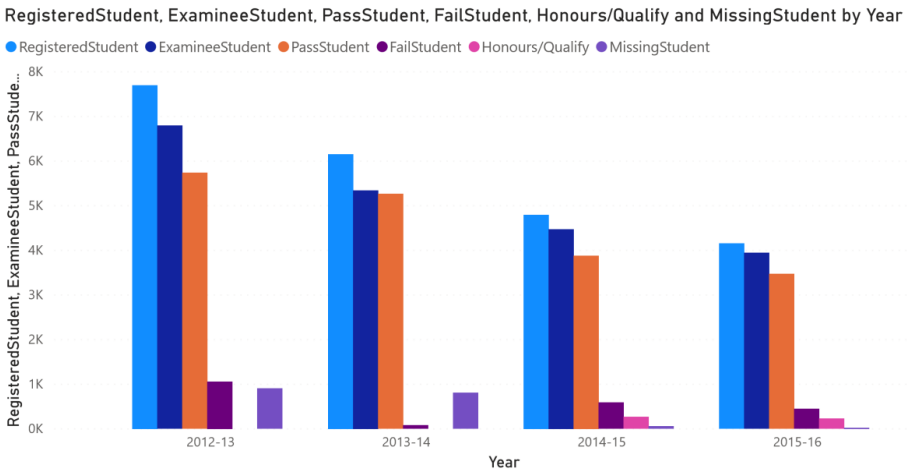

Figure 5 2012-2013 to 2015-2016 Period of Attending the University Student

Table 2 2013-2014 Intake Student Condition of Pass/Fail and Missing Condition

2013-2014 to 2016-2017 Period of Attending the University Student Condition							
Year	**Registered**	**Examinee**	**Pass**	**Fail**	**Honours/ Qualify**	**Missing**	**Missing Percent(%)**
2013-14	8114	6608	4052	2556		1506	18.56
2014-15	6037	5334	3782	1552		703	11.64
2015-16	5076	5075	3498	1577	404	1	0.019
2016-17	4303	4279	3598	681	393	24	0.55
	Total	Missing				2234	30.78

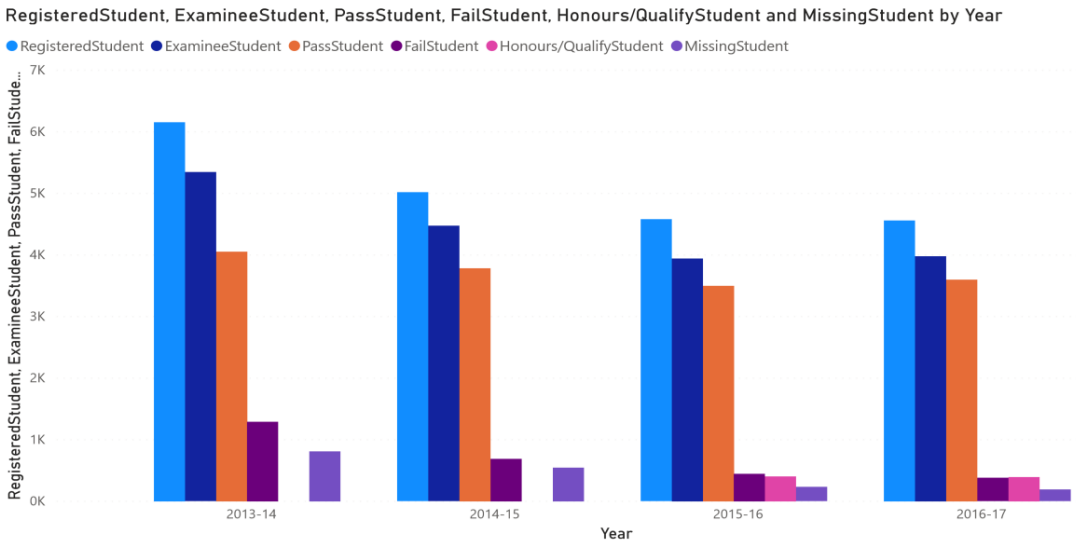

Figure 6 2013-2014 to 2016-2017 Period of attending University Student

Table 3 2014-2015 Intake Student Condition of Pass/Fail and Missing Condition

2014-2015 to 2017-2018 Period of Attending the University Student Condition							
Year	**Registered Students**	**Examinee Students**	**Pass Students**	**Fail Students**	**Honours/ Qualify**	**Missing Students**	**Missing Percent(%)**
2014-15	8146	6802	5768	1034		1344	16.49
2015-16	6196	5458	4365	1093		738	11.91
2016-17	5309	5286	4210	1076	404	23	0.43
2017-18	4920	4895	3598	1297	393	25	0.50
	Total Missing					2130	29.35

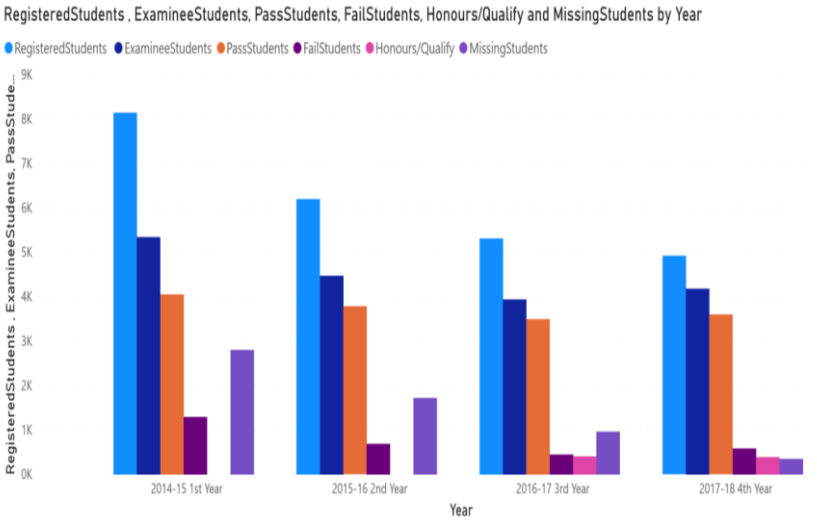

Figure 7 2014-2015 to 2017-2018 Period of Attending the University Student

So, (2250) students in 2012-2013 to 2015-2016 study period of graduate, (2234) students in 2013-14 to 2016-2017 study period of graduate and (2134) students in 2014-2015 to 2017-2018 study period of gradate are missing their graduate. As a analyze result these missing students who are resigned, transfer, and pass away student show in Figure (8) and (9). Mean value of student data who study period (2012-2013 to 2015-2016, 2013-2014 to 2016-2017, 2014-2015 to 2017-2018) of graduate are show in table (4). In this research of among these three study period of graduate, (7333) students are mean value of registered students and (2204) students are average missing students.

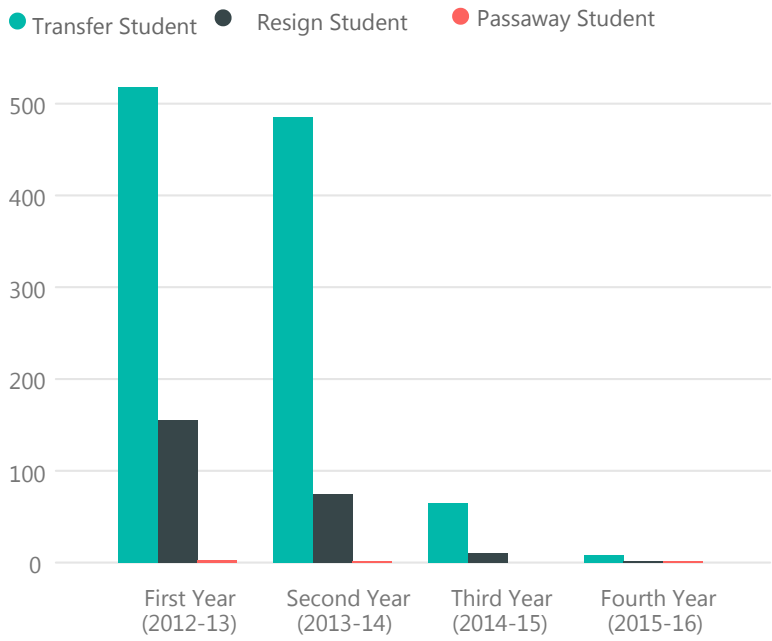

Figure 8 Missing Student of Dagon University (2012-2013 Intake)

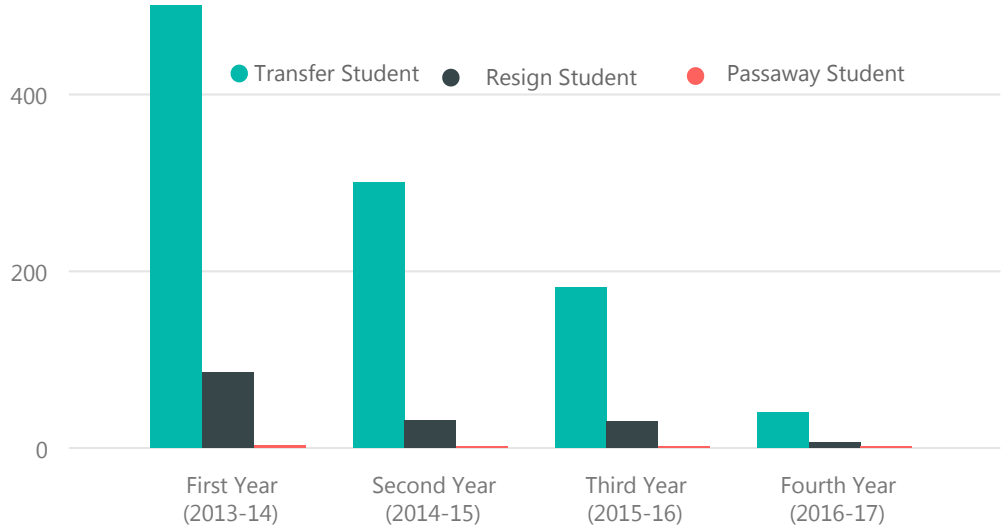

Figure 9 Missing Student of Dagon University (2013-2014 Intake)

Most missing students are first year student and they transfer to the other university. So, I take the sampling data from these students with some questions for their missing condition. Sample Questions to Missing Students are:

1. Which university to transfer?

☐ UDE ☐ Private ☐ Foreign

2. Why want to transfer other university?

☐ Economic ☑ Job ☐ Others

3. Why want to resign the university

☐ Economic ☐ Job ☐ Fail/punish

As the result of sampling questions, most students transfer to UDE (University of Distance Education) answer for No 1, most student answer for No 2 is Job and question No 3 answer is economic.

Table 4 Mean Values of Registered and Missing Student's Data

Means Values of Registered and Missing Student's Data		
Study Period of Graduate	**Registered Student**	**Missing Student**
2012-2013 to 2015-2016	7700	**2250**
2013-2014 to 2016-2017	6153	2234
2014-2015 to 2017-2018	8146	2130
Means Values	7333	2204.67

The SIRM system design and development is to create the academic decision makers with a tool for organized information access, enable assessment of academic statistical data for evaluation of part of the academic decision. These facts can be achieved and the following objectives are available:

- This system is a computerized software unlike most current systems, which necessitate manual data extraction and evaluation,
- Create the general database for adaptation to other universities.
- Design, implementation and analysis of a software system suitable for general university environments using the semester based education system
- Implement of intelligent user interfaces for easy navigation and gathering of required information from the databases,
- Demonstration of powerful techniques for achieving the aims of the project.

As the result of program, decision maker can analyze the relating issues that should be taken into consideration in the field of decision processes. There is students' enrollment, ranking of university, infrastructure, extra curriculum activities, health facility, faculty management, scholarship program, career guidance, hostel facility, transportation, and library and parking facility.

Conclusion

This research, analyze the decision support by using the university student data with intake result management system. This system has been developed by using Microsoft visual studio 2015, SQL server database and Power BI software are used to implement this proposed system. This paper focuses on the problem of offering reliable decision support to the process of student who is studied period to graduate by using result tables and graph of Dagon University student's data. In this paper discussed in different ways such registration students, pass, fail, and missing student for decision of university decision maker in Dagon University. SIRM system with DSS provides support for decision making for effectiveness system of decision maker in a university.

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THE USE OF QUICK RESPONSE CODE APPLICATION FOR ACADEMIC RECORD

Win Win Shwe[1*]

Abstract

This paper concentrates on developing Quick Response Code application for academic record. Academic record includes student enrollment record, student attendance record and student exam record. Student records are maintained by academic departments throughout the University and are used to provide documentation of an undergraduate or graduate student's academic progress within a specific department or program. The purpose of this paper is to create an efficient students' enrollment and their attendance system by using Quick Response code technology. Quick Response code is two dimensional barcodes that are used to encode and decode information. QR code can contain information such as text, URL links, automatic SMS messages, or just about any other information that can be embedded in a two-dimensional barcode. This encoded data can be decoded by scanning the barcode with a mobile device and a web cam attached to a computer that is equipped with a camera and QR reader software.

Keywords: QR Code, Students' Academic Record, Student Attendance Record.

Introduction

Technology and trends rapidly increase day by day and has affected the education area. In the digital era, ICT has become an integral part of every economic, social activity and education sector. Computers are widespread and many everyday-objects come equipped with computer technology. Mobile phones are equipped with high-resolution color displays, wireless access to the Internet, and respectable processing power and memory. Nowadays, different kinds of codes are used in order to store, retrieve and manage information. As new technology rapidly evolves, there are various ways of capturing academic record which include student enrollment record, attendance record and exam record. Students' record is one of the important issues for most of the education institutes like classes, school, college, universities, etc. A proper record needs to be maintained by teachers and administrator. Manual attendance record system is not efficient and requires time to arrange record and to check the attendance of each individual student. The proposed system is intended to replace the manual model.

Objectives

Objectives of this research are as follows:

- To study the concept of QR code which involves the structure of QR code, the characteristics of QR code, different types QR code and the features of different QR code
- To develop a fast and efficient academic record management system
- To analyze the benefits of using QR code for academic record management system
- To implement well-organized students' record management system by developing an application that helps teachers and administrators to monitor and review in the students' database with date and time

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Proposed System

The purpose of the proposed system is to provide the academic record which include student enrollment record with student name, class, academic year and QR code, student attendance record with students' QR code, name, class and present or absent. This system is also to provide a fast and efficient student's academic record management system for class in real time to store the data. The proposed system developed by scanning the QR code using a webcam attached to a computer. Subsequently the data is recorded in a database for retrieval and reporting purposes.

System Requirement

This proposed system requires minimum hardware specification of Intel core i5 with RAM size of 4GB and minimum hard disk capacity of 500 GB. The required software used for development process of the QR codes scan and Web based program requires an operating system Microsoft Windows 7 or Server with 32 bit. A web based WAMP server (PHP, MySQL & Apache) with an additional QR code scanner application. The development system creates with Laravel (PHP Framework), HTML, CSS, and Java script as front end and MySQL server as back end.

QR Code

A QR Code is a two-dimensional barcode introduced by the Japanese company Denso-Wave in 1994. This kind of barcode was initially used for tracking inventory in vehicle parts manufacturing and is now used in a variety of industries. QR stands for "Quick Response" as the creator intended the code to allow its contents to be decoded at high speed.

A QR Code is a matrix code developed and released primarily to be a symbol that is easily interpreted by scanner equipment. It contains information in both vertical and horizontal directions, whereas a classical barcode has only one direction of data (usually the vertical one). Compared to a 1D barcode, a QR Code can hold a considerably greater volume of information: 7,089 characters for numeric only, 4,296 characters for alphanumeric data, 2,953 bytes of binary (8 bits) and 1,817 characters of Japanese Kanji/Kana symbols. QR Code also has error correction capability. Data can be restored even when substantial parts of the code are distorted or damaged. In the QR Code standard, corners are marked and estimated so that the inside-code can be scanned. The barcode recognition process has 5 steps: (1) edge detection, (2) shape detection, (3) identification of barcode control bar, (4) identification of the barcode orientation, dimensions and bit density using the control bar, and (5) calculating the value of the barcode. [3] A QR code shows in Figure (1).

Figure 1 QR Code

QR Code Structure

QR code structure is very important to for encoding and decoding QR code because this is the main features of the process. There are various version of QR code from version 1 until version 40. Each version has a different module configuration or number of modules.

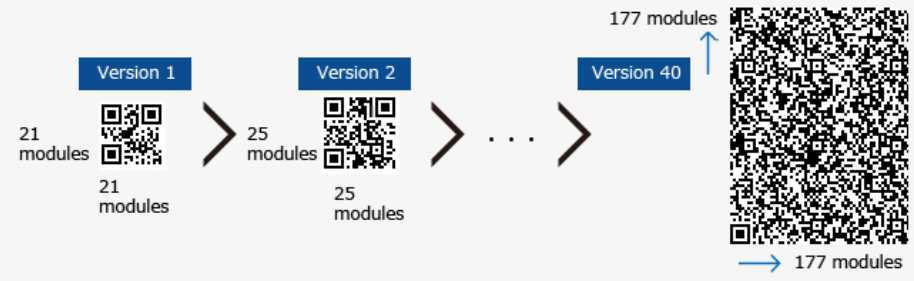

Figure 2 Version of QR Code

Module configuration refers to the number of modules contained in a symbol, commencing with Version 1 (21 rows and 21 columns) up to Version 40 (177 rows and 177 columns) shown in Figure (2). Each version thereafter increases by 4 rows and 4 columns. The largest version is version 40 with results in the 31,329 needed to encode the 3kb of data.

Characteristics of QR Code

(1) High Storage Capacity

A QR code symbol can store up to 7,089 characters of information, which is a huge amount as compared to 1-D barcode.

(2) Encodable Character Set

Numeric data (Digits 0-9)

Alphanumeric data (upper case letters A-Z; Digits 0 - 9; nine other characters: space, : % * + - / _ $)

Kanji characters

(3) Small Printout Size

The information in QR code is stored in both horizontal and vertical directions. Due to this feature, for the same amount of data, space acquired by QR code is one fourth times less than the space acquired by 1-D barcode.

(4) 360 Degree Reading

QR code is readable from any direction. This feature is provided by the finder patterns present at three corners of the symbol. The finder pattern helps to locate the QR code.

(5) Capability of Restoring and Error Correction

If the part of code symbol is damaged or dirty, data can be recovered. The error detecting procedure can focus on the region of correct information.

Implementation of the Proposed System

The main feature of this system is that store student enrollment record and attendance record, exam record and seminar attendance record and make excel report from attendance data. This is very simple system, there are three main modules which include admin, teacher and student. Student can only register, while teacher able to check all students' data. In this system admin and teacher password has been store in encrypted form.

The proposed system will need first step enroll from each student. In the registration form, the student enter email, password, confirm password, name, father's name, Roll No, academic year, class, date of birth and then click the register button. Student's registration page is shown in Figure (3). After that the system will generate QR code each student shown in Figure (4).

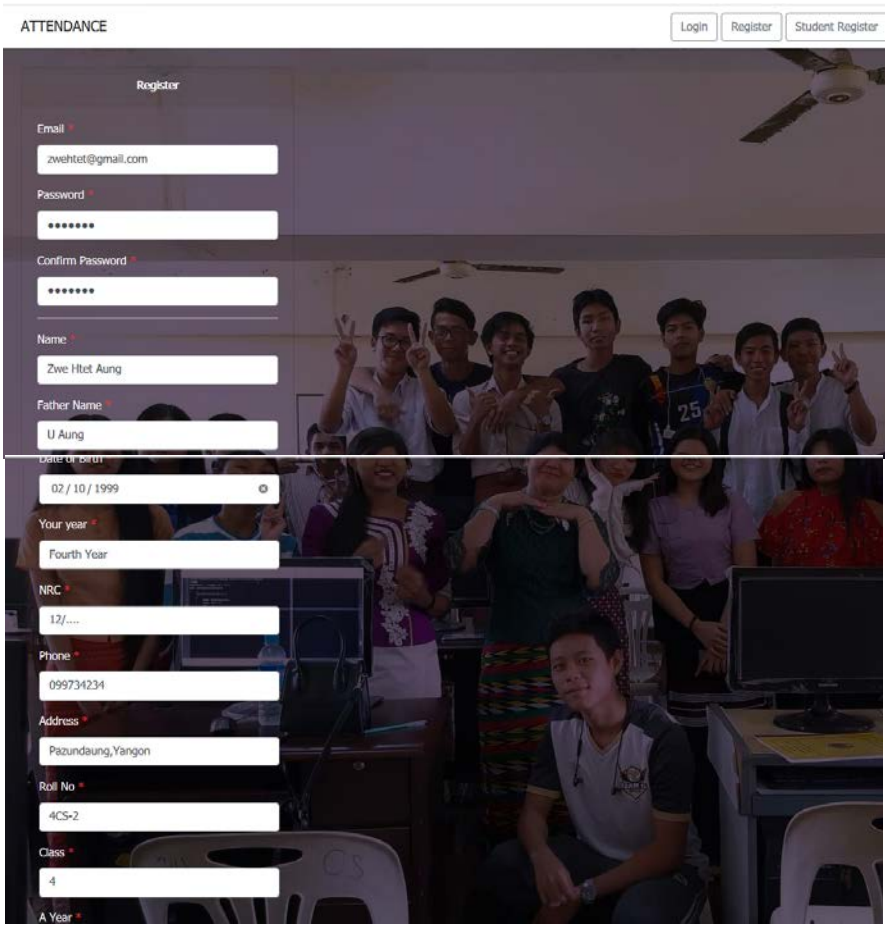

Figure 3 Registration Page for Student

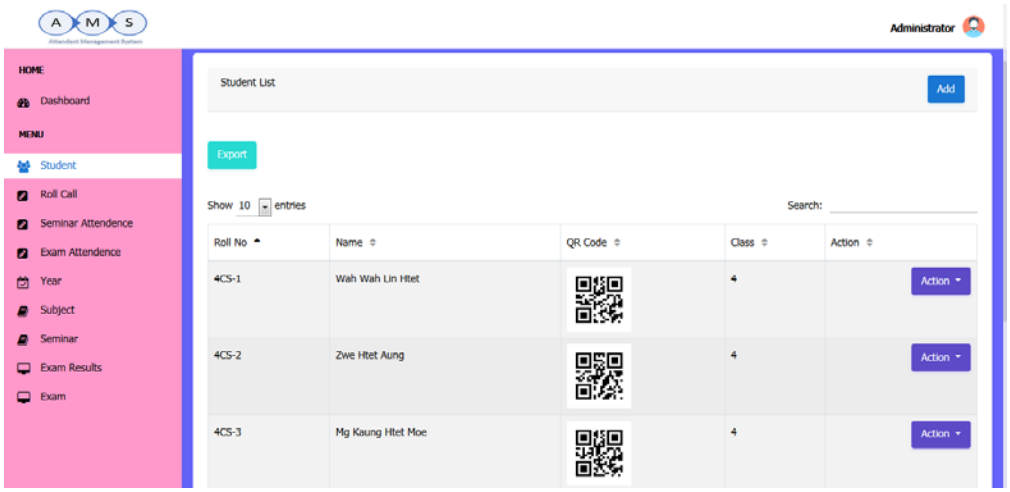

Figure 4 Registration Page for Student

The next step of this system is the teacher needs to register for each lecture and each module in a classroom. In the registration form, the teacher insert teacher's name, email address, password and confirm password and then click the register button. After the registration process success, the teacher can login now. Register page shows in Figure (5).

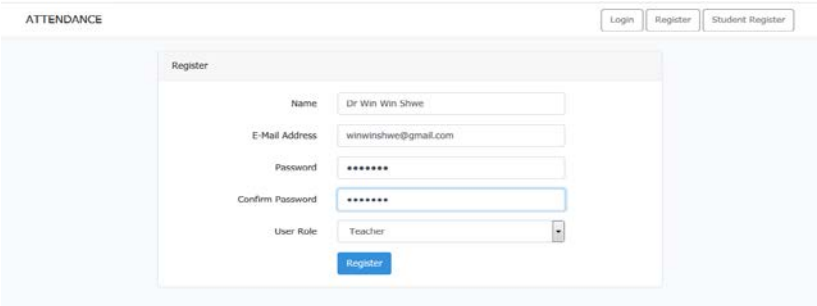

Figure 5 Registration Page for Teacher

A teacher who has been given an email address and password simply enters that information and selects the Login button. Figure (6) is shown in login page.

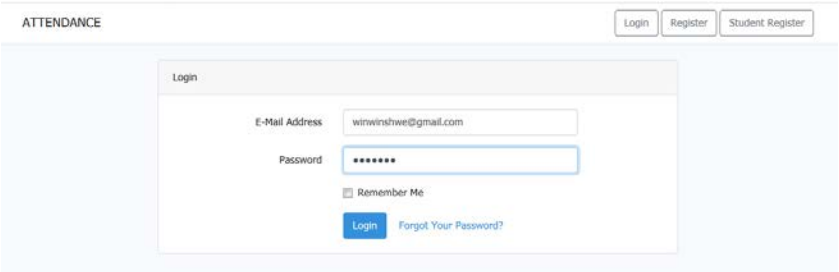

Figure 6 Login Page

After successful login teacher will view the home page of Student Attendance. There are four categories in this page, Student, Roll call, Year and Subject shown in Figure (7) Firstly, The teacher will set lecture class to record attendance.

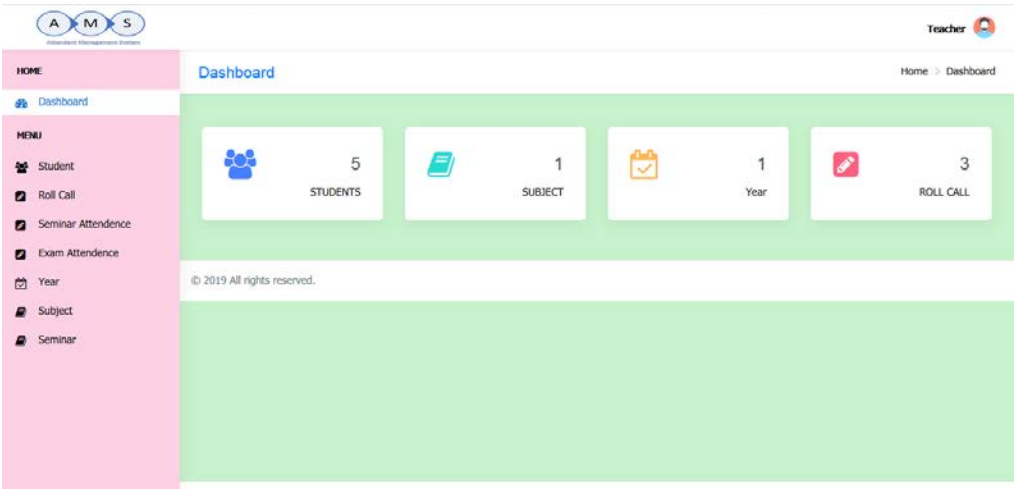

Figure 7 Student Attendance Page

In the next page, the teacher can check students' attendance. And also student need to scan QR code reader with their id card. Students select his or her Roll No, and Subject and choose web cam attached to the computer. And then click scan button. If the student information is correct, scan success message will appear. Figure (8) and figure (9) are shown in QR code scan process.

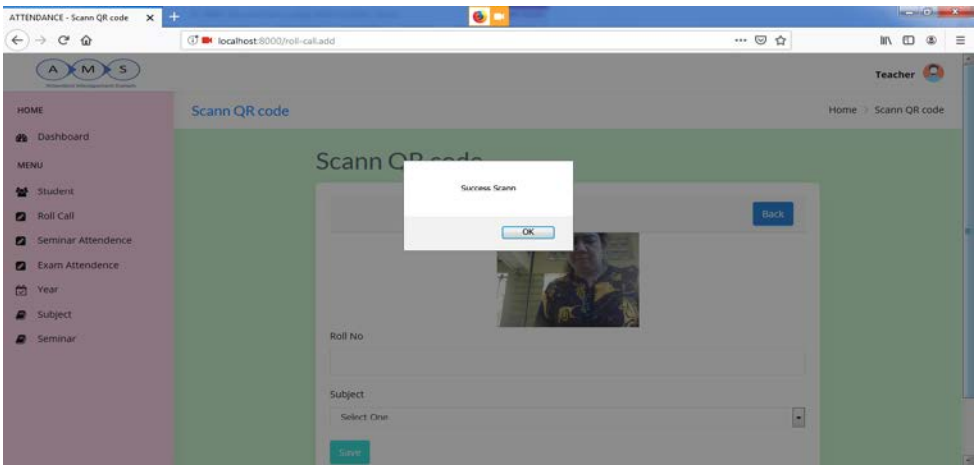

Figure 8 Student Roll Call Scan Page

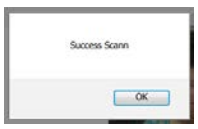

Figure 9 Student's Roll Call Scan Successful Page

Results and Discussion

QR Code based Students' Record Management System" is developed for taking and storing the student's data, attendance of the students in the classroom and attendances of students in seminars and exams. In this system, the teacher who has to handle the subjects, will be responsible to mark all attendance records of the students. An accurate report based on the student data is generated easily.

Advantages of QR Code based Students' Record Management System are follows: It can

- Provide better security by using QR code
- The system is easy to maintain and cost effective
- Generate the result quickly and no use of paper work
- Provide accurate and efficient data for academic record
- User friendly

It is rather convenient for storing academic record using QR code. Among the academic records, a Students' Record Management System is created testing students' record by using only QR code. It is found out that the intended data are able to be searched through a QR code. As an administrator, it is easy to store records and he or she is able to update them when necessary. For the sake of teachers, they will also find QR code is more convenient than manual to search lecture attendances, seminar attendance and exam attendance easily.

Conclusion

The Students' Record Management System is to provide the benefits of using QR code. QR Code is the most famous 2D barcode in the world. QR codes are easy to generate and use. QR technology is open source technology, available free of cost and simple implementation process and user friendly technology. Users do not need special knowledge for using QR code, only required smart phones like iPhone, Android phone, and webcam attached to a computer etc. with QR code scanner. In addition, the computerized processes will be performed more quickly and accurately than the manual system. There will be no paper work involved; all processes are performed by computer. This will help protect against loss of data.

Acknowledgement

First of all I would like to thanks to Dr Win Naing, Rector of Dagon University, who offers to me opportunity to carry this research. I would like to thanks to Dr Nwe Nwe Win, Professor (Rtd), Head of Computer Studies Department, University of Yangon who provides me with valuable advice, support and guidance during this research. I wish to acknowledge with extreme thanks to Dr Daw Yi Mon Win, Associate Professor, Head of Computer Studies Department, Dagon University who encouraged succeeding this research completely.

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MOLECULAR IDENTIFICATION OF CELLULOLYTIC BACTERIAL ISOLATES FROM THE GUT OF THE TERMITE *MACROTERMES GILVUS**

Phyo Nandar Win[1], Moe Moe Aye[2], San Maung Maung Theint[3], Khin Myat Myat Zaw[4], Khin Htwe Yee[5], Thida Lay Thwe[6]

Abstract

The present work was conducted to investigate the identification and phylogenetics of the cellulolytic bacteria from the gut of termite. The termite specimens were collected from termite mounds in campus of University of Yangon and Hlawga Wildlife Park in Yangon Region. Study period was from June, 2018 to December, 2018. Six gram negative bacterial isolates from the termite gut were identified by 16S rRNA gene sequencing. Phylogenetic analysis and BLAST homology and similarity search of GenBank based on mitochondrial 16S rRNA gene sequences indicated that all six were *Pseudomonas putida* (99%-100% homology).

Keywords: 16S rRNA gene, Phylogeny, *Pseudomonas putida*

Introduction

Termites are classified into lower and higher termites, and they contain diverse microbes in their gut. Lower termite has protists and bacteria in their gut, although there is less information about the bacteria. However, higher termites lack protists and contain only prokaryotes (Ohkuma and Brune, 2011). The ability of termites to digest cellulose was collaborated with mutualistic symbiotic action of various microorganisms in the digestive tract (Ohkuma, 2003). Eutick *et al* (1978) stated the ability of termites, especially the caste of workers, in degrading cellulose is supported by the presence of cellulolytic bacteria and other enzymes in the digestive tract of termites. As a higher termite, *Macrotermes gilvus* possesses bacteria in its gut. These bacteria function as a second source of cellulolytic enzymes. The bacteria that have been identified from termite gut belong to the species of aerobes and facultative or strict anaerobes (Ramin *et al*., 2008). There were many previous works on gut of termites regarding their gut microflora and microfauna consisting cellulolytic and non- cellulolytic bacteria, protozoa or protists and fungi whose molecular identification had been done based on 16S rRNA gene sequences compared to related references in Gen Bank through phylogeny and Blast search (Wenzel *et al*., 2002; Mathew *et al*., 2012; Pourramezan *et al*., 2012; Upadhyaya *et al*., 2012; Ferbiyanto *et al*., 2015; Shinde *et al*., 2017). This study focused on molecular phylogeny and identification of the isolated cellulolytic bacteria from the gut of the higher termite *Macrotermes gilvus*, to identify selected cellulolytic bacteria based on 16S ribosomal RNA (rRNA) gene sequences and to determine the phylogenetic and similarity relationship of the cellulolytic bacterial species.

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[*] Best Paper Award Winning Paper in Zoology (2019)

Materials and Methods

Bacterial isolation

Termites were collected from termite mounds in campus of University of Yangon and Hlawga Wildlife Park in Yangon Region. Termites were washed with distilled water and dried on filter papers. Subsequently the specimens were sterilized externally by 70% ethanol and washed in distilled water for 1 min. Each termite specimen was then put on a glass slide and separated into head and body by a forceps. After removing the heads, the bodies (n= 10) were put into a 10 mL test- tube and crushed with a glass rod and 1mL of sterile distilled water was added into the tube. This mixture was centrifuged at 4000 rpm for 10 min to remove large debris. The supernatant was serially diluted ten fold down to 10^{-5} level. And then 1 mL supernatant of each dilution was transferred to a Petri-dish and 20mL of autoclaved plate count agar (PCA) was added to the supernatant, and mixed by gentle rotation and allowed to solidify according to pour plate method (Dubey and Maheshwari, 2002). The plates were incubated at 37°C overnight. Grown bacteria were purified on Carboxy methyl cellulose (CMC) plate medium. Morphological determination of bacterial colony was based on Handbook of Microbiology (Bisen and Verma, 1998).

Biochemical identification and *in vitro* cellulolytic activity tests of isolates

KB009A HiCarbo™ Kit and KB009C HiCarbo™ Kit (HiMedia, India) were used to determined biochemical reactions for identification of the isolated bacteria. The clear zone called halo zones around colonies against the red color of Congo red indicated positive for cellulolytic activity. Cellulolytic activity was measured as a diameter of clear zone after the CMC plate was poured by 1% congo red reagent. The clear zone sizes, measured in diameters (mm), reflected degree of cellulolytic activity (Lu *et al*., 2004).

Genomic DNA extraction

DNA extraction was done using PureLink® Invitrogen Genomic DNA Mini kit, according to the manufacturer's instructions.

PCR amplification of 16S rRNA gene

The 16S rRNA gene of the bacterial isolates was amplified using universal primers, 20F (5′-AGA GTT TGA TCA TGG CTC -3′) and 1500 R (5′-GGT TAC CTT GTT ACG ACT T-3′) (Weisburg *et al*., 1991). Polymerase chain reaction (PCR) was performed in a Thermal- cycler (Proflex PCR System, ProFlex™ Base Block 33). A total volume of 20µL with mixed components of 10µL of master mix, 1µL of forward primer, 1µL of reverse primer, 2µL of DNA samples, and 6 µL of distilled water were used for PCR amplification. Thermo cycling conditions were set up as follows: initial denaturation at 94°C for 4 min, denaturation at 94°C for 40 sec, annealing and extension at 55°C for 1 min and 72°C for 1 min and 10 sec, respectively, and final extension was carried out at 72°C for 10 min. The whole process was carried out in a total of 35 cycles. The amplified products were fractionated on 1% agarose gel (stained with ethidium bromide) together with DNA ladder (100 bp) as size marker. The PCR products in the gel were visualized with a UV transilluminator and photographs of the gel was taken under UV light as records.

Sequencing PCR of 16S rRNA gene

The amplified 16S rRNA gene fragments were utilized for sequencing reactions in PCR, using the same primers of 20F and 1500R (Weisburg *et al*., 1991). A total volume of 20μL with mixed components of 2μL of 5X sequencing buffer, 4μL of Big dye kit, 1μL of forward/ reverse primer, 6μL of DNA samples, and 7μL of distilled water were used for PCR reaction. The sequencing reaction was carried out in a thermal-cycler (Proflex PCR System, ProFlex^{TM} Base Block 33). PCR conditions were initial denaturation at 96°C for 1 min, denaturation and annealing at 96°C for 10 seconds and 50°C for 5 seconds, respectively. Extension was carried out at 60°C for 4 seconds. The whole process was carried out in a total of 30 cycles.

DNA sequencing

ABI 3500 Genetic Analyzer autosequencer (Applied Biosystems) was used for DNA sequencing. The dried up PCR products were prepared by adding 20μL HiDi formamide, mixing well with pipetting. Subsequently the solution was transferred to the 96 wells plate and set in the autosequencer for sequencing. The sequenced data were downloaded from the ABI 3500 computer onto DVD discs and laptop computer using MEGA 7 software, and the sequences were aligned and analyzed.

Sequence analysis and phylogenetic analysis

Sequence analysis was done on 16S rRNA gene marker. Sequence alignment was performed with the ProSeq software. Then, the forward and reverse sequences of both samples were aligned using pairwise alignment tool in BioEdit. BLAST (Basic Local Alignment Search Tool) was used to search for similarity in the nucleotide sequence database of GenBank.

Phylogenetic analysis trees were constructed using Molecular Evolutionary Genetic Analyses (MEGA-7) software with Maximum-Likelihood (ML) and Neighbor-Joining (NJ) methods at 1000× bootstraps.

DNA extraction, PCR reaction, NanoDrop measurement, Gel electrophoresis, and DNA sequencing and analysis were all conducted in the Molecular Biology Laboratory, Zoology Department at Yangon University.

Results

Morphological and biochemical characteristics of gut cellulolytic bacteria

Six bacteria were isolated from the gut of worker *Macrotermes gilvus*, i.e. isolate code 1ND 7, 1ND 9, 1ND 12, 8ND 2, 8ND 7 and 10ND 3. All bacterial isolates were Gram negative and rod shaped (Plate 1). All bacterial colonies were cream colour and undulated.

All bacterial isolates were motile and showed positive reactions to biochemical assays of citrate utilization, and catalase activity except isolate 1ND 9. They all showed negative reactions to biochemical assays of methyl red, Voges-Proskauer, gas and indole production, urease and gelatinase activities, fructose, raffinose, melibiose, rhamnose, cellobiose, melezitose, α- Methyl-DMannoside, xylitol and sorbose tests. However, isolates 8ND2, 8ND 7 and 10ND 3 showed positive reactions to biochemical assays of xylose and mannose tests.

Results of congo- red assay for cellulolytic activity

Congo- red assay of all the isolates was done *in vitro* to determine their cellulolytic activity. The isolates that digest cellulose as 1% CMC in the media, produces clear zones. The diameter of clear zones were measured which corresponded to the cellulose digesting ability of the respective isolates. Cellulolytic activity test showed that 10ND3 isolate had the largest cellulolytic index (3.28) and 8ND 7 isolate had the smallest cellulolytic index (2.14) (Plate 2, Table 1).

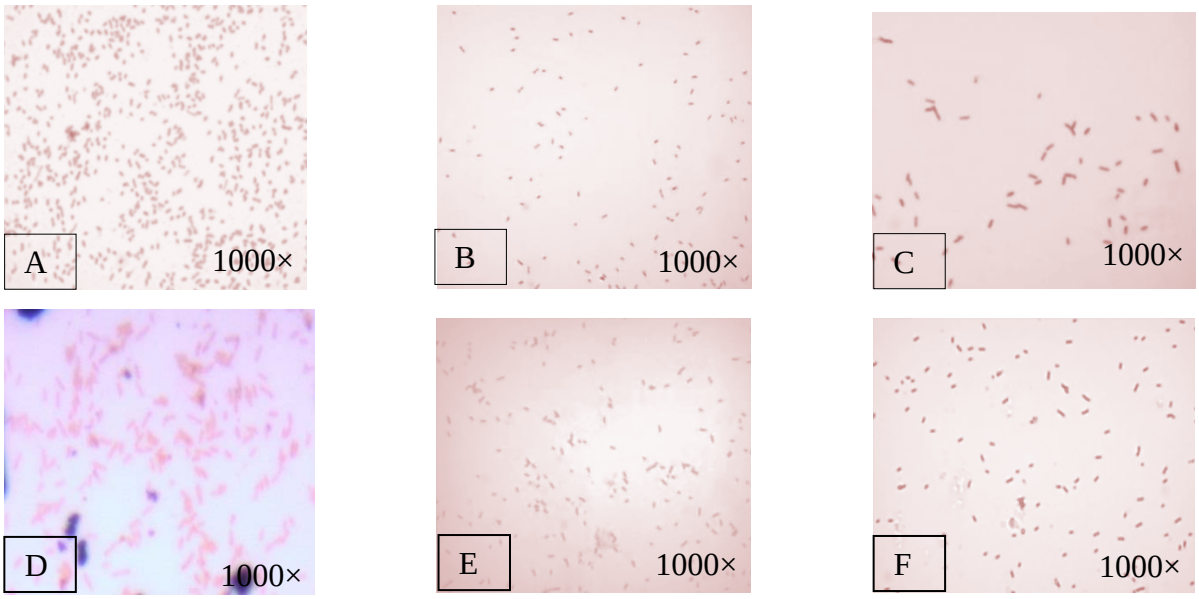

Plate 1 Gram staining of isolated cellulolytic gut bacteria. (A) 1ND 7, (B) 1ND 9, (C) 1ND 12, (D) 8ND 2, (E) 8ND 7 and (F) 10ND 3 bacterial isolates

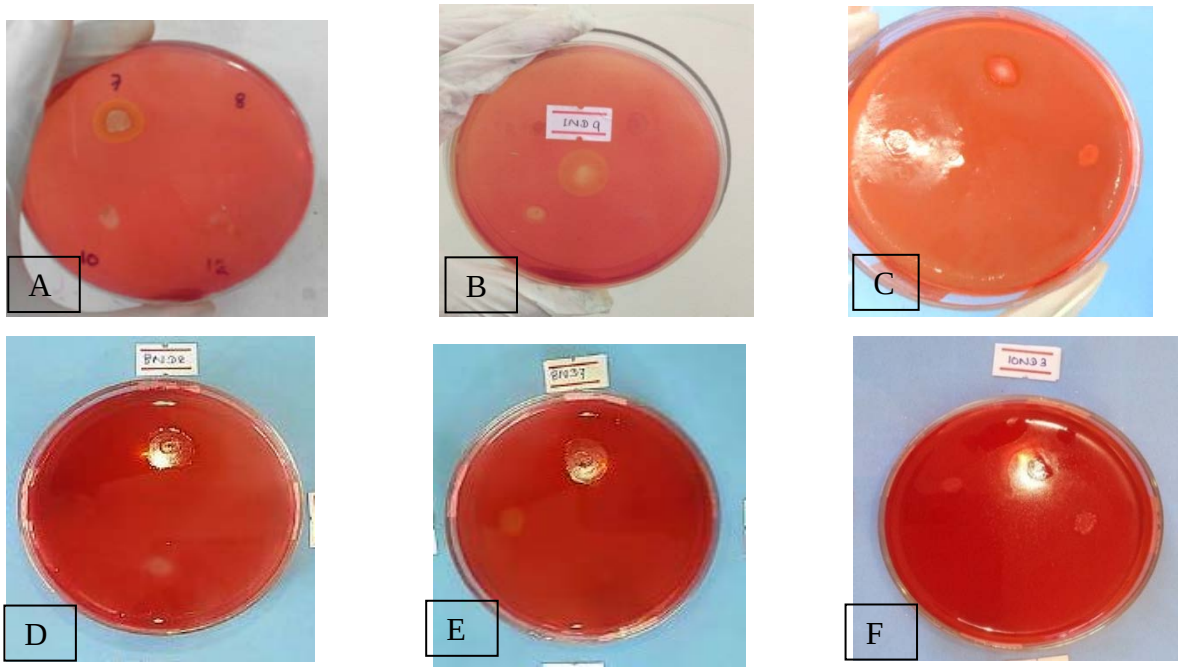

Plate 2 Clear zones are seen against the red colour of Congo red for the positive cellulolytic test. (A) 1ND 7, (B) 1ND 9, (C) 1ND 12, (D) 8ND 2, (E) 8ND 7 and (F) 10ND 3 bacterial isolates

Table 1 Cellulolytic index of the isolated gut bacteria

Sr. No.	Isolate code	Isolate species (Tentative ID)	Zone diameter (Zd) (mm)	Colony diameter (Cd) (mm)	Clear zone diameter (mm)	Ratio Zd/Cd
1.	1ND7	*Pseudomonas putida*	21	9	12	2.33
2.	1ND9	*Pseudomonas putida*	17	6	11	2.83
3.	1ND12	*Pseudomonas putida*	16	7	9	2.28
4.	8ND2	*Pseudomonas putida*	22	7	15	3.14
5.	8ND7	*Pseudomonas putida*	15	7	8	2.14*
6.	10ND3	*Pseudomonas putida*	23	7	16	3.28**

(**) = Highest, (*) = Lowest activity index

BLAST sequence similarity search for cellulolytic bacterial isolates

The similarity search of reference sequences in GenBank for isolate identification confirmed the cellulolytic isolates 1ND7, 1ND9, 1ND12, 8ND2, 8ND7 and 10ND3 as *Pseudomonas putida* (Genus ID similarity of 100%; Species ID similarity of 99% - 100%).

Table 2 Gen Bank BLAST search results for molecular similarity for identification

Sr. No.	Isolate code	Species (Confirmed ID)	Genus Identification (%)	Species Identification (%)
1.	1ND7	*Pseudomonas putida*	100%	99%
2.	1ND9	*Pseudomonas putida*	100%	100%
3.	1ND12	*Pseudomonas putida*	100%	100%
4.	8ND2	*Pseudomonas putida*	100%	100%
5.	8ND7	*Pseudomonas putida*	100%	100%
6.	10ND3	*Pseudomonas putida*	100%	100%

16S rRNA gene, 630 bp alignment (Gram negative)

Phylogenetic relationship of the gram- negative isolates obtained from the termite

ML tree analysis of six identified cellulolytic Gram- negative isolates (1ND9, 8ND7, 8ND2, 1ND12, 10ND3 and 1ND7) revealed all six isolates formed a distinct cluster (99% confidence) in a clade (100% confidence) also containing a different cluster of reference *Pseudomonas putida* in ML tree (Figure 1). Likewise, similar results were also found in NJ tree (Figure 2). The results for ML and NJ tree indicated the six isolates of the present study to be *Pseudomonas putida*. The Gram-positive out group of reference *Bacillus* spp. and *Cellulomonas* spp. diverged clearly indicating the six isolates to be Gram- negative.

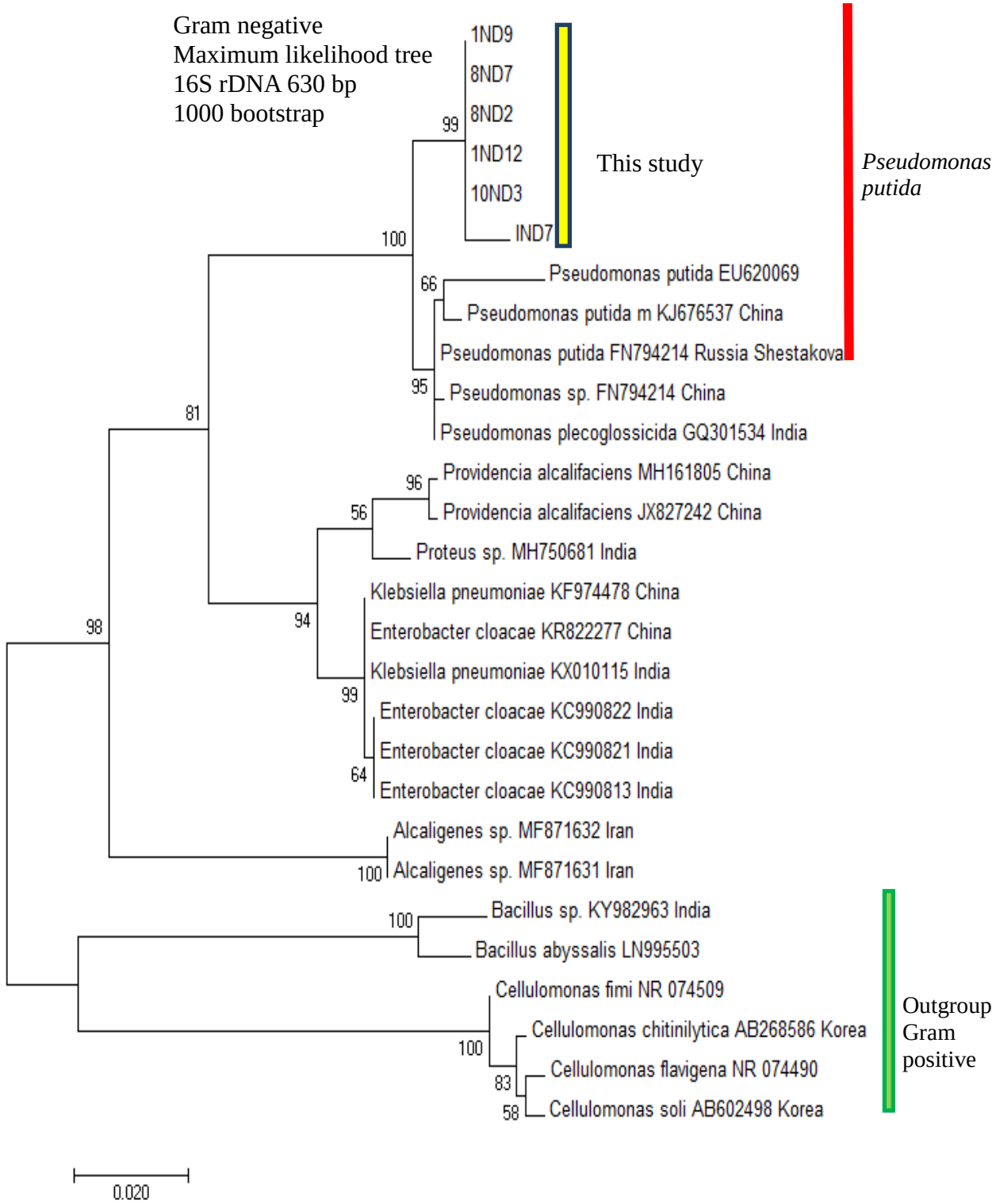

Figure 1 Molecular phylogenetic analysis by Maximum likelihood method for cellulolytic Gram negative isolates 1ND9, 8ND7, 8ND2, 1ND12, 10ND3 and 1ND7 using 630 aligned basepairs. Scale bar indicate nucleotide mutation/ site/ m yr.

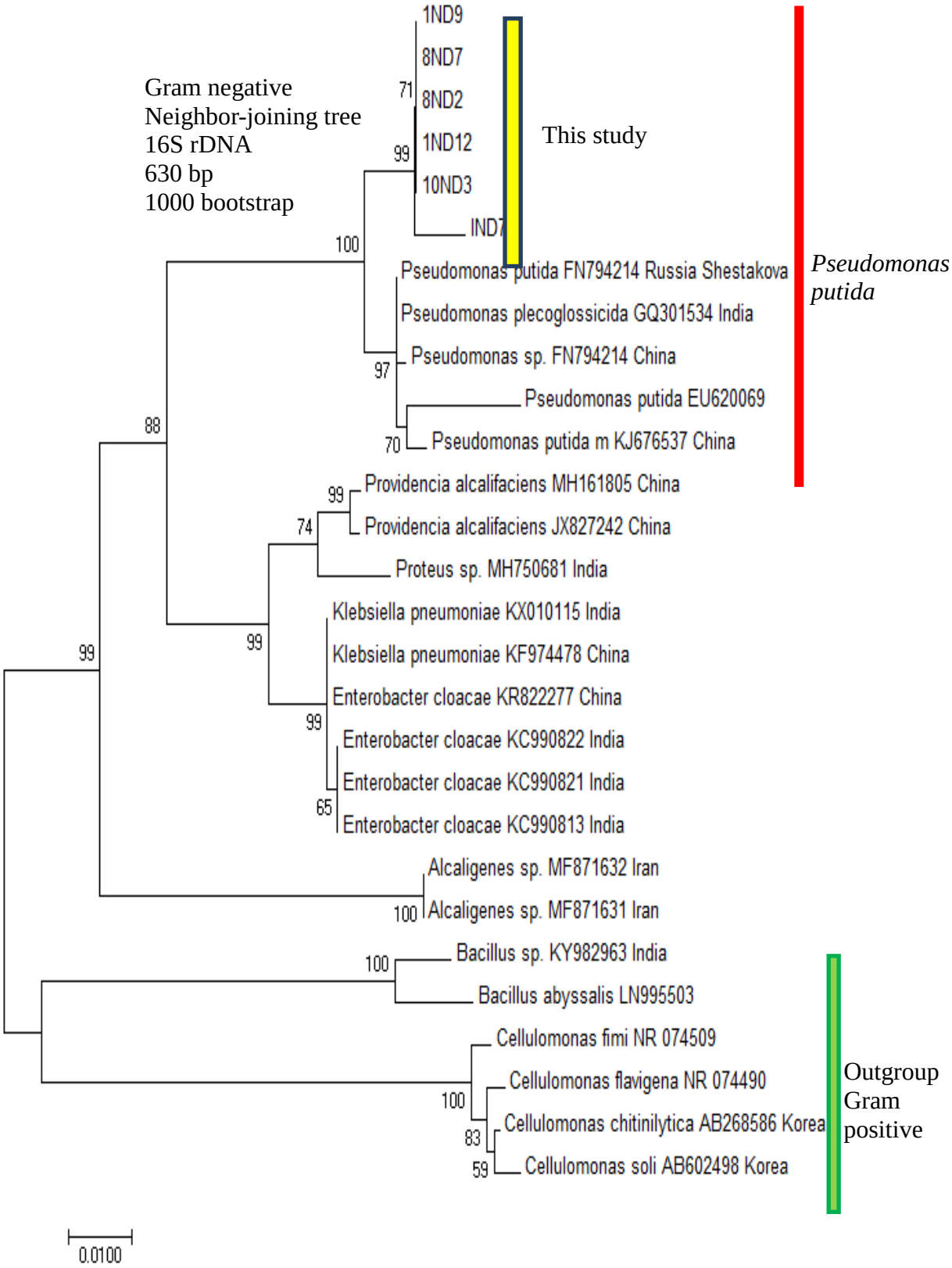

Figure 2 Molecular phylogenetic analysis by Neighbor-joining method for cellulolytic Gram negative isolates 1ND9, 8ND7, 8ND2, 1ND12, 10ND3 and 1ND7 using 630 aligned basepairs. Scale bar indicate nucleotide mutation/ site/ m yr.

Discussions and Conclusion

Termites thrive in great abundance in the terrestrial habitats by recycling cellulose from plants and wood. Termites have their own cellulolytic mechanism, but also harbor gut microbes which assist them to degrade cellulose (Upadhyaya *et al*., 2012).

The presence of discoloration of congo red around the colony on CMC agar indicates the cellulase activity of the bacteria (Lu *et al*, 2004). The diameter of clear zone is a measure of degree of cellulose digesting ability of bacterial strains (Upadhyaya *et al.*, 2012). The cellulase activity of the isolates can be known by measuring the ratio of the total zone diameter to colony diameter on the agar plate.

Regarding *in vitro* cellulolytic activity, highest activity was found in *Pseudomonas putida* (isolate code 10ND3) with Zd/Cd ratio of 3.28 and *Pseudomonas putida* isolate (8ND7) showed the lowest cellulolytic activity at ratio of 2.14. Out of these 15 only 2 bacterial isolates showed zone diametre to colony diametre ratio (Zd/Cd) below 2.0. Out of 15 isolates, 11 isolates showed cellulolytic activity in the range of Zd/Cd ratio 2.0 - 3.0 and only 2 which were showing highest activity above 3.0 (Nidhi Kakkar, 2015). Our finding was similar to the previous study.

The molecular identification was done for the six gram negative cellulolytic bacteria isolates from termite gut. Based on molecular identification of 16S rRNA gene, isolates 1ND9, 1ND12, 8ND2, 8ND7 and 10ND3 had 100% similarity with *Pseudomonas putida* and 1ND7 has 99% similarity with *Pseudomonas putida*.

According to Weisburg *et al* (1991) the amplification of 16S rRNA gene sequences with primer 20F and 1500R showed 1500bp DNA amplicons. In this study, the results revealed that, the primers could successfully isolate 16S rRNA gene but only partially (~850bp) with only 630bp successful alignment. Phylogenetic and Blast similarity analyses of the 16S rRNA gene sequences revealed the presence or occurrence gut bacteria *Pseudomonas* sp. and *Bacillus* sp. in *Macrotermes* sp. termite (Muwawa *et al*., 2016); *Bacillus megaterium* and *Pseudomonas spingomonas* in the termite *Zootermopsis angusticollu* (Wenzel *et al*., 2002); *Bacillus megaterium* in *Macrotermes gilvus* (Ferbiyanto *et al*., 2015).

The results of the present phylogenetic analysis indicated isolates 1ND7, 1ND9, 1ND12, 8ND2, 8ND7 and 10ND3 were closely clustered with reference *Pseudomonas putida.* Some members of the genus *Pseudomonas*, such as *P. fluorescens*, have been reported to produce cellulase enzymes (Hall, 1995). *Pseudomonas putida* was reported to be found in the gut of termites based on 16S rRNA gene phylogenetic and Blast analyses in India (Shinde *et al.*, 2017).

Species such as termites and crayfish produce their own cellulases that are different from those produced by their indigenous microflora (Ohkuma, 2003).

The results of the present study, it is hoped, would contributed to application of cellulase producing bacteria in various industries like production of fruit juices, biofuels, detergents, alcohols and other fermentation technologies.

Hence, the data generated in the present study could serve as a basis for further microbiological and molecular phylogenetic research on cellulolytic gut bacteria and their various species of termite host.

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POPULATION VARIATION OF PLANT-PARASITIC NEMATODES INFECTED IN RICE FIELD FROM KYAING PHAUNG VILLAGE, KYAING TONG TOWNSHIP

Naing Naing Oo[1], Moe Moe Aye[2], Cho Sin Win[3], Nyein Nyein San[3]

Abstract

In Myanmar, rice is the national food crop. Rice production needed for local consumption as well as for export. Root-rot disease occurred in some rice growing areas of the country; however, information about the disease was very limited. Therefore, the present study was conducted two rice farms (Hmawbe- 3) according to different situation. One was sandy and flooding area (Site- I) and another one was muddy and non-flooding area (Site-2) from the Kyaing Phaung Village during the rainy season of 2018. The soil and roots samples were randomly collected (one center and four corners) from both farms fortnightly. The collected samples were taken to the laboratory of Zoology Department, Kyaing Tong University. The nematodes extraction was done from soil and roots separately by Whitehead Tray method. The extracted nematodes were examined using the stereo and compound microscopes and indentified down to genus level. Total seven genera of plant- parasite nematodes were recorded from both study sites. Among them the number of *Hirschmenniella oryzae* (root-rot nematode) were highest during July and gradually reduced on September however *Meloidogynes* spp. (root-knot nematode) were sudden rise until the harvesting time in both study sites. Therefore, it may be assumed that the root damage nematodes were distributed around the rice field of Kyaing Phaung village. The present findings would be helpful towards the management of agriculture including the rice and other crops at the East Shan State.

Keywords: Rice field, nematodes, Hmawbe-3, plant-parasitic

Introduction

Rice is the dominant staple food crop in the developing countries. Almost 90 percent of rice is produce and consumed in Asia, and 96 percent in developing countries (FAO, 2004). In Myanmar, rice is the stable food for all people. Rice production needed for local consumption as well as for export. However, rice crop is subjected to a number of pests and diseases and plant parasitic nematodes are generally regarded as potentially serious constraints to crop productively. Among the rice diseases, nematode infestation can result in yield losses of up to 30 percent in general (Doberman and Fairhurst, 2000).

Over 150 species of nematodes parasitize rice. Some have a geographically restricted distribution, while others occur throughout the rice-growing regions of the world. Nematodes parasites on rice may be divided into foliar parasites and root parasites. Injury from foliar parasites produces distinctive symptoms, while above ground symptoms of root damage can be difficult to diagnose. Most nematode species are specific to a particular rice growing environment; however, some species occur across a range of environments. Communities of several potential pest species of nematodes can occur in the same field, which complicates management decisions. In the dynamic and hydrologically heterogeneous conditions of small rice farms, nematode communities may be particularly diverse (DFID, 2004).

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Nematodes can cause significant yield losses through direct pathogenic effects; for instance, by suppressing seedling establishment and growth, and yield. Nematodes can interact with other soil biotic and abiotic factors, and influence rice-weed competition. Nematode attack can induce symptoms of water stress and intensify symptoms under low water availability, reducing a crop's ability to recover from drought stress (DFID, 2004).

The main rice growing areas of Myanmar are divided into three regions according to different types of soils and climates. They are upper Myanmar, lower Myanmar and delta region (Ye Goung, Khin Win and Win Htin, 1978).

The nature of East Shan state was included in upland region. Especially, Kyaing Tong region have low rainfall and plenty of mountain ranges around the area. It has also cultivated rice plant as main crop for local consume. In the past, emphasis had been placed on nematode disease of rice such as Ufra, white tip, root-knot in Myanmar. But information of the nematodes disease from Kyaing Tong region was poor. Of these regards;

- To record the root nematodes and other plant- parasitic nematodes from the rice field
- To compare the nematodes population from different situation of rice field
- To investigate the population fluctuation of plant parasitic nematodes during the study period

Materials and Methods

The recent survey was carried out from June to December, 2018 in Kyaing Phaung village, Kyaing Tong Township, East of Shan State. In the hmawbe-3 rice field, soil and roots were collected fortnightly from two different situation of farms (one was flooding and sandy area and other was no flooding and muddy field).

Sample collection

Rice plants with roots and soils sample were collected from both rice fields. Soil and roots samples were taken randomly from five different sites (four corners and one center) at a depth of about 15 cm. Collected samples were placed in each plastic bag, attached with labels containing sampling date, locality, and sites number. Samples are stored in a cool place.

Nematode extraction from soil samples

Nematodes were extracted by using the Whitehead tray Method (Whitehead, 1965). Randomly collected soil samples from each field were thoroughly mixed and 100 ml was taken out for nematode extraction. Firstly, 100 ml of soil subsample was spread in a thin layer over a muslin cloth in a plastic sieve. The sieve was placed in a plastic tray. About 250 ml of tap water was carefully added down from the edge of the tray until the soil layer looked wet. Then the set of nematodes extracted tray was place about 24 hours.

Collecting, Counting and Identification of extracted nematodes

Secondly, the sieve was removed and about 250 ml of nematode suspension in the tray was poured into a glass beaker (300 ml Pyrex) and left for 2-3 hours to settle the nematodes at the bottom of beaker. After which upper portion of suspension was discarded and remaining 25 ml of nematode suspension in the beaker was thoroughly shaken and 1 ml of the nematode

suspension was taken by pipette and added into a counting dish where rice nematodes was examined and counted under dissecting microscope. Then these nematodes were identified under compound microscope according to Chitwood, (1950) Gooedy, Bridge, Hunt, (2002).

Nematode extraction from rice roots

Infected rice root samples were washed with tap water and cut into small pieces about 1 mm long then mixed together. The mixture of root was taken for extraction of nematodes by Whitehead tray method. Extraction, identification and counting were done like the soil samples procedure.

A. Study site-1

B. Study site-2

C. Rice plant Sample

D. Infected root

E. Nematodes extraction from soil

F. Nematodes extraction from root

Plate 1. Study sites, rice samples and nematodes extraction

Results

Total seven genera of plant-parasitic nematodes belonging to two orders and seven families were recorded during study period. Out of them, five genera of plant-parasitic nematodes were recorded from rice root samples in both study sites and four genera of plant-parasitic nematodes from soil samples of site-1 while five genera of plant-parasitic nematodes in soil samples of site-2 were recorded (Plate 1).

Among them, *Hirschmanniella oryzae* and *Meloidogyne* spp. were recorded from roots throughout the study period in both sites (Plate 1A, 1B).

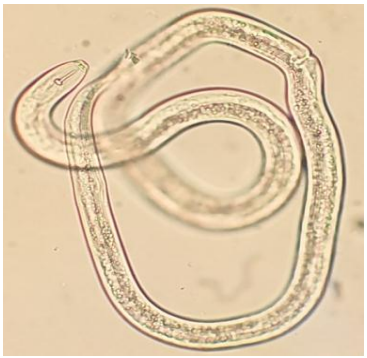
The number of *Hirschmanniella oryzae* from roots were gradually increase until the harvesting time in site-1 , although the number of them were more reduced in harvesting time than the initial of transplanting time in site-2. The peak numbers of them were recorded in 45 days after transplanting in both sites (Fig 3). Almost the recorded numbers were juvenile, some were female and males were rare.

The numbers of *Meloidogyne* spp. were alternated increase and decrease almost the roots samples in both sites. However the populations of them were peak in harvesting time at roots sample of site-1 (Fig 4). All recorded *Meloidogyne* spp. were only juvenile stage.

Moreover in the root samples of both sites, *Tylenchus* sp. and *Helicotylenchus* sp. were also recorded. But *Aphelenchus* sp. was found just only in site-1 and *Aphelenchoides* sp. was recorded only in site-2. All of them were occasionally recorded during study period in both site, therefore the population of them were fewer than the previous mention genera (Table 2,4).

In soil samples, four genera of plant-parasitic nematodes in site-1 and five genera of plant-parasitic nematodes in site-2 were recorded during the study period. Among them *Hirschmaniella oryzae* and *Meloidogyne* spp. were recorded throughout the study period except the 4th sample (60 days after transplanting). However, the populations of them were fewer than the root samples (Table 3, 5).

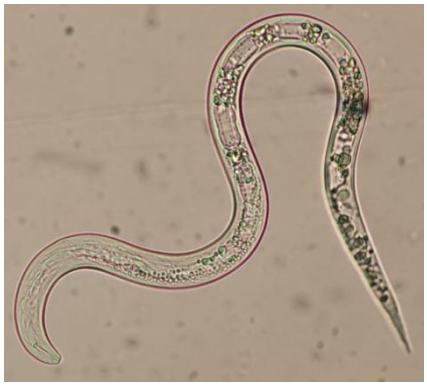
The rest genera, *Tylenchus* sp. and *Criconema* sp. were occasionally found in both sites throughout the study period. The *Helicotylenchus* sp. was recorded only in 45 days after transplanting (3rd sample) at study site-2. The populations of them were very low in number during the recent study.



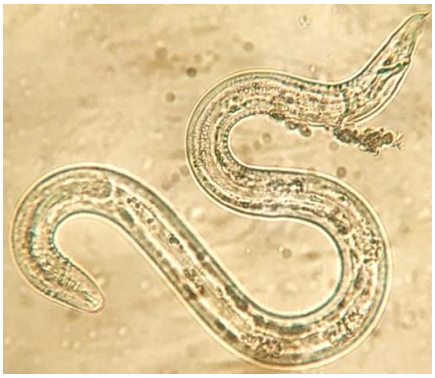
A. *Hirschmeniella oryzae*
Female (100x)

B. *Hirschmeniella oryzae*
Male (100x)

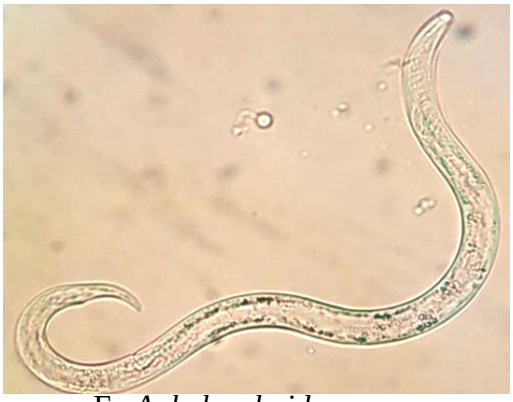
C. *Meloidogyne* sp.
Juvenile (100x)



D. *Meloidogyne* sp.
Juvenile (100x)

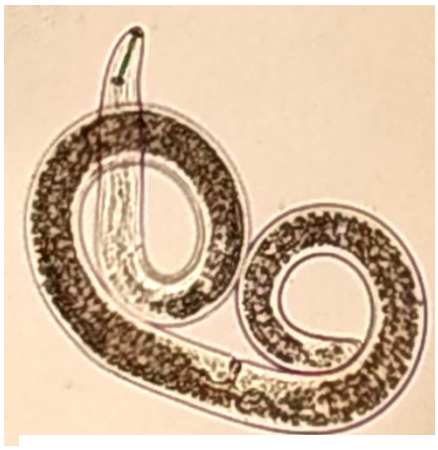


E. *Aphelenchus* sp.
Male (100x)



F. *Aphelenchoides* sp.
female (100x)

G. *Tylenchus* sp.
Male (100x)



H. *Helicotylenchus* sp.
Female (100x)

I. *Criconema* sp.
Female (100x)

Plate 2 Recorded plant-parasitic nematodes from two study sites

Table 1 Systematic position of recorded plant-parasitic nematodes

Sr. No	Class	Order	Family	Genus	Species	Habitat
1.	Phasmidia	Tylenchida	Paratylenchida	*Hirschmanniella*	*oryzae*	Root
2.			meloidogynidae	*Meloidogyne*	spp.	Root
3.			Tylenchidae	*Tylenchus*	sp.	Soil
4.			Hoplolaimidae	*Helicotylenchus*	sp.	Soil
5.			Criconematidae	*Criconema*	sp.	Soil
6.		Aphelenchida	Aphelenchidae	*Aphelenchus*	sp.	Soil
7.			Aphelenchoididae	*Aphelenchoides*	sp.	Soil

Table 2 Recorded numbers of plant-parasitic nematodes from root samples of site-1

Site 1 (Root samples)	1st sample	2nd sample	3rd sample	4th sample	5th sample	6th sample	Total	Mean± SD
Hirschmenniella oryzae	158	417	2550	950	767	962	5804	967.33 ± 837.04
Meloidogyne spp.	0	25	100	33	1008	4938	6104	1017.33± 1959.68
Helicotylenchus sp.	0	100	0	0	0	0	100	16.67 ± 40.82
Tylenchus sp.	0	0	0	0	25	0	25	4.17 ± 10.21
Aphelenchus sp.	0	0	0	8	0	0	8	1.33 ± 3.27
Total	**158**	**542**	**2650**	**991**	**1800**	**5900**	**12041**	

Table 3 Recorded numbers of plant-parasitic nematodes from soil samples of site-1

Site 1 (Soil samples)	1st sample	2nd sample	3rd sample	4th sample	5th sample	6th sample	Total	Mean ± SD
Hirschmenniella oryzae	1	5	8	8	4	63	89	14.88 ± 23.74
Meloidogyne spp.	6	4	83	3	8	692	796	132.67 ± 257.78
Criconema sp.	0	0	42	3	1	0	46	7.67 ± 16.86
Tylenchus sp.	1	0	42	1	2	12	58	9.67 ± 16.45
Total	**8**	**9**	**175**	**15**	**15**	**767**	**989**	

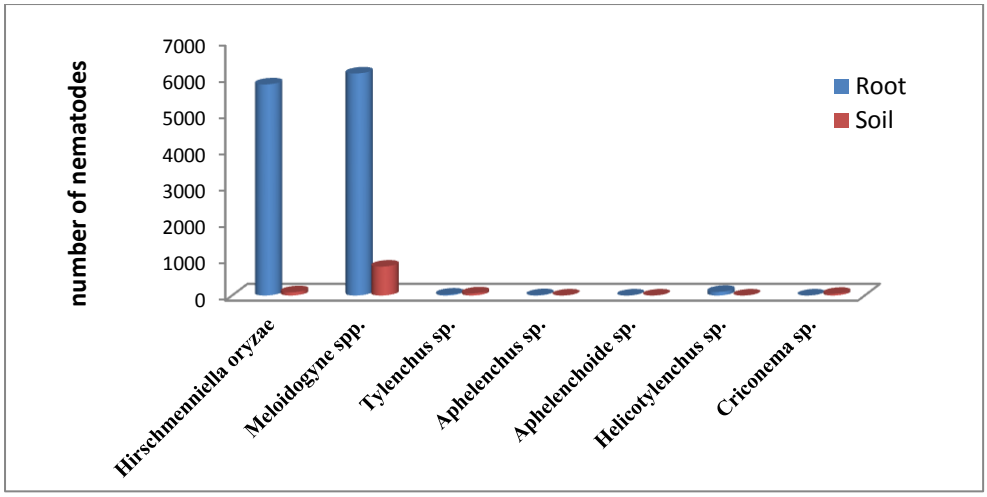


Figure 1 Recorded nematodes population from roots and soil samples of site-1

Table 4 Recorded numbers of plant-parasitic nematodes from root samples of site-2

Site 2 (Root samples)	1st sample	2nd sample	3rd sample	4th sample	5th sample	6th sample	Total	Mean ± SD
Hirschmenniella oryzae	258	930	950	516	901	204	3759	626.50 ± 346.01
Meloidogyne spp.	100	30	275	34	182	141	762	127.00 ± 93.71
Helicotylenchus sp.	0	0	34	0	0	0	34	5.67 ± 13.88
Tylenchus sp.	0	0	0	9	0	0	9	1.5 ± 3.67
Aphelenchoide sp.	0	0	0	0	18	34	52	8.67 ± 14.35
Total	**358**	**960**	**1259**	**559**	**1101**	**379**	**4616**	

Table 5 Recorded numbers of plant-parasitic nematodes from soil samples of Site-2

Site 2 (soil samples)	1st sample	2nd sample	3rd sample	4th sample	5th sample	6th sample	Total	Mean ± SD
Hirschmenniella oryzae	75	34	5	0	7	50	171	28.50 ± 29.94
Meloidogyne spp.	125	50	21	0	18	14	228	38.00 ± 45.66
Helicotylenchus sp.	0	0	34	0	0	0	34	5.67 ± 13.88
Criconema sp.	0	34	5	0	52	14	105	17.50 ± 21.18
Tylenchus sp.	0	25	0	0	1	1	27	4.50 ± 10.05
Total	**200**	**143**	**65**	**0**	**78**	**79**	**565**	

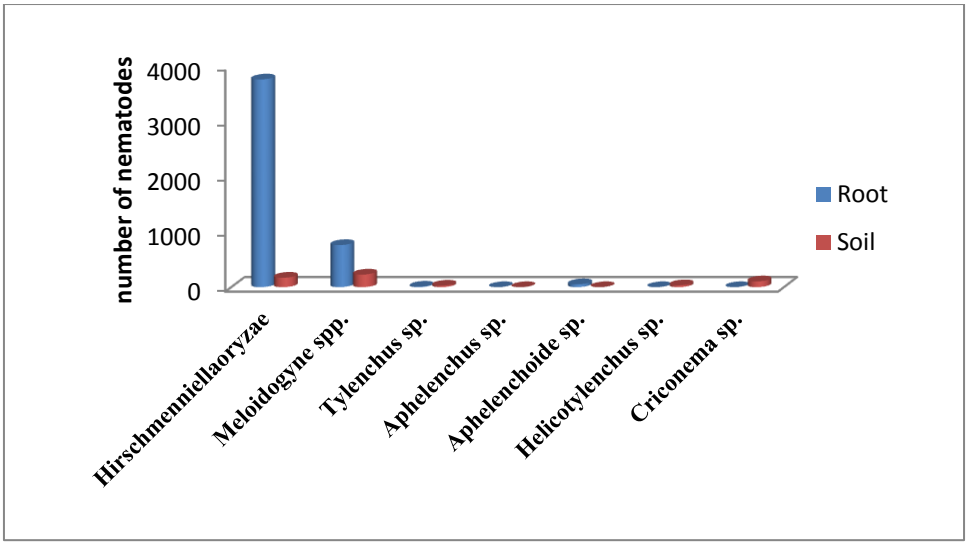


Figure 2 Recorded nematodes population from roots and soil samples of site-2

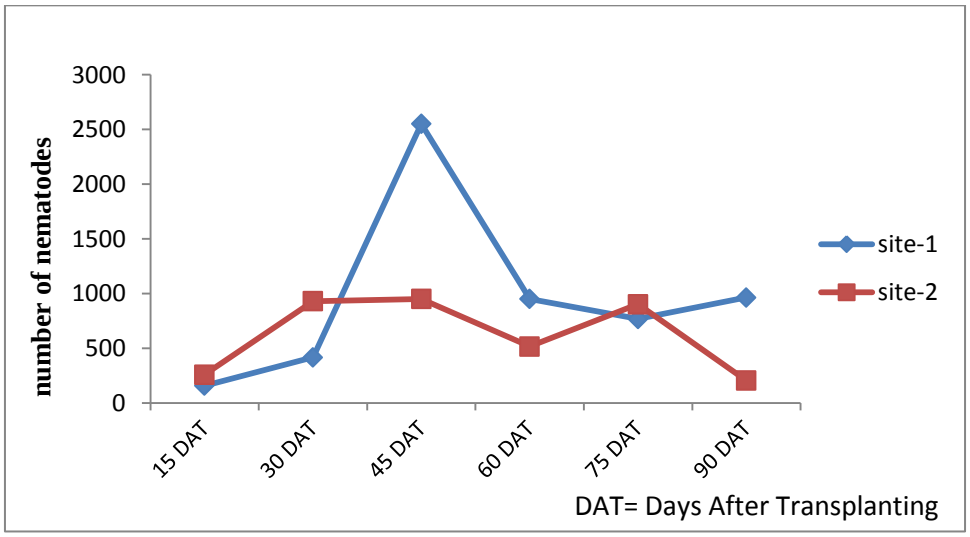


Figure 3 Population fluctuation of *Hirschmanniella oryzae* from rice roots of both study sites

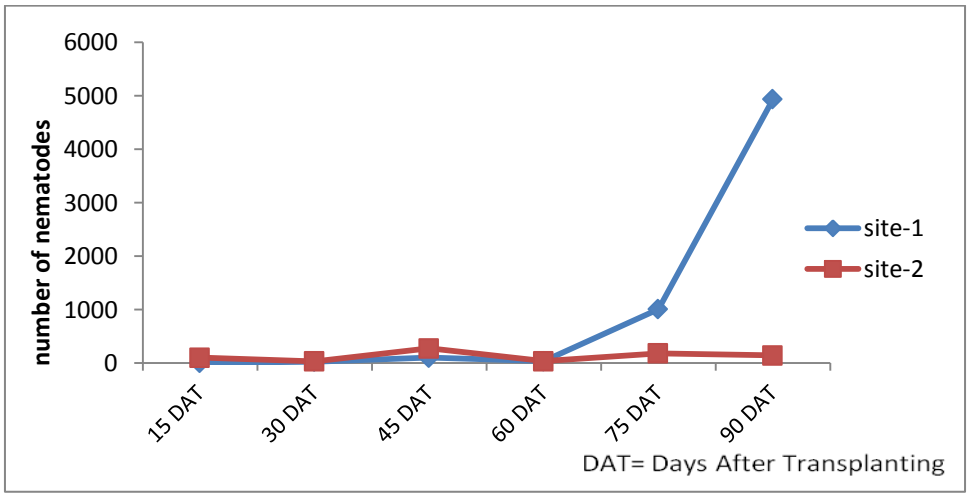


Figure 4 Population fluctuation of *Meloidogyne* spp. from rice roots of both study sites

Discussions

In present study, seven genera of plant-parasitic nematodes were recorded. Among them *Hirschmanniella oryzae* and *Meloidogyne* spp. were abundant in both study sites during the study period. However the total numbers of these two genera were higher in site-1 than in site-2.

It may be assumed that the differences of soil type and field condition were variable the population of nematodes. In site-1, soil type were sandy and flooding condition while in site-2 had muddy and no flooding condition. Therefore, field condition of site-1 was easy to transmit from one to another plant rapidly by swimming or crawling within the rice field.

The present finding agreed with the report of DFID, (2004) it had stated that nematodes can interact with other soil biotic and abiotic factors, and influence rice-weed competition.

H. oryzae are root rot nematodes they particularly infected in the rice root for consume the root sap. In present work, the population of *H. oryzae* rapidly increased until the 3rd sample (45 days after transplanting) in site-1, and then rapidly decreased within next two weeks (4th sample) to harvesting time.

It may be suggested that, the rice roots were highly developed up to 45 days old plants; *H. oryzae* got the fully nutrient from rice roots for multiply their population at that time. The newly hatched juveniles more attacked to root for their food, after that the rice roots became rapidly damage and rot within two weeks. Therefore, their populations in root were decreased until the harvesting time.

The present suggestion was assisted by the report of Yokoo and Su (1966), they found 33% decrease in numbers of *H. oryzae* within 50 days of removal of flooded water. Fortuner and Merny (1979) also stated that, they survive longer in roots than in soil but survival of root populations is shorter in flooded soil due to the more rapid decay of roots

In the present study site-2, the populations of *H. oryzae* were gradually increased along the study period except the harvesting time. Moreover site-2 was reverse situation of mention the previous authors there was no flooding and muddy soil, so population of *H. oryzae* was not significantly differences along the study period.

Similarly *Meloidogyne* spp. is root knot nematodes and they infected on roots of the cultivated crops. In recent work, *Meloidogyne* spp. was gradually increased along the studying time in site-2. However in site-1, the populations of *Meloidogyne* spp. were suddenly increased from the 60 days after transplanting to harvesting time, which was highest in number.

At the same time, the population reduction of *H. oryzae*, and population increasing of *Meloidogyne* spp. were recorded in study site-2. It may be suggested that when the *H. oryzae* infected roots were damage and rot, the rice plants had newly roots developing was occurred at that time because adequate of water supply (flooding). Therefore, it may be favorable condition to *Meloidogyne* spp. for invasion and breeding in the younger roots.

Taylor *et a*l, (1966), assisted to present opinion; they stated that rice root nematodes are well adapted to conditions in marshes and flooded rice paddies. They can infect and reproduce in some sedges and grasses.

According to Mya Mya (1983), *Meloidogyne graminicola* or rice root-knot nematode a sedentary endoparasite, attacks the roots of rice plants and occurs in lowland and upland rice

areas of Myanmar. The present study was agreed with the report of Mya Mya (1983) because the recent study sites were upland area.

Butler,(1913, 1919), who stated that nematode parasites of rice include rice white tip nematode (*Aphelenchoides besseyi*), rice stem nematode (*Ditylenlenchus angustus*), rice root nematode (*Hirschmanniella* spp.), root knot nematode (*Meloidogyne* spp.), rice cyst nematode (*Heterodera oryzae*), stunt nematode (*Tylenchorynchus* spp.), ring nematode (*Criconemoides* spp.) and lance nematode (*Hoplolaimus* spp.)

In recent study, out of the Butler reported rice nematodes, three genera of nematodes *Hirschmanniella oryzae, Meloidogyne* spp. and *Criconemoides* sp. were recorded. However, Criconemiodes sp. was recorded only in soil from both study sites.

The rest of nematodes *Aphelenchus* sp., *Aphelenchoides* sp., *Helicotylenchus* sp. and *Tylenchus* sp. were recorded in both study sites during study period.

According to DFID (2004) report, communities of several potential pest species of nematodes can occur in the same field, which complicates management decisions. In the dynamic and hydrologically heterogeneous conditions of small rice farms, nematode communities may be particularly diverse. Some have a geographically restricted distribution, while others occur throughout the rice-growing regions of the world.

Conclusion

Total seven genera of plant-parasitic nematodes were recorded during study period. Out of them five genera, *Hirschmaniella oryzae, Meloidogyne* spp., *Helicotylenchus* sp., *Tylenchus* sp. and *Aphelenchus* sp. from root samples of site-1, were observed. *H. oryzae*, *Meloidogyne* spp., *Helicotylenchus* sp., *Tylenchus* sp. and *Aphelenchoides* sp. from root samples of site-2 were recorded. Among them *H. oryzae*, and *Meloidogyne* spp. were root damage nematodes and abundantly recorded throughout the studying time. Four genera of nematodes, *H. oryzae*, *Meloidogyne* spp., *Tylenchus* sp. and *Criconema* sp. were recorded from soil samples of site-1 were recorded. Five genera, *H. oryzae*, *Meloidogyne* spp., *Helicotylenchus* sp., *Tylenchus* sp. and *Criconema* sp. were recorded from soil of site-2. All of the recorded nematodes, except the *Criconema* sp. were found in roots and soil samples of both study sites. *Croconema* sp. was recorded only in soil sample of both sites. The present finding may be concluded that the population of root nematodes such as *Hirschmaniella oryzae* and *Meloidogyne* spp. were more infected on flooded area then the non flooded rice fields. The population fluctuation of them was also more rapid in flooded area than the non flooded rice fields.

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MICROBIAL ANALYSIS AS INDICATORS OF POLLUTION IN WASTEWATER OF HLAINGTHARYA INDUSTRIAL ZONE

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Abstract

The present work was conducted to study the status of some indigenous bacterial species in wastewater from factories of noodles, confectioneries and rice vermicelli in Hlaingtharya industrial zone near Yangon city of Myanmar. The study was done from June to August, 2017 for preliminary studied. All samples were showed *Escherichia coli* positive on 3M petriflim. Among them, four *E.coli* isolates were tested for their antibiotics susceptibility patterns with Ampicillin and their generation time were studied. All *E.coli* isolates showed ampicillin resistance and generation time was between 20 to 22 min. Subsequently, genomic DNA and plasmid DNA profiling those antibiotics resistant *Escherichia coli* strains was done for future reference in further works on pollution estimation in this industrial zone.

Keywords: *Escherichia coli*, petriflim, Genomic DNA, Plasmid DNA

Introduction

Indicator organisms are bacteria that are used as a sign of quality or hygienic status in a food, water, or environment. The definition of the word "indicator," . . . in fact, includes the concept of the indicator organism, i.e., something "so strictly associated with particular … conditions that its presence is indicative of the existence of these conditions"(Merriam-Webster Online, http://www.m-w.com/cgi-bin/dictionary, 2018).

There is an extensive literature which stresses deterioration of water quality. The addition of various kinds of pollutants and nutrients through the agency of sewage, industrial effluents, agricultural runoff etc: into the nature natural water bodies brings about a series of changes in the physiochemical and other characteristics of water, which have been the subject of several investigations (Tiwari and Mishra, 1986 and Khulab, 1989).

The major microbial pathogens in water are bacteria, viruses, fungi and protozoan parasites. Bacteria pathogens are mostly present in feces and a wide variety can be present in wastewater due to fecal contamination. The discharge of untreated or inadequately treated wastewater into the environment can have negative impact on human health due to the release of pathogenic microorganisms into water which could lead to serious health diseases (Rosario *et al*., 2009). Water that is contaminated with microbial pathogens is a medium for several waterborne diseases, such as cholera, typhoid fever, shigellosis, salmonellosis, campylobacteriosis, giardiasis, cryptosporidiosis and Hepatitis A (WHO, 2004). Several pathogenic organisms in contaminated water are the basic causes of gastrointestinal illnesses in human. Some of the pathogens are known to cause several outbreaks of diseases by releasing toxins in the human body (Krauss and Griebler, 2011).

Indicator organisms were first used in the testing of water supplies for sanitary quality. The mid to late 1800s were marked by huge developments in the sciences of public health and microbiology. Indicator organisms in wastewater are organisms whose presence suggests the

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presence of a pathogen in wastewater. The density of an indicator organism is always associated with health hazards and several sources of pollution. It is indicated that for an organism to qualify as an indicator organism of a particular pathogen, it must be continuously and totally related to the source of the pathogen and be abundant enough to provide appropriate and exact mass concentration of the level of pathogen in relation to high risk of illness. Also, an indicator organism should have resistant ability to disinfectants, environmental stress and toxic materials that may be present at the source of the pathogen (Berg, 1978; Galveston Bay Centre, 2002).

Many published standardized methods exist for the detection and enumeration of Enterobacteriaceae, coliforms and *E. coli* in foods including international standard methods like those published by the International Organization for Standardization (ISO). Nowadays, coliforms bacteria isolated from foods that indicate poor hygiene or inadequate processing, process failure and post-process contamination of foods. *E. coli* is commonly used to provide evidence of faecal contamination in certain foods and is used as an index organism for the presence of enteric pathogens such as *Salmonella.* (Muytjens *et al.,* 1988).

The use of *E. coli* as an indicator organism is somewhat restricted by the fact that *E. coli* is not a single species; certain genera of the coliform group such as Proteus and Aerobacter are normally found outside the human intestinal tract in soil; other organisms found in water that do not represent fecal pollution possess some of the characteristics attributed to *E. coli* and *E. coli* identical to that found in humans is also found in the intestinal tract of other warm-blooded animals. However, primarily, studies have shown that *E. coli* is a much better indicator of disease risk than is faecal coliform, EPA has therefore, recommended that *E. coli* be used as a criteria for classifying waters for fresh water contact recreation. (EPA, 1986 and Hoffmann, Sturenburg and Heesemann, 2013) .

This study was aimed at analysis of microbial pathogen indicators as microbial pollutants for contaminated wastewater from three food industries in Hlaingtharya Industrial zone and focus on determination of *E.coli* strain and their antibiotic resistance, growth rate and genomic DNA and plasmid DNA profile of this species. This finding will provide references for future work on pollution estimation especially for the *E.coli* bacteria detection at the molecular level.

Materials and Methods

Sample collection and identification of *E. coli*

The wastewater samples were collected and analysed from the disposal of three factories sites including noodles, confectioneries and rice vermicelli factories in Hlaingtharya industrial zone near Yangon city of Myanmar. This study was done during June to August, 2017 as preliminary research.

The wastewater samples (one liter each) were collected in triplicates per sites. Samples were collected during the day at 9.00 am from each sampling site and collected aseptically in sterile containers and placed in a cooler box and transported to the Microbiology Laboratory of Zoology Department, West Yangon University within two hours after collection. One mL of each diluted samples cultivated and identified on 3M Petriflim^{TM} *E.coli* count plate (Thermofisher Scientific, Austrialia) according to their instruction and enumeration of *E.coli* was done with Plate Reader (TICO, USA).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India, MV1084), according to the Clinical and Laboratory Standards Institute (CLSI, 2009) guidelines. The antimicrobial agent tested and their corresponding concentrations was Ampicillin (25 μg/disk), after incubating the inoculated plates aerobically at 37 °C for 18 to 24 h, the susceptibility of the *E. coli* isolates was measured and the results were recorded in accordance with criteria provided by CLSI. *E. coli* ATCC 25922 was used as quality control organisms in antimicrobial susceptibility determination.

Determination of generation times

Four isolates of ampicillin resistance *E.coli* strains tested for their generation time. Each *E.coli* samples were diluted into 10^1 to 10^{10}, and incubated on nutrient agar and incubated at 37 ^{0}C checked for their growth at every one hour and recoded as bacterial colony/mL. 10^5 dilution factors was used for determination of their generation time and calculation method was followed by Stanley *et al.,* 1994.

Extraction of Genomic DNA

To prepare genomic DNA from cultured *E.coli* bacterial, the bacteria from 3 ml of liquid media were used for extraction of genomic DNA used with Pure Link Genomic DNA Mini Kit (Invitrogen, Life Technologies, 50 preps, USA) according to the manufacturer's instructions (Invitrogen Test kit, Germany). Centrifuged at 16000 Ug for 30 min, and the pellet was used for DNA preparation with the kit mentioned above.

Extraction of Plasmid DNA

To prepare plasmid DNA from *E.coli* bacterial strains, the bacteria from 3 ml of liquid media were used for plasmid DNA isolation according to the manufacturer's instructions (QUIAGEN Plasmid Mini Kit, Germany).

Qualitative and Quantitative analysis

Extracted genomic DNA and plasmids DNA were separated by horizontal agarose gel electrophoresis (100 DVC, 1% agarose gel, 1X TAE bufferin 30 mins). After Etbr staining, DNA was visualised and digitalized images captured under UV light trans illumination (Gel Imager, Thermofisher scientific, Co. Ltd) the molecular weight of each plasmid, Gene Ruler™ 1kb DNA Ladder marker was used to estimate the molecular mass. The Nanodrop (ND 1000, Thermofisher scientific, Germany) was employed to measure optical density at 260 nm and 280 nm for quantity of DNA concentration.

Results and Discussion

Escherichia coli is the best indicator of fecal contamination from human and animal wastes. *E.coli* presence is more representative of fecal pollution because it is present in higher numbers in fecal material. Baudizsova (1997) found that the other thermotolerant and total coliforms were capable of growth in non-polluted river water while *E.coli* was not, and supports a recommendation for *E.coli* to use as the sole indictor bacteria for recent fecal contamination (Tallon, *et.al.,* 2005).

According to this reason, the present research work was conducted to isolate *E. coli* from three food processing factories including noodles, confectioneries and rice vermicelli factory in Hlaingtharya industrial zone near Yangon city as a preliminary research during June to August, 2017. In this study, after incubation on 3M Petriflim^{TM} *E.coli* count plate showed all samples are *E.coli* positive, produce blue to red blue colonies, and give lactose fermentation produce gas. It is due to plate contain Violet Red Bile (VRB) and indicator of glucuronoidase activity.

Table 1 Results of *E.coli* isolates from three different food processing wastewater effluent drainage.

Factory	**Total percentage**
Noodle processing factory	100%
N 1	+
N 2	+
N 3	+
Confectionary processing factory	100%
C 1	+
C 2	+
C 3	+
Vermicelli processing factory	100%
V 1	+
V 2	+
V 3	+

Table 2 Growth as colony count/mL of *E.coli* isolates from three food processing factories. (incubation at 37 ^{0}C)

Isolate Number	**Sampling intervals (hrs)**								
	0:00	**1:00**	**2:00**	**3:00**	**4:00**	**5:00**	**6:00**	**7:00**	**8:00**
1	0	72	102	180*	102	90	82	0	0
2	0	40	100	191*	95	63	49	0	0
3	0	38	61	148*	34	10	30	40	0
4	0	38	60	150*	52	50	49	42	0

(Nutrient agar = 20 mL agar plate + 1 mL sample; Dilution factor 10^{4} ; * = Peak growth)

In noodle factory, mainly uses with egg, flavor, instant pigments, yeast, starch and large volume of water are using for cleaning. In confectionary factory, mainly use with sugar, water, milk, flavoring materials, nuts, vegetable oils, scereals, eggs, instant pigment, baker yeast and liquid sugar. All of these wastes contain mainly nutrient for bacteria. Vermicelli factory use with dry rice flour and yeast are high content with starch and heavy particulates, this will term to more favorable for bacteria growth in their wastewater effluent. All factories produce large amount of waste characterized by high concentration of organic materials and nutrients. Their effluent discharges directly to the environment and there is no proper treatment process. These wastewater are making bad odor in environment.

E. coli bacterial species has a great capacity to accumulate resistance genes, mostly through horizontal gene transfer. In this study, four *E.coli* isolates from three different factories were tested with ampicillin antibiotic. All are showed resistance on this antibiotic. Ampicillin

resistance strains are important in clinical microbiology. They are not only for its effects on human health but also for potential source of transferring the antibiotic-resistant genes to other important pathogenic serotypes through horizontal gene transfer between bacteria and that contributing to the increase of the resistant genes in the environment (Sarina Pignato, *et al.*, 2010).

Table 3 Generation time of *E.coli* isolates from three factories

Tested samples	Initial number of bacteria colony x 10^5/mL	Final number of bacteria colony x 10^5/mL	Generation Time (min)
1	10.20 x 10^5	18.00x 10^5	20.42
2	10.00 x 10^5	19.10x 10^5	22.36
3	6.10 x 10^5	14.80x 10^5	20.52
4	6.00 x 10^5	15.00x 10^5	21.36

Generation times are varies among in bacteria, is controlled by the nature of bacteria species, *E.coli* has every 20 to 30 minutes for generation time (Stanely *et al*., 1994). Four isolates are tested their generation time and all *E.coli* isolates showed between 20.42 to 22.36 minutes of their generation time. These results are reveled with similar to other studies. According to this study, little variation are occurred may be due to the medium that provides energy source and more of biosynthetic intermediate that the cell would otherwise that make to itself.

Clinical isolates of *E.coli* are revealed a greater degree of genomic size variation than the detected among the natural strains that occur in wastewater (Bremnner, *et al.*, 1972). That varies estimate ranging from 3.8 to 4.8 Mb. In this study, all *E.coli* isolates were greater than the 20 to 50 kb (Genomic DNA) and less than the 30 kb (Plasmid DNA). It was dependent on the GC content of the genome as well as the amount of unique sequences; it is difficult to establish reflect actual variation in genome size. The isolation of plasmid DNA from bacteria is a crucial technique in molecular biology and is an essential step in many procedures such as cloning, DNA sequencing, transfection, and gene therapy. These manipulations require the isolation of high purity plasmid DNA. The purified plasmid DNA can be used for immediate use in all molecular biology procedures such as digestion with restriction enzymes, cloning, PCR, transfection, in vitro translation, blotting and sequencing. The purified plasmid DNA can be used for immediate use in all molecular biology procedures such as digestion with restriction enzymes, cloning, PCR, transfection, in vitro translation, blotting and sequencing (Anonymous, 2018).

Table 4 Genomic DNA, Plasmid DNA concentrations and Purity of DNA from Isolated *E.coli* strains (A 260/280)

Tested samples	Genomic DNA (ng/µL)	DNA purity	Plasmid DNA (ng/µL)	DNA purity
1	38.50	1.90	19.60	1.85
2	31.90	1.96	19.10	1.86
3	26.50	1.83	20.20	1.84
4	28.00	1.80	18.40	2.00

To the degree that naturally occurring microbial pathogens become a significant public health concern, completely new test procedures may have to be developed. Furthermore, while *E.*

coli is specific for faecal contamination, there are three inherent problems of using *E. coli* as a confirmation of faecal contamination: (i) it is outnumbered by other types of fecal bacteria making it more difficult to find; (ii) it does not survive for long outside of the gut; (iii) it can be found in pristine environments in the tropics. Therefore, the absence or presence of *E. coli* via a culture test does not absolutely confirm the absence or presence of faecal contamination. The *E. coli* tests used today as an indication of fecal contamination are commonly culture tests although that need to study with the sensitivity test, serotype, molecular test for the pathogen *E.coli* strain (USEPA, 2000). Thus in recent study *E.coli* isolates were used as indicator and further study with the antibiotic resistance test and plasmid profile for the further study of PCR detection for pathogenic strain.

Many studies of antibiotic-resistant bacteria in the aquatic environment but little work has been done to assess the prevalence of drug-resistant bacteria in water and their relationships to antibiotic-resistant microorganisms in untreated source waters. They have found increased rates of resistant bacteria in drinking water within the distribution net by standard plate-count experiments, and have concluded that the treatment of raw water and its subsequent distribution select for antibiotic-resistant bacteria (Armstrong, Shigeno, Calomiris, and Seidler, 1981).

In agreement with these data, increased phenotypic resistance rates were also detected in *E.coli* isolates. Additionally, plasmid profile investigations concerning the underlying resistance mechanisms were performed for further study of antibiotic resistance gene. The occurrence of the ampicillin resistance genes of *E.coli* bacteria confirmed for the influence of the water sources on the study area.

Plate 1. Antibiotic susceptibility patterns of *E.coli* isolates on Muller Hinton agar tested with ampicillin
1-4 = sample number of *E.coli* isolates; C = control sample

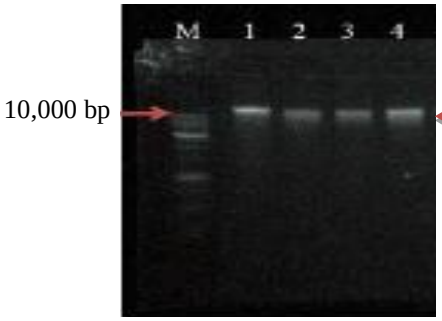

Plate 2 Gel electrophoresis image of extracted genomic DNA from *E.coli* isolates of three sample sites (Lane M = 1Kb plus maker ; Lane 1-4 = *E.coli* isolates)

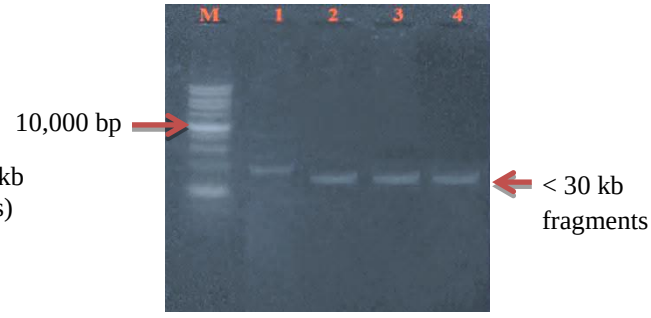

Plate 3 Gel electrophoresis image showing plasmid profiles extracted from *E.coli* isolates of three sample sites (Lane M = 1Kb plus maker ; Lane 1-4 = *E.coli* isolates)

Conclusion

In conclusion it's clear that *E. coli* appears to be the best indicator of bacteriological quality of water, primarily because of the, availability of affordable, fast, sensitive, specific and easier to perform detection methods for *E. coli*. However the fact remains that the life span of *E. coli* in water is short, thus it best determines, recent contaminations. It is therefore important that there is continuous monitoring for *E. coli* to determine the bacteriological quality of water. Indicator organisms continue to serve important functions in microbiological testing programs. Further research is required in order to evaluate the health risks of using reclaimed water harboring antibiotic-resistant bacteria for drinking, agricultural, and recreational purposes**.**

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ANALYSES OF WATER QUALITY IN WATER SAMPLES FROM NATURAL PONDS IN WARTARYA VILLAGE

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Abstract

Bacteriological analysis was done from May, 2013 to September 2015. A total of 21 water samples were collected from natural ponds from different locations in Wartarya Village, Htan-Ta-Pin Township, Yangon Region. There are seven ponds in Wartarya village. All water samples were found to be contaminated with faecal coliforms and the range of faecal coliforms counts were observed between 4 and 1600 MPN/100ml. The minimum amount of faecal coliforns found in the samples was 8 MPN/100ml and the maximum amount was 1600 PMN/100ml in summer. In contrast, the minimum amount of faecal coliforms found in the samples was 4 MPN/100ml and the maximum amount was 50 MPN/100ml in wet season. Some physical parameters of the samples were also recorded. The range of temperature at the sampling sites was from 27˚C to 33˚C, pH from 7.0 to 9.0 and DO from 4 mg/L to 12mg/L. The range of metallic ions such as Fe in the pond waters during summer (May) ranged from 0.22mg/L to 0.26mg/L whereas in the raining season (July) Fe ions varied from 0.27mg/L -0.72mg/L. By contrast, calcium (Ca) varied from 1.20mg/L - 11.46mg/Lin the summer (May) and 0.5mg/L -8.3mg/L during the raining season (July).Lead (Pb) was absent in all pond waters during rainy and summer seasons. All chemical elements present in the water were within the WHO and ICMR permissible limits and thus safe for human consumption. The findings of the current study indicated the presence of acceptable limits of the chemicals, metallic irons concentrations and safety of the waters for drinking and other purposes. However, the studied areas were polluted by faecal coliforms bacteria posing harzard to the populace especially in summer. Hence, the environmental hygiene needs to be improved in Wartarya village. This research highlights that water need to be boiled before drinking or washing.

Keywords: Faecal coliforms , *Escherichia coli* , Physical and chemical parameters and natural ponds

Introduction

Good quality water is essential for all living beings. Besides quality also plays an important role in development of human health. Good quality of water is more essential for the aquatic flora and fauna (Idris and Noor, 2003).

Microorganisms are found everywhere in our environment. They are common in the air, soil and water. There are thousands of species of bacteria on earth (Sadowsky, 2008).

Bacteria are numerous and are a natural component of lakes, rivers and streams. Over 60 genera of bacteria are present in these aquatic systems. Most of these bacteria are harmless to humans. Elevated numbers of these harmless bacteria are associated with increased numbers of harmful bacteria. Consumption of water contaminated with Feces of warm-blooded animals can cause a variety of illnesses (Elizabeth, 2000).

The ever-increasing population has resulted in various types of contamination in most water bodies. The faecal contamination in drinking water sources is a major cause of various water borne infectious diseases, a global problems. The coliform bacteria have been

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internationally used as prime indicator of faecal contamination of water since the beginning of 20^{th} century (Pathak and Gopal, 2005).

Determining the bacterial quality of drinking water is the single most important water quality test. One glass of water containing just a few disease organisms can cause illness. Bacterial contaminants such as *E.coli* and faecal coliform in drinking water represent an acute health risk (Environmental Service, 2010). Total coliforms are a group of bacteria commonly found in the environment. Some coliform bacteria will occur in all ponds, but dangerously high levels may occur in ponds (Anonymous, 2012).

Faecal coliform bacteria are a sub-group of total coliform bacteria. Faecal coliform bacteria are indicators of faecal contamination. Faecal coliform bacteria are found in Feces. The faecal category contains both pathogen (disease causing) and non pathogenic bacteria. An example of one group of faecal coliform bacteria is *Escherichia coli* which was first discovered by Theodor Escherich. *E.coli* is the USEPA (US Environmental Protection Agency) recommended indicator of faecal contamination in fresh water. *E. coli* bacteria originate from the wastes of animals or humans. *E. coli* is the only member of the total coliform group of bacteria. The bacterium *E.coli* is one of the best and most thoroughly studied free-living organisms. Some *E.coli* strains live as harmless commensals in animal intestines. Most *E.coli* actually are important part of a healthy human intestinal tract. Some *E.coli* bacteria are beneficial by producing vitamin K_2 for their host. *E.coli* normally colonizes an infant's gastrointestinal tract within 40 hours of birth. Faecal coliforms and *E.coli* generally do not pose the actual health risk, but the presence of faecal matter, which may carry numerous pathogenic disease causing organisms.

Escherichia coli O_{157}: H_7, may cause illness. Other kinds of *E.coli* are used as markers for water contamination. Faecal coliforms and *E.coli* are used indicators to measure the degree of pollution and sanitary quality of water.

The survival of *Escherichia coli* in natural waters is one of great interest due to the importance of these organisms as indicators of faecal pollution in natural waters (Wcislo and Chrost, 2000).

Escherichia coli in drinking water indicates the water has been contaminated with faecal material that may contain disease causing microorganisms, such as certain bacteria, viruses or parasites. The most common symptoms of waterborne illness include nausea, vomiting and diarrhea. *E. coli* can cause diarrhea, urinary tract infections and other illnesses (Anonymous, 2009).

Faecal coliform density may be conducive to bacterial pathogen regrowth. Faecal coliform analysis remains an effective tool for evaluating potential public health or environmental impact. The presence of any faecal indicators shows that drinking water is potentially unsafe for consumption (Environmental Services, 2003).

Metals are important component of human environment and these may be either beneficial or toxic depending upon their concentration. calcium is naturally present in water. Calcium is a dietary requirement for all organisms apart from some insects and bacteria. Calcium also gives water a better taste. Elementary iron dissolves in water under normal condition. Iron is an essential element in human nutrition. Iron also is an essential trace element in living organisms (WHO, 1996).

A pond used to supply drinking water for humans and animals should be tested for faecal coliforms, *Escherichia coli* and other parameters. Microbiological and Chemical surveillance of drinking water quality is an important of public health management. Thus, the present research was carried out with the aim to assess the pollution of microbial contamination and chemical elements in water of natural ponds in Wartarya Village with the following objectives;

- To investigate occurrence of total faecal coliforms in natural water samples from different study sites
- To isolate and identify *Escherichia coli* as faecal indicator from study area
- To examine their distribution pattern in natural ponds by month
- To analyze some physical parameters of water samples in different sites
- To analyze some metals in parameters of water at different sites.

Materials and Methods

Study area and study site

Water samples were collected in natural ponds from different locations in Wartarya Village, Htan-Ta-Pin Township, Yangon Region. There are seven ponds in the village which are located in different places of the village (Plate 1). Two ponds are in the east, three ponds are in the middle, and two ponds are at the west of the village.

Study period

The study was done from May, 2013 to September, 2015.

Sample collection

A total of twenty-one water samples were collected from natural ponds at different sites in Wartarya Village .Water samples were collected in three successive months at each pond from May to July, 2013. Samples were collected aseptically in sterilized glass bottles with caps from 15 cm (0.5 feet) depth at about 300 cm (10 feet) away from the bank of ponds. Collected samples bottles were transported in an ice box to the Microbiology Laboratory in Department of Zoology, West Yangon University for the laboratory experiments.

Pond 1 (sample collection site 1 in May)

Plate 1: Sampling sites in the study area.

Methods

Determination of faecal coliform bacteria

Faecal coliform bacteria counts were determined by multiple tube method as described by WHO, (1985) and as shown in (Figure 1).

Identification of *Escherichia coli*

Identification of *Escherichia coli* was done as shown in flow chart (Figure 2). The Gram stain was carried out by Bisen and Verma (1998) .

Isolation and identification of *Escherichia coli* and other Gram-negative bacteria

All isolates were gram stained to determine their gram reaction. Physico-chemical tests used to identify the species were as follows: Sugar fermentation test, indole, motility and hydrogen sulphide production (SIM) test, citrate utilization test, urease test, Methyl-Red test, Voges-Proskouer test and catalase test. *Escherichia coli* was identified based on biochemical keys by Bergey's Manual (1975), Cowan and Steel (1975) and Martin (1997).

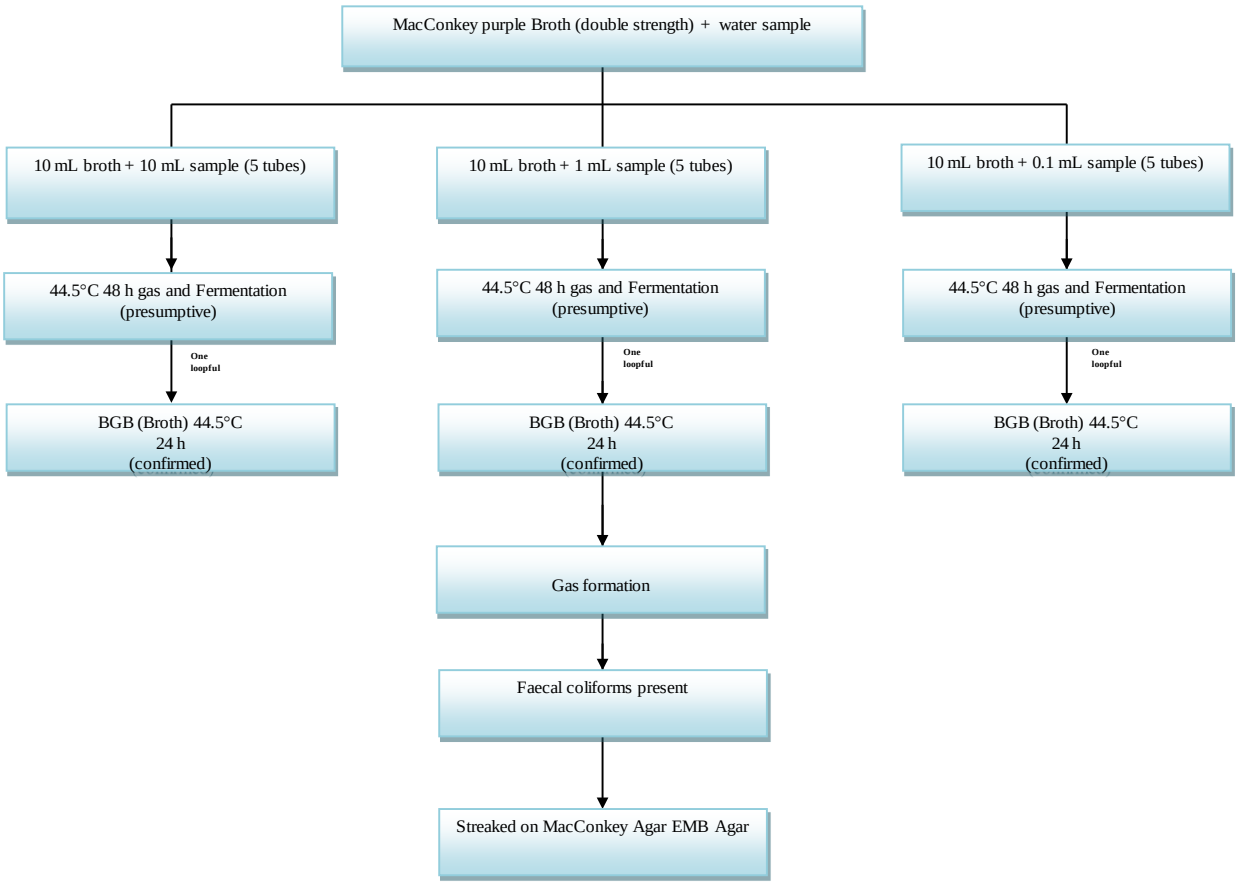


Figure 1 Flow chart for determination of total faecal coliform by multiple tube method (MPN/100 mL) at 44.5°C (WHO, 1985)

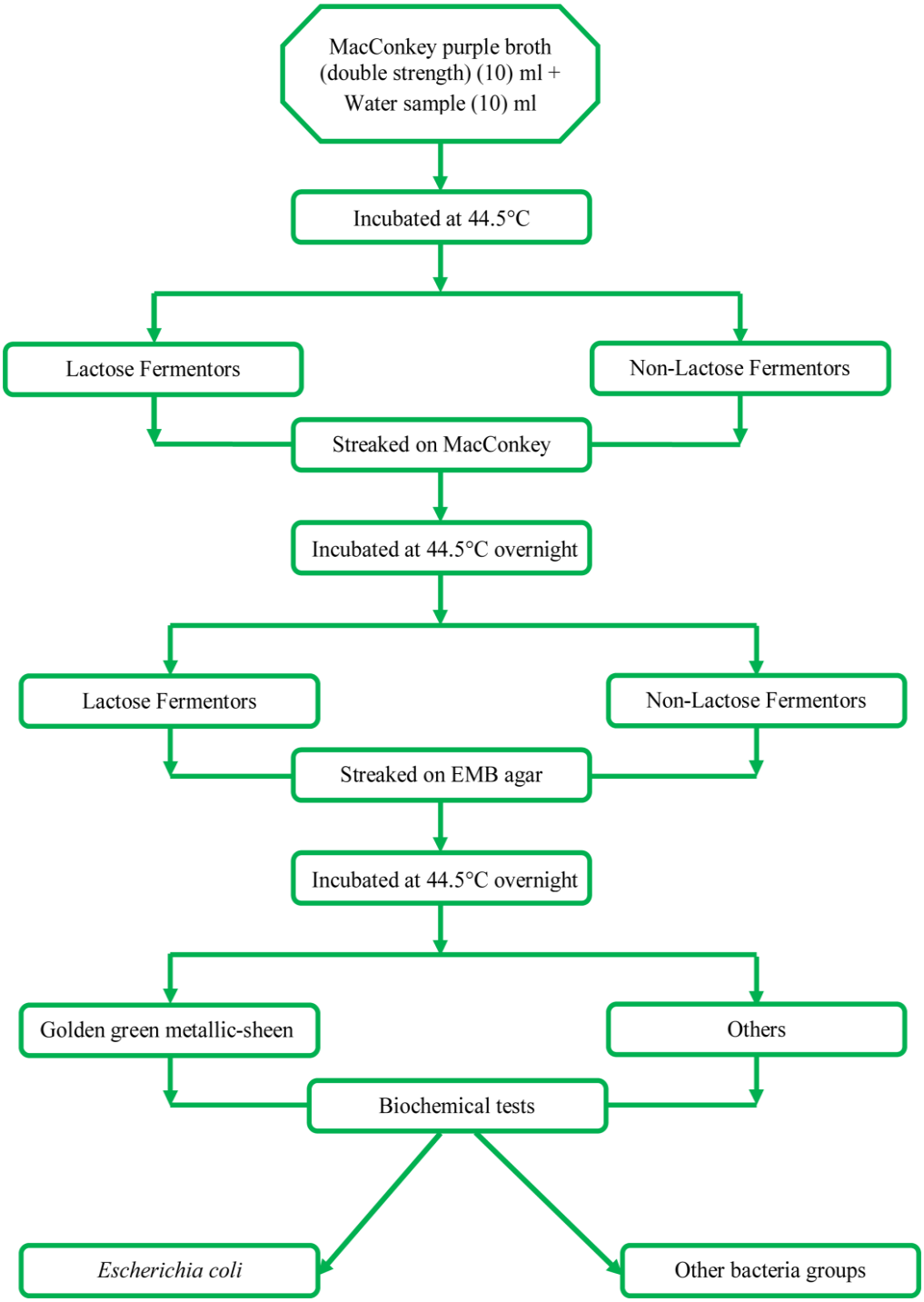


Figure 2 Flow chart for the identification of *Escherichia coli* and other bacterial groups

Measurement of some water physical parameters

Temperature, pH and dissolved oxygen of the water samples were measured as physical parameters as follows:

Temperature

Temperature was measured *in-situ*. A °C thermometer was inserted into the water for 5 minutes. After the 5 minutes, the thermometer was pulled out of the water and the data was recorded.

pH

The values of water pH were measured by pH paper *in situ* .

Dissolved oxygen (DO)

The values of dissolved oxygen were measured by dissolved oxygen kit *in-situ* .

Measurement of some water chemical parameters

Fe, Ca, and Pb of the water samples were measured as chemical parameters. Concentrations of chemical elements were measured by Atomic Absorption Spectrophotometer in Universities' Research Center, University of Yangon.

Results

A total of 21 water samples from different sites of natural ponds in Wartarya Village were tested.

Determination of faecal coliform by multiple tube method

The presence of faecal coliforms in water samples were determined by using double strength MacConkey broth purple. A change in colour from original purple to yellow with the production of gas at 44.5°C for presumptive test (Plate 2).

A loopful of the fermented bacterial culture tube with gas was inoculated into a tube of Brilliant Green Bile Lactose broth and the tubes were then incubated at 44.5°C. The tubes were examined for gas production and the gas tubes were regarded as positive faecal coliform (confirmed test) (Plate 3).

Presumptive faecal coliform (Fc) counts detected in water samples

Presence of faecal coliforms in water samples and their most probable number (MPN) per 100 ml of water samples were determined and the results were shown in Table 2, 3, 4 and 5.

Table 1 shows that 21 samples of natural ponds were found to be contaminated with faecal coliforms 100% and the range of faecal counts were observed between 4 and 1600 MPN/100 ml. Similar range of total faecal coliform count was observed in the samples of ponds 1, 2, 6 and 7. Also they were highest ranged of faecal coliform counts as compare as other different ponds such as pond 3, 4 and 5. The smallest ranged of counts was found in the samples of pond 4.

Table 2 shows that seven samples of natural ponds in May were found to be contaminated with faecal coliforms 100% and the minimum amount of faecal coliforms were found in the samples was 8 MPN/100 ml and the maximum amount was 1600 MPN/100 ml.

Table 3 shows that seven samples of natural ponds in June were found to be contaminated with faecal coliforms 100% and the minimum amount of faecal coliforms were found in the samples was 4 MPN/100 ml and the maximum amount was 30 MPN/100 ml.

Table 4 shows that seven samples of natural ponds in July were found to be contaminated with faecal coliforms 100% and the minimum amount of faecal coliforms were found in the samples was 30 MPN/100 ml and the maximum amount was 50 MPN/100 ml.

Identification of *Escherichia coli*

Escherichia coli isolated were Gram-negative and cells were typically rod-shaped (Plate 4) and about 2.0 μm long and 0.5 μm in width. Each colony of *E.coli* was a circular raised, smooth, dry and rose pink coloured on MacConkey agar (Plate 5). Each colony of *E.coli* was circular, smooth and had golden green metallic-sheen on EMB agar (Plate 6).

Identification of other Gram-negative rods

Each colony of other Gram-negative bacteria (rods) were circular, pale-purple, purple-pink, deep-purple and deep-pink on EMB agar while Gram-negative bacteria were circular, pale-pink and pink on MacConkey agar . All isolated Gram-negative bacteria were short or long rods under the compound light compound at magnification of × 1000 .

Plate 2 Positive reactions of faecal coliform test (presumptive)

Gas →

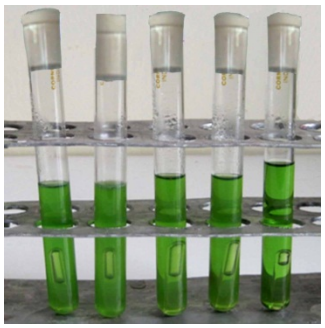


Plate 3 Positive reactions of faecal coliform test (confirmed)

Incidence of *Escherichia coli* and other Gram-negative rods

A total of 21 water samples were collected at different sites from natural ponds. In 11 out of 21 colonies, 52.38% were observed to be contaminated with *Escherichia coli*. Similarly, in 10 out of 21 colonies, 47.62% were observed to be contaminated with other Gram-negative rods bacteria (Table 6). Based on the data there was no significant difference between occurrence of *E.coli* and other Gram-negative rods ($\chi^2 = 0.22$, df = 1, $p > 0.05$).

Table 7 shows that a total of 11 isolate colonies of *Escherichia coli* in samples. The present study shows that the number of *E.coli* colonies obtained in May was 6, in June was 3 and in July was 2. Other Gram-negative colonies observed were 1 isolate number in May, 4 in June and 5 in July (Table 7). Figure 3 shows that colonies of *Escherichia coli* were observed 85.71%, 42.86% and 28.57% in May, June and July respectively. Whereas other Gram-negative rods bacteria were observed 14.29%, 57.14% and 71.43% in May, June and July respectively. There was significantly highest loading of *E.coli* in May ($\chi^2 = 21.496$, df = 2, $p < 0.01$).

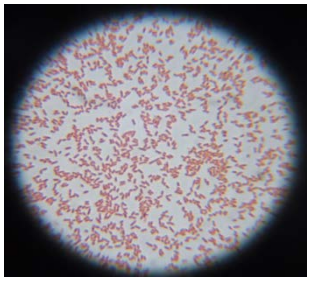


Plate 4 Cell morphology of *Escherichia coli* (× 1000 magnification)

Plate 5 *Escherichia coli* colonies on MacConkey agar

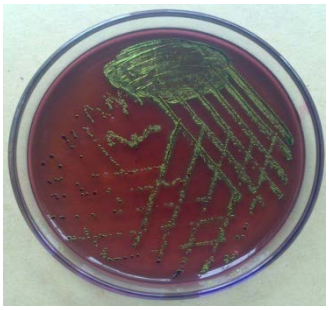
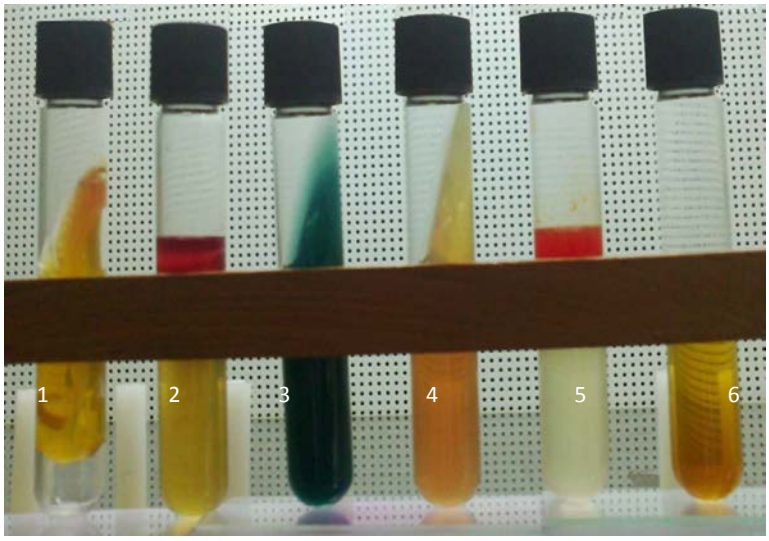


Plate 6 *Escherichia coli* colonies on EMB Agar



Biochemical Test results

1 = Triple Sugar Iron Agar, 2 = Sulphide Indole Motility Agar
3 = Simon's Citrate, 4 = Urease Agar,
5 = Methyl Red, 6 = Voges-Proskauer

Plate7 Biochemical reactions for *Escherichia coli* isolates

Biochemical tests for identification of *E.coli*

A total of 21 colonies were examined for identification by biochemical tests. At the present study showed that 11 colonies of *E.coli* were determined. Confirmation of *Escherichia coli* were done by some biochemical tests Table 5and Plate 7.

Some physical parameters of water samples in natural ponds

The range of temperature observed for the whole study period was from 27°C to 33°C, pH from 7.0 to 9.0 and dissolved oxygen (DO) from 4 mg/l to 12 mg/l Table 8.

The present result observed the mean value of water temperature was 32.57°C, pH was 7.57 and dissolved oxygen (DO) was 6 mg/l in May Table 9.

According to June results, the mean value of water temperature was 28.28°C, pH was 7.92 and dissolved oxygen (DO) was 6 mg/l Table 10.

In July shows that the mean water temperature was 27.71°C, the pH value was 7.43 and dissolved oxygen (DO) was 9.28 mg/l Table 11.

Chemical parameters (Fe , Ca, Pb) of natural pond waters

The present study revealed the presence of Fe and Ca and the absence of Pb in the natural ponds water samples. The mean value of Fe concentration was 0.24 mg/l and Ca was 5.75mg/l in summer. The metals of Pb could not be detected in all ponds (Table 12). Fe concentration ranged from the lowest of 0.22mg/l at pond 4 to the highest of 0.26mg/l at pond 1 in summer. Ca concentration ranged from the lowest of 1.20mg/l at pond 4 to the highest of 20.56 mg/l at pond 2 in summer. The mean value of Fe concentration was 0.84 mg/l and Ca was 20.59mg/l during the month of rainy July. Metals of Pb could not be detected in all ponds during the rainy months too. (Table13). Fe concentration ranged from the lowest 0.27mg/l at pond 6 to the highest of 2.94 mg/l at pond 5 in rainy (July).However, the metals of Fe were surprisingly absent in pond 2 in rainy (July). Ca concentration was the lowest of 0.59mg/l at pond 2 in rainy (July).

Table 1 Presumptive total faecal coliforms counts in some water samples (N=21) from natural ponds for the whole study period

Sr. No	Sites	Number of Water Samples contaminated with faecal coliforms/total number of samples tested	Range of faecal colifom counts (MPN/100 ml)
I	Pond-1	3/3	30 - >1600
II	Pond-2	3/3	30 - >1600
III	Pond-3	3/3	8 - 30
IV	Pond-4	3/3	4 - 30
V	Pond-5	3/3	23 - 50
VI	Pond-6	3/3	30 - > 1600
VII	Pond-7	3/3	30 - > 1600
	Total	21/21 (100%)	4 - > 1600

MPN = Most Probable Number of Cells per 100 ml of water

Table 2 Presumptive total faecal coliforms counts in some water samples (N=7) from natural ponds in May

Sr. No	**Sites**	**Number of Water Samples contaminated with faecal coliforms/total number of samples tested**	**Range of faecal colifom counts (MPN/100 ml)**
I	Pond-1	1/1	>1600 **
II	Pond-2	1/1	>1600 **
III	Pond-3	1/1	8 *
IV	Pond-4	1/1	23
V	Pond-5	1/1	50
VI	Pond-6	1/1	>1600 **
VII	Pond-7	1/1	>1600 **
	Total	7/7 (100%)	

MPN = Most Probable Number of cells per 100 ml of water
** = Maximum count, * = Minimum count

Table 3 Presumptive total faecal coliforms counts in water samples (N=7) from natural ponds in June

Sr. No	Sites	Number of Water Samples contaminated with faecal coliforms/total number of samples tested	Range of faecal colifom counts (MPN/100 ml)
I	Pond-1	1/1	30 **
II	Pond-2	1/1	30 **
III	Pond-3	1/1	8
IV	Pond-4	1/1	4*
V	Pond-5	1/1	23
VI	Pond-6	1/1	30 **
VII	Pond-7	1/1	30 **
	Total	7/7 (100%)	

MPN = Most Probable Number of cells per 100 ml of water
** = Maximum count, * = Minimum count

Table 4 Presumptive total faecal coliforms counts in water samples (N=7) from natural ponds in July

Sr. No	**Sites**	**Number of Water Samples contaminated with faecal coliforms/total number of samples tested**	**Range of faecal colifom counts (MPN/100 ml)**
I	Pond-1	1/1	30 *
II	Pond-2	1/1	30 *
III	Pond-3	1/1	30 *
IV	Pond-4	1/1	30 *
V	Pond-5	1/1	30 *
VI	Pond-6	1/1	50 **
VII	Pond-7	1/1	30 *
	Total	7/7 (100%)	

MPN = Most Probable Number of cells per 100 ml of water
** = Maximum count, * = Minimum count

Table 5 Biochemical characteristics of all isolated *Escherichia coli* from pond water

Bacterial isolates	IMVIC				TSI			SIM			Urcase	Catalase
Escherichia coli N= 11	Indole	MR	VP	Citrate utilization	H_2S	Slant	Butt	Indole	H_2S	Motility		
	+	+	-	-	-	A	AG	+	-	+	-	+

\+ = positive
\- = negative
A = Acid
G = Gas
H_2S = Hydrogen Sulphite production
MR =Methyl red test
VP =Voges-Proskauer test
SIM =Sulphite, Indole, Motility test
TSI = Triple Sugar iron test

Table 6 Occurrence of *Escherichia coli* and other Gram-negative rods (% of total) in water samples (N = 21) from natural ponds

Bacteria	Number of colonies obtained	Percentage (%)
Escherichia coli	11	52.38
Other Gram-negative rods	10	47.62
Total	**21**	**100**

Table 7 Isolated number of bacteria colonies in natural ponds in different months

Bacteria	Number of colonies			Total colonies
	May	June	July	
Escherichia coli	6	3	2	11
Other Gram-negative bacteria	1	4	5	10
Total	**7**	**7**	**7**	**21**

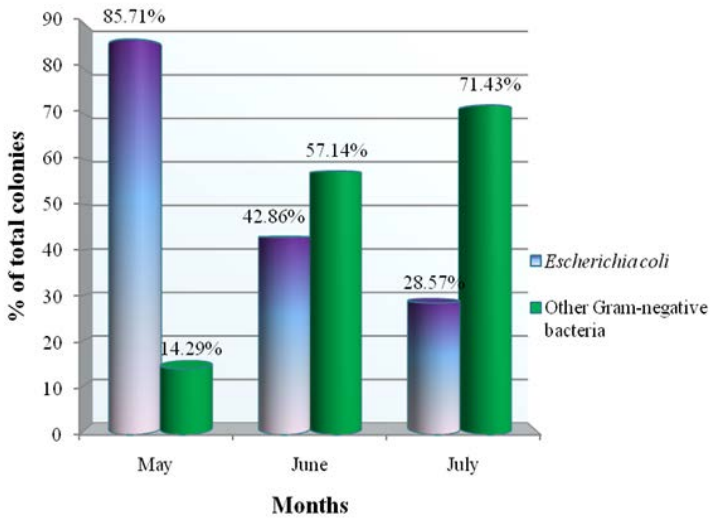


Figure 3 Distribution of *Escherichia coli* colonies and other Gram-negative bacteria colonies (percent of total) in natural ponds at different time in Wartarya Village.

Table 8 Some physical parameters of water samples in natural ponds in different months in Wartarya Village

Sr.no	Sites	Physical parameters			Months
		Temperature (°C)	pH	Dissolved Oxygen (DO) (mg/l)	
I	Pond - 1	32	8.0	6	May
II	Pond - 2	32	8.0	6	
III	Pond - 3	33	7.0	6	
IV	Pond - 4	33	7.5	7	
V	Pond - 5	32	7.0	4	
VI	Pond - 6	33	7.5	7	
VII	Pond - 7	33	8.0	6	
VIII	Pond - 1	28	8.0	6	June
IX	Pond - 2	29	8 0	5	
X	Pond - 3	28	7.0	6	
XI	Pond - 4	29	8.0	7	
XII	Pond - 5	28	8.0	6	
XIII	Pond - 6	28	7.5	4	
XIV	Pond - 7	28	9.0	8	
XV	Pond - 1	28	7.5	12	July
XVI	Pond - 2	28	8.0	8	
XVII	Pond - 3	29	7.0	6	
XVIII	Pond - 4	28	7.0	8	
XIX	Pond - 5	27	7.5	8	
XX	Pond - 6	27	7.5	11	
XXI	Pond - 7	27	7.5	12	
Mean ± SD		29.52 ± 2.29	7.64 ± 0.50	7.08 ± 2.23	

Table 9 Some physical parameters of water samples in relation to the presence of faecal coliforms, *Escherichia coli* and other Gram-negative bacteria among the samples in May

Sr.no	Sites	Physical parameters			Faecal coliform counts (MPN/100ml)	Number of colonies	
		Temperature (°C)	pH	(DO) (mg/l)		*Escherichia coli*	Other Gram-negative bacteria
I	Pond - 1	32	8.0	6	>1600	1	-
II	Pond - 2	32	8.0	6	>1600	1	-
III	Pond - 3	33	7.0	6	8	1	-
IV	Pond - 4	33	7.5	7	23	1	-
V	Pond - 5	32	7.0	4	50	1	-
VI	Pond - 6	33	7.5	7	>1600	-	1
VII	Pond - 7	33	8.0	6	>1600	1	-
Mean		32.57	7.57	6		6	1

(-) = not isolate

Table 10 Some physical parameters of water samples in relation to the presence of faecal coliforms, *Escherichia coli* and other Gram-negative bacteria among the samples in June

Sr. no	Sites	Physical parameters			Faecal coliform counts (MPN/100ml)	Number of colonies	
		Temperature (°C)	pH	(DO) (mg/l)		*Escherichia coli*	Other Gram-negative bacteria
I	Pond - 1	28	8.0	6	30	-	1
II	Pond - 2	29	8 0	5	30	1	-
III	Pond - 3	28	7.0	6	8	-	1
IV	Pond - 4	29	8.0	7	4	-	1
V	Pond - 5	28	8.0	6	23	1	-
VI	Pond - 6	28	7.5	4	30	-	1
VII	Pond - 7	28	9.0	8	30	1	-
Mean		28.28	7.92	6		3	4

(-) = not isolate

Table 11 Some physical parameters of water samples in relation to the presence of faecal coliforms, *Escherichia coli* and other Gram-negative bacteria among the samples in July

Sr. no	Sites	Physical parameters			Faecal coliform counts (MPN/100ml)	Number of colonies	
		Temperature (°C)	pH	(DO) (mg/l)		*Escherichia coli*	Other Gram-negative bacteria
I	Pond - 1	28	7.5	12	30	-	1
II	Pond - 2	28	8.0	8	30	1	-
III	Pond - 3	29	7.0	6	30	1	-
Iv	Pond - 4	28	7.0	8	30	-	1
V	Pond - 5	27	7.5	8	30	-	1
VI	Pond - 6	27	7.5	11	50	-	1
VII	Pond - 7	27	7.5	12	30	-	1
Mean		27.71	7.4	9.28		2	5

(-) = not isolate

Table 12 Average values of chemicals (mg/L) in water samples from study sites in Summer (May)

Chemicals (mg/l)	Sites							
	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6	Pond 7	Mean
Fe	0.24	0.24	0.25	0.22	0.26	0.25	0.23	0.24
Ca	11.46	20.56	1.20	1.37	1.15	2.87	1.69	5.75
Pb	-	-	-	-	-	-	-	-

Absent = -

Table 13 Average values of chemicals (mg/L) in water samples from study sites in Rainy (July)

Chemicals (mg/l)	Sites							
	Pond 1	**Pond 2**	**Pond 3**	**Pond 4**	**Pond 5**	**Pond 6**	**Pond 7**	**Mean**
Fe	0.72	-	0.38	0.36	2.94	0.27	1.25	0.84
Ca	3.90	8.3	1.92	1.64	0.59	2.12	2.12	2.94
Pb	-	-	-	-	-	-	-	-

Absent = -

Discussion

In the present study, the water samples from seven natural ponds of Wartarya Village, Yangon Region were analyzed on faecal coliform, *Escherichia coli* and other Gram-negative bacteria, also physical parameters.

The result indicated that all natural ponds from the study area were found to be contaminated with faecal coliforms.

It may be assumed that faecal coliform bacteria are ubiquitous at different sites of natural ponds environs. In the present study area, all ponds except pond 1 were located near human dwellings. Pond 1 is located near the paddy field and cowshed. So, human sewages could enter the pond and contaminated the natural water bodies. Ahmed (2003) stated that faecal coliform bacteria can enter ponds through direct discharge of waste from mammals and birds. Birds can be a significant source of faecal coliform bacteria.

Especially the result observed that the concentration of faecal coliform counts were higher in summer (May). Similarly, number of *Escherichia coli* bacteria was higher in summer (May). It could be due to the level of waters were shallow in ponds during the summer when most of the ponds except pond 3 were added the waters from the Hlaing River and the tube wells. Therefore, the waters from that of ponds may contain higher contamination of faecal coliforms bacteria and *E.coli*.

Due to Steven and David (2007) stated that *E.coli* level appeared to have higher levels in river during May. Therefore, the present results agree with the previous report (Ahmed 2003) stated that faecal coliforms were higher during summer than during winter in ponds. The abundance of normal bacteria coliforms are greater in the warm months than in the cold months of pond water. However, faecal coliform bacteria were observed in low concentration in pond 3 (site 3) in summer (May). It could be due to presence of herb lotus (Kyar) in that pond and not directly added of water from the river and other tube wells.

Because, Gray (2007) stated that lotus could be used as bacterial reductions. In the present result were reduced faecal coliforms and *E.coli* loading in all ponds in June and July. The reason for this might have been due to decrease of water temperature during the wet or rainy season. Ahmed (2003) stated that bacterial load might be increased with an increase in temperature in the pond water.

During study period, the average of water temperature was 29°C ± 2.29. The average of pH and dissolved oxygen (DO) values were 7.64 ± 0.50 and 7.09 mg/L ± 2.23. FEPA (1991) stated that the maximum limit of water temperature was 35°C for all aquatic organisms. And EPA (1997) mentioned that natural normal water has pH between 6.5 and 8.5; DO level is

between 4 mg/L to 6 mg/L. Therefore, the condition of water temperature and pH were suitable for the survival and propagation of bacteria and aquatic organisms in the study area. Nevertheless, the report of Pond Management Publication (1855) recommended that most pond waters can hold about 10 to 12 mg/L of oxygen. By comparing with the present record observed that the average of DO value was 7.09 mg/L ± 2.23. DO were low in most of the ponds. It could be due to ponds having no out flow, and land locked system. Therefore, the decaying of organic matters dumped in the ponds that will lead to the reduction of DO in the water bodies.

All the data have shown that the bacteria and aquatic organisms could thrive at these physical parameters conditions in the study sites.

The Saskatchewan Drinking Water Quality Standard (2008) stated that the maximum acceptable concentration for coliform in drinking water is zero organisms detectable per 100 ml. But in the present study area, all ponds were loading with *E.coli* 52.38%.

In the present study area, the metals of Fe were observed in all natural ponds in summer. Moreover Fe was higher in summer than in the rainy season and all these indicate that the possible sources of Fe could have been from the aquatic plants and algae as they could have died, fragment and released Fe into the pond waters in the summer or possibly tube well waters were supplemented into ponds for irrigation purposes during summer. Our studies have demonstrated that all pond waters except the pond number 2 showed substantial amounts of the metal Fe. Moreover the pond 2 appeared to be unique as there were no Fe in this pond water and it was located far away from the muddy clay rice fields. Thus our studies conclusively demonstrate that the metal Fe is carried into the pond 2 water during rainy seasons through the running of the rainy muddy clay waters into some ponds while in others Fe is lacking as the rain water is clean and uncontaminated with muddy clay soil.

In the present study, the concentration of Fe was shown to be far less between 0.22mg/L and 0.26mg/L in summer and between 0.27mg/L and 2.94 mg/L in rainy and in these respects, all natural pond waters were shown to contain far less Fe than other reports and thus the natural pond waters were found to be different, less harder and useful for consumption. However Patel and Romani (2003) stated that the values of Fe concentration in well waters was as high as 5.05mg/L. Moreover the values of Fe recorded in the current study were not higher than acceptable limit of 0.3mg/l(WHO,1988) in summer but not in rainy. The values of Fe were not higher than acceptable limit of 1.0mg/L(ICMR, 1983) except pond 5. It was noted that Fe values were higher in rainy than in summer. It was possible that fertilizers employed in the rice field were brought by rain water into the ponds. However Patel and Romani (2003) confirmed that the values of Fe concentration in fertilizer was 10.51mg/L.

The present study revealed the occurrence of the metals of Ca in all pond waters in summer as well as in the rainy seasons indicating natural occurrence of calcium all the ponds thorough out the year. We report a higher Ca values were in summer than in rainy in some ponds and perhaps evaporation and low level of water in ponds during summer may account for the consequent increase in the concentration of the metals of calcium in all pond waters. Another reason could be due to the level of waters were shallow in ponds during summer. So, most ponds were added water from tube wells and Hlaing river. Moreover our studies have shown that the concentration of Ca was found to be between 1.20 mg/L and 20.56mg/L in summer (May). Similarly, the concentration of Ca is between 0.59m/L and 8.30mg/L in rainy (July) and the values of Ca were thus not higher than acceptable limit of 200mg/L (WHO, 1988). A similar

observation was reported by Reginaa and Nabi (2009) who stated that the values of Ca concentration in river water were 42mg/L in May and 36mg/l in July. Patel and Romani (2003) stated the values of Ca concentration in well were 3.0mg/l.

Furthermore our studies have shown the contamination of infectious agents and fecal coliform bacteria in the waters of all natural ponds indicating the possible risks for consumption of the contaminated waters which needs water treatment to make safe drinking waters for human consumption. Moreover our studies have shown that the concentrations of ions / metals present in the water of all ponds were within the acceptable limits and therefore the waters from the natural ponds are safe for human consumption and also useful for other agricultural and domestic purposes.

Nevertheless, the water from all ponds are unsafe to drink due to the people might be develop infections from swallowing contaminated pond water.

Conclusion

The findings of the present work indicated that faceal coliforms and *Escherichia coli* were commonly observed in the water bodies of natural ponds at Wartarya Village both in dry and wet season. In the present study, faecal coliform are dominant in samples. The result proved that faecal coliform are ubiquitous at the Wartarya environs. *E.coli* was found to occur at 52.38% of present investigation. Thus, the water is unsafe to drink.

In particular, the water is required to make a rolling boil for three minutes to kill the bacteria and other organisms. Faecal coliforms and *E.coli* can usually be inhibited in growth or killed by boiling water or by treating with chlorine or washing thoroughly with soap after using with water. But the water does not usually need to be boiled for other household purposes like washing.

In water, faecal coliform bacteria have no taste, smell or colour. They can only be detected through a laboratory test. Hence, the detection of faecal coliform and *E.coli* should be tested per year for faecal pollution and microbiological monitoring of the pond water. However, no pond water related illness had recently been reported in the studied village due to bacteria may be nonpathogenic. So, there is no negative impact on public health in Wartarya Village. Local people could be taking necessary precautions in utilizing the pond waters in Wartarya Village.

These physical parameters indicated that the bacteria and aquatic organisms could thrive well in natural ponds of Wartarya Village. This means natural control of pathogenic bacteria could be occurring in the pond waters through food chains and food webs.

DO were low in most of the ponds, thus dissolved oxygen levels below about 6 mg/l may begin to have detrimental effects on pond life. Hence, the removing of decaying of organic matters should be done annually for all ponds.

The study has demonstrated the occurrences of acceptable levels of Fe and Ca chemical elements in most ponds waters through the year and indicated the usefulness of the water for human consumption, domestic purposes and the waters are safe for the life of aquatic organisms.

The results obtained could be useful to conserve natural ponds and to control occurrence of faecal coliforms bacteria in the ponds and to improve public health in Wartarya Village.

The study further recommends an annual sampling testing and analysis of pond water samples at Wartarya village that for faecal, physical and chemical contaminations.

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ISOLATION OF RHIZOBIA FROM THE ROOT NODULES OF COW PEA AND BLACK GRAM CULTIVATED IN KYAUNGKONE, MYANMAR

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Abstract

Isolation of nitrogen fixing rhizobia was made from the root nodules of Cow Pea and Black Gram during July to November 2018. Root nodules were collected from the cultivated field of Anauksu Village, Kyaungkone Township, Ayeyarwady Region, Myanmar. The aim of this research was to isolate nitrogen fixing *Rhizobium* as starter culture of biofertilizer for peas. Isolation of Rhizobium was made by using Yeast Extract Mannitol agar with Congo red and Bromothymol blue. Identification was based on colony morphology, cell morphology, basic staining reactions and motility. One strain of *Rhizobium* from Cow Pea and one strain of *Bradyrhizobium* from Black Gram were isolated. These bacteria could be used as starter culture for the production of rhizobia biofertilizers.

Keywords: *Rhizobium*, nitrogen fixing, root nodules, Cow Pea, Black Gram, biofertilizer

Introduction

Microbes are essential regulating agents for soil fertility. Microorganisms are indispensable in decay processes and in the transformation of organic substances and humus formation in soil. Bacteria are by far the most numerically abundant soil microorganisms. Among these bacteria, some are symbiotic bacteria in the root of some leguminous plants and some were associated in soil near the root region of some plants and grasses. Bacteria associated with these regions are collectively known as rhizobacteria and some have the ability to fix atmospheric nitrogen.

The rhizobia live freely in the soil and as soon as they come in contact with suitable host plant, they start the process of infection. After infection of appropriate legume, they can cause formation of nodules and participate in the nitrogen fixation. In general they are Gram negative, rod-shaped but varieties of morphological shapes are observed when isolated from the root nodule (Jadhav, 2013). The bacteria belonging to the genera *Rhizobium, Bradyrhizobium, Allorhizobium, Rinorhizobium* and *Mesorhizobium* are able to form nodules on their host plants inside of which they fix-nitrogen (Abo-Aba *et al.,* 2015).

Members of the genus *Rhizobium* have been isolated from nodules of many leguminous plants. *Rhizobium* symbiosis with legumes species is of special importance, producing 50% of 175 million tonnes of total biological N-fixation annually worldwide (Ogutcu *et al.,* 2008).

Allito (2015) studied the population and phenotypic characterization of soybean (*Glycin max*) and haric bean (*Phaseolus vulgaris*) nodulating rhizobia by using yeast extract mannitol agar (YMA). Rhizobia are unique in that they are the only nitrogen fixing bacteria living in a symbiotic relationship with legume and they can be used as biofertilizers that would reduce the need for chemical fertilizers and decrease adverse environmental effects.

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Therefore, the aim of the present work was to isolate the rhizobia bacteria from the rhizosphere of Cow Pea and Black Gram for the production of biofertilizer.

Materials and Methods

Study Period

This research was conducted at the laboratory of Zoology Department, Pathein University during July to November, 2018.

Collection of Samples

Plants and roots of Cow Pea (*Vigna catjang* Walp) and Black Gram (*Phaseolus mungo* L.) were collected from Anauksu Village, Kyaungkone Township, Ayeyarwady Region (Plate 1).

A. A plantation of Cow Pea

B. A sample plant of Cow Pea

C. A plantation of Black Gram

D. A sample plant of Black Gram

Plate 1: Plants of Cow Pea and Black Gram from which rhizobia were isolated

Preparation of Yeast Extract Mannitol Agar (Somesegaram and Hoben, 1985)

The chemical ingredients (Himedia, India) of yeast extract mannitol agar (YMA) were accurately weighed by digital balance. The ingredients were mixed in 1000 mL of distilled water. The YMA medium was added with Bromothymol blue to obtain the BTBYMA medium and similarly the YMA medium was added with Congo red to obtain the CRYMA medium. Then the chemical ingredients were sterilized by autoclaving with pressure of 1.05 kg per cm^2 (15 lb per in^2), temperature of 121ºC and duration of 15 minutes.

Culture of Bacteria

The portions of roots were taken from 5 to 15 cm below the stem base. The root segments were washed in tap water for three times to remove adhering soil particles. From each sample, two to three nodules were picked up and washed thouroughly with sterile distilled water. After washing, nodules were surface sterilized in 95% alcohol for 30 to 40 seconds to remove wax coating and subsequently immersed in 4% sodium hypochlorite for 3 to 4 minutes. Then nodules were immediately washed 5 to 6 times with sterile distilled water to remove traces of sodium hypochlorite. The surface-sterilized nodules (0.5 g) were crushed with the help of a sterile glass rod and 0.5 mL sterile distilled water was added and mixed. The milky suspension was streaked on to BTBYMA medium and incubated under aerobic condition at 30 ± 0.5ºC in the incubator until growth appeared. Single unique colonies were picked up and were streaked on to CRYMA medium. Isolation of pure cultures is made by using streak plate technique of Gillies and Dodds (1968).

Differential Staining Techniques

Gram's staining, capsule staining, acid-fast staining and endospore staining (Bradshaw, 1992) were used to identify the bacteria species.

Detection of Motility by Cultivation in Semi-solid YMA

Motility of the isolated bacteria can be detected in semi-solid agar medium using the method of Gillies and Dodds (1968). Ten milliliter of semi-solid YMA was dispensed in test tubes and they were left to set in the vertical position. A straight wire was inoculated and a single stab down was made in the centre of the tube to about half the depth of the medium. Whether the isolated bacteria were motile or not could be seen easily.

Identification of Bacteria and Plants

Bacterial species identification was followed after Breed *et al.,* (1957) Buchanan, Gibbons (1974) and Holt *et al*. (1994). The sample plants were identified at Botany Department in Pathein University.

Results

All species of Rhizobia were isolated with Yeast Extract Manitol Agar (YMA) containing Bromothymol blue (BTB) and YMA containing Congo red (CR) media. After two to five days inoculations and incubation of nodule suspension on YMA medium with BTB, colonies of bacteria were observed. This is the growth of rhizobia. Two species of rhizobia were isolated, one species of *Rhizobium* from Cow Pea, one species of *Bradyrhizobium* from Black Gram (Table 1).

Rhizobium species from Cow Pea

One species of *Rhizobium* was isolated from root nodules of Cow Pea. After two days of inoculation on YMA media containing BTB, colonies of *Rhizobium* were observed and the media turned into yellow colour. These colonies do not absorb the red colour when incubated on YMA media containing CR. The colony feature of *Rhizobium* from Cow Pea is pale yellow on

BTBYMA and white on CRYMA, convex and circular with entire edge. The diameter of colony on both media is 1.3 to 2.7 mm. Cells are ovoid with 1 to 2 µm width. They are Gram negative, capsulated, not acid-fast and non endospore. They were motile in semi-solid medium. The temperature for culture of this species is 30 ± 0.5ºC (Table 1, Plate 2. A and Plate 3).

Bradyrhizobium species from Black Gram

One species of *Bradyrhizobium* was isolated from root nodules of Black Gram. After five days of inoculation on YMA media containing BTB, colonies of *Bradyrhizobium* were observed and the medium was not changed into yellow colour. These colonies do not absorb the red colour when incubated on YMA medium containing CR. The colony feature of *Bradyrhizobium* from Black Gram is white on both BTBYMA and CRYMA, convex and circular with entire edge. The diameter of colony on both media is 1.2 to 3 mm. Cells are ovoid with 1 to 2 µm width. They are Gram negative, capsulated, not acid-fast and non endospore. They were motile in semi-solid medium. The temperature for culture of this species is 30 ± 0.5ºC (Table 1, Plate 2. B and Plate 4).

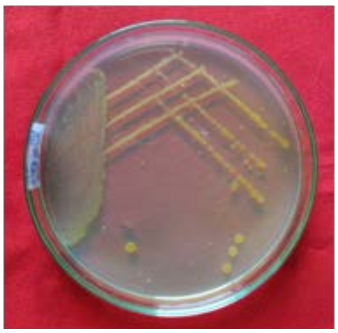
Table 1 Bacterial species isolated from root nodules of Cow Pea and Black Gram

Characteristic features	***Rhizobium* from Cow Pea**	***Bradyrhizobium* from Black Gram**
Colony morphology	Convex, circular, entire edge	Convex, circular, entire edge
Colony size	1.3-2.7 mm	1.2-3 mm
Colony colour	Pale yellow on BTBYMA and white on CRYMA	White on BTBYMA and white on CRYMA
Cell morphology	ovoid	ovoid
Cell size	1-2 µm	1-2 µm
Arrangement	Singly, pair	Singly, pair
Respiration	aerobic	aerobic
Incubated Temperature	30±0.5ºC	30±0.5ºC
Motility	Motile	Motile

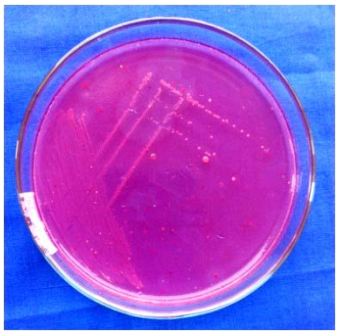
A. Motility of *Rhizobium* from Cow Pea

B. Motility of *Bradyrhizobium* from Black Gram

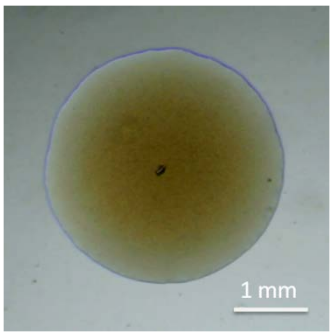
Plate 2: Motility test of isolated bacteria species in semi-solid medium



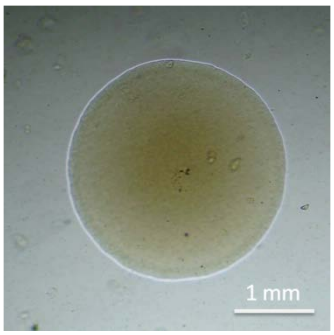
A. *Rhizobium* colonies of Cow Pea growth on BTBYMA medium



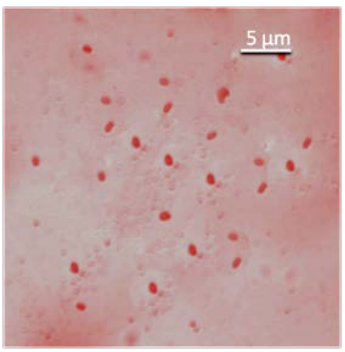
B. *Rhizobium* colonies of Cow Pea growth on CRYMA medium



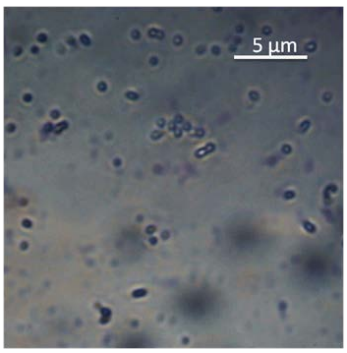
C. Single colony of *Rhizobium* on BTB medium



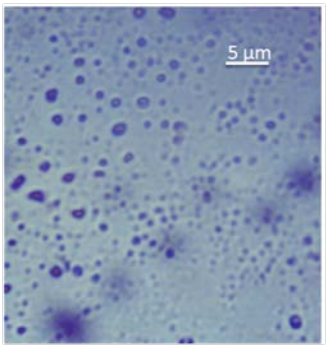
D. Single colony of *Rhizobium* on CRYMA medium



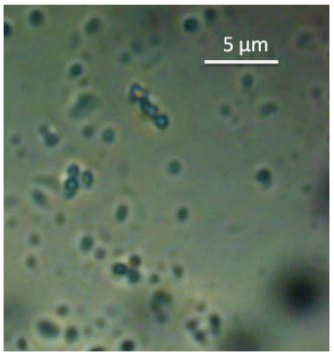
E. Gram staining of *Rhizobium*



F. Capsule staining of *Rhizobium*



G. Acid-fast staining of *Rhizobium*

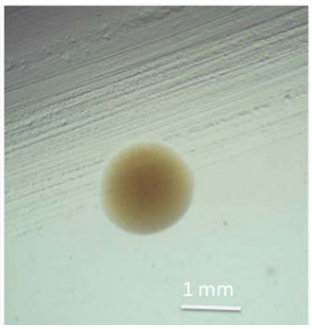


H. Endospore staining of *Rhizobium*

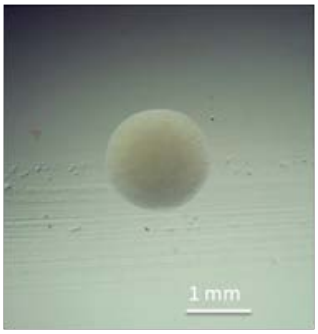
Plate 3: Colony morphologies and staining reaction of *Rhizobium* species from Cow Pea

A. *Bradyrhizobium* colonies of Black Gram growth on BTBYMA medium

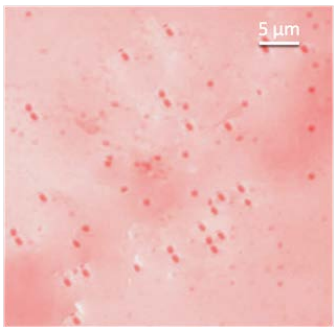
B. *Bradyrhizobium* colonies of Black Gram growth on CRYMA medium



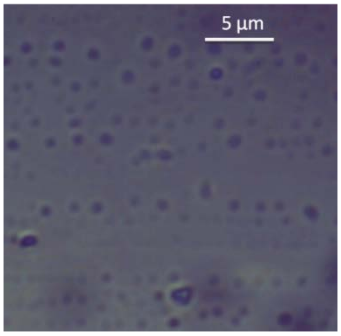
C. Single colony of *Bradyrhizobium* on BTB medium



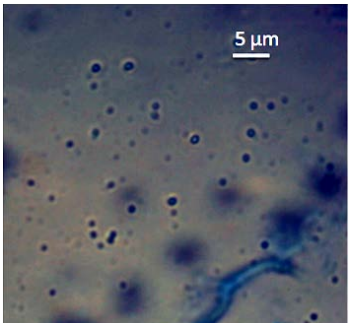
D. Single colony of *Bradyrhizobium* on Congo Red medium



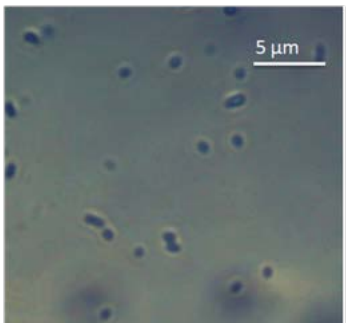
E. Gram staining of *Bradyrhizobium*



F. Capsule staining of *Bradyrhizobium*



G. Acid-fast staining of *Bradyrhizobium*



H. Endospore staining of *Bradyrhizobium*

Plate 4: Colony morphologies and staining reaction of *Bradyrhizobium* species from Black Gram

Discussion

To find out the rhizobia species (N_2 fixing bacteria) from inside root nodules of Cow Pea and Black Gram, yeast extract mannitol agar (YMA) medium with bromothymol blue or Congo red was used. Yeast extract mannitol agar (YMA) is the special medium used to grow *Rhizobium* (Neeraj *et al.,* 2009). Initial characterization of rhizobia involves observation of colonies growing in YMA containing Congo Red and bromothymol blue indicators (Bala *et al.,* 2011)

Sharma *et al.* (2010), Kaur *et al.* (2012) and Ahmed and Abdelmageed (2015) also used YMA with Congo red and YMA with bromothymol blue media to isolate and distinguish *Rhizobium* and *Bradyrhizobium* from pea and bean.

Therefore, these media were used in this work to isolate and characterize *Rhizobium.* Identification of the isolated bacteria were made based on cell and colony morphologies, basic staining reactions, motility and growing temperature. Two species of rhizobia from Cow Pea and Black Gram were isolated.

In this investigation, one of the isolated species from root nodules of Cow Pea changed the medium colour to yellow after two days incubated on YMA medium containing bromothymol blue. After five days incubation on YMA containing BTB, isolated bacteria from Black Gram nodules did not changed the medium colour into yellow.

Distinct colonies of fast-growing rhizobia begin to appear within 3-5 days, while those of slow-growers require 7-10 days to appear. Colonies of slow-growing rhizobia are characterized by a blue colouration, which indicates alkaline reaction on BTB. A yellow colour, indicating acid reaction, is produced by fast-growing rhizobia (Bala *et al*., 2011). Similarly, Sharma *et al*. (2010) classified the rhizobia as fast (medium turn yellow) and slow growers (medium turn blue) based on the yeast extract mannitol agar with bromothymol blue.

The characteristic of fast growing rhizobia is changing the YMA-BTB medium to yellow colour due to acid production (Ahmed and Abdelmageed, 2015). Therefore, in this study, isolated rhizobia from Cow Pea are fast grower, and the isolated species from Black Gram are slow grower. Distinct colonies of fast-growing rhizobia begin to appear within 3-5 days, while those of slow-growers require 7-10 days to appear.

In this work, the colonies of all isolated species do not absorb the red colour when inoculated on CRYMA medium. Typical Rhizobia colonies should show little or no Congo red absorption (Bala *et al*., 2011).

Pseudo-nodule forming bacteria *Agrobacterium* utilized Congo red but *Rhizobium* strains didn't utilize Congo Red. This test is essential to differentiate *Rhizobium* and *Agrobacterium* (Deshwal and Chaubey, 2014).

Therefore, the isolated rhizobia were not *Agrobacterium* and they may be *Rhizobium* or *Bradyrhizobium*.

In this research, colonies of all isolated strains are 1.2-3 mm in diameter and cells are gram negative, non endospore, not acid-fast and capsulated. They were motile in semi-solid medium. *Rhizobium* colonies were large (2-4 mm in diameter) mucilaginous, circular, convex with smooth edges, glistening translucent or white in YMA medium (Holt *et al*., 1994).

Rhizobium from the root of fresh rice plants are Gram negative, aerobic and rod-shape bacteria (Zhang *et al*., 2011).

The *Rhizobium* colonies were entire, opaque with regular margin, milky white, translucent, circular in shape, shiny, raised (convex), sticky consistency and 2-4 mm in diameter. They were also aerobic, non spore forming, gram negative rods and motile (Pawar *et al.*, 2014).

The coloration of colonies was milky-white translucent with a circular shape, with regular borders, shiny and raised after 3 to 5 day of growth on YMA medium at 28ºC. Colony diameter ranging from 2 to 5 mm and cells were motile, gram negative and rod shaped bacteria. All these features are characteristics of *Rhizobium* strains (Ahmed and Abdelmageed, 2015).

Therefore, *Rhizobium* species isolated from Cow Pea in this work was similar with the above reports and other standard characteristics of the isolates indicated that the isolated microorganisms were *Rhizobium* species.

The *Rhizobium japonicum* (Syn. *Bradyrhizobium japonicum*) was Gram negative, aerobic, non-spore forming and motile rods. In general, the colonies were 3.1 mm in diameter, circular, convex, whitish pink and glistening with entire margin (Gachande and Khansole, 2011).

Colony morphology of isolated bacteria from Black Gram was white on both BTBYMA and CRYMA; convex, circular, entire edge, 1.2-3mm in diameter and cells are gram negative, non endospore, not acid-fast, capsulated and motile. Therefore, the characteristics of isolated bacteria from Black Gram indicated that they were *Bradyrhizobium* species.

Conclusion

In this work, isolated bacteria from Cow Pea and Black Gram were nearly the same with the some characters of *Rhizobium* and *Bradyrhizobium*. Therefore, this research work could be expected to provide some basic information for the preparation and production of *Rhizobium* biofertilizers.

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INVESTIGATION ON HUNTING, TRAPPING AND THE IMPACT IMPOSED ON MAMMALIAN WILDLIFE IN THE ENVIRONS OF INHKAI BUM MOUNTAIN RANGE, KACHIN STATE

Hpaw Bwe[1] and Bawk Ra[2]

Abstract

Trading, hunting and trapping status on wild mammals were investigated from Inhkai Bum mountain range, from June 2016 to June 2018. Total of (20) types of traps were utilized by local people, eight types of traps were advanced made with iron and the rest (12) traps were traditional bamboo traps. Cruel iron trap types are locally more applied than bamboo types found in this area. A total of (35) mammal species were recorded, among them ten species were enormously target species by local hunters either for the purpose of meat or medicinal uses. These species included *Manis pentadactyla* (Pangolin), *Ursus thibetanus* (Asian black bear), *Catopuma temminckii* (Asian golden cat), *Anyx cinerea* (Small claws otter), *Sus scrofa* (Wild pig), *Bos frontalis* (Gaur), *Rusa unicolor* (Sambar), *Muntiacus muntjac* (Muntjac), *Atherurus macrourus* (Asiatic brush tail porcupine), and *Petaurista petaurista* (Red giant flying squirrel). Black bear, Asian golden cat and Otter were trapped by iron traps. Chinese pangolin and brush tail porcupine were trapped by bamboo traps, Wild pig, Gaur, Samber, Muntjac and Red giant flying squirrel were hunted with Tumi- gun by local peoples. Population of Black bear and Gaur species declined during June 2015 to June 2018, but Otter and Chinese pangolin population increased during the study period. The data from two years study indicated that although the demands on the body parts of wild species varied with the time, there is a constant demand of pangolin in the market. In the present study Pangolin species are more hunted and exploited by local people.

Keywords: mammals, trap types, hunting activities, Kachin State

Introduction

The present study focused on Inhkaibum mountain range Myitkyina Township located in the Kachin State, since no previous study on the mammals of this area has ever been attempted. Nevertheless, Rabinowitz and Saw Tun Khaing (1998) recorded 21 species of mammals from a remote region of Northern Myanmar. Moreover, Than Zaw *et al*. (2008) reported 18 small carnivores in Myanmar. The review is based on data from camera-trap surveys, between 1999 and 2005, supplemented by examination of wild mammal remains in hunting camps, villages and markets and other incidental information.

Nowadays, overexploitation of wildlife hunting for the commercial trade has resulted in significant declines and local extinctions for several wildlife species (Nooren and Claridge 2001; Anon 2005), both within and outside protected areas in south-east Asia (Bennett *et al*., 2000; Kaul *et al*., 2004). It directly affects the natural environment in that it throws off natural predation and population growth of the wildlife.

Over the centuries human beings have exploited carnivores, either for fur and meat or for the secretions that they produce from the scent glands. Many medium-sized carnivores are also threatened such as tiger, leopard, sun bear, and musk deer. Similarly, pangolin species are experiencing victim, because of their keratin scales.

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Rao *et al*. (2005) examined hunting patterns in tropical forests adjoining the Hkakaborazi National Park in north Myanmar. They used strip transect and camera trap surveys to generate relative weekend market. They also reported that poaching of large mammals including wild cats, clouded leopard, marbled cat and gaur are subject for live trade.

On the other hand, natural resources such as wood, fish and wildlife are also wrench by demands from Myanmar's neighbours, China, India, Thailand and Bangladish (Myint Aung *et al*., 2004). Many rural people consume and trade wildlife and the country's common border with China is powerful driver of wildlife hunting (Yiming *et al*., 2000). Worldwide, tens of millions of mammals each year are trapped legally. Additionally, an unknown number of animals are trapped illegally and, moreover, for every target animal captured; a varying number of non-target animals are injured or killed (Lossa *et al.,* 2007).

In additionally, many resources in Myanmar are currently under open access, as they belong to the state and lack rules to regulate exploitation, this particularly the case for forestry and fisheries (David *et. al*, 2015). Kachin State is fairly representative of many areas in Myanmar in which small and large mammal are found and should thus provide a baseline for country wide recommendations about the protection of these little known species.

Therefore, the present study seeks to the way of hunting and trapping situations on wildlife mammal species by local people in Inhkaibum mountain areas. Moreover, it investigated in more preference target species on hunting activities.

The reason for studying traditional traps is that the researcher wants to know how much do the local people's traps affect the decreasing number of wildlife mammals. And the researcher also wants to reveal the techniques of traditional bamboo traps practiced by Kachin tribal people.

Materials and Methods

Study area

The area of this study is in Myitkyina Township which is located on the west bank of Ayeyawady, and in the southeast of Kachin State, (24°31′ to 26° 12′ N and 96° 40′ to 97° 32′ E) InHkai Bum Mountain range was average altitude sea levels in 2000 meter. It can be divided into hill forests, evergreen forest and mixed deciduous according to the types of forest. Moreover, in this mountain range bearing between Sumpara bum mountain areas including Bumpha Bum Wildlife sanctuary and Chin dwin regions (Thamonthi Wildlife sanctuary). Thus, according to this mountain range topography was may be available seasonal local migration corridor for wild mammals tracing. Present study conducted in four study sites (Fig. 1).

Site I (Nam Jim)

This site is situated at 25° 31' 21.19" N and 97° 24' 25.98" E at the elevation of 551 m above sea level. This site includes densely growing trees and cultivation open lowland. This study area is 2.36 km^2, including four villages namely Nam Jim, Ding Galu, Aung Myae and Ahhi sha.

Site II (Nawng Nan)

This site is located in the west bank of Ayeyawaddy River and north part of Nam Jim village. It lies at 25° 34' 15.59" N and 97° 29' 47.57"E, at the elevation 780 m above sea level. The vegetation of the study area comprises secondary forest and cultivation area. This study area is 4.56 km^2 comprising nine villages namely, Gaw nan, Maw tung, La myan, Naung nan, La bang rosana, 10 mile village, 8 mile village, Yin Kaw and kawahka.

Site III (Ar Lam)

It lies at 25° 38' 40.90" N and 97° 30' 10.81" E. This has an average height of 538 m above sea level, located of the east part Myitkyina. This site includes lowland cultivation area, bamboo forest, mountain forest and secondary forest. This study area is 3.38 km^2. The area allocated five villages, Ar Lam, Sharawng kahtung, Dun gan, Lamung zup and U byit.

Site IV (Tanphre)

This site is located near the Myitsone confluence of Ayeyarwaddy. It lies at 25° 43' 3.72" N and 97° 29' 13.78" E at the elevation 610 m above sea level. It mainly comprises secondary forest, paddy field and agricultural area. The original mix-deciduous forests were still present. However, many kind of un-exported tree are still remaining for hewing the export trees. This study area is 3.35 km^2 and contains only three villages, namely Kying hkran, Tang hpre and Tiyan zup.

Data Collection

All the information's of species in this study were collected from various habitats of Myitkyina and its environs; down-hill forest and Inhkaing Bum Mountain. For account on the species composition of study sites, the researcher accompanied with local hunter want through the jungle paths monthly. Survey was conducted every twice in a month at each of four study sites. In additionally, interview survey on hunting information of data collection was based on interviews with local traders, indigenous people hunters and also survey in markets. Random survey method was practiced in collecting data. A total of (135) people were interviewed with local residents in (21) villages during the study periods, villager from each village were invited to obtain information on wildlife hunting data such as hunting quantities and techniques (Appendix 1). Data analysis is prepared in Microsoft Excel Programmed based on field information. The data from two years study periods was used for comparison**.** Species identification was followed after U Tun Yin (1993), Martin *et al.* (2001), Francis (2008).

Results

During the study period, a total of 35 species of mammal belonging to nine genera and five families and six orders namely Insectivora, Pholidota, Primate, Canivora, Artiodactyla and Rodentia were recorded (Table 1).

Among the four study sites, study Site IV represented the highest species composition of 27% and the lowest of 23% was Site II. Order wise, species composition of mammals was found to be the highest in Carnivore and Rodentia (34.28%) each, followed by Primate (11.43%)

Artiodactyla (5.71%) and the lowest was observed under Scandentia and Pholidita 5.71 % in each (Fig.2;3)

In the present study 20 types of traps were utilized, among them eight types of traps were modern trap made with iron and the rest of 12 traps were traditional traps by local peoples (Table 2 and 3; Fig 4 and 5)

Two year comparison on the price rate of target parts of wild animal species are presented in Table 4.

Table 1 List of mammal species recorded in the study area

Sr. No.	Family	Genus	Species	Common name
1	Tupaiidae	*Tupaia*	*T. belangeri* (Wagner, 1841)	Tree shrew
2	Erinaceidae	*Neotetracus*	*N. sinensis* (Trouessart,1909)	Gymnure
3	Manidae	*Manis*	*M. Pantadactyla* (Linnaeus, 1766)	Chinese pangolin
4			*M. Javanica* (Desmarest, 1822)	Sunda pangolin
5		*Nycticebus*	*N. bengalensis* (Lecèpède, 1800)	Slow loris
6	Cercopithecidae	*Macaca*	*M. mulatta* (Zimmermann,1780)	Macaques
7		*Trachypithecus*	*T. shortridgei* (Wroughton,1915)	Shortridge's langur/ leaf monkey
8	Hylobatidae	*Hoolock*	*H. hoolock* (Harlan, 1834)	Gibbon
9	Canidae	*Cuon* (Hodgson, 1838)	*C. alpinus* (Pallus, 1811)	Wild dog
10	Ursidae	*Ursus*	*U. thibetanus* (G. Cuvier, 1823)	Asian black bear
11			*H. malayanus* (Raffles, 1821)	Sun bear
12	Felidae	*Catopuma*	*C. temminckii* (Vigors & Horsfield, 1827)	Asia golden cat
13	Felidae	*Prionailurus*	*P.bengalensis* (Kerr, 1792)	Leopart cat
14	Mustelidae	*Martes*	*M. flavigula* (Boddaert, 1785)	Yellow-throated marten
15		*Arctonyx*	*A. collaris* (Cuvier, 1825)	Hog badger
16	Herpestidae	*Herpestes*	*H. Javanicus* (Goffroy saint-Hilair, 1818)	Javan mongoose
17		*Amblonyx*	*A. cinerea* (Illiger, 1815)	Small-claw-otter
18	Viverridae	*Viverricula*	*V. indica* (Geoffroy Saint-Hilaire, 1803)	Small-Indian palm civet
19		*Arctogalida*	*A. trivirgata* (Gray, 1832)	Three strip palm civet
20		*Paradoxurus*	*P. hermaphroditus* (Pallus, 1777)	Common palm civet
21	Cervidae	*Muntiacus*	*M. muntjak* (Zimmermann, 1780)	Barking deer
22		*Rusa*	*R. unicolor* (Kerr, 1792)	Samber
23	Suidae	*Sus*	*S. scrofa* (Linnaeus, 1758)	Wild boar/ Wild pig
24	Sciuridae	*Callosciurus*	*C. caniceps* (Gray, 1842)	Grey-bellied squirrel
25			*C. finlaysonii* (Horsfield, 1824)	Variable squirrel
26			*C. erythraeus* (Pallas, 1779)	Pallas's squirrel
27		*Tamiops*	*T. mcclellandii* (Horsfield, 1840)	Himalayan/ Burmese strip squirrel
28	Muridae	*Bandicota*	*B. indica* (Bechstein, 1800)	Lesser bandicoot
39		*Rattus*	*R. rattus* (Linnaeus, 1758)	House rat
30			*R. norvegicus* (Berkenhout, 1769)	Norway rat
31			*R. adamanensis* (Blyth. 1860)	Sikkim rat
32		*Diomys*	*D. crumpi* (Thomas, 1917)	Crump's mouse
33	Spalacidae	*Cannomys*	*C. badius* (Hodgson, 1841)	Lesser bamboo rat
34	Hystricidae	*Atherurus*	*A. macrourus* (Linnaeus, 1758)	Brush tail porcupine
35		*Hiystrix*	*H. brachyura* (linnaeus, 1758)	porcupine

Table 2 Utilizing of trapping categories, quantity and hunting success at In Hkai Bum Mountain range.

Sr. No.	Study site	Village	Trap categories (apply frequency)			Trapping effort	Trap success
			Bamboo trap	Iron trap	* Other		
1	Site - I	Nam Jim	7	8	1	201	176
2		Ding Galu	9	5	1	211	100
3		Aung Myae	6	5	-	198	97
4		Ah hi sha	7	4	2	212	84
5	Site - II	Gaw Nan	2	4	2	74	47
6		Maw tung	4	5	2	60	30
7		La myan	2	4	1	70	57
8		Naung Nan	3	4	-	54	32
9		La bang Rosana	2	5	-	75	45
10		10 mile	4	4	-	84	43
11		8 mile	3	3	-	98	42
12		Yin hkaw	4	4	-	100	30
13		ka wa hka	3	6	-	123	29
14	Site - III	Ar Lam	5	4	2	130	120
15		Sha rawng kahtung	4	3	1	131	107
16		Dun Gan	5	5	3	210	171
17		Lamung Zup	5	6	2	202	174
18		U byit	4	5	3	230	170
19	Site - IV	Kyinghkag	2	6	3	206	156
20		Tang hpre	2	6	3	205	170
21		Ti Yanzup	4	6	2	202	160
Total			**89**	**102**	**28**	**3076**	**2040**

* Other: Hand net, tumi gun, with bow and arrow utilizing

Table 3 Monthly frequency of trapping and hunting activities of one year round in all study sites

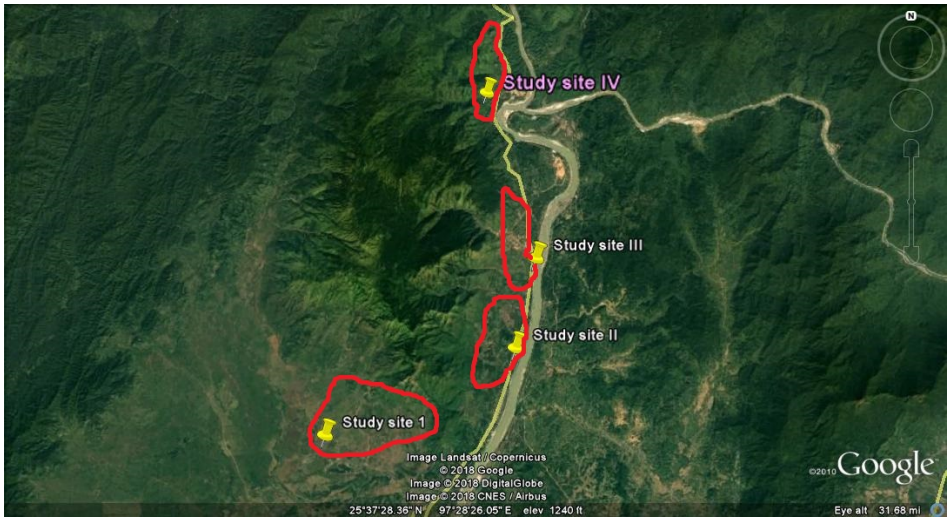
Site	* Type	Jan	Feb	Mar	Apr	May	Jun	July	Agu	Sep	Out	Nov	Dec	Total
Site I	T	4	2	1	2	2	-	-	-	-	1	2	2	14
	H	2	1	2	2	2	1	2	1	2	3	4	1	14
Site II	T	2	3	4	4	5	-	1	-	-	-	2	3	23
	H	4	1	2	3	2	2	1	1	2	2	3	2	17
Site III	T	2	3	3	4	2	-	-	-	1	-	4	1	19
	H	2	1	2	2	2	1	1	1	2	2	5	2	16
Site IV	T	4	1		5	5	1	-	-	-	2	2	3	20
	H	2	2	2	3	4	1	2	1	2	3	1	3	17
Total		**21**	**14**	**16**	**25**	**24**	**6**	**7**	**4**	**9**	**13**	**23**	**17**	

* Poaching type: T= Trapping, H= Hunting by gun, hand net

Table 4 Price rates of hunter targeted parts of wild mammal species (2016-2017)

Sr. No.	Specie	Common name	Parts/categories	Unit	Cost in local (Ks-)*	
					2016	2017
1	*Manis pantadactyla*	Asia Pangolin	scale	Kg	520,069	520,069
2	*Manis javanica*	Sunda pangolin	scale	kg	489,476	489,476
			live	kg	550,660	550,660
3	*Nycticebus bengalensis*	Asian slow loris	gall bladder	g	1,856	1,000
			live	kg	140,000	60,000
3	Trachypithecus shortridgei	Shortridge's langur	skin	one	85,000	45,000
4	*Hoolock hoolock*	Hoolock gibon	skull	one	35,000	35,000
			live	individual	120,000	120,000
5	*Ursus thibetanus*	Asiatic black bear	gall bladder(brown)	g	4,290	4,100
			gall bladder(gold)	g	9,177	9,177
6	*Helarctos malayanus*	Sun bear	gall bladder	g	4,290	2,200
			leg	Kg	122,369	48,948
			canine	one	30,000	15,000
7	*Catopuma temminkii*	Asian Golden cat	whole skin	one	50,000	20,000
			leg	one	50,000	20,000
8	*Neofelis nebulosa*	Clouded leopard	whole skin	one	120,000	40,000
			canine	one set	50,000	30,000
			leg	pair	50,000	20,000
9	*Prionailurus bendalensis*	Leopard cat	whole skin	one	50,000	30,000
10	*Actogalidia trivirgata*	Small tooth civet	whole skin	one	25,000	25,000
			meat	kg	15,908	15,000
11	*Aonyx cinerea*	Small claw otter	whole skin	one	80,000	45,000
12	*Sus scrofa*	Wild pig	meat	kg	18,355	18,355
			canine	one	30000	25000
13	*Bos frontalis*	Gaur (wild Ox)	meat	kg	30000	30000
			horn	kg	48,948	25,000
14	*Rusa unicolor*	Samber	meat	kg	36,711	36711
			horn	kg	24,474	12,000
			meat	kg	20000	5000
			leg	pair	90,000	50,000
15	*Muntiacus muntjak*	Red muntjac	meat	kg	12,237	10,000
			skin	one	5000	2000
			hoof	Kg	24,474	5,500
16	*Petaurista petaurista*	Red flying squirrel	skin	one	80,000	30,000
17			bladder	g	1,836	1,836
18	*Atherurus macrourus*	Brush tail porcupine	stomach	g	2500	2500
19	Hystrix brachyura	Malay porcupine	stomach	g	1,836	1,800
			Spine	one	15,000	3,800

* Ks = Myanmar Kyats



(**Source:** Google map)

Figure 1 Four study sites of Inhkaibum mountain range area, Myitkyina environs

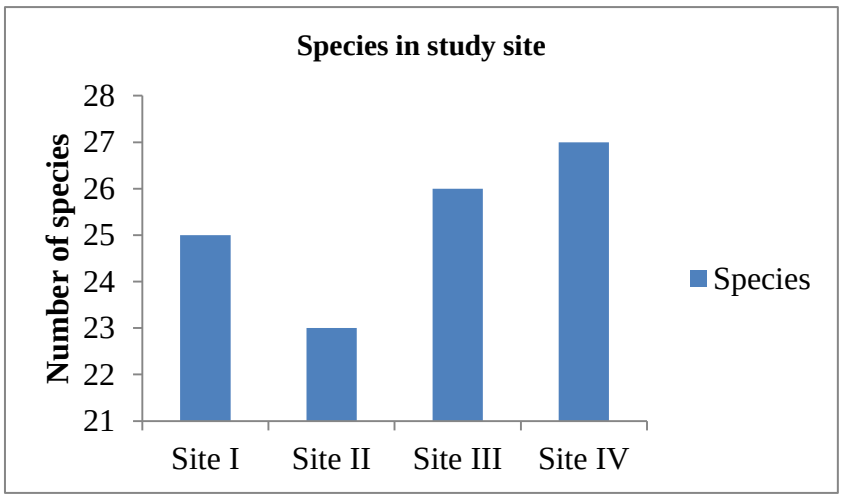


Figure 2 Composition on the number of species in study sites

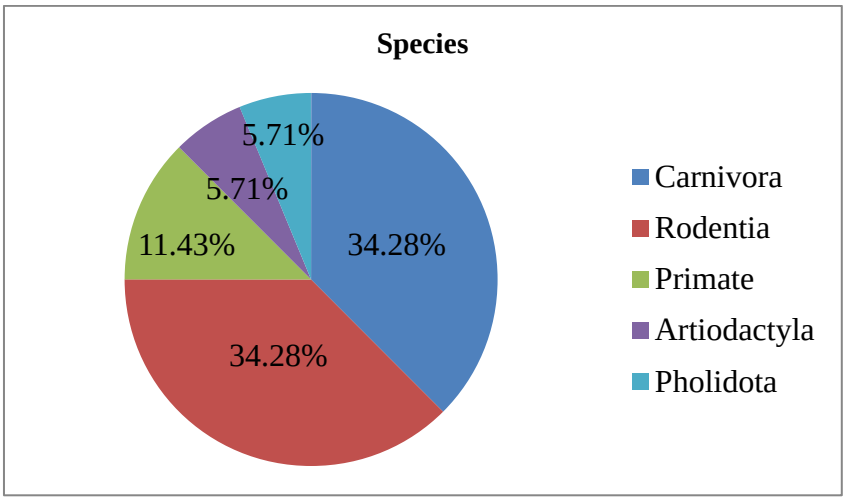


Figure 3 Composition of mammal species in different orders

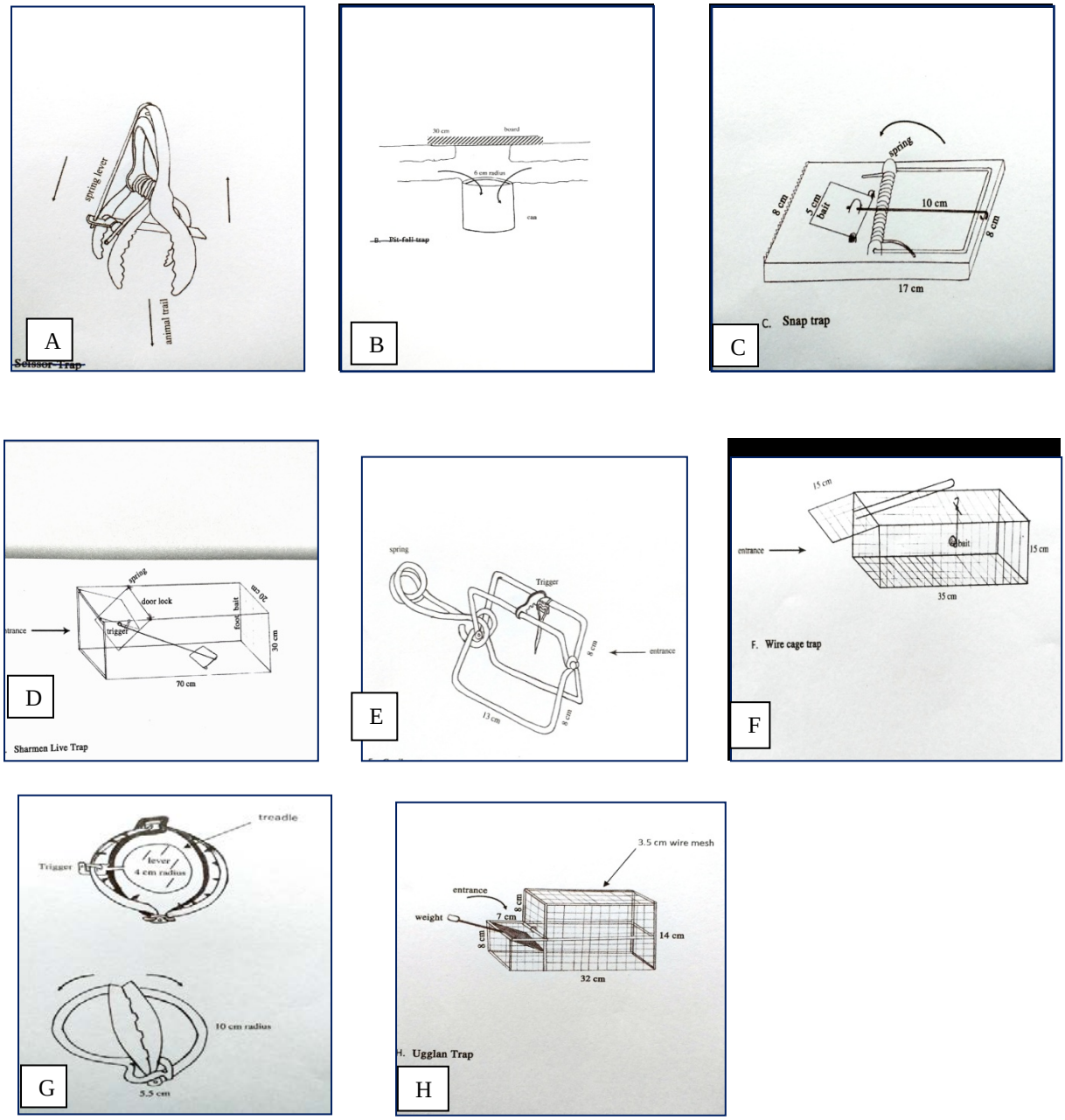


Figure 4 Local and indigenous people use iron made trapping gears **A**- Scissor trap **B**- Pit fall trap **C**- Snap trap **D**- Shaman's live trap **E**- Conibear trap **F**- Wire cage trap **G**- Tiger mount trap **H**- Ugglan trap

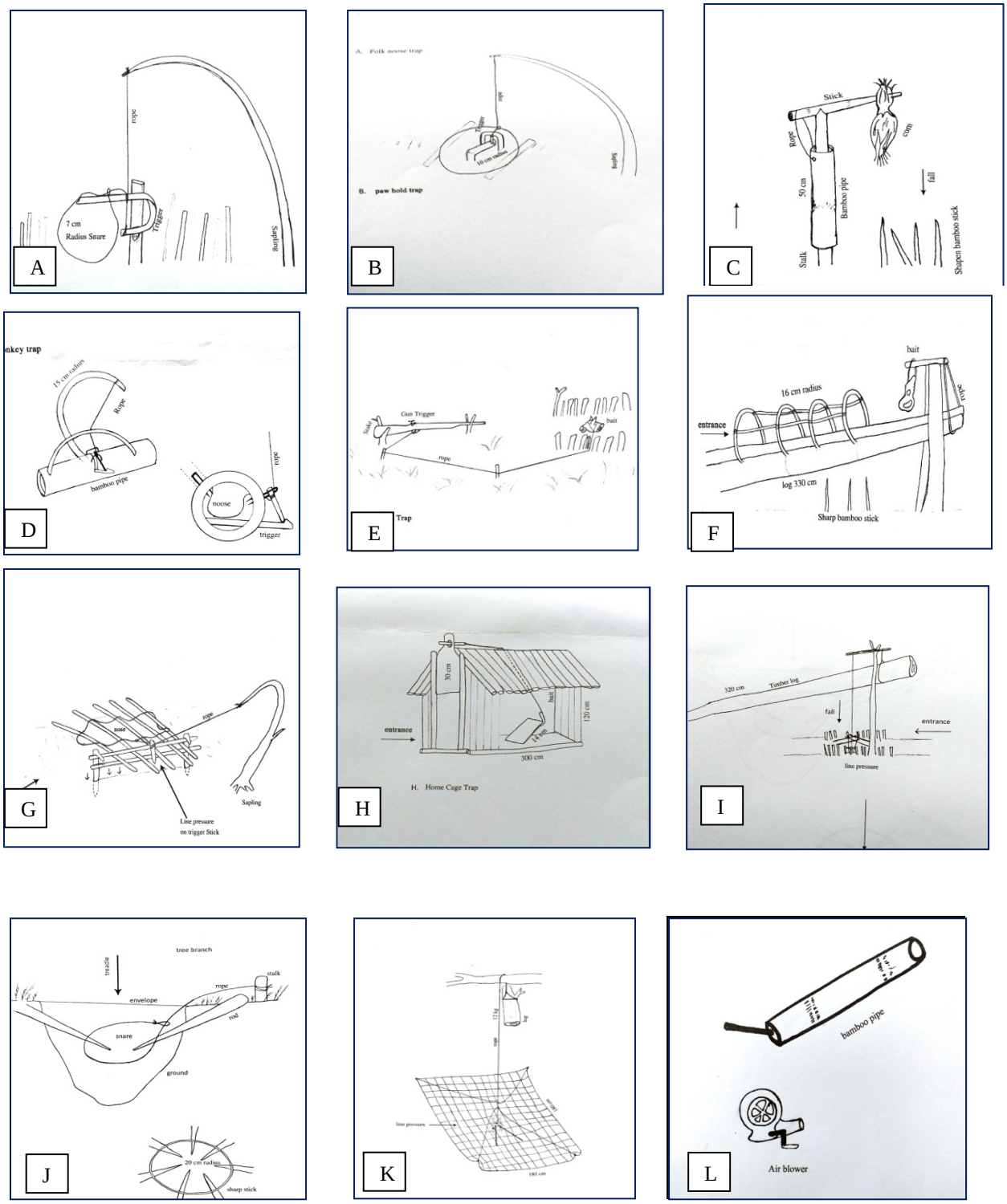


Figure 5 Local and indigenous people use bamboo made trapping gears **A**- Folk noose trap **B**- Paw hold trap **C**- Monkey trap **D**- Bamboo pipe trap **E**- Gun trap **F**- Pull basket-work trap **G**- Treadle spring snare **H**- Homelike cage trap **I**- Heavy timber log trap **J**- Apachi noose trap **K**- Transplant net trap **L**- Smoke out

Discussion and conclusion

The natives do hill-side cultivation in the rainy season. They grow rice to harvest in autumn. They mostly trap wild animals during the game season in late winter and summer. Thus, the number of animals' would not be decreased tactically as they have the time for reproduction and breeding. However, if the animals are over harvested for the horn, skin, skull, bladder and the animal as whole in illegal trade; they surely would become threatened in a near future.

From the overall results, it revealed that the highest frequency of poaching and hunting activity were encountered during March and April and dwindled to lowest during the peaked rainfall in September, during cultivated and harvested times (Table 3).

Yiming, 2000 explained that wildlife in Myanmar is threatened by illegal and unregulated hunting for domestic and international trade.

Nevertheless, researcher also encounter local market, where highly demands mammal species was throughout a year poaching by hunter (Table 4). According to collected data in Site IV Tanghpre area was high trapping effort among four study sites, it is assumed that this site may be due to the high species composition (Figure 2).

During the study recorded with respect to the local hunter preference trapping gear on the mammal species, it was revealed that, highly utilized tiger mount trap was in all study sites, followed by commonly used other iron trapping gears (Table 2).

Hpaw Bwe (2015) reported that (39) species of mammals were found in this mountain area, twelve species of small mammal such as, bat, mouse and wood rats were included. In this study, local hunters and peoples do not target these species. They hunt large mammals solely for the purpose of making traditional medicine, trading and for bush meat. However, in relation to traps and hunting patterns used by the hunter were that might influence among wildlife mammals fauna.

The demand for hunting wild mammal species depends upon the market. According to Hpaw Bwe, 2015, the pangolins and bears were mostly captured. This result is the same as the result of the present study. This is because their parts of the body are much more highly demanded in local market than borderline pass trade.

In normal, several of local people do not use iron made traps in catching animals. This is because it is difficult to construct those traps and also expensive. They preferably use traditional traps made of local materials without the involvement of iron materials, however, success in hatchability was not affected. On the other hand, greedier they are, the cruelled capturing methods they use. This is because people in study site among many different villages use tiger mount snap traps to capture mammals since it is the only way to get much more money for their living livelihood.

On the whole, it was found out that local people trapping effort on small mammals was for the purpose of controlling cultivation pests (Hpaw Bwe, 2015), However, overexploitation capture by trapping medium and large mammals was both for the purpose of consumption and illegal export, such as clouded leopard, samber and black bear parts. Especially the Pholidota, *Manis pentadactyla* (Chinese Pangolin) is highly sought, both for consumption and use in local medicine practice and have a demand in illegal trade across the border.

It was also known that when harvested time, rats, mice and inedible species were trapped not for the purpose of reselling, consuming or blending in folk medicine, but only captured to prevent from destroying the plantation, agricultural products, domestic stock, farm and damage of species for poultry such as civet mongoose. The local people hunting activities of preference target on market demand wild mammal species in this area.

Between two types of the entrapping method, live trap and death trap methods, it is found that the local people use death traps. Moreover, they sometimes use bow and arrow, gun in catching animals. Just as Sherman live traps, the natives can't afford to use metal meshes, wire cages, tiger mouth traps in every case because they are very expensive. Thus, they use them just for catching (illegal trade) species.

Among the recorded species, ten species were target species by local hunters for the purpose of meat and medicinal used. These species were *Manis pentadactyla*, *Ursus thibetanus*, *Catopuma temminckii*, *Anyx cinerea*, *Sus scrofa*, *Bos frontalis*, *Rusa unicolor*, *Muntiacus muntjac*, *Petaurista Petaurista*, and *Atherurus macrourus*. Among the target species, except of *Sus scrofa* (wild pig) and *Muntiacus muntjac* (red muntjac) the remaining all species were decreasing in world population trend (CITES 2009). According to 2015 IUCN red list, *Manis pentadactyla* Pangolin are considered as endanger species and followed by vulnerable species were *Ursus thibetanus*, *Anyx cinerea*, *Bos frontailus*, and *Rusa unicolor*. In this present work, according to sighting and questionnaires base survey the populations of these species were gradually decreased in Inhkai bum mountain range.

In the present study, the risk of overexploitation by poaching due to weak enforcement of wildlife laws, they have encouraged hunting among poor local people communities.

Thus it appeared that, the study area, Myitkyina Township embody mammals that are at risk by world population trend and by local standard some are threatened by over exploitation and illegal trade across the border, so that there is a need to safeguard the sustainability of the mammals in Myitkyina Township for the future generations to come.

To determine relationship between wild mammals occurrence and associated with local people hunting activities are needed to further more detail survey for long term in this areas.

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Appendix I

Interview survey for hunting profile

General Hunting Interview

Location (village): _____ Interviewee _____

Position N: _____

E: _____

1. How often do you hunt? _____
2. What time taken for hunting? _____
3. What kind of weapons do you use for hunting? _____
4. What kind of your preference target animals? _____
5. In each hunt, how many animals do you usually get? _____
6. What are your purposes for hunting? _____

ABUNDANCE OF RED-EARED SLIDER TURTLE *TRACHEMYS SCRIPTA ELEGANS* (WIED, 1839) AND THEIR POTENTIAL IMPACTS ON THE NATIVE TURTLE SPECIES IN THE TEMPLE PONDS, YANGON ENVIRONS

Kyi Thar Khaing [1], Khin War War [2], Naing Naing Oo[3], Aye Aye Thint[4], Hlaing Hlaing Thin Kyi[1] and Khin Thida Shwe[5]

Abstract

Species abundance of turtles and tortoises in the temple ponds, Yangon environs were observed in the study work. The study was conducted from 2015 to 2018. Distinctive characters and morphometric data of studied species were given systematically. Classification and identification of the individual specimens were also recorded with the sex. *Trachemys scripta elegans* (Red-eared slider turtle) is one of the world's most invasive species. They originated from North America and they have been considered as invasive species. The research designed was based on field study. It was observed on population status, the pet trade and distribution of the red-eared sliders turtle species. The population size of recorded turtles and tortoises were calculated for the sex and age groups (males, females, and juveniles) based on carapace length. *Morenia ocellata* and *Lissemys scutata* were the highest populations and the second highest population *T. s. elegans* were found to be recorded in all temple ponds. The population size of Red-eared slider (RES) turtle was composed of 6hatchlings, 10juveniles, 62adults (25males and 37females) with a percentage of 21% immature and 79% mature individuals. In Yangon, a large number of hatchling Red-eared sliders were sold at pet shops. The present study was the first attempt to record and present the current distribution and status of this invasive freshwater turtle.

. **Keywords**: red-eared sliders turtle, invasive species, population, abundance, pet

Introduction

Invasive Alien Species (IAS) are non-native species in a specific ecosystem whose introduction and subsequent establishment impact negatively on the economy, agriculture, biodiversity and/or animal and human health. They include animals, plants, fungi and microorganisms introduced from their original habitat and have the ability to outcompete native species for food and habitat. When invasive exotic species have persisted for a long time they may eventually be recognized by the public as "natural" or "native" due in part to the phenomenon of "shifting baselines" (Knowlton and Jackson, 2008). Invasive species are a major [1]threat to biodiversity (Simberloff *et al.* 2013) and are an ongoing concern for conservation practitioners (Kuebbing and Simberloff, 2015).

Little is known about the status of IAS in Myanmar but a few IAS have been observed throughout the country introduced by water, air and/or land transport (NBSAP Myanmar, 2011). The study species *Trachemys scripta elegans* (Red-eared slider turtle) was included on the list of invasive alien species that are one of the ''100 worst invasive alien species'' according to the International Union for Conservation of Nature (IUCN) (Lowe *et al.,* 2000; Cadi and Joly, 2003;

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Kikillus *et al*., 2010), which means that concrete action at the Union level is required to prevent their introduction, establishment or spread.

Trachemys scripta elegans RES is a semiaquatic turtle of the family Emydidae, order Testudines. It is one of the subspecies of the pond sliders *T. scripta*. The species *T. scripta* contains three subspecies: *T. s. elegans* (red-eared slider), *T. s. scripta* (yellow-bellied slider), and *T. s. troostii* (Cumberland slider) (Seidel, 2002), is native to the south-eastern USA and northeastern Mexico (Van Dijk *et al.* 2012). RES, an intentionally introduced species, is considered to be among the most common reptile pets traded worldwide (Salzberg, 1995, 1998; Lowe *et al.*, 2000; Telecky, 2001; Reed and Gibbons, 2003). In recent decades millions of hatchlings of this species, a popular pet, have been exported from USA farms to many countries. RES became very popular because of their small size, their simple husbandry requirements, and their reasonably low price. The colorful and small hatchlings are popular until they become adults that are more difficult to care for. Often, these unwanted pets are liberated into the local freshwater area where they become established as competitors and carriers of disease and parasites, with negative consequences for native turtle species (Pearson *et al.*, 2015).

RES now occurs in most temple ponds ecosystems throughout Myanmar. Although this species is well known and popular with the general public, few studies have investigated its impact on local ecosystems and native species, or its invasive status. The research paper was conducted at Yangon environ, by the following objectives; to determine the current range of the RES in Yangon environ, to record the species composition of native turtle and tortoise species in some turtle ponds, to examine abundance of turtle species in the temple ponds, to investigate the impact of invasive RES turtle at temple ponds in Yangon environs.

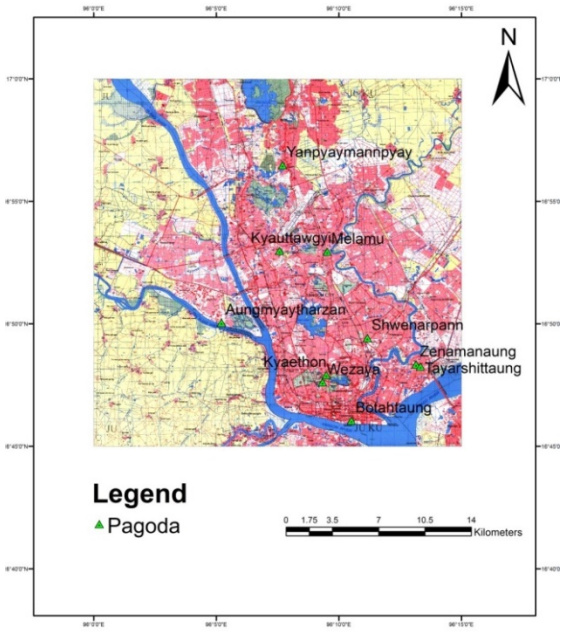
Materials and Methods

Study sites and study period

Ten artificial turtles ponds in pagodas and twelve pet shops in Yangon environ were selected for the present research design (Table1, Fiuger.1, Plate 1 and 2). Data collections were carried out from 2015 to 2018.

Table 1 Ten artificial turtles ponds of pagodas in Yangon environs

Sr No.	Study sites			
	Pagoda	Location	Pond type	Dimension (m)
1	Botahtaung	16˚ 46' N and 96˚ 10' E	Concrete	50×50×3
2	Zenamanaung	16˚ 48' N and 96˚ 12' E	Natural	80×80×3
3	Melamu	16˚ 53' N and 96˚ 09' E	Concrete	45×40×2
4	Kyaethon	16˚ 47' N and 96˚ 09' E	Concrete	60×60×2
5	Mahar Wezaya	16˚ 46' N and 96˚ 09' E	Concrete	50×50×2
6	Kyauttawgyi	16˚ 46' N and 96˚ 12' E	Semi-natural	50×50×3
7	Aungmyaytharzan	16˚ 19' N and 96˚ 05' E	Natural	80×80×2
8	Yanpyaymannpyay	16˚ 42' N and 96˚ 06' E	Concrete	45×45×2
9	Tayarshittaung	16˚ 48' N and 96˚ 13' E	Concrete	50×50×3
10	Shwenarpann	16˚ 49' N and 96˚ 10' E	Concrete	8×8×3



***Source:** Geography Department (YU)

Figure1 Location map of the study sites

Plate1 Artificial turtle pond of pagoda

Plate 2 Pet shop

Collection of the specimens

The present study has performed the techniques of direct sighting visual encounter survey (VES) and mark-recapture method for the abundance of turtle species in all different ponds. Basking turtle observation was the easiest to make on sunny days (between 7:00 am and 4:00 pm). The basking turtle was counted by visual eyes and photographic records were taken. Another survey was conducted by hand capturing turtles with the net. The hand capturing method survey determined the turtle species and approximate size class (hatchling, juvenile, adult).

Visual encounter surveys were conducted in 12 pet shops in Yangon between June 2017 and June 2018. Hatchling RES displayed in plain view or hidden in pet shops were photographed when permitted by pet shop owners. Interviews with pet shop owners were conducted to determine the price, quantity, source, and distribute of this species.



(A)Visual encounter survey (B) Hand capturing method (C) Using the net method

Plate 3 Collection of the specimens by various methods

Identification

Identification of the studied species was according to Smith (1931); CITES Identification Guide Turtles and Tortoises (1996); Win Maung and Win Ko Ko (2002) and Kalyar *et al.*, (2018).

Measuring of the specimens

Measurements were taken from the maximum carapace length (CL), carapace width (CW), plastron length (PL), plastron width (PW), height (H) and body weight (BW). Hatchling, <= 50 mm plastron length; Sub adult, 51 mm – 100 mm; and Adult,= > 101 mm plastron length for red-eared sliders were recorded (Cagle, 1946; Gibbons and Lovich, 1990 and Gibbons, *et. al.*, 1981).



(A) (B) (C)

Plate 4 Measurements of the specimens

Relative abundance

The relative abundance of the species was evaluated using the following formula (Bisht *et al.*, 2004) and (Bibby, Jones and Marsden, 1998).

$$\text{Relative abundance (RA)} = \frac{\text{Number of individual of a species (n)}}{\text{Number of individuals of all the species (N)}}$$

Dominance index

Analysis of the dominance index was done according to the method of Kumar and Sivaperuman (2005).

$$\text{Dominance index} = \frac{\text{No.of individual of each species}}{\text{Total number of individuals of all species}} \times 100$$

Community dominance index

A simple community dominance index was calculated as follows (Mc Naughton, 1968).

Community dominance index = percentage of abundance contributed by the two most abundant species

$$= 100 \times \frac{y_1+y_2}{y}$$

Where:

y_1= abundance of most abundant species
y_2= abundance of second most abundant species
y= total abundance for all species

Ordinal categories of abundance

Abundance category	Abundance score	Ordinal scale
< 0.1	1	Rare
0.1-2.0	2	Uncommon
2.1-10.0	3	Frequent
10.1-40.0	4	Common
40.1 +	5	Abundant

Results

Systematic position

The systematic position of the studied species was according to CITES Identification Guide Turtles and Tortoises (1996); Win Maung and Win Ko Ko (2002) and Kalyar *et al*., (2018).

Phylum - Chordata
Subphylum - Vertebrata
Class - Reptilia
Subclass - Anapsida
Order - Testudines
Suborder - Cryptodira
Family - Emydidae
Genus - *Trachemys*
Species - *T. scripta*
Subspecies - *T. s. elegans* (Wied, 1839)
Common name - Red-eared slider turtle
Vernacular name - Leik Parni or Na ywet ni leik
Type - Hardshell turtle

Description

The carapace is rounded or oval and flattened (especially in the male), has a dark green background with light and dark, highly variable markings (Plate5). The plastron is a light yellow with dark, paired, irregular markings in the center of most scutes. The plastron is highly variable in pattern. The plastron is slightly broader at the anterior than the posterior (Plate6). A red stripe

or red patch present behind each eye. Head, neck, and legs are green with many yellow lines. Juveniles have brighter markings and color. Toes are webbed and all bear foreclaws.

Sexual dimorphism

Males have longer claws on their front feet than the females. The male's tail is thicker and longer, it has a dark-colored retractable sexual organ known as their penis, inside the tail. The male's plastron is slightly concave, while that of the female is completely flat. The male is smaller than the female.

(A) (B) (C) (D) (E) (F)

Plate 5 Carapace color variation of Red-eared slider turtle (Young-Adult)

(A) (B) (C) (D) (E) (F)

Plate 6 Plastron patterns variation of Red-eared slider turtle (Young-Adult)

The research surveys confirmed the presence of *T. s. elegans* RES in all study sites (10 temple ponds) of Yangon environs. The total captured number of RES was found about 78 individuals from different temple ponds. The male and female sex ratio of the population was 1:1.5. The population size of RES was composed of 6-hatchlings, 10-juveniles, 62-adults (25-males and 37-females) with a percentage of 21% immature and 79% mature individuals (Fiuger 2 and 3). Mean value with the range of morphometric measurements for male, female, juveniles and hatchlings were shown in Table 2.

Market field surveys confirmed the presence of Red-eared sliders was sold in high numbers at the pet shops in Yangon (Plate7). Most hatchling specimens were observed in the pet shops, the average sold about 30> number per week. According to the interviews, many adult specimens had been released in the turtle ponds by their owners. Hatchlings RES prices ranged from 3000Ks to 25000Ks/ individual. The species was on the large scale and increasing levels of pet market activity in the Yangon environs.

Table 2 Morphometric measurements of *T. s. elegans* (Red-eared slider turtle)

Sex	Parameter					
	CL (cm)	CW (cm)	PL (cm)	PW (cm)	H (cm)	BW (g)
Males (n=25)	18.45±4.90	15.28±4.64	15.41±4.17	12.43±4.03	7.49±1.89	1135.29±641.44
Females (n=37)	21.12±3.40	18.90±4.18	18.98±2.32	11.50±1.97	8.08±1.05	2119.41±391.44
Juveniles (n=10)	7.48±0.55	6.37±1.20	6.61±0.74	4.91±0.80	3.72±0.40	121.50±17.96
Hatchlings (n=6)	3.35±0.35	3.25±0.08	2.73±0.23	1.70±0.19	1.27±0.18	40.67±4.71

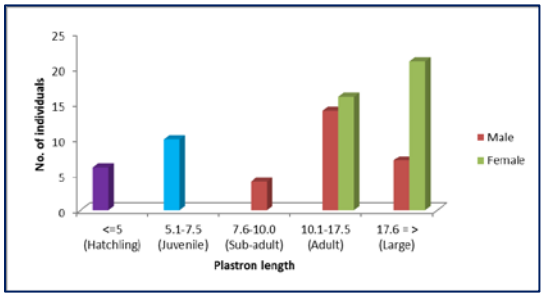


Figure 2 Plastron length on different life stages

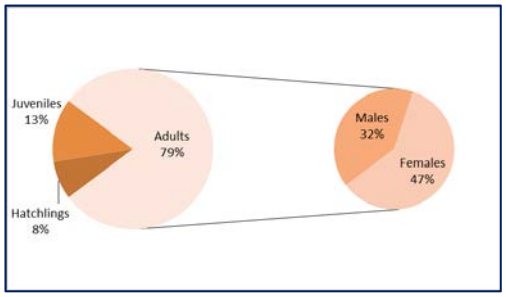


Figure 3 Percentage of immature and mature RES

(A) (B) (C)

Plate 7 Hatchlings RES at the pet shops in Yangon environs

Species composition and conservation status

A total of 11 species were recorded representing two species of tortoises and nine species of freshwater turtles belonging to four families, Testudinidae (two species), Trionychidae (five species), Geoemydidae (three species), Emydidae (one species) under Order Testudines. *Indotestudo elongata* (Yellow tortoise), *Manouria emys* (Asian Brown tortoise), *Amyda ornata phayrei* (Burmese softshell turtle), *Lissemys scutata* (Myanmar flap shell turtle), *Lissemys punctata* (Indian flap shell turtle), *Nilssonia formosa* (Myanmar peacock softshell turtle), *Chitra vandijki* (Myanmar narrow-headed softshell turtle), *Morenia ocellata* (Myanmar eyed turtle), *Cyclemys fusca* (Myanmar brown leaf turtle), *Batagur baska* (Northern mangrove terrapin), and *Trachemys scripta elegans* (Red-eared slider turtle) were found from different study sites in Yangon environs (Table 2, Fiuger.4). Among them, six species were observed as the endemic species.

As the conservation status according to the IUCN Red List (2015), two species were critically endangered (CR), three were endangered (EN), two were vulnerable (VU), one was

nearly threatened (NT), two were least concerned (LC), and one was data deficient (DD). According to CITES (2015), out of 11 species, three species were listed in Appendix I, five species as Appendix II and three were not listed. All of the species were listed as the protected species under MWPL (1994) (Table3, Fiuger.5).

Table 3 Recorded tortoises and turtles at the temple ponds in Yangon environs

Order	Family	Genus	Species/Subspecies	Common name
Testudines	Testudinidae	*Manouria*	*M. emys*	Asian Brown Tortoise
		Indotestudo	*I. elongata*	Yellow Tortoise
		Lissemys	*L. scutata*	Myanmar Flapshell Turtle (Endemic)
			L. punctata	Indian Flap shell Turtle
	Trionychidae	*Nilssonias*	*N. formosa*	Myanmar Peacock Softshell Turtle (Endemic)
		Amyda	*A. ornata phayrei*	Burmese Softshell Turtle (Endemic)
		Chitra	*C. vandijki*	Myanmar Narrow-headed Softshell Turtle (Endemic)
	Geoemydidae	*Morenia*	*M. ocellata*	Myanmar Eyed Turtle (Endemic)
		Cyclemys	*C. fusca*	Myanmar Brown Leaf Turtle (Endemic)
		Batagur	*B. baska*	Northern Mangrove Terrapin
	Emydidae	*Trachemys*	*T. scripta elegans*	Red-eared Slider Turtle

Table 4 National and International protection/ conservation status of recorded species

Species	Conservation Status			
	IUCN Red List (2012)	CITES (2010)	MWL (1994)	MFL (1993)
M. emys	Endangered	Appendix I	Protected	Not Listed
I. elongata	Endangered	Appendix II	Protected	Not Listed
L. scutata	Data Deficient	Appendix II	Protected	Protected
L. punctata	Least Concerned	Appendix II	Protected	Protected
N. formosa	Endangered	Not Listed	Protected	Not Listed
A. o. phayrei	Vulnerable	Appendix II	Protected	Protected
C. vandijki	Critically Endangered	Appendix II	Protected	Protected
M. ocellata	Vulnerable	Appendix I	Protected	Protected
C. fusca	Nearly Threatened	Not Listed	Protected	Protected
B. baska	Critically Endangered	Appendix I	Protected	Protected
T. s. elegans	Least Concerned	Not Listed	Protected	Protected

IUCN (International Union for Conservation of Nature and Natural Resources), CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), MWL (Myanmar Wildlife Law), and MFL (Myanmar Fisheries Law)

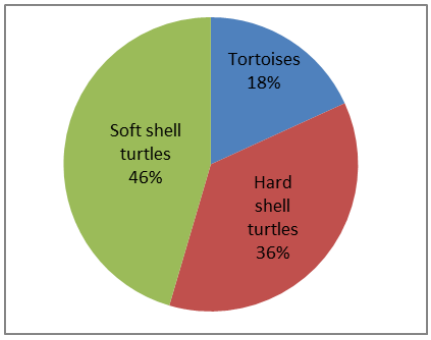


Figure 4 Percentage shell types of the recorded species

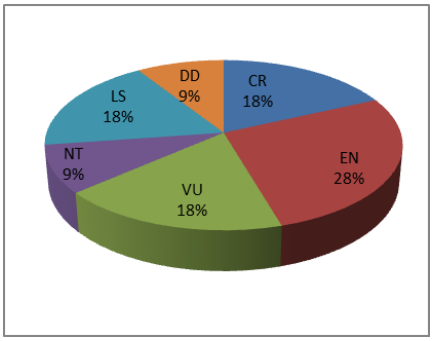


Figure 5 Conservation status of the recorded species

Abundance of species

According to the survey, *M. ocellata* (Myanmar eyed turtle) and *L. scutata* (Myanmar flap shell turtle) were the highest populations and the second highest population was *T. s. elegans* (Red-eared slider turtle) in all temple ponds. The lowest population of four species, *M. emys* (Asian Brown tortoise), *C. vandijki* (Myanmar narrow-headed softshell turtle), *C. fusca* (Myanmar brown leaf turtle), and *B. baska* (Northern mangrove terrapin) were observed (Fiuger.6).

The calculated community dominance index indicated that the common (CDI=21.11) ordinal scale of turtle species were recorded in Yangon environs. An ordinal category of recorded turtle and tortoise species in Yangon environs was shown in Table5. The mean value with the range of carapace length and body weight for the recorded species were shown in Table 6.

Table 5 Ordinal categories of recorded turtle and tortoise species in Yangon environs

Sr. No.	Species	Relative abundance index	Dominance index	Ordinal scale*
1	*M. emys*	0.0034	0.34	Rare
2	*I. elongata*	0.0135	1.35	Uncommon
3	*L. scutata*	0.3041	30.41	Common
4	*L. punctata*	0.010	1.01	Uncommon
5	*N. formosa*	0.0439	4.393	Frequent
6	*A. o. phayrei*	0.0304	3.04	Frequent
7	*C. vandijki*	0.0034	0.34	Rare
8	*M. ocellata*	0.3209	32.09	Common
9	*C. fusca*	0.0034	0.34	Rare
10	*B. baska*	0.0034	0.34	Rare
11	*T. s. elegans*	0.2635	26.35	Common

*Abundance score and Ordinal scale: < 0.1 (Rare), 0.1-2.0 (Uncommon), 2.1-10.0 (Frequent), 10.1-40.0 (Common), 40.1 + (Abundant)

Table 6 Mean number of carapace length and bodyweight of the recorded species

Sr.No	Species	No. of individuals	CL (cm) Mean±SD	BW (kg) Mean±SD	Males	Females	Juveniles
1	*M.emys*	1	60	37	-	1	-
2	*I. elongata*	4	22.5±2.6	2±0.26	-	4	-
3	*L.scutata*	90	15.8±4.9	0.85±0.54	35	40	15
4	*L.punctata*	3	17.4±2.5	0.96±0.57	1	2	-
5	*N.formosa*	13	50.9±13.4	17.9±10.9	3	9	1
6	*A. o. phayrei*	9	61.2±6.2	26.3±8.2	3	6	-
7	*C. vandijki*	1	61	23.08	-	1	-
8	*M. ocellata*	95	15.5±4.8	0.80±0.54	38	47	10
9	*C. fusca*	1	20	1.32	-	1	-
10	*B.baska*	1	58	33.9	-	1	-
11	*T. s. elegans*	78	16.22±6.46	1.19±0.84	25	37	16

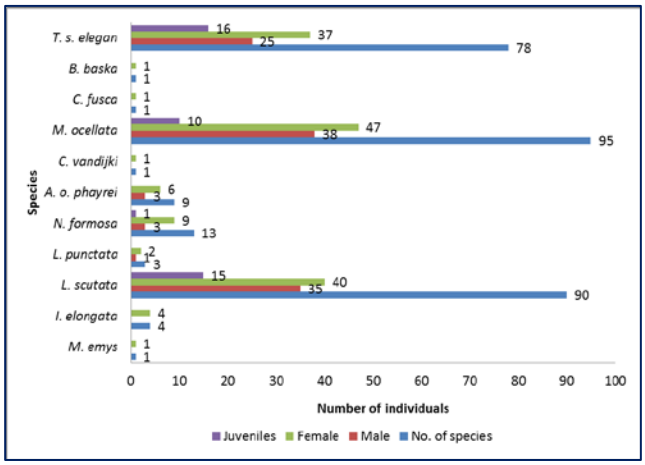


Figure 6 Comparison between the individual numbers of species recorded from different sites

Impacts of Red-eared sliders on the native turtle species

During the study periods, the impacts of the red-eared sliders on native turtles were competition for nesting and basking sites.

Basking turtle observation

According to the survey, the turtles spent most of their time basking between 8:00 and 16:00 hrs. There were 48 photo records for the basking sites during the study times; the number of RES was most abundant than the other native turtle species on the basking area (Table7 and 8, Plate8).

Table 7 Comparison between the numbers of basking native and RES turtle species

Groups	Count	Sum	Average	Variance
M.ocellata	48	141	2.938	19.592
L.scutata	48	214	4.458	18.594
T.s.elegans	48	226	4.708	28.722
Other	48	7	0.1458	0.170

Table 8 Comparative value on the numbers of basking native and RES turtle species

Source of Variation (ANOVA)	SS	Df	MS	F	P-value	F crit
Between Groups	632.625	3	210.875	12.575	1.576	2.653
Within Groups	3152.625	188	16.769			
Total	3785.25	191				

F>Fcrit (12.575>2.653); reject the null hypothesis (null hypothesis H0:µ1= µ2= µ3); significantly differences within groups.

(A) (B) (C)

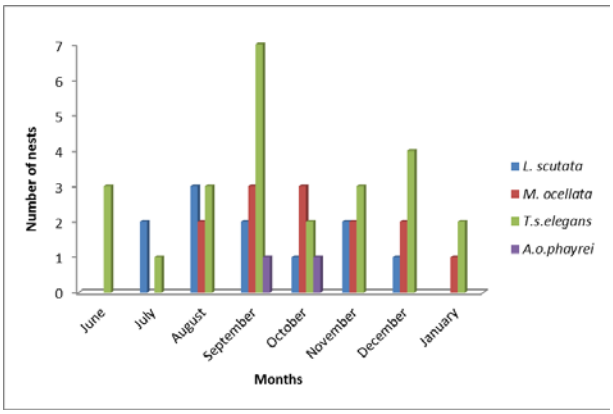
Plate 8 Competition for the basking sites between RES and native species

Nesting turtle observation

Four species of turtles *M. ocellata* (Myanmar eyed turtle), and followed by *L. scutata* (Myanmar flap shell turtle), *A.o.phayrei* (Burmese softshell turtle) and *T.s.elegans* (Red-eared slider turtle) were recorded as egg-laying species and their nests from the nest sites of turtle ponds during the breeding seasons. The nesting season was observed from June 2015 to January 2016 (Fiuger.7). The clutch size of *L.scutata* was 3-12 eggs (5.25 ± 2.19eggs), *M.ocellata* was 2-11 eggs (6.50 ± 4.03eggs), *T.s.elegans* was 2-21 eggs (8.25 ± 5.19eggs) and *A.o.phayrei* was only one egg of a single nest (Table9, Fiuger.7, and Plate9).

Table 9 Comparison between the clutch size of RES and native turtle species

Sr. No	Species	Mean	Std. Dev.	Maximum	Minimum
1	*M. ocellata*	6.5	4.03	11	2
2	*L. scutata*	5.25	2.19	12	3
3	*T. s. elegans*	8.25	5.19	21	2
4	*A. o. phayrei*	1	0	1	1



Fiuger 7 Comparison between the numbers of nest recorded from different months

(A) *T.s.elegans* (B) *L.scutata* (C) *M.ocellata*

Plate 9 Comparison between the nest of RES and native turtle species

Discussion

There are 27 species of native freshwater turtles and tortoises in Myanmar (Kalyar *et al*., 2018), including several species classified as rare and threatened in Myanmar. According to the results, the most population number of *T. s. elegans* (Red-eared slider turtle) was found to be recorded in all temple ponds. Moreover, a large number of hatchling Red-eared sliders were sold at pet shops and there was no monitoring to a higher proportion of RES sales. The recent surveys indicated that the occurrence of RES in these numbers posed a threat to the native turtle populations.

In the present study, a total of 78 individuals RES (6-hatchlings, 10-juveniles, 62-adults) were recorded in the study ponds. Therefore, it can be assumed that the RES populations are successfully reproducing in the temple ponds. Accordingly, the population under study was composed of 21% immature and 79% mature individuals. It is believed this composition reflects a healthy population with enough adults for continuous reproductions.

Species of native turtles and tortoises were also observed during the study periods. Among them, the two critically endangered species *Chitra vandijki* (Myanmar narrow-headed softshell turtle) and *Batagur baska* (Northern mangrove terrapin) were recorded. The most common species were *M. ocellata* (Myanmar eyed turtle) and *L. scutata* (Myanmar flap shell turtle) in all temple ponds. During the study periods, the abundance of RES was common species but the individual number size was smaller than that of the native turtle species *M. ocellata* and *L. scutata.*

RES has several attributes that seem to confer a competitive advantage over locally native turtle species because they mature at a younger age, are more aggressive, have higher fecundity and have a larger adult body size (Scalera, 2006). A range of studies provides evidence that red-eared sliders can compete successfully with native turtles for food, nesting sites and basking sites (Scalera, 2006). Basking sites are a key resource for evaluating the competition between aquatic turtle species because these sites are critical for proper thermoregulation, which directly influences vital physiological parameters like disease control as well as growth and reproductive rates (Ernst and Lovich, 2009).

During the course of study, RES and native turtle species were competition for basking places, because most of the basking sites were a restricted resource in the ponds. Most of the study sites, the turtles were in an overcrowded space with a ratio of 1-2 turtles/m2 and have limited food, basking, and nesting sites. In the present study, the highest population numbers of RES have been recorded in most basking sites.

The sliders usually lay eggs from April to July in their native distribution, and the development of eggs depends on moisture and temperature (Ernst *et al.,* 1994). Females could lay up to six clutches every year, varying 2-30 eggs in a clutch; the incubation period is 59 - 112 days and is extended in low temperatures (Ernst *et al.,* 1994; Bringsøe 2006). During the study periods, nesting occurrence was high from September to December. Differences in clutch size, number, shape, and dimension of eggs between the RES and native turtle species were observed. Comparative analysis of the nesting data, the clutch sizes of RES was larger than that of the native turtle groups. Existing RES in all turtle ponds causes unbalancing the ecology of those ponds because their potential impacts basking and breeding behaviors that cause reduced growth rates, and reproductive rates of native turtle species.

Conclusion

Most of the Red-eared slider turtles occurred in the temple ponds ecosystems throughout in Yangon. Thus, the present study was the first attempt to record and present the current distribution and status of this invasive freshwater turtle. This paper highlights the need for further research to assess the impacts of *T. s. elegans* on native species and proactive efforts to prevent its further spread.

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BENEFICIAL SERVICES OF WETLANDS AND THEIR INDICATOR BIRD SPECIES IN WETLAND AREAS OF AYEYARWADY REGION

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Abstract

The present research conducts with the beneficial services of wetlands and their indicator bird species in wetland areas of Ayeyarwady Region. Wetlands are critical habitats for wetland dependent bird species. These habitats are facing rapid degradation due to anthropogenic activities that affect the wetland indicator bird distribution by changing their habitats. Distance sampling point count method (Buckland *et.al*., 2004) was applied. Vegetation types were recorded by using the visual estimation. Vegetation covers were categorized by peripheral and mosaic by (Semeniuk *et al*.,1990). Field surveys were carried out from May 2017 to April 2018. During the survey period, 101wetland indicator bird species were recorded including three globally threatened bird species and four near- threatened bird species. These bird species were also indicate the wetland habitats.

Keywords: wetlands, globally threaten, birds, species, habitats, vegetation, visual estimation

Introduction

Wetlands provide many benefits to society – such as fish and wildlife habitats, natural water quality improvement, flood storage, shoreline erosion protection, opportunities for recreation and aesthetic appreciation, and natural products for our use at little or no cost. Wetlands are among the most productive ecosystems in the world, comparable to rain forests and coral reefs. They also are a source of substantial biodiversity in supporting numerous species from all of the major groups of organisms – from microbes to mammals. Physical and chemical features such as climate, topography (landscape shape), geology, nutrients, and hydrology (the quantity and movement of water) help to determine the plants and animals that inhabit various wetlands. Wetlands can be thought of as "biological supermarkets." They produce great quantities of food that attract many animal species. Many animals need wetlands for part or all of their life cycle. Numerous species of birds and mammals rely on wetlands for food, water, and shelter, especially while migrating and breeding. There are many studies and researches on wetlands for delineate the wetlands in many parts of the world. In some country, planning to do the project that transform farmlands into wetlands. The current situations of wetlands are still poorly known and the concept is still in its infancy in Myanmar. The objectives of research were to examine the functions and values of wetland, to record the wetland indicator bird species and their habitat characteristics (vegetation cover) and to observe the vegetation types (i.e. emergent, submerged and free-floating).

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Materials and Methods

Study area

Ayeyarwady region (Ayeyarwady Delta) lies between north latitude 15° 40' and 18° 30' approximately and between east longitude 94° 15' and 96° 15'. Data were recorded in wetlands of two districts (Maubin and Myaungmya).

Study sites

Maubin District

1. Maubin Township (Site I)

   (North latitude 16° 41' and East longitude 95° 32')

2. Nyaungdon Township (Site II)

   (North latitude 17° 07' and East longitude 95° 31')

Myaungmya District

1. Wakhema Township (Site III)

   (North latitude 16° 45' and East longitude 95° 14')

Study period

The survey was conducted from May, 2017 to April, 2018.

Field data collection

The present research was conducted in some wetlands of Ayeyarwady Region by field survey. The research data were collected the functions and values of wetland, diversity of the wetland indicator bird species, habitats and vegetation types. Distance sampling point count method (Buckland *et al*., 2004) was applied for this research. Recorded wetland indicator bird species were identified by Robson, 2011. The vegetation types were categorized by emergent and submerged using the visual estimation. Habitats (vegetation cover) were collected by rapid assessment by level 4 of Asian Wetland Inventory Handbook (Finlagson *et al.*, 2002). Field data were carried out three days per trip and couple times every month during the research period.

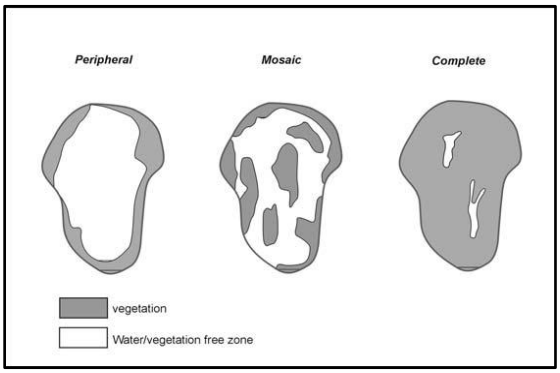

Figure 1 Categories of vegetation cover (Semeniuk *et al*.,1990)

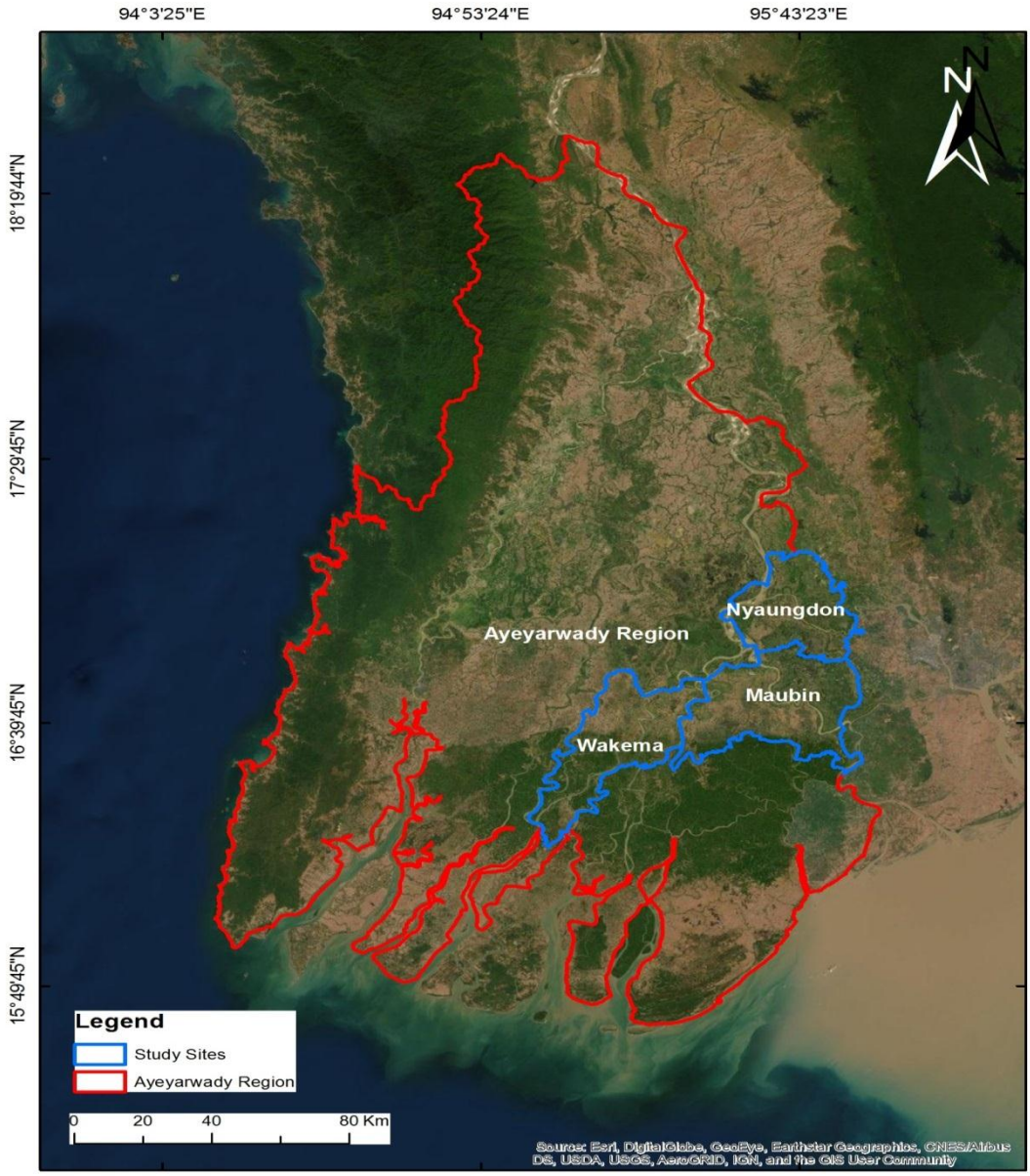

Figure 2 Map of the study sites

Results

Beneficial services of wetland in three sites

According to the result, all of three study sites were provided benefit for local peeople who depends on wetlands and biodiversity especially bird species by many ways. These wetlands provided wildlife resources or wild foods (snakes, turtles, and mollusks), fisheries and aquatic resources, agricultural resources (rice, vegetables, and crops), and food resources for animals (grass, rice straw, bush and shrubs). Additional, water transportation, recreation and tourism, and education and research were supported by these wetland sites (Plate II, III, IV).

Among three study sites, site I (Maubin) and site III (Wakhema) were more provided wetland beneficial services than site II (Nyaungdon). These two study sites were wide wetland area, low impact and good vegetation types and cover for local people and animals than site II.

According to the result, wetlands (site I and site III) were look like nearly natural wetlands. Most of the beneficial values for local people and animals including bird species were getting from these wetlands. The beneficial values of wildlife resources, fisheries and aquatic resources, agricultural resources and food resources for animals were more observed in these two sites. These two wetlands provided ecosystem services, research site for biodiversity and ecotourism site.

Recorded wetland indicator bird species in three study sites

A total of 101 wetland indicator bird species belonging to 49 families under 15 orders were recorded in three study sites (Table 1). Three globally threatened bird species (Yellow – breasted Bunting, Sarus Crane, Jerdon's Babbler, and five near-threatened species (Oriental Darter, Painted Stork, Black- headed Ibis and Asian Golden Weaver) were observed during the study period (Plate I).

Maubin District

Recorded wetland indicator bird species in Maubin Township (Site I)

Study site was chose in Thae Phyu village. A total of 79 wetland indicator bird species were recorded in Maubin Township (Table 2). According to IUCN Red list, one vulnerable bird species of Sarus Crane and five near- threatened bird species of Asian Golden Weaver, Oriental Darter, Spot- billed Pelican, Black- headed Ibis and Painted Stork were recorded during the study period. The highest near- threatened bird species numbers were recorded in site I. Most of the bird species were dependent on this wetland throughout the year. Most of the time of their life spent in wetland for foraging, roosting, breeding, and rearing their young.

Recorded wetland indicator bird species in Nyaungdon Township (Site II)

Study site was chose in Natse village. A total of 43 wetland indicator bird species were recorded in **Nyaungdon** Township (Table 2). In this site, two vulnerable bird species (Sarus Crane and Jerdon's Babbler) and two near- threatened bird species (Oriental Darter and Asian Golden Weaver) were recorded during the study period. The lowest species numbers and second highest globally threatened bird species were recorded in this site.

Myaung Mya District

Recorded wetland indicator bird species in Wakhema Township (Site III)

Study site was chose in Shwelaung village. A total of 82 wetland indicator bird species were recorded in Wakhema Township (Table 2). In this study site, three globally and four near-threatened bird species were recorded. There were one critically endangered (Yellow Breasted Bunting), two vulnerable bird species (Sarus Crane and Jerdon's Babbler) and four near-threatened bird species (Asian Golden Weaver, Painted Stork, Black- headed Ibis and Spot-billed Pelican) recorded during the study period. The highest globally threatened bird species were recorded in this site. According to the data, the rediscovered bird species of Jerdon's Babbler was recorded in site III. This species had last been recorded in 1941 and rediscovered in May, 2014 (Kathy Khine, 2019). Highest population was recorded in this site and follow after by Nyaungdon (site II).

Vegetation cover and vegetation types in three study sites

The vegetation covers were categorized by peripheral and mosaic. In site I and site II were mosaic while site III was peripheral. According to the data of vegetation types, emergent plants and submerged plants were observed in three study sites. In emergent plant, there are two kinds of type such as bottomed rooted emergent and free floating emergent. *Neptunia oleracea, Limnocharis flava, Eichhornia crassipes,* and *Pistia stratiotes* were free floating emergent plant. *Eleocharis dulcis* and *Nymphoides indica* were bottom rooted emergent plant. *Utricularia aurea* and *Ipomoea aquatic* were submerged plant.

Table 1 Recorded wetland indicator bird species in Ayeyarwady Region

Sr.no	Scientific name	Common name	IUCN Status
1	*Dendrocygna javanica*	Lesser Whistling-Duck	
2	*Tachybaptus ruficollis*	Little Grebe	
3	*Mycteria leucocephala*	Painted Stork	Near-threatened
4	*Anastomus oscitans*	Asian Openbill	
5	*Threskiornis melanocephalus*	Black-headed Ibis	Near-threatened
6	*Plegadis falcinellus*	Glossy Ibis	
7	*Ixobrychus sinesis*	Yellow Bittern	
8	*Ixobrychus cinnamomeus*	Cinnamon Bittern	
9	*Ixobrychus flavicollis*	Black Bittern	
10	*Nycticorax nycticorax*	Black-crowned Night-Heron	
11	*Ardeola grayii*	Indian Pound-Heron	
12	*Ardeola bacchus*	Chinese Pond-Heron	
13	*Bubulcus coromandus*	Eastern Cattle Egret	
14	*Ardea cinerea*	Grey Heron	
15	*Ardea purpurea*	Purple Heron	
16	*Ardea alba*	Great Egret	
17	*Mesophoyx intermedia*	Intermediate Egret	
18	*Egretta garzetta*	Little Egret	
19	*Pelecanus philippensis*	Spot-billed Pelican	Near-threatened
20	*Phalacrorax niger*	Little Cormorant	
21	*Anhinga melanogaster*	Oriental Dater	Near-threatened
22	*Pernis ptilorhynchus*	Oriental Honey-Buzzard	
23	*Elanus caeruleus*	Black-shouldered Kite	
24	*Milvus migrans*	Black Kite	
25	*Milvus lineatus*	Black-eared Kite	
26	*Amaurornis phoenicurus*	White-breasted Waterhen	
27	*Gallicrex cinerea*	Watercock	
28	*Gallinula chloropus*	Common Moorhen	
29	*Grus antigone*	Sarus Crane	Vulnerable
30	*Vanellus cinereus*	Grey-headed Lapwing	
31	*Hydrophasianus chirurgus*	Pheasant-tailed Jacana	
32	*Metopidicus indicus*	Bronze-winged Jacana	
33	*Rostratula benghalensis*	Greater Painted-Snipe	
34	*Actitis hypoleucos*	Common Sandpiper	

Sr.no	Scientific name	Common name	IUCN Status
35	*Tringa glareola*	Wood Sandpiper	
36	*Glareola maldivarum*	Oriental Pratincole	
37	*Chlidonias leucopterus*	White-winged Tern	
38	*Chlidonias hybrida*	Whiskered Tern	
39	*Columba livia*	Rock Pigeon	
40	*Streptopelia tranquebarica*	Red Collared-Dove	
41	*Streptopelia chinensis*	Spotted Dove	
42	*Psittacula alexandri*	Red-breasted Parakeet	
43	*Clamator coromandus*	Chestnut-winged Cucukoo	
44	*Cacomantis merulimus*	Plaintive Cuckoo	
45	*Eudynamys scolopacaceus*	Asian Koel	
46	*Centropus sinensis*	Greater Coucal	
47	*Centropus bengalensis*	Lesser Coucal	
48	*Glaucidium cuculoides*	Asian Barred Owlet	
49	*Cypsiurus balas*	Asian Palm-Swift	
50	*Coracias benghalensis*	Indian Roller	
51	*Halcyon smyrnensis*	White-throated Kingfisher	
52	*Alcedo atthis*	Common Kingfisher	
53	*Merops orientalis*	Little Green Bee-eater	
54	*Mecops philippinus*	Blue-tailed Bee-eater	
55	*Megalaima haemaccephala*	Coppersmith Barbet	
56	*Dendrocopos analis*	Spot-breasted Woodpecker	
57	*Oriolus chinensis*	Black-naped Oriole	
58	*Oriolus xanthornus*	Black-hooded Oriole	
59	*Artamus fuscus*	Ashy Woodswallow	
60	*Aegithina tiphia*	Common Iora	
61	*Rhipidura albicollis*	White-throated Fantail	
62	*Dicrurus macrocercus*	Black Drongo	
63	*Corvus splendens*	House Crow	
64	*Corvus japonensis*	Large-billed Crow	
65	*Dendrocitta vagabunda*	Rufous Treepie	
66	*Lanius cristatus*	Brown Shrike	
67	*Cinnyris asiaticus*	Purple Sunbird	
68	*Cinnyris jugularis*	Olive-backed Sunbird	
69	*Dicaeum cruentatum*	Scarlet-backed Flowerpecker	
70	*Ploceus philippinus*	Baya Weaver	
71	*Ploceus hypoxanthus*	Asian Golden Weaver	Near-threatened
72	*Amandava amandava*	Red Avadavat	
73	*Lonchura punctulata*	Scaly-breasted Munia	
74	*Lonchura atricapilla*	Chestnut Munia	
75	*Passer domesticus*	House Sparrow	
76	*Passer montanus*	Eurasian Tree-Sparrow	
77	*Anthus rufulus*	Paddyfield Pipit	
78	*Motacilla alba*	White Wagtail	
79	*Motacilla tschutschensis*	Eastern Yellow Wagtail	

Sr.no	Scientific name	Common name	IUCN Status
80	*Emberiza aureola*	Yellow-breasted Bunting	Critically Endangered
81	*Acridotheres fuscus*	Jungle Myna	
82	*Acridotheres tristis*	Common Myna	
83	*Acridotheres burmannicus*	Vinous-breasted Myna	
84	*Gracupica contra*	Asian Pied Starling	
85	*Sturnus malabaricus*	Chestnut-tailed Starling	
86	*Saxicola maurus*	Eastern Stonechat	
87	*Saxicola caprata*	Pied Bushchat	
88	*Ficedula albicilla*	Taiga Flycatcher	
89	*Copsychus saularis*	Oriental Magpie-Robin	
90	*Alauda gulaula*	Oriental Skylark	
91	*Pycnonotus blanfordi*	Streak-eared Bulbul	
92	*Pycnonotus jocosus*	Red-whiskered Bulbul	
93	*Pycnonotus cafer*	Red-vented Bulbul	
94	*Hirundo rustica*	Barn Swallow	
95	*Phylloscopus fuscatus*	Dusky Warbler	
96	*Chrysomma altirostre*	Jerdon's Babbler	Vulnerable
97	*Chrysomma sinense*	Yellow-eyed Babbler	
98	*Cisticola juncidis*	Zitting Cisticola	
99	*Orthotomus sutorius*	Common Tailordbird	
100	*Prinia hodgsonii*	Grey-breasted Prinia	
101	*Prinia inornata*	Plain Prinia	

Table 2 Recorded number of wetland indicator bird species in two Districts

District	Name of site	Number of order	Number of family	Number of species
Maubin	Maubin (site I)	12	33	79
	Nyaungdon (siteII)	9	18	23
Myaung Mya	Wakhema (site III)	13	41	82

Habitat utilization of wetland indicator species

During the study period, bird species used by various habitats types in seasonally. Foraging, nesting, and roosting in diverse habitats such as marsh swamp, lotus swamp, paddy fields, reed bed, open water and terrestrial tree. Nesting sites of some species used in flooded paddy field and some used in reed bed and terrestrial tree were observed. Some waterbirds species used both aquatic and terrestrial for nesting sites. One near–threatened bird species of Asian Golden Weaver used reed bed and some terrestrial tree for nest during the research period. On the other hand vulnerable species of Sarus Crane used flooded paddy fields for nest site.

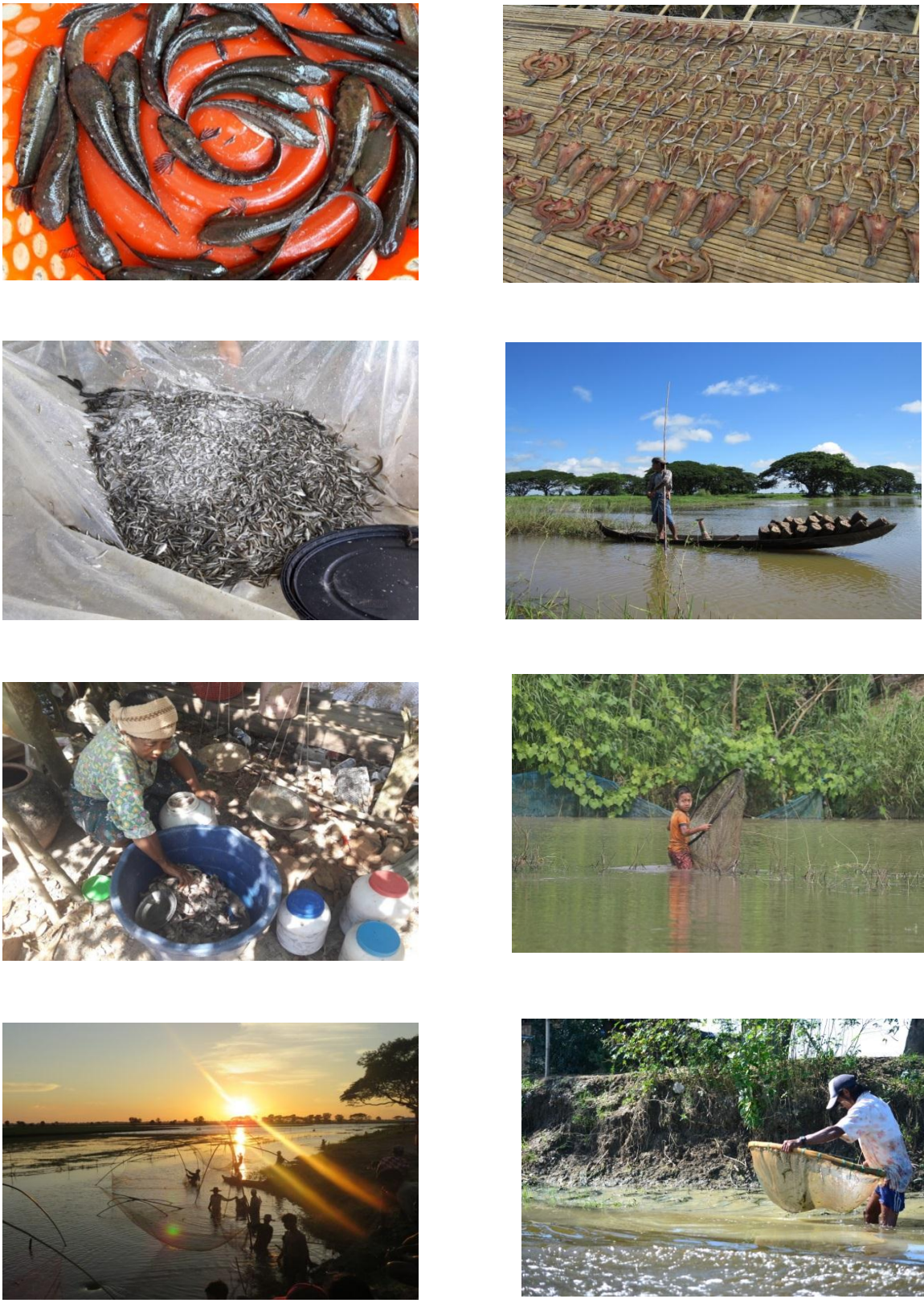

Plate I. Fisheries and aquatic resources








Plate II. Wetland's functions and values








Plate III. Ecotourism and Research

A. Yellow- breasted Bunting

B. Jerdon's Babbler

C. Sarus Cranes

D. Painted Stork

E. Purple Heron

F. Asian Golden Weaver

G. Indian Pound Heron

H. Red- collared Dove

Plate IV. Recorded wetland indicator bird species in three study sites

Discussion

According to the results, many resources can be obtained from wetlands in Wakhema and Maubin site for local people who live on these wetlands area. It may be assumed that these two study sites were invaluable supporting to local people livelihood. Some bird species foraged for food in wetland soils. Some feed on water column, some feed on the vertebrates and invertebrates that live on submerged and emergent plants. Widespread use of wetlands and their resources were common among diverse bird species. Birds have daily and seasonal dependence on wetlands for food and other life supporting systems (Stewart, 2001). According to the data of vegetation types, emergent plants and submerged plants were observed in three study sites. Some plants were useful for local people and some plants were suitable food for bird species. The plant species, *Eleocharis dulcis* (Water Chestnut) was vital food for Sarus Crane (Vulnerable) when wetlands were dry out. The plant species of *Nymphoides indica* (Water Liliy) was observed in abundant in three study sites. Almost all lily plant' parts were used for food, medicine, wrapping materials and provided small income for local people. According to the result, all of three study sites may be sufficient and suitable provided for not only bird species but also local people who depends upon the wetlands and also express the good condition of wetland habitats. It may be supposed that, wetlands provided the beneficial values to local people and bird species as well.

Conclusion

Wetlands are critical part of natural environment in Ayeyarwady region. All wetlands provided many societal benefits such as food and habitat for fish and wildlife (including threatened and endangered species), water quality improvement, flood storage, economically beneficial natural products for human use, and opportunities for recreation, education, and research. Wetlands serve as excellent study sites to learn about vegetative structure, ecological functions, natural ecological processes, biodiversity, and plant-animal interactions.Wetland birds had some unique features that enable them survive better in their environment. These adaptations make birds better equipped as a group to exploit wetland resources. Wetlands birds perform important functions in the ecosystem as main vectors maintaining biotic connection between catchments for aquatic plant and invertebrates, but also reflect the ecosystem functionality of the habitat. Birds are performed as environmental indicators.

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DIFFERENT TYPES OF FOODS FORAGED BY VARIOUS BIRD SPECIES IN PAKOKKU ENVIRONS, MAGWAY REGION

Yadanar Myo[1], Thant Zin[2], Sann Sann Htay[3]

Abstract

Different types of foods foraged by various birds were investigated in four study sites of Pakokku environs during July 2015 to June 2016. A variety of foods including animal matters and plant matters was consumed by different bird species. The bird fauna in the study area was represented with 69 carnivorous species (54.77%), 53 omnivorous species (42.06%) and four herbivorous species (3.17%) according to the foods taken. The carnivorous birds were predominant due to the abundance of food sources for these birds to forage. The environs of Pakokku provide different food sources that are important for the existence of bird species inhabited in that area.

Keywords: Foods, birds, Pakokku environs

Introduction

Birds are the ideal bio-indicators and useful models for studying a variety of environmental problems (Newton, 1995). Birds are useful ecological indicators by which evaluate successful maintenance of biotic integrity. Birds are sensitive to environment changes, respond rapidly to changes, and are abundant within various landscape classes (Glennon, 2005).

Different birds have different food habits. Birds like parrots and pigeons eat seeds and fruits. Birds like crows and ducks eat both plant and flesh of other animals. Birds like eagles and vultures eat the flesh of other animals.

Birds in their diversity constitute part of the natural environment and play functional roles such as agents of flower pollination and seed dispersal, source of food chain and agents in breaking seed dormancy (David *et al*., 2015). Seed dispersal is one of the most important ecological processes carried out by birds in tropical forests that have been modified by land-use changes. In some tropical forest up to 90% of tree species are dispersed by animals, mainly mammals and birds. Therefore, loss of seed dispersers, such as frugivorous birds, can affect plant regeneration and impact heavily of forest structure and phenotypic and genotypic characteristics of plant species (Menezes *et al*., 2016).

In addition to importance as pollinators, birds in their glide, consume hundred of insects, many of which are considered as pests. Hence birds play a critical role in reducing and maintaining populations of insects in natural systems. In a paddy field, the role of birds in pest control is enormous.

Pakokku is located in the western part of the central dry zone, on the west bank of the Ayeyawady River. Pakokku has a mostly flat topography, except for some low mountains in the western area of the township and is characterized by a hot, dry climate. Areas by the river experience floods, which away from the river drought and access to water is a chronic problem.

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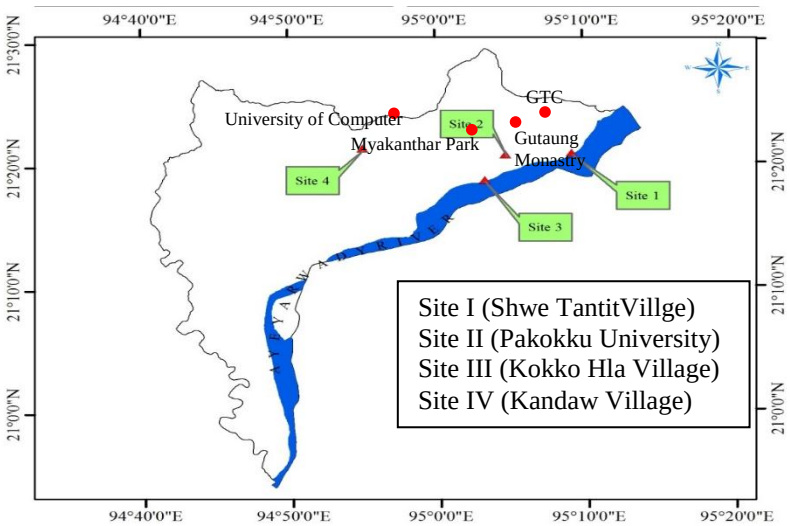
Although Pakokku is located in the central dry zone, some areas are inhabited with trees that grow sparsely in some places and moderately in some habitats. Some areas are agricultural fields and bushes. Some fruiting trees and trees with thick foliage are major food sources for frugivorous and insectivorous birds.

Myanmar is a country rich with diversified bird species. Most researchers concentrate their studies on species composition and occurrence of birds in different areas of Myanmar. The research works relating to the foods of birds are very rare compared to other areas of research. This research aims to fill the gap required in some research areas of bird ecology. This research was conducted to investigate the different kinds of foods foraged by various bird species in Pakokku environs and to determine the feeding habit of birds in Pakokku environs based on the foods taken.

Materials and Methods

Study area

Pakokku is situated about 30 km away from the northwest of Bagan and lies at the sides of Ayeyawady River in Magway Region. Pakokku lies between latitude 20° 20' to 21° 30' North and longitude 94° 40' to 95° 20' East and located in the dry zone of central Myanmar. Four study sites were located in the study area as Shwe Tantit Village environs (Site I), Pakokku University (Site II), Kokko Hla Village environs (Site III) and Kandaw Village environs (Site IV) (Fig. 1). The large woody trees, paddy fields, bushy area, medium and tall trees, cultivated area, garden, wetland with submerged plant, flood plain, Kyi village Dam and some portions of Ayeyawady River were included in Site I. The large woody trees, medium and tall trees, artificial pond, bushy area and buildings were found in Site II. The large woody trees, medium and tall trees, paddy fields, flood plain, horticultural land, Kokko Hla village Dam and nearby Ayeyawady River were occurred in Site III. The less number of large woody trees, medium and tall trees, and bushes, and artificial pond and agricultural fields were contained in Site IV.



Source Google Earth, 2013

Figure 1 A map of Pakkoku Township showing the study sites

Study period

The present study was conducted from July 2015 to June 2016.

Study design

In terms of monitoring the foods foraged by different bird species, an appropriate transect lines were drawn in each study site. Birds eating the foods were viewed by a pair of binocular while traversing the transect routes. Observations were taken from 6:30 AM to 10:30 AM. In each occasion of bird watching, bird species and the foods taken were recorded. The photos of birds together with their foods were taken immediately after viewing the birds. Binoculars, camera and note book were used during the field study. Each study site was visited twice per month.

Identification and classification of species

The identification of birds was made by referring to taxonomic descriptions given by Robson (2015; 2016). Classification of birds was followed after Birdlife International (2015).

Different types of foods foraged

Feeding of birds was investigated by viewing with binocular and recording with digital camera. Foraging incidence of birds such as on the ground, tree, in flight, water etc. were recorded. Feeding habit of birds were determined and arranged based on the kind of foods eaten such as carnivorous, omnivorous, and herbivorous.

Results

Site I

In this study site, a total of 102 bird species with different feeding habits was recorded. Among the bird species recorded, 49 species were carnivorous, 49 species were omnivorous and four species were herbivorous. (Table 1, 2, 3 and Plate 1)

In carnivorous species, 28 species were terrestrial birds and 21 species were water birds. In omnivorous group, 34 species were terrestrial species and 15 species were water birds. All herbivorous species were terrestrial species. (Table 3)

Site II

In Site II, a total of 64 bird species with different feeding habits was observed. Among them, 33 species were carnivorous, 29 species were omnivorous and two species were herbivorous. (Table 1 and Plate 1)

In carnivorous species, 31 species were terrestrial birds and two species were water birds. In omnivorous group, all 29 species recorded were terrestrial species and all herbivorous species found were terrestrial species. (Table 3)

Site III

In the case of Site III, a total of 86 bird species with different feeding habits was recorded. Among the avifaunal group in this site, 47 species were carnivorous, 37 species were omnivorous and two species were herbivorous. (Table 1 and Plate 1)

In carnivorous species, 32 species were terrestrial birds and 15 species were water birds. In omnivorous group, 28 species were terrestrial species and nine species were water birds. All herbivorous species were terrestrial species. (Table 3)

Site IV

Regarding with Site IV, a total of 58 bird species with different feeding habits was recorded. Among the bird species, 28 species were carnivorous, 28 species were omnivorous and two species were herbivorous. (Table 1 and Plate 1)

In carnivorous species, 26 species were terrestrial birds and two species were water birds. In omnivorous group, 27 species were terrestrial species and only one species was water bird. All herbivorous species were terrestrial species. (Table 3)

Pakokku environs

When consideration was made on the whole study area, a total of 126 bird species with different feeding habits was recorded. Among the bird species recorded, 69 species were carnivorous, 53 species were omnivorous and four species were herbivorous. (Table 1 and Plate 1)

Among them, one piscivorous, two avivorous and 36 insectivorous birds were recorded. These birds and the rest of the species that ate different animal matters are included in carnivorous group. Among the bird species group that ate different animal matters, nine species feeding on fish and insect, two species on fish, insect and worm, one species on fish and tadpole, three species on fish, insect and tadpole, two species on rat, fish and insect, six species on mollusk and insect, one species on insect and worm, one species on lizard and insect, three species on rat and insect and two species on mollusk, fish and insect were recorded. (Table 2 and Plate 1)

Among the omnivorous group that prefer different diets, one species feeding on mollusk, vegetation and fish, one species on mollusk, vegetation, fish and insect, five species on mollusk, vegetation and insect, one species on vegetation, grain and insect, one species on grain, mollusk, insect and worm, two species on vegetation and insect, five species on grain, vegetation and insect, four species on insect and fruit, one species on worm, vegetation and insect, 11 species on insect and nectar, six species on insect, nectar and fruit, two species on dead animal, insect, fruit, grain and disable bird, one species on dead animal, seven species on grain and insect, four species on grain, insect, nectar and fruit and one species on grain, insect and fruit were recorded. (Table 2 and Plate 1)

Among the herbivorous group feeding on fruits and grains or seeds, one species of frugivorous and three species of granivorous were recorded. (Table 2 and Plate 1)

In Pakokku environs, the bird fauna was represented with 54.77% carnivorous birds, 42.06 % omnivorous birds and 3.17 % herbivorous birds. (Fig. 2)

According to the different feeding habits, omnivorous birds eat insects, young bird, fish, molluscs, dead animal, nectar, seed and fruit. Carnivorous birds eat fish, worm, rat, lizard, young bird, tadpole, molluscs and insect (cricket, bee, ant, locust, butterflies, moth, flies, termite and beetle). Herbivorous birds feed on nectar, leaves, fruit, seed, flower, sunflower, wildflower, grass, weed seed, rice, barley, corn and millet. (Table 1)

Table 1 Feeding habit of bird species in Pakokku environs during July 2015 to June 2016

Sr. No.	Scientific Name	Foraging incidence	Type of foods	Feeding habits			Sites			
				Carni.	Omni.	Herbi.	I	II	III	IV
1.	*Dendrocygna javanica**	Sh	Moll./Veg./Fish		+		+		+	
2.	*Tadorna ferruginea**	Sh/W	Moll./Veg./Fish/Insc.		+		+		+	
3.	*Anas zonorhyncha**	Sh	Moll./Veg./Insc.		+		+		+	
4.	*Tachybaptus ruficollis**	Sh/W	Fish/Insc.	+			+			+
5.	*Anastomus oscitans**	Sh/G	Fish/Insc.	+			+		+	
6.	*Plegadis falcinellus**	Sh/G	Fish/Insc.	+			+		+	
7.	*Ixobrychus sinensis**	Sh	Fish/Insc.	+			+			
8.	*Ixobrychus cinnamomeus**	Sh	Fish/Insc.	+			+			
9.	*Ardeola grayii**	Sh/G	Fish/Insc.	+			+		+	
10.	*Ardeola bacchus**	Sh/G	Fish/Insc.	+			+	+	+	
11.	*Bubulcus coromandus**	Sh/G	Fish/Insc./Worm	+			+	+	+	+
12.	*Ardea cinerea**	Sh/G	Fish/Tad.	+			+		+	
13.	*Ardea purpurea**	Sh/G	Moll./Fish/Insc.	+			+			
14.	*Ardea alba**	Sh/G	Fish/Insc./Tad.							
15.	*Mesophoyx intermedia**	Sh/G	Fish/Insc./Worm	+			+		+	
16.	*Egretta garzetta**	Sh/G	Fish/Insc.	+			+		+	
17.	*Phalacrocorax niger**	Sh	Fish/Insc.	+			+		+	
18.	*Phalacrocorax carbo**	Sh	Fish (Pisci.)	+			+		+	
19.	*Falco tinnunculus*		Rat/ Insc.	+				+		
20.	*Elanus caeruleus*	F/T	Rat/Fish/Insc.	+			+			
21.	*Butastur teesa*	T	Rat/Fish/Insc.	+			+			
22.	*Buteo buteo*	F/T	Dis.birds (Avivo.)	+					+	
23.	*Aquila nipalensis*	F/T	Dead animal		+		+			
24.	*Gallirallus striatus**	Sh/G	Veg./Gra./Insc.		+		+			
25.	*Amaurornis phoenicurus**	Sh/G	Gra./Moll./Insc./Worm		+		+		+	
26.	*Porzana pusilla**	Sh/G	Insc.	+			+			
27.	*Porzana fusca**	Sh/G	Moll./Veg./Insc.		+		+			
28.	*Gallicrex cinerea**	Sh/G	Moll./Veg./Insc.		+		+			
29.	*Porphyrio poliocephalus**	Sh/G	Moll./Veg./Insc.		+		+		+	
30.	*Gallinula chloropus**	Sh/W/G	Moll./Veg./Insc.		+		+		+	
31.	*Fulica atra**	Sh/W	Veg./Insc.		+		+		+	
32.	*Himantopus himantopus**	Sh/G	Gra./Veg./Insc.		+		+		+	+
33.	*Vanellus cinereus**	Sh/G	Moll./Insc.	+			+		+	
34.	*Vanellus indicus**	Sh/G	Veg./Insc.		+		+		+	
35.	*Charadrius hiaticula**	Sh/G	Insc./Worm	+			+		+	
36.	*Chardrius dubius**	Sh/G	Moll./Insc.	+			+		+	
37.	*Rostratula benghalensis**	Sh/G	Gra./Veg./Insc.		+		+			
38.	*Gallinago gallinago**	Sh/G	Worm/Veg./Insc.		+		+			
39.	*Tringa ochropus**	Sh/G	Moll./Fish/Insc.	+			+		+	
40.	*Tringa stagnatilis**	Sh/G	Moll./Insc.	+			+			
41.	*Columba livia*	G	Gra./Veg./Insc.		+		+	+	+	+
42.	*Streptopelia orientalis*	G	Gra. (Grani.)			+	+			
43.	*Streptopelia decaocto*	G	Gra. (Grani.)			+	+	+		+
44.	*Streptopelia chinensis*	G	Gra. (Grani.)			+	+	+	+	+
45.	*Treron phoenicopterus*	T	Fruit (Frugi.)			+	+		+	
46.	*Clamator jacobinus*	T	Insc.	+				+		+
47.	*Cuculus canorus*	F	Insc.	+			+	+	+	+
48.	*Cacomantis merulinus*	T	Insc.	+			+	+	+	+
49.	*Cacomantis sepulcralis*	T	Insc.	+			+	+		
50.	*Centropus sinensis*	T	Insc./Fruit		+		+			
51.	*Centropus bengalensis*	T	Insc.	+			+	+	+	+
52.	*Tyto alba*	T	Rat (Avivo.)	+				+		+
53.	*Athene brama*	F/G	Rat/Insc.	+				+	+	+
54.	*Coracias benghalensis*	F/G	Liz./Insc.	+			+	+	+	
55.	*Halcyon smyrnensis*	F/Sh	Fish/Tad./Insc.	+			+	+	+	+
56.	*Alcedo atthis*	F/Sh	Fish/Tad./Insc.	+			+		+	

Sr. No.	Scientific Name	Foraging incidence	Type of foods	Feeding habits			Sites			
				Carni.	Omni.	Herbi.	I	II	III	IV
57.	*Merops orientalis*	F	Insc.	+			+	+	+	+
58.	*Merops philippinus*	F	Insc.	+			+	+	+	+
59.	*Upupa epops*	T/G	Insc.	+			+	+	+	+
60.	*Megalaima haemacephala*	T	Insc./Fruit		+		+	+	+	+
61.	*Dendrocopos analis*	T	Insc.	+				+		
62.	*Oriolus chinensis*	T	Insc./Nect./Fruit		+		+	+		
63	*Artamus fuscus*	T	Insc.	+			+	+	+	+
64.	*Argithina tiphia*	T	Insc.	+			+	+	+	+
65.	*Dicrurus macrocercus*	T/F	Insc./Nect.		+		+	+	+	+
66.	*Dicrurus leucophaeus*	T/F	Insc./Nect.		+		+	+	+	+
67.	*Dicrurus aeneus*	T/F	Insc./Nect.		+		+		+	+
68.	*Dicrurus remifer*	T/F	Insc./Nect.		+			+		+
69.	*Corvus splendens*	G	De an./ Insc./ Fruit/Gra./Dis.birds		+		+	+	+	+
70.	*Corvus macrorhynchos*	G	De an./ Insc/Fruit/Gra/ Dis.birds		+		+	+	+	+
71.	*Lanius cristatus*	T/F	Insc.	+			+	+	+	+
72.	*Lanius collurioides*	T/F	Insc./Fruit		+		+	+	+	+
73.	*Lanius schach*	T/F	Rat/Insc.	+				+	+	+
74.	*Cinnyris asiaticus*	T	Insc./Nect.		+		+	+	+	+
75.	*Cinnyris jugularis*	T	Insc./Nect.		+			+		
76.	*Ploceus manyar*	T	Gra./Insc.		+		+		+	
77.	*Ploceus philippinus*	T	Gra./Insc.		+		+		+	+
78.	*Amandava amandava*	T	Gra./Insc.		+		+			
79.	*Lonchura punctulata*	T/G	Gra./Insc.		+		+	+	+	+
80.	*Passer domesticus*	T/G	Gra./Insc./Nect./Fruit		+		+	+	+	+
81.	*Passer flaveolus*	T/G	Gra./Insc./Nect./Fruit		+		+	+	+	+
82.	*Passer montanus*	T/G	Gra./Insc./Nect./Fruit		+		+	+	+	+
83.	*Anthus hodgsoni*	G	Insc./Gra.		+		+	+	+	
84.	*Anthus rufulus*	G	Insc./Gra.		+		+	+	+	+
85.	*Anthus similis*	G	Insc./Gra.		+		+			
86.	*Motacilla alba*	G	Insc.	+			+	+	+	+
87.	*Motacilla cinerea*	G	Moll./Insc.	+			+		+	+
88.	*Motacilla flava*	G	Moll./Insc.	+			+			
89.	*Motacilla citreola*	G	Moll./Insc.	+			+		+	
90	*Acridotheres grandis*	T	Insc./Nect.		+			+		
91.	*Acridotheres fuscus*	T/G	Gra./Insc./Nect./Fruit		+		+	+	+	+
92.	*Acridotheres tristis*	T/G	Insc./Nect./Fruit		+		+	+	+	+
93.	*Acridotheres burmannicus*	T/G	Insc./Nect./Fruit		+		+	+	+	+
94.	*Gracupica nigricollis*	T	Gra./Insc./Fruit		+		+			
95.	*Sturnus malabaricus*	T	Insc./Nect./Fruit		+		+	+	+	
96.	*Sturnus vulgaris*	T	Insc./Fruit		+				+	
97.	*Luscinia calliope*	T	Insc.	+					+	
98.	*Luscinia svecica*	T	Insc.	+			+			
99.	*Phoenicurus frontalis*	T/G	Insc.	+			+	+	+	+
100.	*Saxicola ferreus*	T/G	Insc.	+			+	+	+	+
101.	*Saxicola maurus*	T	Insc.	+			+		+	
102.	*Saxicola caprata*	T/G	Insc.	+			+	+	+	+
103.	*Eumyias thalassinus*	F	Insc.	+					+	
104.	*Ficedula albicilla*	T/G	Insc.	+				+		+
105.	*Copsychus saularis*	F/G	Insc.	+				+	+	+
106.	*Mirafra assamica*	G	Gra./Insc./veg.		+		+	+	+	+
107.	*Mirafra microptera*	G	Gra./Insc./veg.		+		+	+	+	+
108.	*Pycnonotus blanfordi*	T/G	Insc./Nect./Fruit		+		+	+	+	+
109.	*Pycnonotus brunneus*	G	Insc.	+				+		
110.	*Pycnonotus cafer*	T	Insc./Nect./Fruit		+			+	+	+
111.	*Hirundo rustica*	F	Insc.	+				+		+

Sr. No.	Scientific Name	Foraging incidence	Type of foods	Feeding habits			Sites			
				Carni.	Omni.	Herbi.	I	II	III	IV
112.	*Cecropis daurica*	F	Insc.	+			+	+		+
113.	*Chrysomma sinense*	T	Insc./Nect.		+		+	+		+
114.	*Turdoides gularis*	F/G	Insc.	+				+	+	+
115.	*Phylloscopus borealis*	T	Insc.	+				+		
116.	*Phylloscopus armandii*	T	Insc.	+			+	+	+	+
117.	*Locustella certhiolar*	F	Insc.	+			+		+	
118.	*Megalurus palustris*	T	Insc.	+			+			
119.	*Orthotomus ruficeps*	T	Insc.	+				+		+
120.	*Orthotomus sutorius*	T	Insc./Nect.		+		+	+	+	+
121.	*Cisticolar juncidis*	F	Insc.	+			+		+	
122.	*Prinia rufescens*	T	Insc.	+					+	
123.	*Prinia hodgsonii*	T	Insc./Nect.		+		+	+		+
124.	*Prinia flaviventris*	T	Insc.	+					+	
125.	*Prinia inornata*	T	Insc./Nect.		+		+	+	+	+
126.	*Prinia polychroa*	T	Insc.	+					+	
Total number of species				69	53	4	102	64	86	58

+ Present * Water bird

Carni. = Carnivorous Omni.= Omnivorous Herbi.= Herbivorous Insc. = Insect F = in flight

T = Tree W = Water G = Ground Nect. = Nectar Sh = Shallow water

Pisci = Piscivorous Liz. = Lizard Frugi = Frugivorous Dis. birds = Disable birds Veg. = Vegetable

Avivo = Avivorous Moll. = Molluscs Grani = Granivorous De. an. = Dead animal Tad. = Tadpole

Table 2 Number of bird species in different feeding habits of birds in Pakokku environs

Sr. No.	Feeding habit	Food types	Number of bird species
1.	Carnivorous	Fish, insect	9
		Fish, insect, worm	2
		Fish, tadpole	1
		Fish, insect, tadpole	3
		Rat, fish, insect	2
		Disable bird (Avivorous)	1
		Insect (Insectivorous)	36
		Mollusc, insect	6
		Insect, worm	1
		Lizard, insect	1
		Rat, insect	3
		Rat (Avivorous)	1
		Mollusc, fish, insect	2
		Fish (Piscivorous)	1
2.	Omnivorous	Mollusc, vegetation, fish	1
		Mollusc, vegetation, fish, insect	1
		Mollusc, vegetation, insect	5
		vegetation, grain, insect	1
		Grain, mollusc, insect, worm	1
		Vegetation, insect	2
		Grain, vegetation, insect	5
		Insect, fruit	4

Sr. No.	Feeding habit	Food types	Number of bird species
		Worm, vegetation, insect	1
		Insect, nectar	11
		Insect, nectar, fruit	6
		Dead animal, insect, fruit, grain, disable bird	2
		Dead animal	1
		Grain, insect	7
		Grain, insect, nectar, fruit	4
		Grain, insect, fruit	1
3.	Herbivorous	Fruit (Frugivorous)	1
		Grain (Granivorous)	3

Table 3 Different feeding habit of bird species in each study site

Species	Study sites				
	I	**II**	**III**	**IV**	**All**
Carnivorous	49	33	47	28	69
- terrestrial bird species	28	31	32	26	48
- water bird species	21	2	15	2	21
Omnivorous	49	29	37	28	53
- terrestrial bird species	34	29	28	27	38
- water bird species	15	-	9	1	15
Herbivorous	4	2	2	2	4
- terrestrial bird species	4	2	2	2	4
- water bird species	-	-	-	-	-

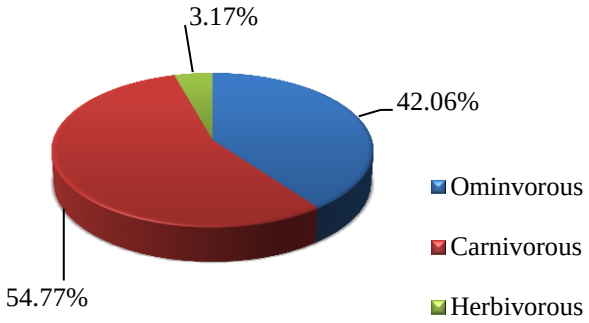
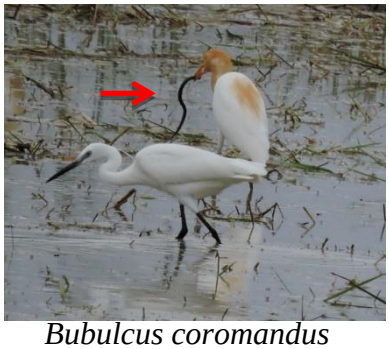
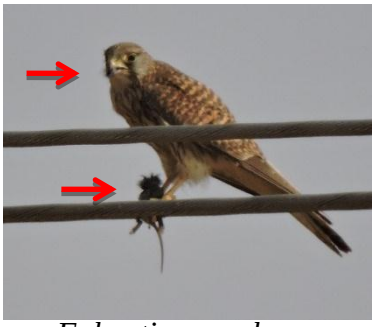


Figure 2 Different feeding habits of birds in Pakokku environs during July 2015 to June 2016



Bubulcus coromandus
eating worm

Egretta garzetta
foraging cricket



Falco tinnunculus
having rat

Phoenicurus frontalis
taking insect

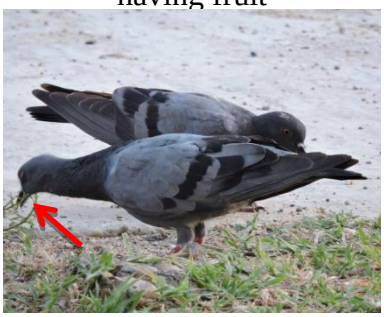
Upupa epops
eating insect

Acridotheres burmannicus
taking nectar

Acridotheres fuscus
having fruit

Pycnonotus blanfordi
eating fruit

Cinnyris asaticus
taking nectar



Columba livia
having leaves

Treron phoenicopterus
eating fruit

Streptopelia chinensis
taking grain

Plate 1 Different types of food foraged by various birds recorded in the study area

Discussion

Throughout the study period from July 2015 to June 2016, a total of 126 bird species having different feeding habits were identified and recorded from four study sites in the environs

of Pakokku. Among them, 53 species were omnivorous, 69 species were carnivorous and four species were herbivorous. Feeding habits were determined based on the kinds of foods taken.

Different bird species foraged on various foods available in each study site. The number of foraging species varied among the study sites relating to habitats. In Site I of Shwe Tantit village environs and Site III of Kokko Hla village environs where a variety of microhabitats with land and water sources available are found more number of terrestrial birds and water birds than other sites. Carnivorous birds are species which feed on animal matters. Sixty-nine species of carnivores were found in the study area. Among them, one piscivorous, two avivorous and 36 insectivorous species were included. These birds and the rest of species that ate different animal matters are included in carnivorous group. Carnivorous birds ate fish, worm, rat, lizard, young bird, tadpole, mollusks and insect (cricket, bee, ant, locust, butterflies, moth, flies, termite and beetle) in the study area. Insectivorous birds are included in the category of carnivores.

Erwin (1982) stated that the correlation of insectivores was positive relationship under closed canopy, where the vegetation was dense; with higher tree density and higher basal area. This could be due to abundant insects as a result of moist conditions and dense foliage. In the present study area, due to occurrence of many tree species, 36 species of insectivorous birds were recorded and they are dominant in the foraging groups.

Chettri *et al*. (2005) stated that the omnivores were significantly related to open canopy habitat as well as habitat with better stratification where resource availability (insects, seeds, fruits and small mammals) was high. In the study area, 53 bird species were recorded as omnivorous. Omnivorous birds ate insects, young bird, fish, mollusks, dead animal, nectar, seed and fruit in the study area.

Moreover, due to seasonal availability of fruiting trees and vegetative matters in the study area, four species of herbivorous birds were observed. Among them, one species was frugivorous and three species were granivorous. Herbivorous birds fed on nectar, leaves, fruit, seed, flower, sunflower, wildflower, grass, weed seed, rice, barley, corn and millet in the study area.

According to the bird diet types, there are 12 different types of dietary habits in the world bird fauna. Among them, seven types of feeding habits are recognized in different foraging bird species in pakokku environs during study period.

Fruiting trees provided and attracted different insect species and fruit eating birds. Similarly, flowering trees also produced diverse flowers that attracted nectarivore bird species to nip the nectar and prey on insects. In addition, the occurrence and richness of food resources such as nectar, leaves, fruits, seeds, insects (locust, moths, butterflies, crickets, flies, ants, termites, and beetles), reptiles (lizards and snake), mammals (rats), amphibians and birds are also a key factor that affects diversity and richness of bird species.

The species richness of birds is mainly due to the occurrence of many insectivorous and omnivorous birds, with a few species of herbivorous birds. Plant species richness is the most important determinants and however positive effect for many insectivorous. In the present study, insectivorous birds dominated in the bird community. Pakokku environs with a variety of microhabitats support the diverse bird species.

Conclusion

The avifauna of Pakokku environs was represented with different feeding habits mostly of carnivorous and omnivorous because of availability of food sources in the area. Pakokku environs is crucial alternative habitat for diverse population of water and terrestrial birds and therefore promote high species diversity and density due to their wide diversity of habitat characteristics and increased food availability.

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FORAGING PATTERN OF SOME BIRDS IN WAIMAW TOWNSHIP AND ITS ENVIRONS, KACHIN STATE

Bauk Ra,[1] Hpaw Bwe[2]

Abstract

A total of 52 species belonging to 17 families were selected for observation of foraging pattern during the May 2017 to April 2018. They are categorizied into 18 groups. The present study highlight the foraging pattern of there birds species based on the food eaten and habitat use. In present study, seven substrates (Above canopy (ABC), the upper understory (UUN), unshaded canopy (USC), emergent leaves (EME), lower understory (LUN), Shaded canopy (SHC) and ground (GRO)) were categorized in Waimaw environs. Among 15 groups preferably utilized upper understory (UUN) level for foraging and 13 groups, the unshaded canopy (USC) level were mostly utilized for foraging opportunities on there environ because vegetation offered suitable food in this area.

Keywords: Foraging type, habitat use, Waimaw environs.

Introduction

Kachin State is Myanmar's northernmost province. The highest mountain of Myanmar, Hkakaborazi lies in this state. There are also other mountains in this area. These mountain are descendents of the Himalaya mountain ranges. Tropical deciduous forests and evergreen forests grow in the Kachin State, lies on the north of Tropic of Cancer, therefore the climate is warm, temperate and wet. Capital of Kachin State is Myitkyina. Waimaw Township is about 3.22 km far from Myitkyina. It is located near the bank of Ayeyarwaddy River. This area possesses diversity of habitat. Birds are used various kind of habitats. Depending upon the habitats different kinds of birds are evolved.

Foraging is searching for wild food resources. It affects an animal's fitness because it plays an important role in an animal's ability to survive and reproduce. Foraging theory is a branch of behavioral ecology that studied the foraging behavior of animals in response to the environment where the animal lives (Danchin, *et al.*, 2008).

Waimaw Township is selected as a study area. It lies 908.9m above sea level. It lies between latitude 25°22' N and 25°44′ N and 25°44′N and between longitude 97°12′ E and 97°24' E. It has an area of 1883.17 km^2. It is 35. 4 Km long from East to West, and 94.95 km from Sout to North. The area included consisting paddy fields, plantations, cultivated land, grass land and abundant leafy vegetation in some part of the township forms good habitats for various kinds of birds. The aim and objectives of this research are to investigate the foraging pattern of some birds species in Waimaw Township

Materials and Methods

The field work was conducted starting from May 2017 to April 2018 in different area of Waimar Township in Northern Kachin State. The method used in this research is point count method. The birds were observed from 6:30 am to 9:30 am in the morning and from 3:00 pm to 6:00 pm in the evening. However, special emphasis was given to record the foraging pattern.

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When an individual or group was encountered a standardised observation was made that described the activity and precise position of the birds and instantly recorded on photographs. Foraging observations were classed according to O'Donnell and Dilks (1994).

Foraging activity

(a) glean searching for and taking food from the surface of a substrate when the bird is not on the wing;

(b) hawk searching for and taking food when both prey and bird are in flight;

(c) hover searching for and taking food when the prey is on the substrate and the bird is in flight;

(d) probe penetrating into the substrate while searching for prey, most commonly in soil, little or rotting wood;

(e) rip ripping the substrate and exposing another surface,

(f) scan use of a vantage point to look for prey where the bird stops, looks and flies to another perch if no prey are sighted (O'Donnell and Dilks, 1994).

Level within the study sites (Stratum)

A measure of the foraging level the terrestrials bird was using within sampling sites: ground (GRO), lower understorey (LUN), upper understorey (UUN), shaded (within) canopy (SHC), unshaded (on top of) conopy (USC), in emergent leaves (EME), and above canopy (ABC) in flight. (O' Donnell and Dilks, 1994).

Results

Foraging pattern of studied species

52 species belonging to 17 families were selected for observation of food and foraging pattern. They are categorized into 18 groups.

Table 1 Groups of bird with representative species in the study area

Group	(Family)		Representative Species	Common Name
I	Pigeon and Dove (Columbidae)	1	*Columba livia*	Rock Pigeon
		2	*Streptopelia orientalis*	Oriental Turtte-Dove
		3	*S. chinensis*	Spotted Dove
		4	*Chalcophaps indica*	Emerald Dove
II	Kingfisher (Alcedinidae)	1	*Halcyon smyrnensis*	White-throated Kingfisher
		2	*Alcedo atthis*	Common Kingfisher
		3	*Ceryle rudis*	Pied Kingfisher
III	Roller (Coraciidae)	1	*Coracias benghalensis*	Indian Roller
IV	Bee-eater (Meropidae)	1	*Merops orientalis*	Little Green Bee-eater
		2	*M. Philippinus*	Blue-Tailed Bee-eater
		3	*M. leschenaulti*	Chestnut-headed Bee-eater
V	Barbet (Megalaimidae)	1	*Megalaima lineata*	Lineated Barbet
		2	*M. asiatica*	Blue-throated Barbet

Group (Family)		Representative Species		Common Name
		3	*M. haemacephala*	Coppersmith Barbet
VI	Drongo	1	*Dicrurus macrocercus*	Black Drongo
	(Dicruridae)	2	*D. leucophaeus*	Ashy Drongo
		3	*D. aeneus*	Bronzed Drongo
VII	Treepie	1	*Pica pica*	Black-Billed Magpie
	(Corvidae)	2	*Dendrocitta vagabunda*	Rufous Treepie
		3	*D. formosae*	Grey Treepie
		4	*D. frontalis*	Collared Treepie
VIII	Shrike	1	*Lanius oristatus*	Brown shrike
	(Laniidae)	2	*L. schach*	Long-tailed shrike
		3	*L. tephronotus*	Grey-backed shrike
IX	Sparrow	1	*Passer domesticus*	House Sparrow
	(Passeridae)	2	*P. montanus*	Eurasian Tree Sparrow
X	Pipit & Wagtail	1	*Anthus roseatus*	Rosy Pipit
	(Motacillidae)	2	*A. rufulus*	Paddy Field Pipit
		3	*Motacilia alba*	White Wagtail
		4	*M. citreola*	Citrine Wagtail
XI	Myna & Starling	1	*Acridotheres fuscus*	Jungle Myna
	(Sturnidae)	2	*A. albocinctus*	Collared Myna
		3	*A. tristis*	Common Myna
		4	*A. burmannicus*	Vinous-Breasted Myna
		5	*Gracupica nigricollis*	Black-collared Starling
		6	*G. contra*	Asian Pied Starling
XII	Thrush	1	*Monticola rufiventris*	Chestnut-Bellied Rock Thrush
	(Muscicapidae)	2	*Myophonus caeruleus*	Blue Whistling Thrush
XIII	Chat & Forktail	1	*Saxicola Ferreus*	Grey Bush chat
	(Muscicapidae)	2	*S. maurus*	Eastern stonechat
		3	*S. caprata*	Pied Bushchat
		4	*Enicurus scouleri*	Little Forktail
XIV	Tit(Paridae)	1	*Parus monticolus*	Green-backed Tit
XV	Bulbul	1	*Pycnonotus jocosus*	Red-whiskered Bulbul
	(Pycnonotidae)	2	*P. cafer*	Red-Vented Bulbul
		3	*Hypsipetes leucocephalus*	Himalayan Black Bulbul
		4	*Pycnonotus finlaysoni*	Stripe-throated Bulbul
XVI	Swallow	1	*Hirundo rustica*	Barn Swallow
	(Hirundinidae)	2	*H. smithii*	Wire-Tailed Swallow
		3	*Cecropis striolata*	Striated swallow
XVII	Tailorbird	1	*Orthotomus sutorius*	Common Tailorbird
	(Cettiidae)			
XVIII	Prinia	1	*Prinia crinigera*	Striated Prinia
	(Cisticolidae)			

Pigeons and Dove (**Columbidae**)

Four species were recorded under this family, Rock Pigeon *Columba livia*, Oriental Turtle-Dove *Streptopelia orientalis*, Spotted Dove *Streptopelia chinensis* and Emerald Dove *Chalcophaps indica*. Pigeons fed almost entirely by gleaning in the unshaded canopy (20.8%), emergent leaves (20.1%), ground (18.2%), shaded canopy (15.6%), upper urderstorey (12.8%), and lower understorey (12.5%) (Table 2, Fig 1).

Kingfisher (**Alcedinidae**)

Under this family, three species were recorded White-throated kingfisher *Halcyon smyrnensis*, Common kingfisher *Alcedo atthis* and Pied kingfisher *Ceryle rudis*. Kingfisher fed almost entirely by hovering in the above canopy (39.3%) emergent leaves (25.7%), upper understorey (20.3%) and lower understorey (14.7%) (Table 2, Fig 1).

Roller (**Coraciidae**)

Only one species in this family recorded Indian Roller *Coracias benghalensis*. Roller fed almost entirely by hovering in the emergent leaves (48.9%), ground (33.3%) and upper understorey (17.8%) (Table 2, Fig 1).

Bee-eater (**Meropidae**)

The representative of this family, Little Green Bee-eater *Merops orientalis*, Blue-Tailed Bee-eater *Meropsphilippinus* and Chestnut-headed Bee-eater *Merops leschenaulti* were hawking in the above canopy (44.3%), emergent leaves (35.2%) and unshaded canopy (20.5%) (Table 2, Fig 1).

Barbet (**Megalaimidae**)

Under this family, three species were record Lineated Barbet *Megalaima lineata*, Blue-throated Barbet *M. asiatica* and Coppersmith Barbet *M. haemacephala*. Barbet fed almost entirely by ripping the unshaded canopy (56.2%), Shaded canopy (33.3%), upper understorey (10.5%) (Table 2, Fig 1).

Drongo (**Dicruridae**)

Three species were recorded under this family, Balck Drongo *Dicrurus macrocercus*, Ashy Drongo *D. leucophaeus* and Bronzed Drongo *D. aeneus*. Drongo fed almost entirely by gleaning in the emergent leaves (34.9%), unshaded canopy (25.6%), above canopy (20.1%), ground (10.1%) and upper understorey (9.3%) (Table 2, Fig 1).

Treepie (**Corvidae**)

Under this family, five species were recorded Black-billed Magpie *Pica pica*, Rufous Treepie *Dendrocitta vagabunda*, Grey Treepie *D. formosae* and collared Treepie *D. frontalis*. Treepie fed almost entirely by scanning in the above canopy (42.7%), emergent leaves (31.8%), shaded canopy (13.9%) and lower understorey (11.6%) (Table 2, Fig 1).

Shrike (**Laniida**e)

Shrike were represented in the study area by three species, Brown Shrike *Lanius cristatus*, long-tailed shrike *L. schach* and Grey-backed shrike *L. tephronotus*. These shrike used by scanning in the shaded canopy (35.1%), lower understorey (33.3%) and upper understorey (31.6%) (Table 2, Fig 1).

Sparrow (**Passeridae**)

Two sparrow species, House sparrow *Passer domesticus* and Eurasian Tree sparrow *P. montanus* were recorded in the study area. Both sparrows fed mainly by gleaning. Sparrows were observed throughout emergent leaves levels to the ground. (Table 2, Fig 1).

Pipit and Wagtial (**Motacillidae**)

This group comprised four species, *Pipit (Anthus roseatus and Anthus rufulus*) and *Wagtail* (*Motacillia alb*a and *Motacilia citreola*) were recorded. Both bird species fed mainly by probing but there two species search for food in different ways: Pipit fed in the paddy field and Wagtail in aquatic shallow. A wide range of ground (46.3%), upper understorey (33.4%) and lower understorey (20.3%) both species were used (Table 2, Fig 1).

Myna and Starling (**Sturnidae**)

Six species of introduced Myna & Starling were found in study area: Jungle Myna *Acridotheres fuscus*, Collared Myna *A. albocinctus*, Common Myna *A. tristis*, vinous-Breasted Myna *A. burmannicus*, Black-collare Starling *Gracupica nigricollis* and Asian Pied Starling *G. contra*. Feeding observations of the Myna and Starling were probing. When they fed in the ground and all foraging level in the study area (Table 2, Fig 1).

Thrush (**Muscicapidae**)

Thrush were represented in the study area by two species recorded Chestnut-Bellied Rock-Thrush *Monticola rufiventris* and Blue Whistling-Thrush *Myophonus caeruleus*. Thrush fed mainly by gleaning in the shaded canopy (26.9%), unshaded canopy (22.1%), upper understorey (20.35%), ground (16.35%) and above canopy (14.3%) (Table 2, Fig 1).

Chat and Forktail (**Muscicapidae**)

This group comprised four species, Grey Bushchat *Saxicola ferreus*, Eastern Stonechat *S. maurus*, Pied Bushchat *S. caprata* and Little Forktail *Enicurus scouleri*. Both species fed mainly by gleaning in the lower understorey (39.6%), upper understorey (33.6%), shaded canopy (20.7%) and unshaded canopy (6.1%) (Table 2, Fig 1).

Tit (**Paridae**)

One species were found in study area: Green-backed Tit *Parus monticolus*. Tit fed almost entirely by scanning in the shaded canopy (32.1%), lower understorey (25.3%), unshaded canopy (22.7%) and upper understorey (19.9%) (Table 2, Fig 1).

Bulbul (**Pycnonotidae**)

This group comprised four species, that Red-whiskered Bulbul *Pycnonotus jocosus*, Red-vented Bulbul *P. cafer*, Himalayan Black Bulbul *Hypsipetes leucocephalus* and Stripe-Throated Bulbul *Pycnonotus finlaysoni*. Bulbul foraging was by gleaning in the lower understorey (28.7%), unshaded canopy (24.5%), emergent leaves (20.2%), shaded canopy (16.4%), upper understorey (10.2%) (Table 2, Fig 1).

Swallow (**Hirundinidae**)

Three species were recorded under this family. Barn Swallow *Hirundo rustica*, Wire-Tailed swallow *H. smithii* and Striated swallow *Cecropis striolata*. The native insectivorous species mainly fed by hovering in above canopy (48.3%), emergent leaves (32.9%) and unshaded canopy (18.8%) (Table 2, Fig 1).

Tailorbird (**Cettiidae**)

Common Tailorbird *Orthotomus sutorius* was recorded under this family. Tailorbird fed almost entirely by hovering in the unshaded canopy (28.4%), upper understorey (21.4%), shaded

canopy (21.2%), emergent leaves (18.3%) and lower understorey (10.7%) (Table 2, Fig 1).

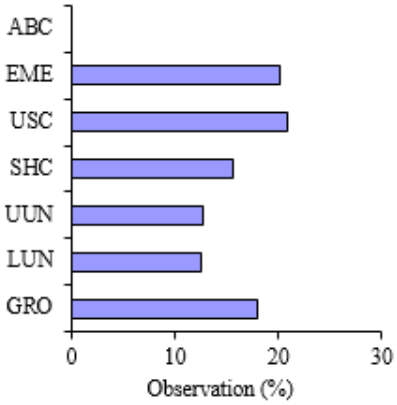
Prinia (**Cisticolidae**)

Striated Prinia *Prinia crinigera* was recorded under this family. Prinia fed mainly by hovering at upper understorey (32.1%), lower understorey (30.4%), emergent leaves (21.1%) and unshaded canopy (16.4%) (Table 2, Fig 1).

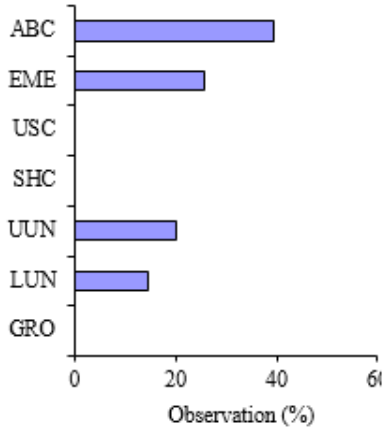
Table 2 Percentage of foraging layers utilized by terrestrial birds

Sr. No	Group Name	No. of observation	ABC	EME	USC	SHC	UUN	LUN	GRO
1.	Pigeon and Dove	385	-	20.1	20.8	15.6	12.8	12.5	18.2
2.	Kingfisher	20	39.3	25.7	-	-	20.3	14.7	-
3.	Roller	14	-	48.9	-	-	17.8	-	33.3
4.	Bee-eater	31	44.3	35.2	20.5	-	-	-	-
5.	Barbet	10	-	-	56.2	33.3	10.5	-	-
6.	Drongo	22	20.1	34.9	25.6	-	9.3	-	10.1
7.	Treepie	9	42.7	31.8	-	13.9	-	11.6	-
8.	Shrike	88	-	-	-	35.1	31.6	33.3	-
9.	Sparrow	278	-	21.5	18.2	17.2	15.2	14.7	13.2
10.	Pipit and Wagtail	17	-	-	-	-	33.4	20.3	46.3
11.	Myna and Starling	243	15.4	10.6	13.5	14.3	11.7	9.2	25.3
12.	Thrush	13	14.3	-	22.1	26.9	20.34	-	16.36
13.	chat and Forktail	160	-	-	6.1	20.7	33.6	39.6	-
14.	tit	12	-	-	22.7	32.1	19.9	25.3	-
15.	bulbul	63	-	20.2	24.5	16.4	10.2	28.7	-
16.	Swallow	98	48.3	32.9	18.8	-	-	-	-
17.	Tailorbird	10	-	18.3	28.4	21.2	21.4	10.7	-
18.	Prinia	12	-	21.1	16.4	-	32.1	30.4	-
	Total individual	**1485**							
	Total group		**7**	**12**	**13**	**11**	**15**	**12**	**7**

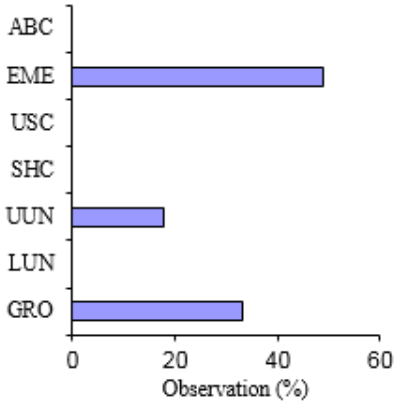
Foraging layer : ABC = above canopy, EME = emergent leaves,
USC = unshaded canopy, SHC = Shaded canopy,
UUN = upper understorey, LUN = lower understorey,
GRO = ground, N = number of birds
(-) = zero observations



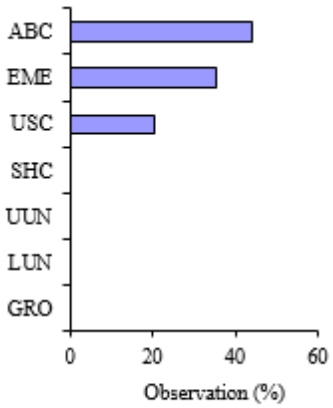
(A) Pigeon and Dove



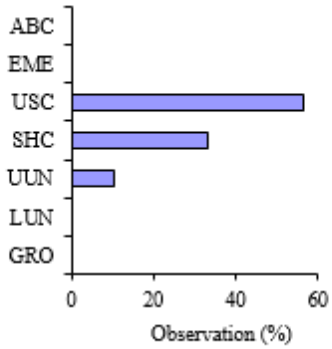
(B) Kingfisher



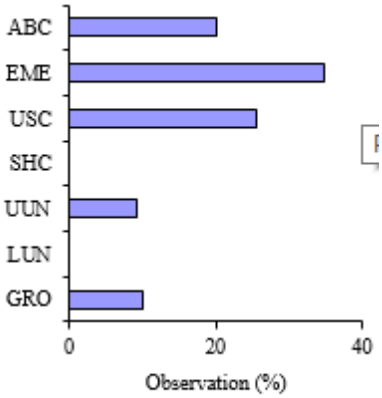
(C) Roller



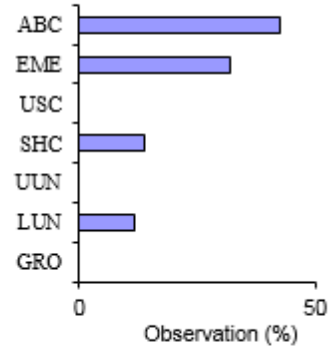
(D) Bee-eater



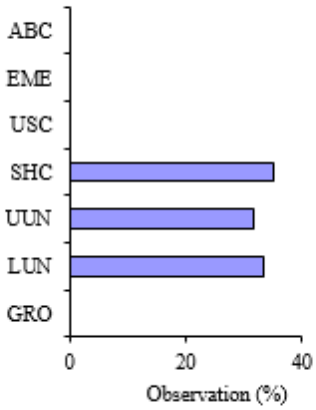
(E) Barbet



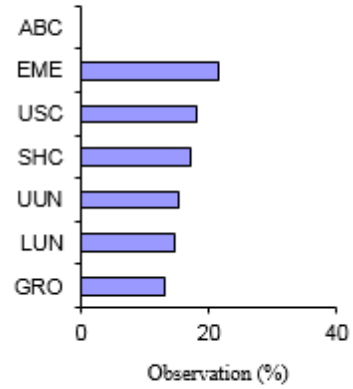
(F) Drongo



(G) Treepie



(H) Shrike



(I) Sparrow

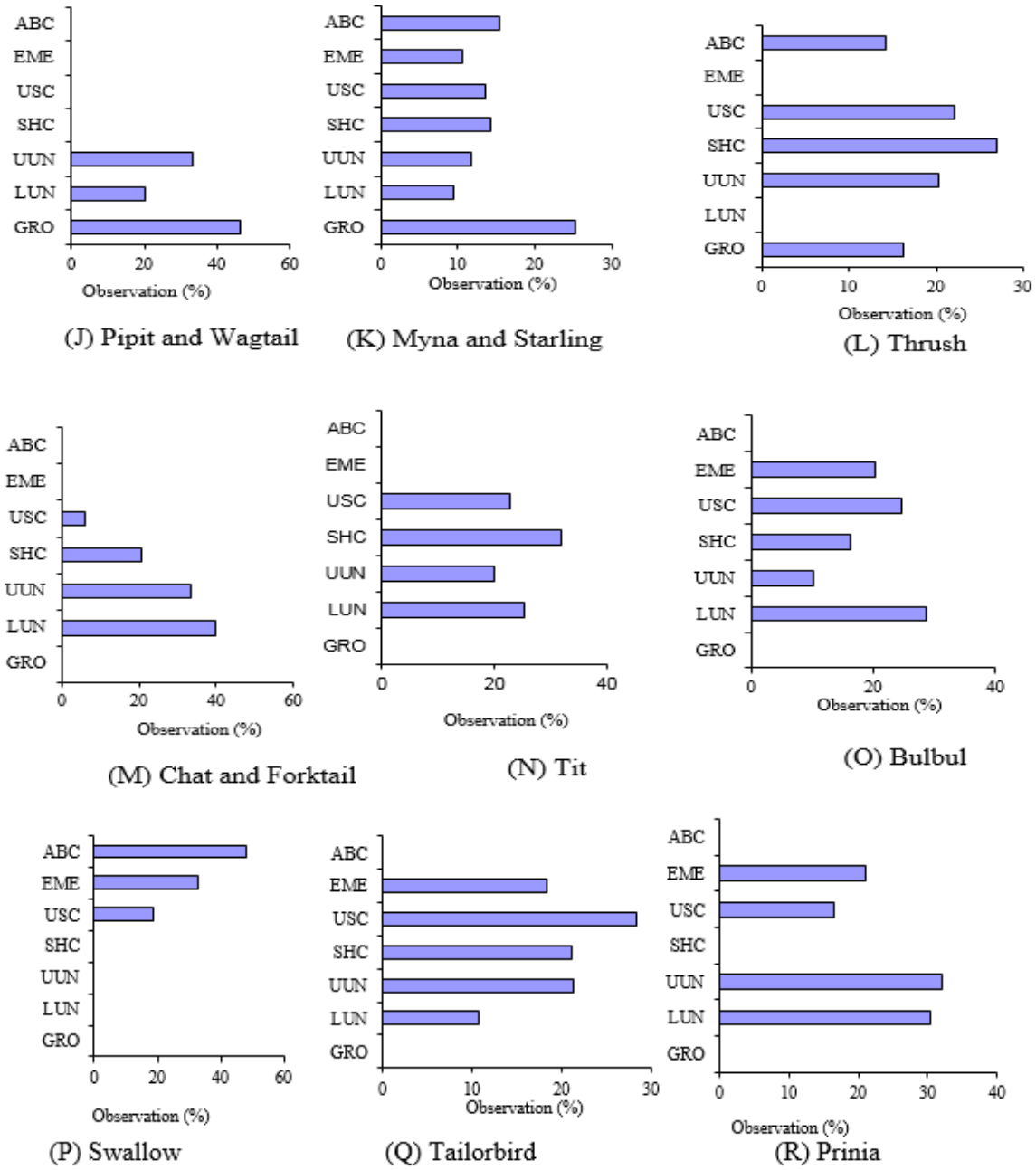


Figure 1 Percentage of foraging layers utilized by terrestrial birds Foraging layer : ABC = above canopy, EME = emergent leaves, USC = unshaded canopy, SHC = Shaded canopy, UUN = upper understorey, LUN = lower understorey, GRO = ground, N = number of birds

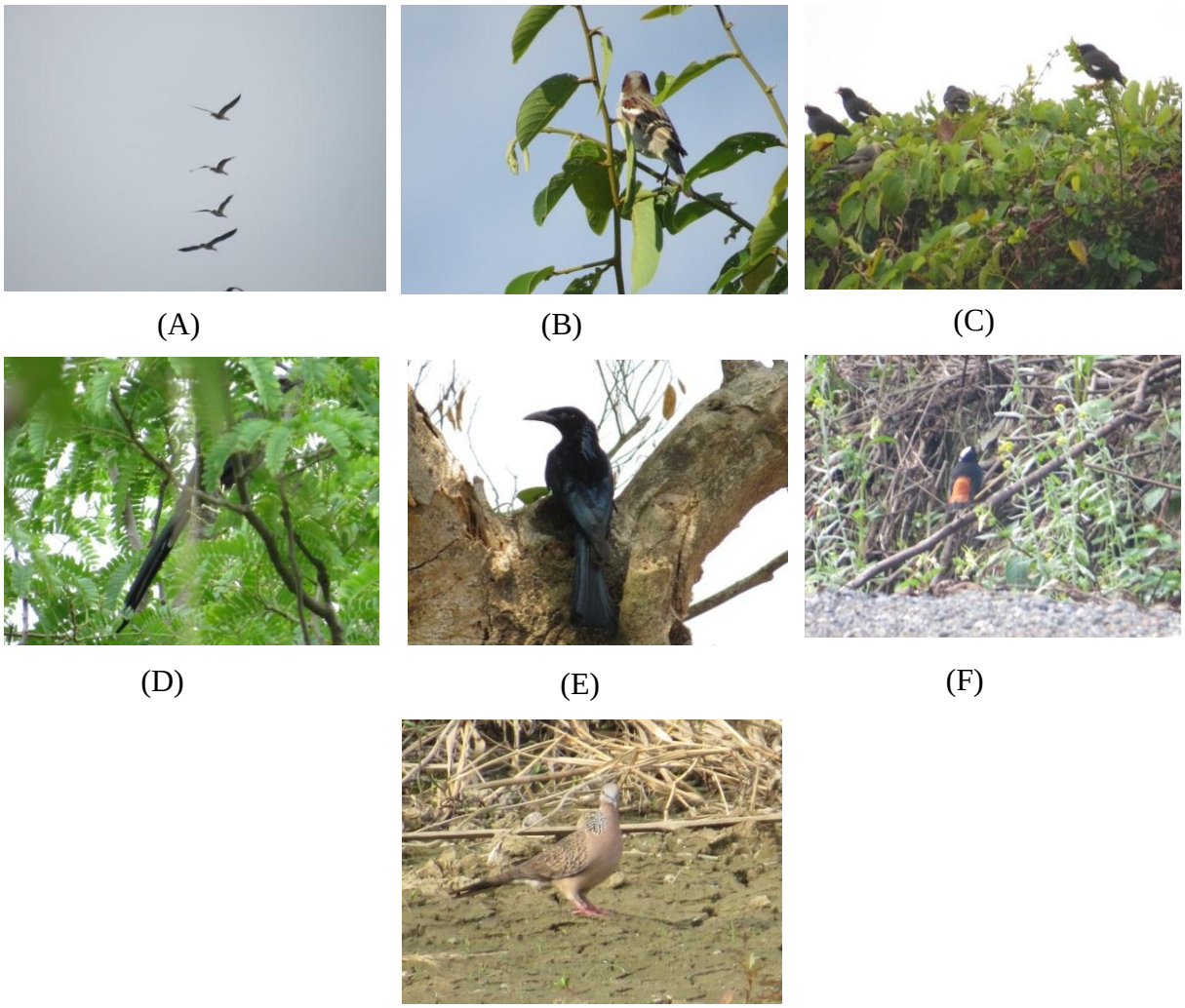


Plate 1 Foraging layers by terrestrial birds
A. Above canopy
B. Emergent leaves
C. Unshaded (on top of) conopy
D. Shaded (within) canopy
E. Upper understorey
F. Lower understorey
G. Ground

Discussion and Conclusion

In present study, among the observed seven substrates were taken into consideration and included upper understorey (UUN), unshaded canopy (USC), emergent leaves (EME), lower understorey (LUN), shaded canopy (SHC) and ground (GRO) were mostly utilised for foraging opportunities on these envrions because vegetation offered suitable food in this area. A larger number of terrestrial birds species were observed to assemblage in these substrates. *Saxicola ferreus* (Grey Bushchat) and *Enicurus scouleri* (Little Forktail) frequently used upper understorey and lower understorey. Barbet and Tailor bird mostly used unshaded canopy. Noske (1995) discovered that the Arctic Warbler and the Ashy Tailorbird were very similar in foraging behaviour with a mean overlap of 70% in substrates used for foraging.

In the present study, a total of 52 species forage on the ground and out which some species Pipit and Wagtail, Myna and Starling, Roller, Pigeon and Dove and Thrush used this substrate. Therefore, both insectivores and granivores used this substrate. Sultana (2002) reported that the height and height related characteristics separated the ground foragers from these species and formed three distinct foraging environments (ground, plants and air). The complexity of foliage layer provided supporting substrates.

Sultana and Hussain (2010) pointed out that, a large number of bird species fall under the plant guild because plant offers a greater variety of microhabitats for the birds to find suitable food for them.

In present study birds mostly use seven foraging activity to obtain food and closely related species used the same basic searching method. Pigeon and Dove forages by gleaning on the substrate. Myna and Starling were probing. Searching patterns are largely a function of the morphological and perceptual traits of each species, which allow the birds to move through the foliage, locate, detect and capture the prey in specific ways. The feeding methods are move specialized in each species not with standing the habitat structure. Resource partitioning reduces the effect of competition by decreasing the amount of overlap between the competing species (Wiens, 1989).

The same groups belonging to different species such as Myna and Starling (Sturnidae) have shown mostly similarity in foraging. It may be suggested that the morphological character was more or less similar. Hutto (1981) suggested that a group of species, which were similar in their morphological adaptation, formed assemblage. For example, all the pheasant species were ground foragers but other bird species with different morphologies also utilized the same general searching mode and procured similar types of prey e.g. thrushes.

Robinson and Holmes (1984), revealed that closely related species that share similar morphological traits are likely to show similar foraging maneuvers.

Therefore, food and feed habit of birds were more likely to reflect changes in foraging maneuvers.

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SEASONAL OCCURRENCE OF SOME BUTTERFLY SPECIES IN AHLON ENVIRONS, MONYWA TOWNSHIP

Khin Aye Mar[1]

Abstract

A total number on the collection of 1611 individuals of butterfly species belonging to 42 butterfly species and 25 genera representing four families of order Lepidoptera were recorded from Ahlon environs in Monywa Township from December, 2017 to September, 2018.Altogether 884 individuals with 42 butterfly species in wet season, 333 individuals with 22 butterfly species in cool season, 394 individuals with 30 butterfly species in hot season were recorded from Ahlon environs. Among them, *Cepora nerissa*, *Appias libythea*, *Ixias pyrene*, *Catopsilia pomona*, *C. pyranthe*, *Danaus chrysippus*, *Junonia lemoniass* and *Eurema hecabe* were found as the most abundance butterfly species. Pieridae and Nymphalidae species were recorded as dominant families in this area.

Keywords: Butterflies, abundance, and seasonal occurrences

Introduction

Insects are found in all types of environments and they occupy little more than two thirds of the known species of animal in the world. The butterflies and moths belong to the order Lepidoptera within the class Insecta. The butterflies are most beautiful, attracting individual for humans. In term of indicator organisms for biodiversity studies on butterflies is the excellent choice as they are common almost everywhere attractive and easy to observe. The butterfly diversity is high in the tropic compared to temperate regions of the world (Gowda *et al*., 2011).

The use of butterflies as indicator species as biological measure of ecosystem health motivates the quest to determine the best data collection in methods and model of population dynamics to aid in conservation and management of butterflies (Thomas, 2005). Life of butterflies mainly depends upon the all plants and water. They distribute geographically and seasonally (Scudder *et al*., 2007).

The Myanmar weather is divided into cool season (October to February), hot season (March to May), and wet season (June to September). There are more butterflies in wet and cold seasons than those of dry season in Ahlon environs. This paper investigated the butterfly species found in Ahlon environs and related the seasonal abundance of butterfly in nature.

Materials and Methods

Study site and study period

The butterfly specimens were collected from two study sites of Ahlon environs 11.27 km away from Monywa. Ahlon area is located of Latitude 20° 55' 0.8" N and Longitude 95° 14' 35.5" E. Site I is located beside the railway road of Ye U. Site II is situated near Chindwin Bridge (Plate 1).

Field method

Data collection was carried out in two study sites. Butterflies were collected by using transect method. A transect line was dawn in measurements 800 m in length with 5m on either

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side. Observation of butterflies was made walking along the transect route at a constant place according to Pollard, (1977). The collection of butterflies was done twice a month during day time at 7:30 to 11:00 am because they are lovers of sunshine.

Identification

The butterfly specimens were identified and classified according to Binghan (1907), Pinratana (1977-1983), Corbet (1992), Borror *et al.*, (1992) and Kinyon (2003).

Analysis of the data

The collected data were analyzed as following;

$$\text{Relative abundance} = \frac{\text{no.of individuals of a species}}{\text{Total no.of individuals of all the spec ies in a particular site}}$$

Uncommon (uC) = having relative abundance less than 0.01

Common (C) = having relative abundance greater than equal to 0.01 and less than 0.05

Very common (vC) = having relative abundance equal to 0.05 and above (Bisht, *et al.*, 2004)

Plate 1 Location map of study area

Result and Discussion

A total number of butterfly species belonging to 42 butterfly species and 25 genera under four families of order Lepidoptera were recorded from Ahlon environs in Monywa Township.

Table 1 Monthly abundance of butterflies studied in Ahlon Environs (Site I and site II), Monywa Township

Species	Cool season			Hot season			Wet season			Total
	Dec	Jan	Feb	March	April	May	June	July	Aug	
Papilio polytes romulus	3	1	1	0	0	3	5	2	3	18
P. demoleus malayanus	4	4	7	0	1	2	8	5	10	41
G. agameman	3	3	2	5	0	0	0	2	4	19
Delias pasithoe parthenope	1	0	0	2	0	0	3	0	4	10
D. descombesi eranthos	0	0	0	4	0	0	1	0	2	7
Leptosia nina nina	10	5	3	8	10	3	5	10	10	64
Cepora nerissa phryne	0	0	0	0	0	0	0	1	2	3
Cepora nerissa dapha	12	6	0	10	0	3	17	20	22	90

Species	**Cool season**			**Hot season**			**Wet season**			**Total**
	Dec	**Jan**	**Feb**	**March**	**April**	**May**	**June**	**July**	**Aug**	
Appias lyncida	0	0	0	0	0	0	7	4	2	13
Appias libytheca olferna	10	10	10	22	10	10	25	25	20	142
Ixias pyrene verna	15	6	12	16	7	0	15	14	10	95
Catopsilla pomona crocale	0	0	0	0	0	0	8	8	4	20
C. pomona pomana	13	6	0	16	0	20	25	19	17	116
C. pyranthe pyranthe	12	6	0	0	6	4	15	15	15	73
pareronia anais	1	1	1	0	1	0	2	1	0	7
Eurema simulatrix	0	0	0	0	0	0	10	3	12	25
E. hecabea	17	8	4	36	0	0	30	25	35	155
Danaus chrysippus	12	6	11	10	8	5	10	10	15	87
D. genutia	5	4	6	5	8	11	7	10	6	62
D. limniace	0	0	2	1	0	0	2	0	3	8
Euploea midamus	0	0	0	0	0	0	1	2	0	3
Melanitis leda	0	0	0	3	2	1	1	0	5	12
M. phedima	0	0	0	0	0	0	0	3	2	5
Mycalesis visala	3	0	1	1	0	0	2	5	0	12
M. intermedia	0	0	0	0	3	0	5	7	5	20
Ypthima horsfieldii	0	0	0	0	0	0	6	6	9	21
Acracea terpsicore	18	8	5	0	0	10	11	12	8	72
Ariadne ariadne	0	0	0	5	0	7	5	1	2	20
Cethosia cyane	0	0	0	3	0	0	0	3	2	8
C. perthesilea	0	0	0	2	0	3	2	1	0	8
Junonia atlites	0	0	0	0	4	0	2	0	3	9
J. lemonias	7	8	2	18	2	10	14	12	12	85
J. almana	0	0	0	0	0	0	3	2	5	10
J. hierta	0	0	0	0	0	0	1	2	4	7
J. orithya	0	0	0	0	0	0	5	4	7	16
Hypolimnas misippus	2	2	0	0	3	0	5	3	2	17
H. bolina	0	0	0	0	0	0	7	10	5	22
Neptis hylas	3	3	0	6	9	2	0	4	7	34
Arhopola muta	0	8	1	4	4	6	9	5	10	47
Castalius rosimon	4	3	0	12	2	5	10	11	6	53
Chilade pandava	0	0	0	0	0	0	8	5	4	17
Chilades pandava	8	4	0	8	10	2	14	7	5	58

Table 2 Abundance of butterflies found in Site I and Site II, Ahlon Environs

Species	Sites		Individuals	RA	Abundance Code
	I	II			
Papilio polytes romulus		18	18	0.011	C
P. demoleus malayanus		41	41	0.025	C
G. agameman		19	19	0.011	C
Delias pasithoe parthenope		10	10	0.006	uC
D. descombesi eranthos		7	7	0.004	uC
Leptosia nina nina	35	29	64	0.039	C
Cepora nerissa phryne		3	3	0.001	uC
C. nerissa dapha	54	36	90	0.055	vC
Appias lyncida	10	3	13	0.008	uC
A. libytheca olferna	71	71	142	0.088	vC
Ixias pyrene verna	59	36	95	0.058	vC
Catopsilla pomona crocale	11	9	20	0.012	C
C. pomona pomana	66	50	116	0.072	vC
C. pyranthe pyranthe	44	29	73	0.045	vC
pareronia anais		7	7	0.004	uC
Eurema simulatrix tecmessa	18	7	25	0.015	C
E. hecabea	84	71	155	0.096	vC
Danaus chrysippus	49	38	87	0.054	vC
D. genutia genutia	34	28	62	0.038	C
D. limniace limniace		8	8	0.004	uC
Euploea midamus chole		3	3	0.001	uC
Melanitis leda		12	12	0.007	uC
M. phedima ganopati		5	5	0.003	uC
Mycalesis visala		12	12	0.007	uC
M. intermedia		20	20	0.012	C
Ypthima horsfieldii	6	15	21	0.013035	C
Acracea terpsicore	38	34	72	0.044693	C
Ariadne ariadne		20	20	0.012415	C
Cethosia cyane euanthes		8	8	0.004966	uC
C. perthesilea methypsea		8	8	0.004966	uC
Junonia atlites atlites		9	9	0.005587	uC
J. lemonias lemonias	42	43	85	0.052762	vC
J. almana almana		10	10	0.006207	uC
J. hierta hierta		7	7	0.004345	uC
J. orithya ocyale		16	16	0.009932	uC
Hypolimnas misippus		17	17	0.010552	C
Hypolimnas bolina jacintha		22	22	0.013656	C
Neptis hylas	23	11	34	0.021105	C
Arhopola muta maranada	23	24	47	0.029174	C
Castalius rosimon	30	23	53	0.032899	C
Talicada nyseus	7	10	17	0.010552	C
Chilades pandava	30	28	58	0.036002	C
	734	**877**	**1611**		

uC = Uncommon, 16 C = Common, 18 vC = Very Common, 8

Discussion

Butterfly species and the number of individual in Ahlon environs were found change of seasonally. According to the results, 21 species of butterflies in cool season, 30 species of butterflies in hot season and 42 species of butterflies in wet season were recorded in the study area. The high numbers of butterfly species were found in wet season because their feeding plants were most abundant in this season. Among the study species, *Cepora nerissa*, *Appias libythea*, *Ixias pyrene*, *Catopsilia pomona*, *C. pyranthe*, *Danaus chrysippus*, *Eurema hecabe* and *Junonia lemonias* were very common species found in study area. Family Nymphalidae was found as dominant species in study area.

In the present study, the butterfly species of *Appias lyncida*, *Captopsilia pomona*, *Eurema simulatrix*, *Ypthima horsfieldii*, *Talicada nyseus* were not found in cool and hot seasons and the rest species were found in three seasons in Site I. Only 20 butterfly species were found in study area of Site I because the large trees were found in this area.

In the present study of Site II, the butterfly species, *Cepora nerissa dapha*, *Appias lyncida*, *Captosilia pomona*, *Eurema simulatrix*, *Euplooea midamus*, *Melanitis phedima*, *Ypthima horsfieldii*, *Junonia almanac*, *J. hierta*, *j. orithya*, *Hypolimnas bolina*, and *Chilades pandava* were not found in cool and hot seasons. *Delias descombesi*, *Melanitis leda*, *Mycalesis intermedia*, *Ariadne ariadne*, *Cethosia cyane auanthes*, *C. penthesilea methypsea*, and *Junonia atlites atlites* were not found in cool season. The rest butterfly species were found in all seasons. A total number of 42 butterfly species were found in Site II because trees, bushes, flowering plants, and their feeding plants were more abundance in this area.

Butterfly diversity varies with seasons. *Melanitis leda* and *Junonia almana* from family Nymphalidae, wet season butterflies were more active in general than in the dry season. The adult butterflies were spents long period during the dry season (Brakefield and Larsen, 1984).

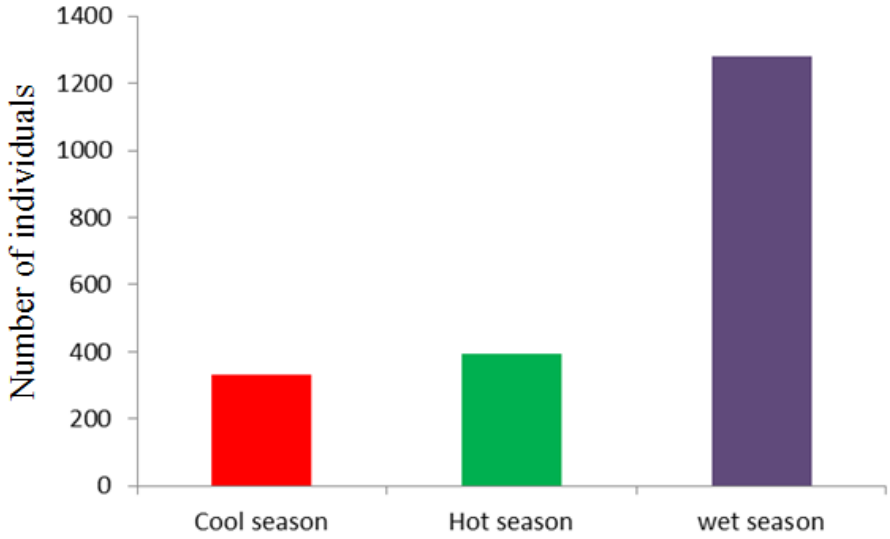

Figure 1 Seasonal occurrence of butterfly species in study area


(A)*Papilio polytes*


(B) *Papilio demoleus*


(C)*Graphium Agamemnon*


(D) Delias pasithoes


(E) *Laptosia nina*


(F)*Cepora nerissa dapha*


(G) *Appias libythea*


(H) *Ixias pyrene*


(I)*Captopsilia pomon*

Plate 1 Recorded butterfly species of families Papilionidae and Peridae


(A) *Pareronia anais*


(B) *Eurema simulatrix*


(C) *Eurema hecabe*


(D) *Danaus chrysippus*


(E) *Danaus limniace*


(F) *Euploea midamus*


(G) *Melanitis leda*


(H) *Mycalesis intermedia*


(I) *Ypthima horsfieldii*


(J) *Acraea terpsicore*


(K) *Ariadne ariadne*


(L) *Cethosia cyane*


(M) *Junonia atlites*


(N) *Junonia lemonias*


(O) *Junonia almana*

Plate 2 Recorded butterfly species of families Pieridae and Nymphalidae

(A) *Hypilimnas*

(B) *Neptis hylas*

(C) *Arhopola muta*

(D) *Castalius rosimon*

(E) *Talicada nyseus*

(F) *Chilades pandava*

Plate 3 Recorded butterfly species of families Nymphalidae and Lycaenidae

Conclusion

Butterfly species were mostly found in the area where many of trees, shrubs and flowering plants were grown. In this area, Butterfly species are more abundance found in wet season than cool and hat seasons because they are sensitive to change in the habitat and climate which influences their distribution and abundance.

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COLOUR PREFERENCE AND BITING BEHAVIOR OF MOSQUITOES ON THE SPECIFIC PARTS OF THE CATTLE BODY

Ma Nandar Moe Oo[1], Tin Lay Mon[2], Mg Mg Mya[3]

Abstract

A monthly field study was conducted to determine the preferred biting behavior of mosquitoes on different specific parts of different coloured cattle body in Taikkyi Township, Yangon Region from March 2017 to November 2018. Outdoor cattle bait mosquitoes collection was done using WHO sucking tube from 18:00 hours to 24:00 hours for 3 days. *Anopheles* and *Culex* mosquitoes were collected for 45 minutes in every hour on the specific parts of the cattle body. Collected mosquitoes were placed in specific body parts labeled paper cups. Next day morning species identified was done all collected mosquitoes according to different mosquito identification key. Result revealed that the highest number (n=1798) of mosquitoes were collected in September followed by n= 1409 in March and lowest was collected in n=226 in May by cattle bait collection. The biting behavior of mosquitoes were found to be the highest (n=2592, 34.39%)on black cattle, followed by (2566,34.05%) on white cattle lowest was observed (2378,31.56%) on Brown coloured cattle. The highest number of biting behavior on specific part of body was found Elbow from Bracelets (1611, 21.38%) followed by Knee from Anklebone (1108, 14.70%), Fingertip (846,11.41%), Tiptoe (887,11.77%) lowest was observed on Head (108,1.43%). When compared with specific parts of different parts of different coloured cattle was found the highest number of mosquitoes were collected from Elbow to Bracelets (n=552,21.51%) white, 522,21.95% on Brown and 537, 20.72% on Black cattle followed by 549 21.18% on Tiptoe of black cattle, 395, 16.61% and 430, 16.59% on Knee to Anklebone of Brown and black cattle body parts. Lowest mosquitoes were observed (13, 0.51%), (47,1.98%) and (48,1.85%) on Head of all White, Brown and Black coloured cattles. In conclusion biting behavior of mosquitoes were observed differ to different parts of the cattle body. Tiptoe, fingertip, Elbow from Bracelets and Knee from Anklebone were mostly bitten by mosquitoes. Mosquitoes were found highly attack to Elbow from Bracelets and Knee from Anklebone followed by tiptoe, fingertip of the cattles. Although very low on Head.

Introduction

Mosquitoes are members of family Culicidae of order Diptera. The family is a large and abundant group that occurs throughout temperature and tropical region of the world. It consists of three subfamilies (1) Anophelinae (2) Culicinae (3) Toxorhychitinae. There are 3500 species of mosquitoes in the world (Harbach 1994) and 49 species (34 Anopheles, ten Culex, two Aedes, two Mansonia and one Armigeres) in Myanmar. Mosquitoes are the single largest group of insects, which serve as intermediate hosts in the transmission of many important human diseases carry number of vector borne diseases, They are including malaria, dengue hemorrhage fever (DHF), yellow fever, filariasis, chikungunya and Japanese encephalitis (JE) (Wharton, 1951), And now recently found Zika virus which cause the microcephaly in fetus (Kuno *et al*., 1998). Mosquitoes biting behavior is most important to eliminate and control the mosquitoes and vector borne diseases. Understanding biting distribution of potentially infectious (parous) mosquitoes at various hours of the night would be useful in establishing the likely impact of bed nets on malaria transmission. However, this behavior is likely to vary across ecological settings and among mosquito populations.

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Therefore the present study focus the biting habit of mosquitoes. Biting or feeding preferences and host behavior have an important influence on the local epidemiology of vector borne diseases. For example, malaria prevalence may be less in communities where the principal malaria vector is not strictly anthropophilic and where cattle range between the villages and major breeding sites (8 Kelin *et al*., 1991). In some areas of Brazil, observations indicate that local populations of mosquito species are zoophilic, while in others they are anthropophilic (2 Deane, 1988). It is now apparent that these differences are often species differences within species complexes (Deane, 1988). Mosquito blood feeding behavior consists of several phases: the search for potential hosts, attraction to hosts, attacking, feeding, and resting. Of these phases, much attention has focused on attacking behavior of mosquitoes because of its practical importance. Little is known about outdoor biting behavior. Even the dynamic of indoor biting and infection risk of sleeping of household occupants. A recent study, Anopheline biting sites indicate that certain species have a preference for specific parts of the body. *Anopheles gambia* Giles S.S. shows a preference for foot and angle areas (Jong and Knils 1995).

Studied explore the colour preferred and biting behavior sides of cattle body in outdoor condition and demonstrated the strong preference for feeding on the cattle body. The works focused the attention on monthly variation in host colour preference biting behavior of Anopheline and Culicine mosquitoes from Takkyi Township Yangon Region as important vector, *Anopheles* and *Culex*. The opportunities of this study was for behavioral targeting to reduce biting risk and vector borne infections.

Objects of the study

To record the species composition of mosquitoes in studied area

To determine the biting habit of *Anopheles* and *Culex* mosquitoes

Materials and methods

Study site

Monthly mosquitoes sample were collected in 10 cows from Taikkyi Township, Yangon Region.

Study period

The study was done from March to November 2018.

Sample collection method: Outdoor cattle bait mosquitoes collection was done using WHO sucking tube from 18:00 hours to 24hours for 3 days from March to November 2018. *Anopheles* and *Culex* mosquitoes were collected for 45 minutes in every hour on the specific parts of the White, Brown and Black cattle body. Hourly collected mosquitoes were placed in specific body parts labeled paper cups individually. Next day morning all collected mosquitoes were morphologically classified the species.

Species identification

The species identification of all collected mosquitoes according to the body parts were identified by Harrison BA. and Scanlon JE. (1975), Reid 1967 and Myo Paing 1990)

Analysis

Monthly collected entomological data were analyzed by according to specific parts of three different coloured cattles by Microsoft excel software.

Results

Systematic position of Studied species of Anopheles

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: Culicidae

Genus: Anopheles

Species: 1. *An. annularis* (Vander Wulp, 1884)

2. *An. vagus* (Donitz, 1902)

3. *An. barbirostris* (Vander Wulp, 1884)

4. *An. aconitus*

5. *An. fluviatilis*

6. *An. philippinensis*

Table 1 Monthly collection of mosquitoes from different parts of cattle body

	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Total
Total Mosquito bites	1409.00	456.00	226.00	483.00	642.00	845.00	1798.00	1244.00	433.00	7536
Mean	469.67	152.00	75.33	161.00	214.00	281.67	599.33	414.67	144.33	2512
± SD	58.86	67.20	35.12	10.58	15.10	32.58	207.81	52.37	31.34	116.77

Table 1: shows that the highest number (n=1798) of mosquitoes were collected in September followed by n= 1409 in March and lowest was collected in n=226 in May.

Table 2 Biting behavior of mosquitoes against different coloured of cattles

Coloure of cattle's	Total collected	Percentage (%)
White	2566	34.05
Brown	2378	31.56
Black	2592	34.39
Total collected mosquitoes	7536	100.00
mean	2512	0
SD	116.77	0

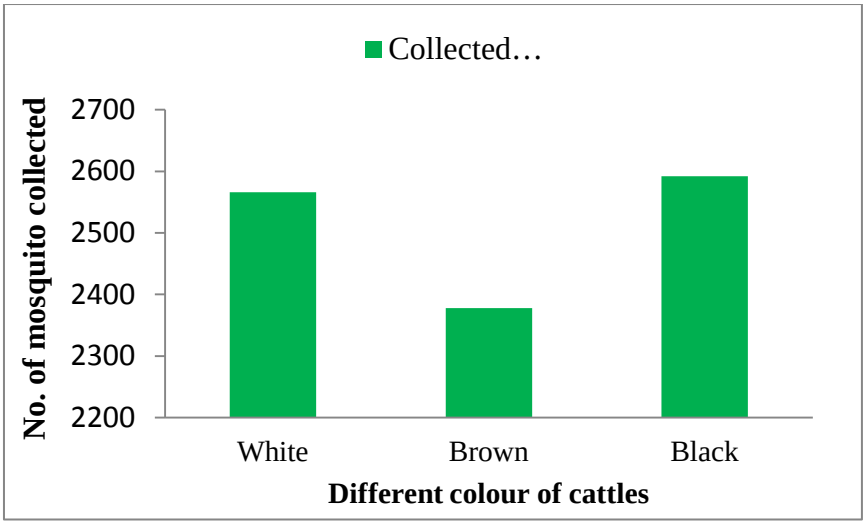

Table 2 and Fig 1. Shows that the biting preference of mosquitoes on different coloured of cattles was found that the highest number of mosquitoes were bitten 2592(34.39%) to black coloured cattle followed by 2566 (34.05%) bitten to white coloured cattle and lowest bitten was observed 2378(31.56%) to Brown coloured cattle.

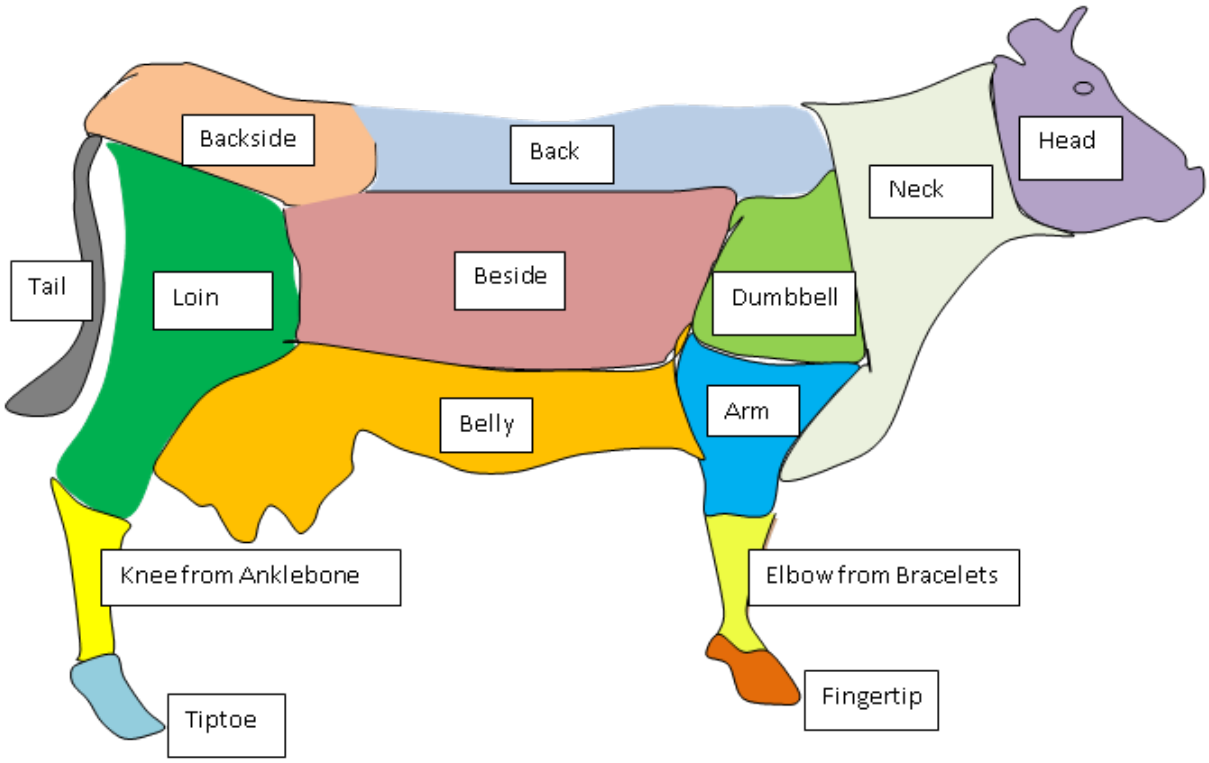

Figure 2 Body divided by specific parts of cattle body

Table 3 Biting preference of mosquitoes according to specific parts of cattles body

Specific parts of body	Different coloured of cattle's			Total collected	Percentage of bite
	White	Brown	Black		
Head	13	47	48	108	1.43
Neck	156	195	69	420	5.57
Dumbbell	165	160	68	393	5.21
Arm	73	94	125	292	3.87
Elbow from Bracelets	552	522	537	1611	21.38
Fingertip	343	159	362	864	11.46
Back	55	150	132	337	4.47
Belly	333	134	51	518	6.87
Beside	222	235	67	524	6.95
Backside	96	54	102	252	3.34
Loin	45	125	52	222	2.95
Knee from Anklebone	283	395	430	1108	14.70
Tiptoe	230	108	549	887	11.77
Total mosquito collected	2566	2378	2592	7536	100.00
Mean	197.38	182.92	199.38	579.69	
SD	±152.85	±135.20	±194.47	±426.69	

Table 3. Shows detail of biting preference of mosquitoes on specific parts of cattle body and found that the highest preference of mosquitoes body part was observed 1611 (21.38%) bite to Elbow from Bracelets and followed by 1108 (14.70%)bite on Knee from Anklebone and lowest was found on 108(1.43%) on Head. Although mosquitoes were found more preference to Finger tip 864(11.46%) and Tip toe 887(11.77%) than the other remaining parts of the cattle's body parts.

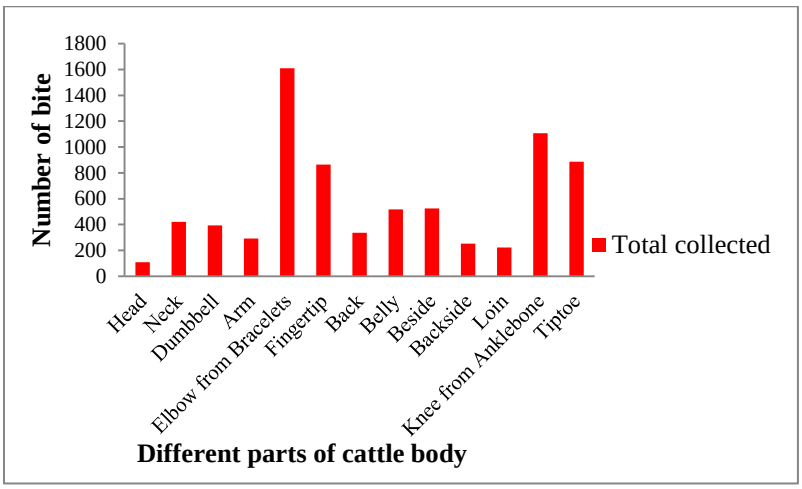

Figure 3 Biting behavior of mosquitoes on specific parts of the cattle body

Fig.3 shows that the highest preference of biting was observe Elbow from Bracelets parts of cattle followed by Knee from Anklebone of body part and lowest biting part was head of the cattle. Fingertip and tiptoe of cattle were found high preference of mosquito biting.

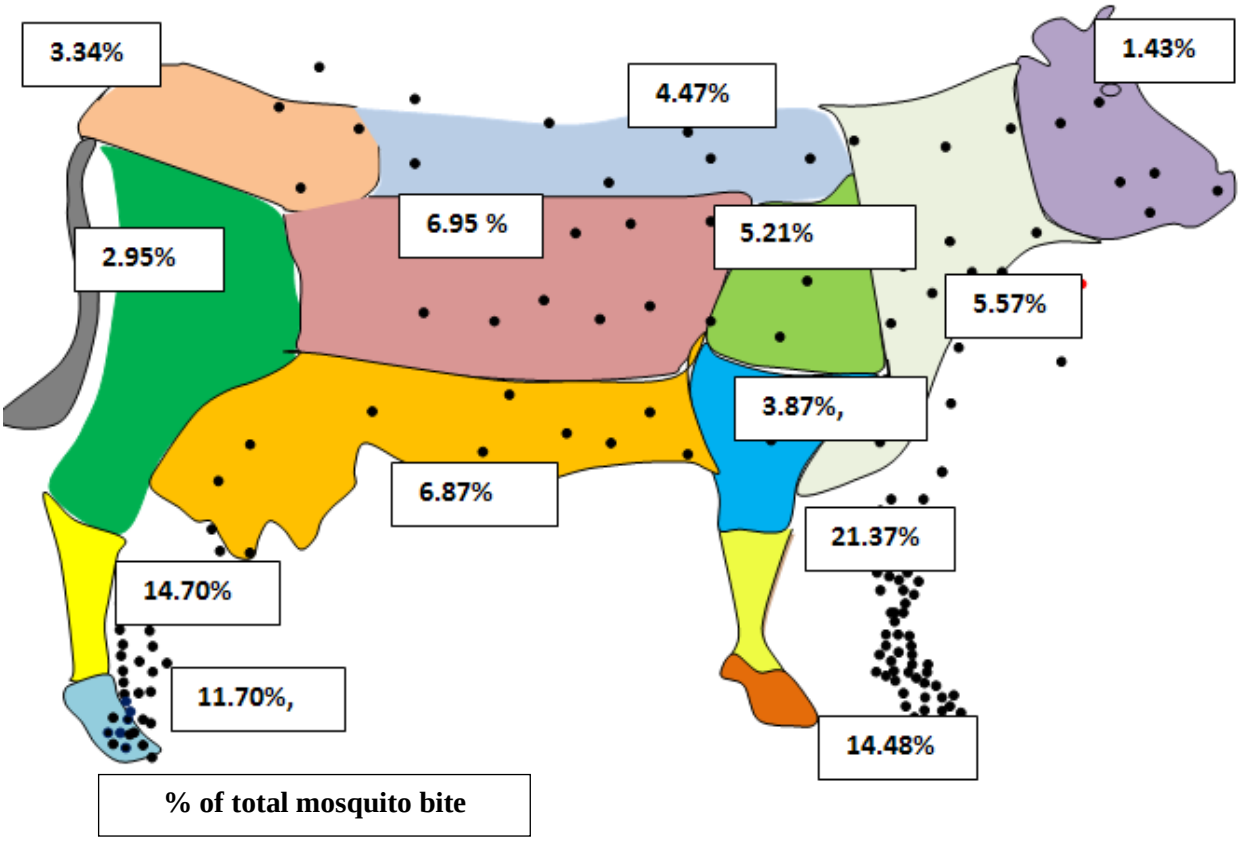

Figure 4 Shows that the biting behavior of mosquitoes on specific parts were found highest 21.37%bite on Elbow from Bracelets followed by 14.70 Knee from Anklebone, 14.48% fingertip, and 11.70% Tiptoe the lowest was found 1.43% on head part of cattle.

Table 4 Colour preference and biting behavior of mosquitoes on different specific part of cattles

Specific parts of body	Different coloured of cattle's					
	White		Brown		Black	
	Average collected	% of bite	Average collected	% of bite	Average collected	% of bite
Head	13	0.51	47	1.98	48	1.85
Neck	156	6.08	195	8.20	69	2.66
Dumbbell	165	6.43	160	6.73	68	2.62
Arm	73	2.84	94	3.95	125	4.82
Elbow from Bracelets	552	21.51	522	21.95	537	20.72
Fingertip	343	13.37	159	6.69	362	13.97
Back	55	2.14	150	6.31	132	5.09
Belly	333	12.98	134	5.63	51	1.97
Beside	222	8.65	235	9.88	67	2.58
Backside	96	3.74	54	2.27	102	3.94
Loin	45	1.75	125	5.26	52	2.01
Knee from Anklebone	283	11.03	395	16.61	430	16.59
Tiptoe	230	8.96	108	4.54	549	21.18
Total mosquito collected	2566	100.00	2378	100.00	2592	100.00
Mean	197.38		182.92		199.38	
SD	±152.85		±135.20		±194.47	

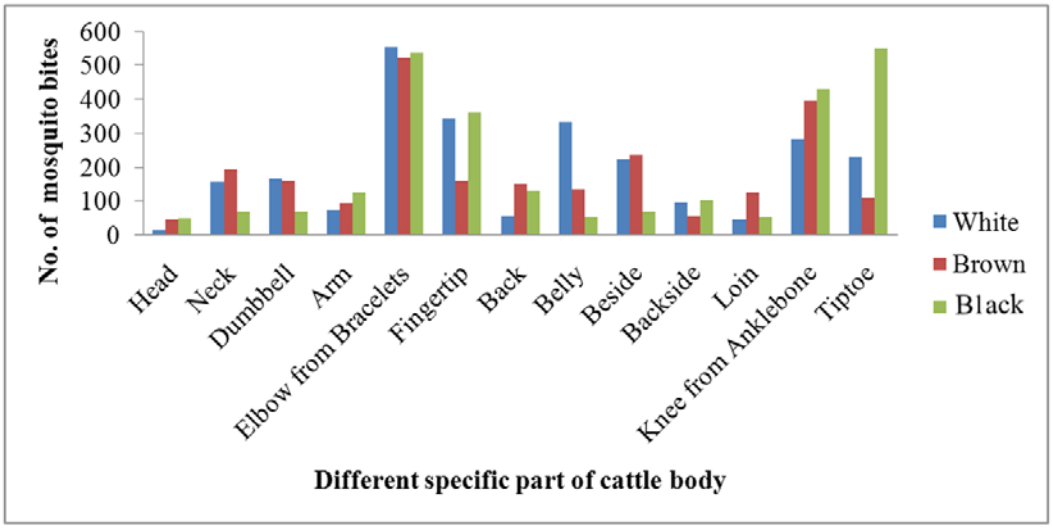

Figure 5 biting behabior of mosquitoes on specific parts of different coloured cattle bodys

Table 4 and Fig.4. shows that Colour preference and biting behavior of mosquitoes on specific part of different coloured cattles body. The result found that mosquitoes were most preference to black coloured cattle (n=2592) followed by white coloured cattle (n=2566) and lowest biting was found to Brown coloured cattle (n=2378). The biting behavior of mosquitoes on specific part of the Black coloured cattle body was found 549 bites to Tiptoe followed by 537 bites to Elbow from Bracelets the lowest was found 48 bites on head. Biting behavior of mosquitoes on different parts of White coloured cattle was found to be the highest 552 bite on Elbow from Bracelets followed by 343 bites on the Fingertip and lowest was found 13 bites on head. Biting behavior of mosquitoes on different parts of brown coloured cattle was found the highest biting on 522 bites on Elbow from Bracelets followed by 395 bites on Knee from Anklebone and lowest was observed 47 bites on head of the brown cattle.

When copared with each other the biting behavior of mosquitoes were observed highest on Elbow from Bracelets (n=1611 and followed by Knee from Anklebone (n=1108)of the all cattle body and the lowest bite was observed on head. Of this the highest biting on Elbow from Bracelets was found to be 552 bites to white followed by 537bites to Black and lowest was found 522 bites to Brown coloured cattle. Although biting on Knee from Anklebone was the second most preference area and found that the highest biting was observed 430 bites on black cattle followed by 395 bite to Brown and lowest bite was observed 283 bite on Knee from Anklebone area of white coloured Cattle. Although fingertip and tiptoe of cattle body were also found high biting parts of bodies. The highest biting on Tiptoe was found 362 bites to Black followed by 343 bite to White and lowest was 159 bite to Brown coloured cattle and the highest biting behavior of mosquitoes on tiptoe was 549 bites to Black followed by 230 bites to White and lowest was observed 108 bites to Brown coloured cattle. More than 150 bites of mosquitoes on different parts of cattle body were found Neck, Dumbbell, Beside and back parts of White and Brown coloured cattle. Although other parts of body were found less than 150 bites to all cattle. Same highest biting behavior of Anopheles mosquitoes on Knee from Tiptoe and Albow to fingertip parts of cattle body was observed in Loikaw (Mya et al., 2017 unpublished data).

Discussion

The host preference of a mosquito species is an important determinant of its vectorial capacity and mosquito species that are highly anthropophilic are often vectors of important human diseases (Takken and Verhulst 2013). In this paper author focus attention on the unusually similar biting behaviour of most important mosquitoes *Anopheles* and *Culex* species. Present study demonstrated that the strong preference for feeding on the different coloured cattle body and found that the highest number of mosquitoes were collected from Black coloured cattle and followed by White coloured and the lowest mosquitoes biting was observed from Brown coloured cattle. When compared the highest preference of biting on specific parts of body was observe Elbow from Bracelets parts of cattle followed by Knee from Anklebone of body part and lowest biting part was head of the cattle. Fingertip and tiptoe of cattle were also found high preference of mosquito biting mean while high number of *Anopheles vagus, An. barbirostris, An. aconitus* and Culex mosquitoes were collected. It may be due to the fact that the high preference biting areas of cattle such as Elbow from Bracelets, Knee from Anklebone, Fingertip and tiptoe of the body parts are very close to the ground and have high odour in these parts of all coloured cattle's. Same finding has been reported by Braack et al., (2015) and revealed that the three species *An. gambiae, An. arabiensis* and *An. funestus* investigated in this study all displayed a strong preference for feeding at the lower leg areas at seated people, such sitting simulating typical outdoor social situations in the evening in rural villages in Africa. Mosquitoes display biting preferences among different sites of cattle body as well as the human body. In addition to height or convection currents, body odour may play a role in the selection of these biting sites (Niels et al., 2016). Differences in attractiveness to mosquitoes also occur between body sites and vary considerably between mosquito species. *Aedes aegypi*, *Ae. simpsoni* and *An. atroparvus*, for example, prefer to bite around the head and shoulder (deLacy et al., 1995, Knols 1996, Haddow 1946)7–9, while *Culex quinquefasciatus* does not seem to prefer any specific body part (Knols 1996)8. Present study observed that colour preference and biting behavior of Anopheles and *Culex* mosquitoes were high on Elbow from Fingertip, Knee from tiptoe of the body. A study in Africa mentioned that *Anopheles gambiae s.s., An. arabiensis* and *An. funestus* are important malaria vectors and bite most frequently on the feet and ankles(deJong et al., 1995, Braack et al., 2015, Dekker et al., 1998) 7,10,11, however, when people lay down, this preference disappears and they may bite anywhere on the body except the head (Braack et al., 2015,Dekker et al., 1998) 10,11. Dekker et al.(1998)11 have suggested that mainly convection currents and partly host odours guide mosquitoes to the feet and ankles, while Braack et al.(2015)10 attribute the selection of biting sites mainly to the height above ground (Braack et al., 2015, Braack et al., 1994, 10,12. In the present study the attractiveness of mosquitoes has differed in different biting site as high biting on angle to fingertip and elbow tiptoe and low biting on head, neck and back of all coloured cattle, although both mosquitoes found highest biting preference on black cattle then white and brown coloured cattles. Other researcher underlying mechanisms behind the variation in attractiveness between individuals to mosquitoes have been studied in detail and have shown that body odours play an important role as well (Qiu et al., 2006, Olanga et al., 2010, Mukabana 2002, Verhulst et al., 2011) 5,13–15. Less is known, however, about the mechanisms behind the variation in attractiveness of different body regions of the same individual. Several studies have investigated the volatile profiles from the human body, resulting in more than 500 compounds that have already been reported (de Lacy et al., 2014, Gallagher et al., 2008,Pann et al., 2007, Bernier et al., 1999) 19–22. However, it is difficult to compare the volatile profiles of the

different body parts due to the use of different sampling and processing techniques across these studies (Dormont 2014) 23. The volatile composition of the skin depends on the type and number of sweat glands and the bacteria that thrive on the products of these glands (Leyden et al., 1981, Stoddart 1990) 24,25. Bacteria convert long chain non-volatile compounds into short chain volatile compounds that are attractive to mosquitoes (James et al., 2004) 26. In addition, the attractiveness of human skin emanations to mosquitoes is correlated with the bacterial diversity and composition of the skin (Verhulst et al., 2011)15.

Conclusion

In conclusion the biting behavior and coloure preference of mosquitoes on cattle are same as human study. The results presented in the current study was not a difference in the attractiveness of *Anopheles* mosquitoes and *Culex* mosquitoes on highest attractive parts of three different coloured Cattles. conclusive evidence for the evolution of colour preference and biting behavioral has often been confounded by methodological issues. However, our preliminary study has demonstrated that biting behavioral of both mosquitoes could have a significant impact on the effectiveness of diseases control. As a result, we propose to improve understanding of both physiological and behavioral in disease vectors.

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PERFORMANCE EVALUATION ON NATURALLY MATED AND ARTIFICIAL INSEMINATION OF QUEEN BEES *APIS MELLIFERA* LINNAEUS, 1758 IN FIELD COLONIES

Nyo Nyo Lwin[1] Padetha Tin[2], Thida Wai[3], Lai Lai Phyu[4]

Abstract

Artificial insemination (AI) known as instrumental insemination, is a practice that is popularity among queen breeders. It provides a valuable tool to control the random mating process and is essential for bee breeding and research requiring specific crosses. Three study sites have been chosen as site (I) in Shan State (Aung Ban), site (II) in Bago Region (near Taungoo University) and site (III) in Mandalay Region (Yamethin). Study period lasted from December, 2017 to December, 2018. The study sites were chosen based on the migratory beekeeping method by flowering plants and seasonal changes condition of Myanmar. Artificial Insemination tool was composed of artificial insemination device, microscope, CO_2 as anesthesia. Artificial Insemination process have inseminated on 30 virgin queen bees in 2018, totally. In site (I) in Taung Lay Lone, Shan State, AI process have inseminated on ten virgin queen bees, but none is survived and oviposition due to severe weather condition. Artificial insemination process has inseminated on ten virgin queen in site (II), of near Taunggoo University, Bago Region. Result has succeeded six AI mated queens in this area. In site (III) of Yamethin, Mandalay Region, AI process have inseminated on ten virgin queen bees have recorded seven AI mated queens. Comparison on the survival rate of Naturally Mated (NM) queen and Artificially Inseminated (AI) queen bees were carried out. The comparison of the oviposition rate on Artificially Inseminated (AI) queen was also done in three study site. The relationship of oviposition rate and mean temperature, mean relative humidity and mean rainfall is carried out in three study sites. SPSS (Statistical Package for Social Science) version 25 was utilized for the statistical tests. The Non-parametric ANOVA test (Kruskal Wallis) was used.

Keywords: Artificial Insemination Technique, Artificially Inseminated and Normally mated Queen bees, Drone

Introduction

Many animals, especially insects, are very effective pollinators. Among them, bees are the most effective and reliable pollinators due to their dependence on flowers for their brood food. Several social and solitary bees can be utilized for the pollination of crops (Sihag, 1997).

Honeybees are the main insects which help in pollination of different species of plants. Honeybees play an important ecological role as pollinators of many plant species, and their products are the basis for a multi-million dollar commercial industry around the world. They are major agricultural pollinators around the world and are the keystone pollinators in tropical ecosystems.

Honeybees are beneficial to both agriculture plant and most wild plants as pollinator. For mankind they provided a lot in agriculture, medicine and food.

Throughout the world, the honeybee is a domestic animal and the great majority of the hundreds of species of bees live in solitary and colony. All honeybee species are social insects.

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In Asia, a total of 11 honeybee species were recorded (Michener, 2000). Some species are being domesticated and employed as pollinator and production of bee product such as honey, beeswax, bee pollen etc; were used in various ways (McGregor, 1973).

In Myanmar, (Petersen, 2005) have reported to have six species of honeybees. Five of them, *Apis dorsata*, *Apis laboriosa*, *Apis cerana*, *Apis florea* and *Apis andreniformis* are indigenous species. One of them, *Apis mellifera*, the European honeybee, a commercial species, large-scale importation of exotic species into Myanmar was done under an FAO project stared in 1979. Under this project, about 500 packages of Italian honeybee *Apis mellifera lingustica* was introduced in 1979 from Australia (Morse, 1982). *Apis mellifera* is now preferred for beekeeping. They are the main bee pollinators worldwide and pollination of plants and production of honey.

A colony of honeybee consists of a queen, several thousand workers and in a certain season of the year- a few hundred drones. Among the members of the colony there is a division of labour and specialization in the performance of biological functions. The structure of the comb of all honeybee species is especially similar: it consists of adjoining hexagonal cells made of wax secreted by worker's wax glands.

The queen, a true mother bee, is the only female that is completely developed sexually. This is a result of a total diet of royal jelly during a developmental period. She is distinguished by her long, slender appearance, due to the full development of the ovaries in her abdomen. In the colony, she is found in the area of the brood nest. The developmental time of the queen, 16 days, is the shortest.

Workers are females that are not fully developed sexually. They do the work of the colony and maintain it in good condition. Workers have special structures and organs which are associated with the duties they perform. The adult worker emerges from the cell 21 days after the egg is laid.

Drones, the males of the colony, are produced from unfertilized eggs. The queen can control whether or not the egg is fertilized as she lays it. The body of the drone is larger than that of the worker or queen. The eyes are large and cover practically the whole head. The end of the abdomen is blunt and is covered with a tuft of small hairs. Drones cannot sting. As the sting is a modified structure of the female genitalia, drones do not have stings. They also do not have any of the structures necessary to collect nectar and pollen. The developmental period of drones is 24 days (Yadav *et al.*, 2017).

Artificial insemination (AI) known as instrumental insemination, is a practice that is popularity among queen breeders. It also becomes more popular as beekeepers realize that traditional technique results in low quality queen bees, unlike the systematic practice of artificial insemination. Controlled mating is essential to achieve the goals of any breeding program. Honey bees present a unique challenge, because queens mate in flight with an average of 15 to 20 drones and therefore mating is difficult to control. Artificial Insemination (AI) provides a valuable tool to control the random mating process and is essential for bee breeding and research requiring specific crosses. Artificial insemination is an essential tool that provides complete control of honey bee mating for research and breeding purposes. The technique requires specialized equipment to anesthetize and immobilize the queen and to collect and deliver semen from the drones.

Artificial insemination is a valuable breeding technique that offers a way to utilize the desirable traits. It allows types of mating that are not possible with natural mating such as mating a queen to a single drone or to a few specific drones, mating mutant queens and drones and mating a queen to her own male offspring (Harbo, 1986a). Naturally, mated queens could mate with drones from unknown origins that could result in bees with undesirable characteristics. Artificial insemination enables bee breeders to design breeding programs in which complete genetic isolation is maintained with the ability to produce consistent, high quality queens selected for a specific trait and high brood viability (Page and Laidlaw, 1982 and Page *et al.*, 1983).

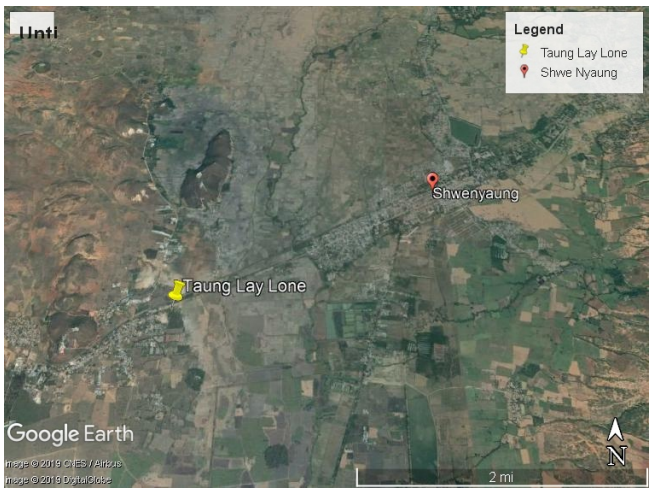
Taking these facts into consideration, the present work was conducted with the following objectives.

- to study in detail on Artificial Insemination of honeybee in Apiculture including field and practical lab works
- to compare on the survival rate of the Naturally Mated queen and Artificially Inseminated queen bees
- to investigate on the oviposition rate of the queen bee by the Artificial Insemination method

Materials and Methods

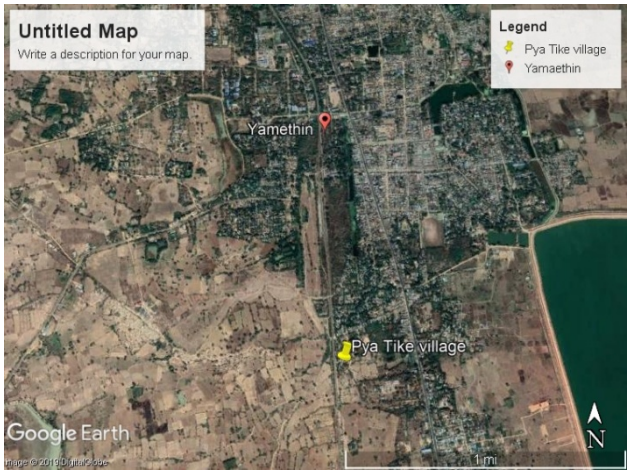
Study Sites

The present study was carried out in an apiary situated in the experimental farms including Site I near Taung Lay Lone village, Shwe Nyaung township, Shan State situated at 20°44'47.50"N and 96°54'19.19"E and Site II near Taunggoo township, Bago Region situated at 18°57'4.18"N and 96°25'57.60"E and Site III near the Pya Tike village, Yamethin, Mandalay Region situated at 20°25'6.63"N and 96° 8'15.57"E (Plate 1).



Site I (Taung Lay Lone, Shan State)

Site II (Taunggoo, Bago Region)



Site III (Pya Tike village, Yamethin, Mandalay)

Plate 1 Map of study sites (Source Google Earth, 2019)

Study Period

The field survey and data analyses were conducted from December, 2017 to December, 2018.

Preparation of Beekeeping

In general, honey beekeeping was conducted with two methods (1) stationary beekeeping (2) migratory beekeeping. In the present study, the migratory method was based on the accessibility of food sources for pollination of crops and production of honey. A single bee colony composed of one queen, approximately one hundred drones and nine thousands worker bees was kept in a wooden box containing six vertical wooden square frames with two parallel steel strings to facilitate the construction of beehive. Requirements for beekeeping – (1) Wooden box (61cm× 43cm× 34cm) (2) Wooden frames (3) Smoker (4) Hive tool (5) Bee brush (6) Bee veil (7) Uncapping knife (8) Extractor (9) Pollen trap (10) container. Data collection was carried out from 10 nucleus colony beehives at three study sites during study period.

Preparation of Project Design

Experimentation was used on the selected from the six days old virgin queen, mature drones and instrument of artificial insemination. There will be three distinct period in a year, wet season and cool season and dry season based upon the migratory method of available designated locations and places in Myanmar as outlined in this proposal. Breeding of virgin queens were prepared in the research apiary field. Before Artificial Insemination process, *Apis mellifera* L. queens were reared in process of queen rearing by the Doolittle grafting method (Doolittle, 1889).

Queen Rearing

One or two days old of young larvae were grafted to queen cups supplied with royal jelly, then transferred to a common cell builder queenless colony. The larvae were originally taken from brood nest frames of selection breeders of the apiary that maintained by the beekeepers at study sites. After maturation, queen cells were individually placed in three frame nucleus colonies. One group of 15 queen cells was randomly selected and allowed to mate naturally. The second group of 15 queen cells was confined to cages and instrumentally inseminated.

Drone Rearing

The strong colonies of honeybee for drone rearing were used. A high population of adult drones depends on a continuous supply of surplus quality pollen coming into the colony. Only pollen is essential for drone rearing. For management of drone rearing, six kinds of wooden frames are prepared for drone rearing (circle-shape, triangle shape, saw- tooth shape, plane sheet foundation, free frame). These frames are kept in the selective colonies respectively.

Procedure

Artificial Insemination (AI) process was begun in apiary and available laboratory.

(a) Ten virgin queen of six days old were selected for Artificial Insemination.

(b) Drones are selected from 16-24 days old.

(c) One day before artificial insemination, each selected virgin queen is treated with CO_2 for 5-7 minutes.

(d) After that, when the queen returned to conscious state, she was replaced into the original hive.

(e) The queen was taken out of the hive on the next day.

(f) The semen was collected from the selected drones.

(g) As the mating time of bee is very specific and different time zone of the native country, care must be taken that the time at which semen collected.

(h) Actually, the semen of 15-20 drone is sufficient for one queen to be inseminated.

(i) The virgin queen was placed in an inverted position keeping the end of abdomen upwards.

(j) Then, the vagina of virgin queen was opened and 1mm of semen was introduced first.

(k) The queen was allowed to rest for 2 minutes, then, observation was made on whether the spermatheca was activated or not.

(l) If the spermatheca was activated, the remaining 1cm of semen was inserted into the vagina.

(m) Then, one third of the wings were cut off to prevent the mating flight.

(n) The queen was placed into the queen cage and she was left for 3 minutes under observation

(o) After that she was replaced back into the original hive

(p) And then, monitoring was done whether the worker bees were accepted the replace queen or not

(q) Care must be taken for duration of insemination which should be not more than 20 minutes so as to avoid the unnecessary damage to the queen.

(r) After about the six days, it was checked whether the eggs are oviposited or not

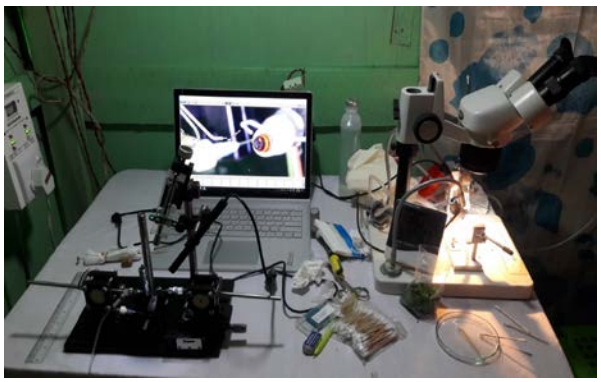
(s) Then, the queen was left in the original hive under observation for 3 months

Data Collection and Identification

Data collection was made and recorded by visiting to apiary near study site during the rearing of queen, drone, selected sample queen and drone. The same experiment was conducted in the three study sites throughout the study period. Collected data were identified according to the methods described by Bingham (1897).

Statistical Methods

Statistical analysis was conducted using SPSS (Statistical Package for Social Science) version 25. The Non-parametric ANOVA test (Kruskal Wallis) was used to test if there were significance differences of survival rates resulted from two methods, Normally Mated (NM) and Artificially Inseminated (AI) queens.



A. Equipment of Artificial Insemination

B. Insemination of Queen bee

C. Equipment of Artificial Insemination

D. Nurse Hive

E. The selected virgin queen is treated with CO_2 before AI Process

F. Collection of Drones

Plate 2 Data collection of experiments and fields

(A) AI queen of after Insemination

(B) AI queen bee and workers who accept the AI queen

(C) Inseminated AI queen covered by queen confider (D) AI mated queen and workers

Plate.6 (E) After Inseminated AI queen (F)Together with AI mated queen and workers

(G) AI Mated Queen (Yamaethin, Nay Pyi Taw)

Plate 3 Recorded on the survival rate of AI Mated Queen

Plate 4 Maintained for Mated queens in field

Results

Classification of *Apis mellifera* Linnaeus, 1758

Kingdom - Animalia
Phylum - Arthropoda
Class - Insecta
Order - Hymenoptera
Family - Apidae
Genus - *Apis*
Species - *Apis mellifera* Linnaeus, 1758
Common name - European honeybee

Survival Rates of Queen bees by Normally Mated (NM) and Artificially Inseminated (AI) Queenbees

In the three study site, total mated queen bees were recorded that 22 Normal Mated **(**NM) and 13 Artificially Inseminated (AI) queens from December, 2017 to September, 2018 (Table. 1 and 2). Comparison of study based on survival rates of queen bee both Normal mated (NM) and Artificially Inseminated (AI) supersede rates and longevity.

In the study site (I) of Taung Lay Lone, Shan State, colonies of 10 Normal Mated **(**NM) and 10 Artificially Inseminated (AI) queens were established in December, 2017. By February 2018, 8 out of the 10 Normal Mated **(**NM) queens were survived whereas no survived due to unfavorable weather condition in Shan State at that time. The process of Artificial Insemination was failed in December, 2017. In the study site (II) of near Taunggoo University, Bago Region, colonies of 10 Normal Mated (NM) and 10 Artificially Inseminated (AI) queens were established in March, 2018. By May 2018, 7 out of the 10 NM queens and 6 out of the 10 AI queens were survived. In site (III) of Yamethin, Mandalay Region, colonies of 10 NM and 10 AI queens were established in July, 2018. By September 2018, 7 out of the 10 NM queens and 7 out of the 10 AI queens were recorded.

The percentage survival rate in three study site were recorded as 22 NM queens out of 30 queens (73.3 %) and AI queens with 13 out of 30 queens (43.3 %) during 3 month study (Figure. 1 and Table. 1, 2, 3). Between study sit (I) Shan State and study site (II) Bago Region, survival rates were higher in Normal Mated (NM) than Artificially Inseminated (AI) queens as well as between Shan State and study site (III) Mandalay Region. They have showed significant differences in study sites (II and III) of relative with study site (I), respectively. At Bago Region, survival rates were slightly higher in NM than AI queens during study period. At Mandalay Region, survival rate was the same level in NM and AI queens in study period.

Table 1 Survival Rates of Normally Mated Queen Bees

Hive Number	Shan State			Bago Region			Mandalay Region		
	Dec, 17	Jan, 18	Feb, 18	March, 18	April, 18	May, 18	July, 18	August, 18	September, 18
H1	√	√	√	√	x	x	√	√	√
H2	√	√	√	√	√	√	√	x	x
H3	√	√	x	√	√	√	√	√	√
H4	√	√	√	√	√	√	√	√	√
H5	√	x	x	√	√	√	√	√	√
H6	√	√	√	√	√	√	√	√	x
H7	√	√	√	√	x	x	√	√	x
H8	√	√	√	√	√	√	√	√	√
H9	√	√	√	√	√	√	√	√	√
H10	√	√	√	√	x	x	√	√	√
Survival Number	10	9	8	10	7	7	10	9	7
Survival Rate (%)	80%			70%			70%		

Table 2 Survival Rates of Artificially Inseminated Queen Bees

Hive Number	Shan State			Bago Region			Mandalay Region		
	Dec, 17	Jan, 18	Feb, 18	March, 18	April, 18	May, 18	July, 18	August, 18	September, 18
H1	√	x	x	√	√	√	√	√	√
H2	√	x	x	√	√	√	√	√	√
H3	√	x	x	x	x	x	√	x	x
H4	√	√	x	√	√	√	√	√	√
H5	√	x	x	√	√	√	√	√	√
H6	x	x	x	√	√	√	x	x	x
H7	√	√	x	√	√	√	√	√	√
H8	√	√	x	√	√	x	√	√	√
H9	x	x	x	√	x	x	√	√	x
H10	√	x	x	√	√	x	√	√	√
Survival Number	8	3	0	9	8	6	9	8	7
Survival Rate (%)	0%			60%			70%		

Survival rates resulted from two different methods, Artificially Inseminated (AI) and Normal Mated (NM) were tested separately for the respective states, Shan, Bago and Mandalay. The survival rates of AI and NM between the Shan states was found to be statistically significant at the level of 0.05. However, the survival rates resulted from two methods in the states, Bago and Mandalay, were not significantly at 0.05 significant level.

Significance difference of the survival rates among the three states, Shan, Bago and Mandalay were tested using non-parametric ANOVA test (Kruskal Wallis) at the level of 0.01.

The significant difference was observed (Table 1 and 2). In order to get the exact specific significance test, further pair wise test was conducted by doing Bonferroni correction on the significance value (Table 1 and 2).

Non-parametric Tests (Method = AI)

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Number is the same across categories of State.	Independent-Samples Kruskal-Wallis Test	.001	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
Shan-Bago	-16.500	5.409	-3.050	.002	.007
Shan-Mdly	-18.000	5.409	-3.328	.001	.003
Bago-Mdly	-1.500	5.409	-.277	.782	1.000

Each row tests the null hypothesis that the sample 1 and sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is 0.05. Significance values have been adjusted by the Bonferroni correction for multiple tests.

The results revealed that the difference between the states, Shan and Bago and Shan and Mandalay were found to be significant. However, the two states between Bago and Mandalay were not significantly different.

Non-parametric Tests (Method = NM)

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Number is the same across categories of State.	Independent-Samples Kruskal-Wallis Test	.537	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

In the case of Normally Mated (NM), the significance differences among the three states were not significantly different at the 0.05 level.

Table 3 Comparison on the Survival Rates of Normally Mated and Artificially Inseminated Queen Bees

Study Sites	Normally Mated	Artificial Insemination Mated
Shan State	8	0
Bago Region	7	6
Mandalay Region	7	7
Totally Survival Queen Number	22	13
Mean ± SD	7.3 ± 0.58	4.3±3.76

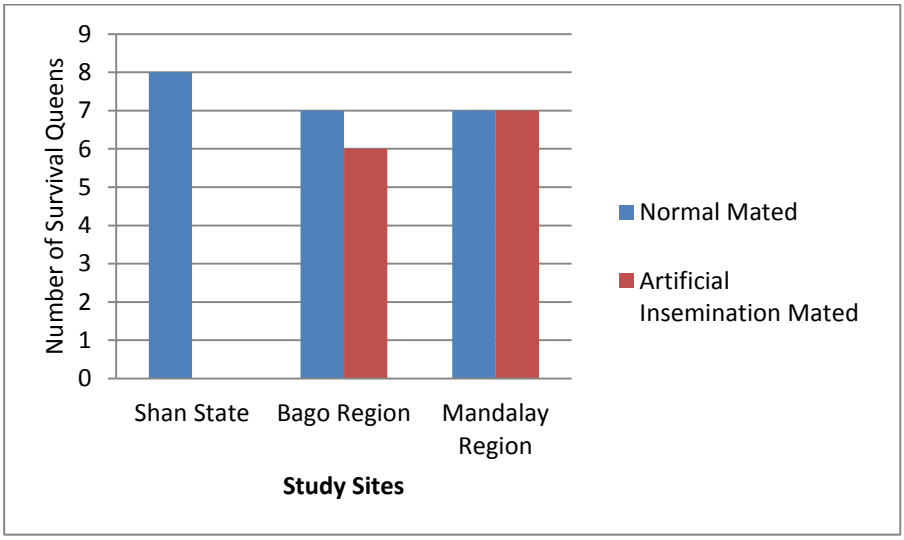


Figure 1 Comparison on the Survival Rates of Normally Mated and Artificially Inseminated Queen Bees

Plate 5 Artificially Inseminated (AI) mated queen laying eggs

Oviposition Rate of Artificially Inseminated queens

In study site (I) in Shan State, 0% oviposition rate were observed in December, 2017 as the weather condition in severely bad after insemination. In Shan State, the process of Artificial Insemination was failed and weather condition is so bad in inserted period.

At study site (II) in Bago Region, the highest percentage of oviposition rate was recorded as 41.6, 52.3, 39.7 and 47.8 in hive number of 2, 4, 6 and 7, respectively after three months (Figure 2 and Table 4).

At study site (III) in Mandalay Region, the highest percentage of oviposition rate was recorded as 51.2, 45.2, 50.3 and 33.5 in hive number of 2,5,8 and 10, respectively after three months (Figure 3 and Table 5).

Table 4 Oviposition Rate of AI mated queens found in Taunggoo, Bago Region

Hive Number	Oviposition Rate (%)		
	March	April	May
H1	20	21.8	23.02
H2	20	32.5	41.6
H3	20	18.9	13.2
H4	23	33	52.3
H5	20	20	18.3
H6	21.5	30.9	39.7
H7	22	31.28	47.8
H8	18	12	0
H9	17.8	13.45	0
H10	13.5	10.39	0
Mean Total Oviposition Rate (%)	19.58	22.422	24.54
Mean Temperature (°C)	37.48	38.35	35.36
Mean Relative Humidity(%)	42.23	43.2	60.58
Mean Rainfall (mm)	0	12.67	19.58

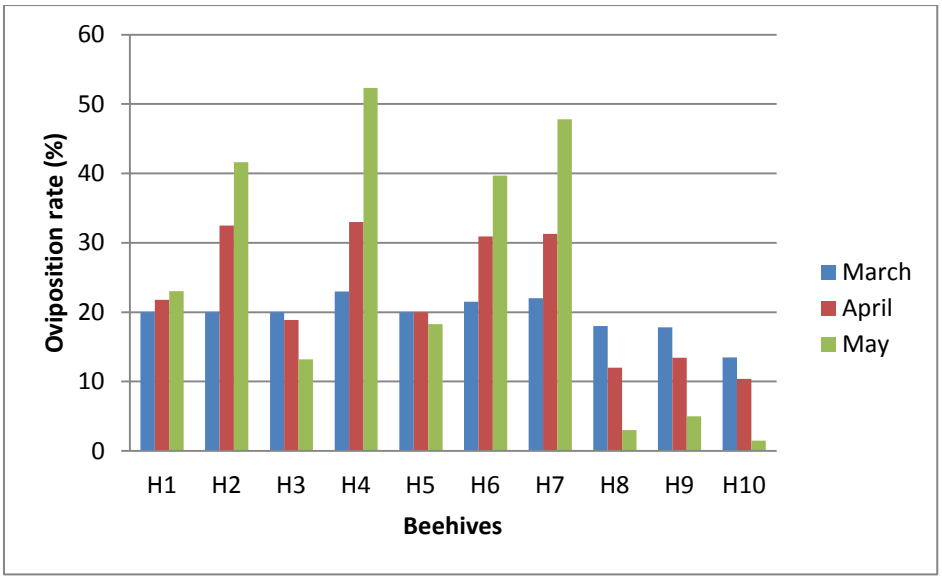


Figure 2 Oviposition Rate of AI mated queens found in Taunggoo, Bago Region

Table 5 Oviposition Rate of AI mated queens found in Yamethin, Mandalay Region

Hive Number	Oviposition Rate (%)		
	July	August	September
H1	19.3	25.57	27.8
H2	20	29.05	51.2
H3	15	11.5	9.23
H4	21	24.89	26.5
H5	25	30.6	45.2
H6	16.5	15	7.1
H7	20	19.5	28.6
H8	20	28.4	50.3
H9	20	14	12
H10	20	27	33.5
Mean Total Oviposition Rate (%)	19.68	22.551	29.143
Mean Temperature (°C)	32.47	32.22	33.22
Mean Relative Humidity(%)	70.09	71.61	68.8
Mean Rainfall (mm)	6.92	11.78	11.7

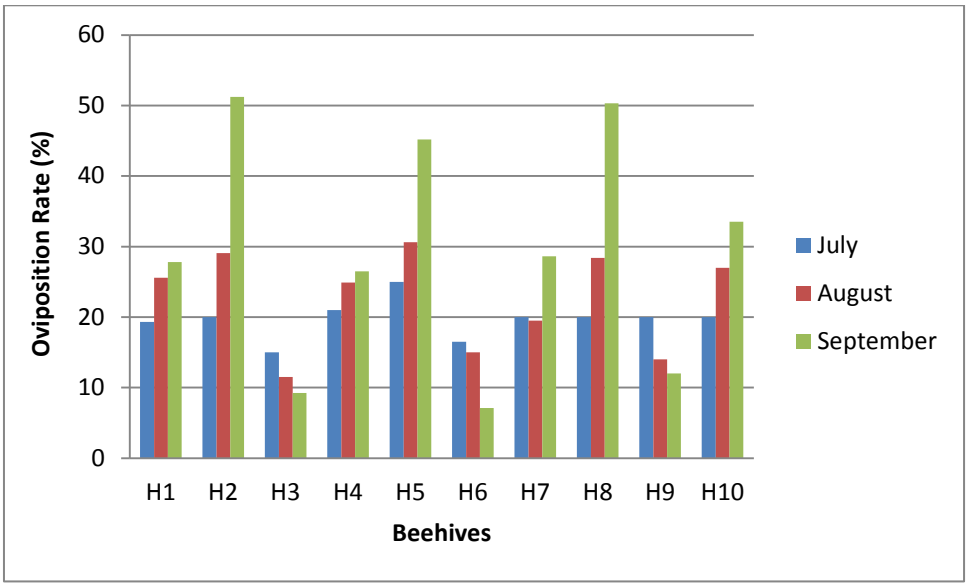
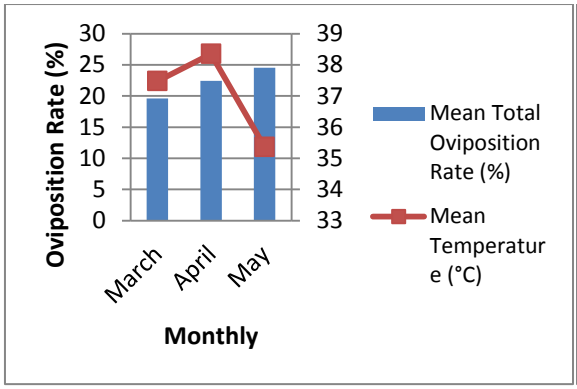


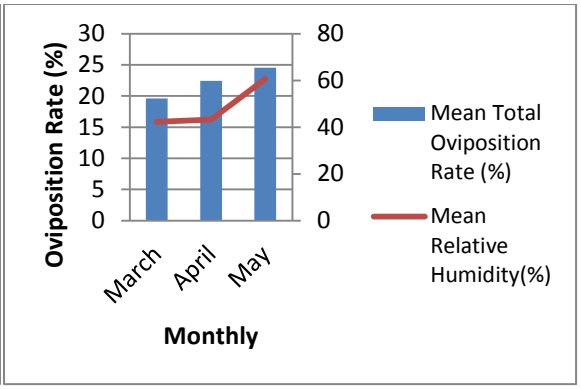
Figure 3 Oviposition Rate of AI mated queens found in Yamethin, Mandalay Region

Relationships between Oviposition Rate of Artificially Inseminated queens and Weather conditions in three Study Sites

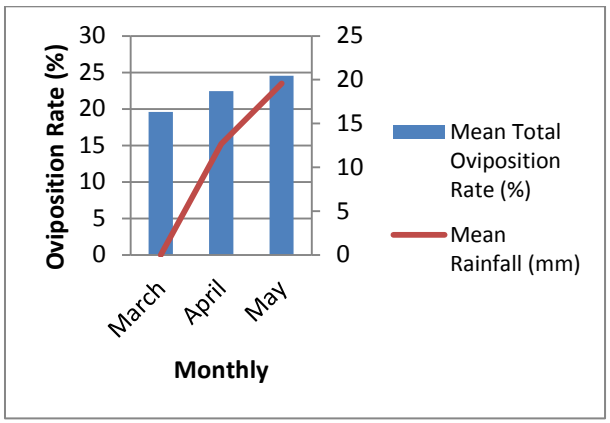
At study site (I) in Shan State as oviposition rate was observed 0 percent due to severe weather condition. At study site (II), in Taunggoo, Bago Region, oviposition rate increased in May as the mean temperature decreased from 37.48 (°C) to 35.36 (°C). Regarding relative humidity, the higher the relative humidity in May was 60.58(%), the greater the oviposition rate, which was 24.54%. For the mean rainfall, the oviposition rate increased to 24.54% as the mean rainfall increased from 0 mm to 19.58mm (Fig 4 and Table 4).



(A) Temperature



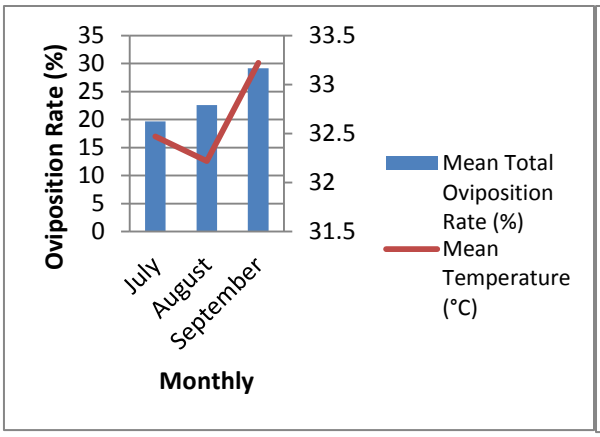
(B) Humidity



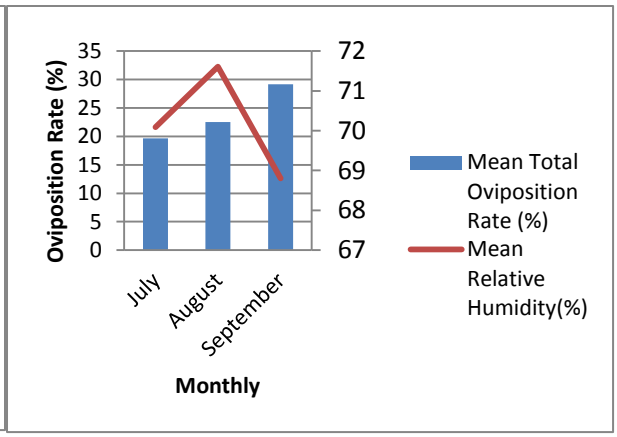
(C) Rainfall

Figure 4 Relationships between Oviposition Rate of Artificially Inseminated queens and Weather conditions at Study Sites (II) in Bago Region

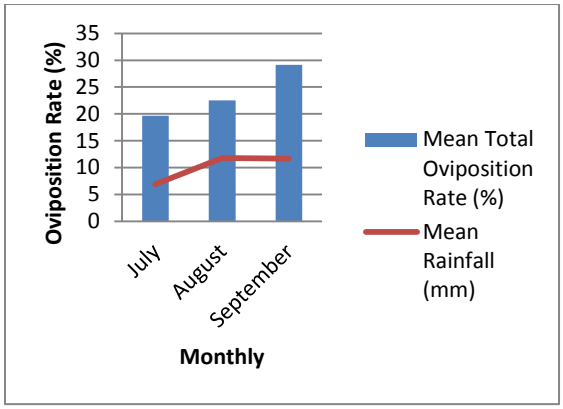
At study site (III) in Yamethin, Mandalay Region, oviposition rate increased in September as the mean temperature increased from 32.47(°C) to 33.22(°C) were recorded. Reguarding relative humidity, the lower the relative humidity in September was 68.8(%), the greater the oviposition rate, which was 29.14%. For the mean rainfall, the oviposition rate increased as well as the mean rainfall increased from 6.92 mm to 11.7 mm (Fig 5 and Table 5).



(A) Temperature



(B) Humidity



(C) Rainfall

Figure 5 Relationships between Oviposition Rate of Artificially Inseminated queens and Weather conditions at Study Sites (III) in Yamethin, Mandalay Region

Discussion

In study period, the results of this study indicate similarity in performance levels of field colonies headed by Normally Mated (NM) and Artificially Inseminated (AI) queens.

At study sites (II) and (III), the survival rate Normally Mated (NM) queens and Artificially Inseminated (AI) queens more or less similar in their field performance. The results revealed that the difference between the states, Shan and Bago and Shan and Mandalay were found to be significant. However, the two states between Bago and Mandalay were not significantly different. In the case of Normally Mated (NM), the significance differences among the three states were not significantly different at the 0.05 level. However, the survival rate of Normally Mated (NM) queens is slightly higher than of Artificially Inseminated (AI) queens. At study site (I) in Shan State, no survival rate in both Normally Mated (NM) and Artificially Inseminated (AI) queens due to severe weather condition. This indicates that the weather condition is greatly influence on the behaviour of drones and Normally Mated (NM) queens which inhibit mating. It is also influence on Artificially Inseminated (AI) queens as they are sensitive to ambient temperature, humidity and rainfall.

Although NM queens showed higher oviposition rates no significant differences were found between the two groups. NM queens showed a slightly higher survival rate than AI queens during the three month study.

In study sites (II) of Bago Region, the highest number of oviposition rates as 41.6, 52.3, 39.7 and 47.8 in hive number of 2, 4, 6 and 7, respectively after three months were recorded. In study sites (III) of Mandalay Region, the highest number of oviposition rates as 51.2, 45.2, 50.3 and 33.5 in hive number of 2,5,8 and 10, respectively after three months were recorded. In study site (I) of Shan State, the process of Artificial Insemination was failed in December, 2017.

Nelson and Laidlaw (1988) found that brood area and honey weight were similar in colonies headed by NM and AI queens. NM queens were significantly heavier than AI queens only on arrival; two months later no differences were found. Wilde (1989) found that only in the first year of a two-year study, NM and AI queens were similar in their production of brood and

honey. In the second year, AI queens produced significantly higher brood and honey than NM queens.

In this study, Artificial Insemination queens were recorded to have many problems and changeless with initial introduction and acceptance. The results indicates that the optimum temperature for high oviposition rate assume to be between 33.22 °C and 35.36 °C depending on the locality and food sources. Regarding relative humidity and rainfall, the more relative humidity and rainfall, the higher the oviposition rate of both NM and AI queens in both study sites (II) and (III) were recorded.

Artificial Insemination technique is widely employed in queen breeding programs for the improvement of honeybee races to have best colony performance. Because of conflicting reports in the literature, research is still needed to study factors that affect rearing, insemination and introduction of AI queens that would lead them to have better evaluations. The present study has been undertaken to compare the performance of naturally mated and instrumentally inseminated queens for survival and oviposition rate.

Nelson and Laidlaw (1988) suggested that AI queens perform as successfully as NM queens and these findings contrary to this might be due to the lack of some special beekeeping and/ or insemination procedures given to AI queens. Conditions that had led to have AI queens exceed NM queens in field performance (Wilde, 1989; Szalai, 1995) should be utilized and improved.

The present study was conducted only within short duration due to migratory behavior of honeybee, limited time for available food sources and sensitive to ambient weather conditions. However, the main aim of the present study is to promote the high quality queens to small scale beekeeping of area by Artificial Insemination technique.

Conclusion

Field performance of AI queens is influenced by many factors, problems and changeless. Nevertheless, the present investigation shows that successful insemination and high performance levels can be achieved when careful attention is given to queen rearing conditions, pre- and post-insemination treatments, semen doses and quality and technique of insemination utilized. Controlled mating is essential to improve honeybee-breeding stock and to maintain high performance in field colonies. Artificial insemination is an essential tool that provides complete control of honey bee mating for research and breeding purposes. This research of breeding high quality of queen bee by the Artificial Insemination techniques that one can apply to promote the quality of Myanmar honey product. As part of the research study, the proposer believes that this work will contribute to the local beekeepers and Department of Apiculture, Ministry of Agriculture, Livestock and Irrigation in Myanmar. Artificial insemination is a viable and insured method that bee breeders and beekeepers can rely on for that purpose.

Acknowledgements

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MORPHOLOGY AND LIFE CYCLE OF THE PIERID BUTTERFLY SPECIES, *DELIAS HYPARETE INDICA* WALLACE, 1867

Khin Mi Mi Oo[1]

Abstract

The colour of head is deep black and rounded in shape. Two compound eyes lie on either side of the head. Long coiled proboscis, 13 to 15 mm in length, lies between the eyes that is creamy white colour. Labial palps tapered towards the tip have three segments named basal, middle and terminal with white hairs and 3.3 to 3.5 mm in length. Black antenna, which has 17 to 20 mm in length has 35 to 36 segments. In male the shape of forewing is more or less triangular. The base colour of upperside of forewing is white. Black colour veins are network on it. The hindwing is more or less rounded shaped or oval-shaped. The base colour of upperside is pale orange white. Weak veins are present on it. In the female, the forewing is mostly triangular in shape. The base colour of upper surface of forewing is black and white. The hindwing is mostly oval-shaped. On the upper surface, various colours are present with black coloured veins. Life cycle of the Pierid butterfly, *Delias hyparete indica* Wallace, 1867 was studied during the study period from June 2017 to May 2018 in Hpa-an University in Kayin State. The mated female mostly laid the eggs on the leaves of *Dendrophthoe pentandra* (L.) Miq. and *Loranthus pentapetalus* Roxb. A single batch of eggs laid by the mated female consisted of 31 to 45 eggs. The entire life cycle from egg to the emergence of adult from the pupa lasted for 31 to 38 days. The various developmental stages and the time taken for each stage were recorded and presented with tables and figures. Life span of male was shorter than that of female.

Keywords: Pierid butterfly, morphology, life cycle, developmental stages

Introduction

Butterflies are beautiful elegant creatures attracting the onlookers to see them flitting from flower to flower, in an apparently aimless way, fluttering and dancing as they go. However, most people know very little about the life of the butterfly. Most butterflies are inherently very suitable for conservation by breeding programmes in protected environments. An adult female will lay dozens, perhaps hundreds of ova. Even under "natural" conditions in the wild, almost all of these will fail to reach the adult stage. If habitats and food plants are destoryed, all butterfly species are likely to become extinct, Lewis (1985). Kunte (2000) stated that coloration and venation patterns on the wings are the principal diagnostic features of butterflies. In addition fold their wings erect over their body, partly or completely covering the hindwings with the forewings.

The relationship between butterfly species and the plants plays an important role in an ecosystem. Flowering plants need butterfly species for pollination and the butterflies require suitable plant species to serve as their host plants to complete their life cycle (Carter, 1992). The species *Delias hyparete indica*, family Pieridae is one of the most attractive, fragile and interesting species distributed throughout the year in Myanmar. Every butterfly species varies in the complexity of life. *Delias hyparete indica* was selected to study morphology and different developmental stages in this research.

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Material and Methods

Study area and period

Hpa-an University Campus with the coordinates of 17˚21′43.2″ N and 97˚40′25.9″ E, at Hpa-an Township, Kayin State was chosen as study area. The study period lasted from June 2017 to May 2018. (Fig 1)

Specimen collection

The adult butterfly specimens were captured aided by a butterfly net from selected natural environments. The captured butterflies were gently transferred into plastic basket laid with flowers and leaves to avoid mechanical injuries. The eggs, larvae and pupae were also collected from the host plants and placed in boxes to rear them in captivity.

Rearing process in captivity

The eggs on the leaves of the host plant were kept separately in rearing boxes (10cm x 8cm) at the temperature of 30˚C and humidity between 70% to 80% until the first larva hatched from the egg. The newly hatched larvae were individually placed in a cleaned rearing box with moistened leaves of the host plant. The larvae were reared in this manner until the larvae stopped feeding before transforming into pupae. The newly formed pupae attached to the substratum of the box or to a branch of the host plant (kept in the rearing box) were kept separately in thoroughly cleaned rearing boxes until the adults emerged from them. The duration taken to transform from one stage to the other was recorded to determine the duration of the entire life cycle.

Identification of the adult butterfly

Identification of the butterfly species is based on wing venation, parts of the head and legs accordin to Bingham (1907) and Talbot 1939).

Preparation of wing venation and the required parts for identification

The 10% potassium hydroxide solution was prepared by mixing 10g of potassium hydroxide crystals in 100 ml of water keeping for one night. Head parts and legs were disarticulated from the body and examined under a dissecting microscope. Scaled photographs were taken with a stereomicroscope (DN-117M).

Figure 1 Satellite map of study site (**Source** Google earth, 2018)

Results

Life cycle of Pierid Butterfly species, *Delias hyparete indica* **Wallace, 1867**

In Pierid butterfly *Delias hyparete indica*, development from egg to adult had four life stages, the egg, five larval stages, pupa and adult stages.

Egg

The recorded number of eggs varied with the type of host plant species. The eggs in a single batch were recorded as 37 to 45 eggs on mistletoe of mango plant and 23 to 35 eggs on mistletoe of wood-apple (Thanakha in Myanmar) plant (Fig 2). The size of fleshly laid eggs ranges from 1.05 to 1.20 mm in length and 0.62 to 0.73 mm in width. The egg is short-necked flask-shaped and the colour is white with longitudinal ridges (Plate 3 A and Table1). The first larva was hatched out from the egg within five to six days after being laid.

First larval stage

The white colour gradually turned orange within one day. The size of newly hatched first larva ranged from 1.00 to 3.00 mm in length and 0.50 to 1.00 mm in width. The first larva stage lasted for three to four days after hatching (Plate 3 B, Fig 2 and Table 1).

Second larval stage

The size of the second larva ranged from 3.00 to 4.00 mm in length and 1.00 to 1.50 mm in width. The larva molted into the third stage within two to three days when the outer covering could no longer accommodate the increased size (Plate 3 C, Fig 2 and Table 1).

Third larval stage

The size of the third larva ranged from 4.00 to 10.00 mm in length and 1.50 to 2.00 mm in width. The body was similar in colour to that of the second larva and the head was also black. The third larva molted after five to six days to transform into the fourth stage (Plate 3 D, Fig 2 and Table 1).

Fourth larval stage

The size of the fourth larva ranged from 10.00 to 21.00 mm in length and 2.00 to 3.00 mm in width. The body colour differed from the host plant leaves and became brighter. The duration of the fourth larva lasted from four to five days before molting into the fifth larva stage (Plate 3 E, Fig 2 and Table 1).

Fifth larva stage

The size of the fifth larva ranged from 21.00 to 35.00 mm in length and 3.00 to 5.00 mm in width. The fifth larva stops feeding after five to six days preparing to pupate by attaching with the last segment to the cover of the box (Plate 3, Fig 2 and Table 1).

Prepupating stage

The size of prepupa ranged from 21.00 to 23.00 mm in length and 4 to 5.00 mm in width. It lasted only one day (Plate 3 G, Fig 2 and Table 1).

Pupal stage

The size of the pupa ranged from 18.00 to 25.00 mm in length and 5.00 to 6.00 mm in width. The adult emerged from the pupa usually after six to seven days (Plate 3 H, Fig 2 and Table 1).

Adult stage

The whole wing span is between 72 to 88 mm in length. The colour of head is deep black and rounded in shape. Two compound eyes lie on either side of the head. Long coiled proboscis, 13 to 15 mm in length, lies between the eyes that is creamy white colour. Labial palps tapered towards the tip have three segments named basal, middle and terminal with white hairs and 3.3 to 3.5 mm in length. Black antenna, which has 17 to 20 mm in length has 35 to 36 segments. Its apex is club-shaped. Three segments, prothorax, mesothorax and metathorax are present in thorax. Its upper and lower sides are deep black colour. Legs are well-developed from it and creamy yellow coxa, trochanter and femur are present. Segmented tibia is dark brown with minute hairs and tapers towards the tip terminating with claws. In abdomen, dorsal side is black, ventral and dorsal sides are creamy-white. A pair of valves tapered at the posterior end of the abdomen.

In male the shape of forewing is more or less triangular. The base colour of upperside of forewing is white. Black colour veins are network on it. The hindwing is more or less rounded shaped or oval-shaped. The base colour of upperside is pale orange white. Weak veins are present on it. In the female, the forewing is mostly triangular in shape. The base colour of upper surface of forewing is black and white. The hindwing is mostly oval-shaped. On the upper surface, various colours are present with black coloured veins (Plate 2 and Table 1).

Recorded host plants

Host plant of *Delias hyparete indica* were recorded as *Dendrophthoe pentandra* (L.) Miq. (Thayetkyibound) and *Loranthus pantapetalus* Roxb. (Thanakhakyibound) under family Loranthaceae (Plate 1).

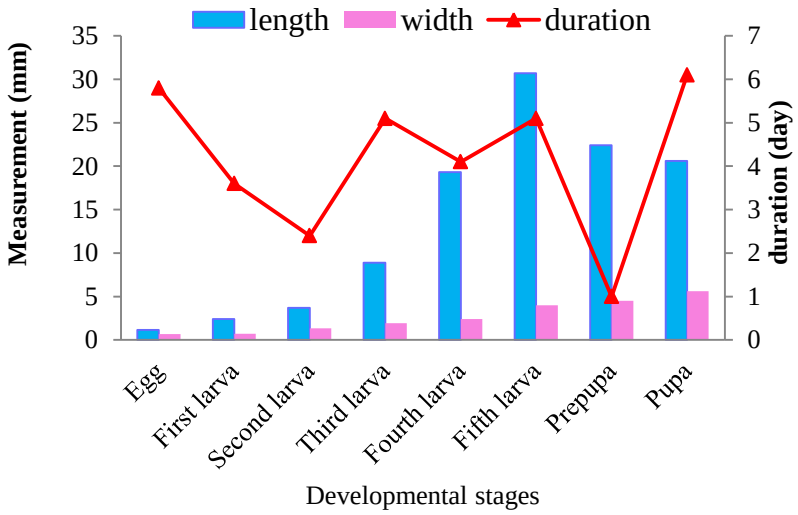


Figure 2 Measurement and duration of various stages (egg to pupa) of life cycle

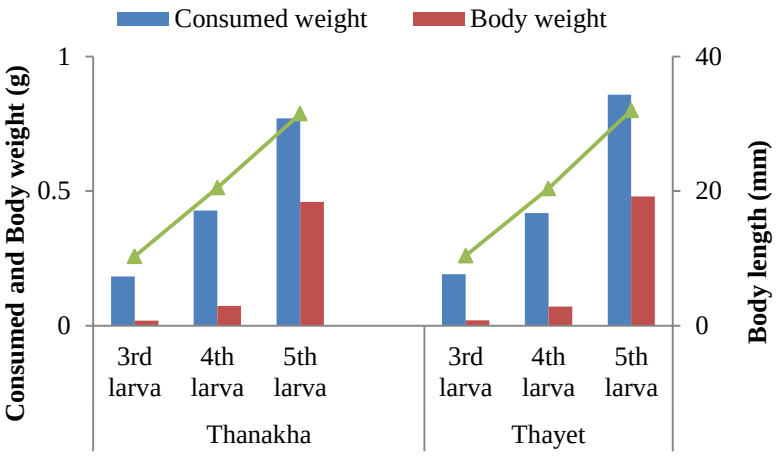


Figure 3 Relationship of consumed weight, body weight and body Length according to host plants Thanakhakyibound and Thayetkyibound

(A) *Dendrophthoe pentandra* (L.) Miq. **(**Thayetkyibound**)**

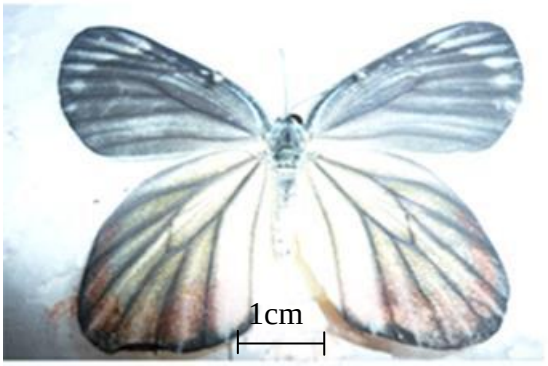
(B) *Loranthus pentapetalus* Roxb. **(**Thanakhakyibound**)**

Plate 1 Host plants of *Delias hyparete indica*

(A) Wing venation

(B) Upperside (male)

(C) Underside (male)



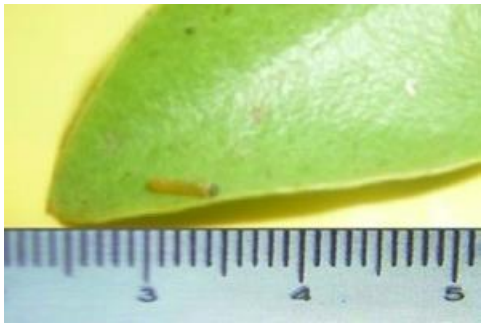
(D) Upperside (female)

(E) Underside (female)

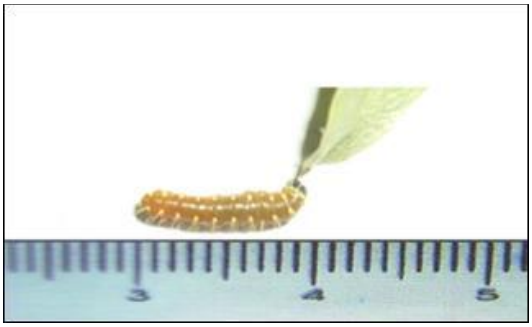
Plate 2. Wing venation and external morphology of *Delias hyparete indica*

(A) Egg (60 ×)

(B) First larval stage



(C) Second larval stage



(D) Third larval stage

(E) Fourth larval stage

(F) Fifth larval stage

(G) Prepupa

(H) Pupa

Plate 3 Developmental stages of *Delias hyparete indica*

(A) Protruding head of the adult from the split end of the pupa

(B) Partially emerged adult

(C) Emerged adult attached to the skin of pupa

(D) Emerged adult with hardened wings ready for flight

Plate 4 Emergence of adult from the pupa

Table 1 Measurement of various stages from egg to adult and duration of life cycle of *Delias hyparete intica*

Sr. no	Stage	No Observed	Min (mm)	Max (mm)	Mean±SD	Duration (days)		Mean±SD
						Min	Max	
1	Egg	10				5	6	5.8 ± 0.42
	Length		1.05	1.20	1.14 ± 0.05			
	width		0.62	0.73	0.68 ± 0.03			
2	1st larva	10				3	4	3.60 ± 0.52
	Length		1.00	3.00	2.40 ± 0.70			
	width		0.50	1.00	0.71 ± 0.23			
3	2nd larva	10				2	3	2.40 ± 0.52
	Length		3.00	4.00	3.70 ± 0.48			
	width		1.00	1.50	1.35 ± 0.24			
4	3rd larva	10				5	6	5.10 ± 0.32
	Length		4.00	10.00	8.90 ±1.85			
	width		1.50	2.00	1.95 ± 0.16			
5	4th larva	10				4	5	4.10 ± 0.32
	Length		10.00	21.00	19.30 ± 3.50			
	width		2.00	3.00	2.40 ± 0.52			
6	5th larva	10				5	6	5.10 ± 0.32
	Length		21.00	35.00	30.70 ± 4.56			
	width		3.00	5.00	4.00 ± 0.94			
7	Prepupa	10				1	1	1.00 ± 0
	Length		21.00	23.00	22.40 ±0.70			
	width		4.00	5.00	4.50 ±0.53			
8	Pupa	10				6	7	6.10 ± 0.32
	Length		18.00	25.00	20.60 ± 2.59			
	width		5.00	6.00	5.60 ± 0.52			
9	Adult							
	Male							
	Length	10	72	88	79.20±7.30	1	2	1.50±0.53
	Famale							
	Length	10	68	86	74.00±8.38	1	2	1.80±0.42

Discussion

The aspect of morphology and life cycle of the pierid butterfly species *Delias hyparete indica* from Hpa-an University in Kayin State was conducted during June 2017 to May 2018. Club-shaped antennae of *D. hyparete indica* is one of the differences between moths and studied butterfly species. Prominent orange patches on the margins of hindwings of male distinguished it from the female where the orange patches along the margin of hindwings are comparatively faint. This morphological characters are in agreement with the descriptive accounts as reported by Kunte (2000).

The eggs were found on either the upper or lower surfaces of young leaves of the host plants during study period. The mated female usually laid 23-45 eggs depending on the host plants.

The complete life cycle of *D. hyparete indica* lasted for a period of 31 to 38 days. Like other butterfly species, four stages namely egg (five to six days), larva (19-24 days), pupa (seven

to eight days including prepupa) and adult (10-16 days) are included. Metabolic process in the pupa (resting stage) is related to the process of development into the adult took place in the pupa.

Aung Cho (2007) stated that *Eurema hecabe* (Linnaeus, 1758), one of the species in family Pierdae lasted for a period of 20-27 days to complete the life cycle. The longest period was recorded in the pupa which took seven to eight days before the emergence of the adult. The duration of each developmental stage was recorded as two to three days before transforming into the pupa. The increase in size with minor development could occurred within two to three days in the previous stages of the development. The number of egg in one batch was 25-60 depending on its host plants. The mated female laid its eggs not only on the upper surface but also on the lower surface of the host plants.

Thida Swe (2007) stated that *Hebomonia glaucippe* (Linnaeus, 1758) one of the species of the family pieridae laid 17-37 eggs on their host plants and the life cycle lasted about 28-34 days.

Conclusion

The study species of *Delias hyparete indica* completed its all developmental stages in Hpa-an University Campus. Grassland, cultivated area, open area, scattered tree, human habitation, areal area and dense tree in the study site serve as good habitats of butterfly species. The present study provides as the university record of butterfly and also provides as a baseline information for further study.

Acknowledgements

I am greatly indebted to Dr. Myint Myint Kyi, Professor and Head of Zoology Department, Hpa-an University for her valuable advice and Dr. Khin Lay Nwe, Associate Professor of same Department, for her valuable suggestions.

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SEASONAL VARIATIONS OF ZOOPLANKTON SPECIES AT THE KANTHARYAR LAKE OF HLAWGA WILDLIFE PARK, MINGALARDON TOWNSHIP, YANGON REGION

Hsu Mon Aung[1], Yee Yee Htwe[2], Yee Yee Lwin[3]

Abstract

Seasonal variations of zooplankton species at the Kantharyar Lake of Hlawga Wildlife Park, Mingalardon Township, Yangon Regionwas conducted during June, 2017 to January,2019. A total of 52 zooplankton species of two phyla, four classes, seven orders, 23 families and 36 genera were collected from the study site. The rotifers and copepods were dominant species in the lake. The abundance of zooplankton was highest in the wet season and the lowest in the cold season. Among the species, *Brachionus falcatus* was the most abundant and *Calanoides carinatus* was least abundance in study sites. During the study period the maximum number of zooplanktons were recorded in July and the minimum number of zooplankton in January.

Keywords: zooplankton, seasonal distribution, weather parameter, correlation

Introduction

Freshwater is the most essential requirement for life and yet comprises only < 1% of the Earth's surface water. Water is the key substance for the survival of all organisms in this globe (Bera *et al.*, 2014). Zooplankton is drifting microorganisms movement by the water current that are importance in the fresh water and marine water ecosystems of biosphere. Plankton is a part of aquatic life, which is composed of tiny organisms, living and drifting in the direction of water current. It is the main source of food for most fauna of lotic and lentic water ecosystem. Freshwater zooplankton is an important biological component in aquatic ecosystem, whose main function is to act as a primary and secondary links in the food chain (Sebastian *et al.*, 2014). Zooplankton play a vital group of organisms that transfer energy from the nutrient cycle, the algae, to the higher trophic levels such as fish. Zooplankton constitute important food item of many omnivorous and carnivorous fish fry and prawn fry because it supply the necessary amount of proteins required for rapid growth and development of different organs of fish (Mozumder and Naser, 2009).

They eats step by step to become the higher energy flow from aquatic ecosystem. Three kinds of namely rotifers (Phylum Rotifera), copepods and cladocerans, (Phylum Arthropoda)are the members of zooplanktons. Three major zooplankton groups dominate freshwater ecosystems (rotifer, copepoda and cladocerans). Rotifers have widely been used as biological indicators in studies due to their sensitivity to different levels of water quality parameters (Radix *et al.*, 2002). Copepods are used as biological indicators for certain ecosystem (AItaff and Chandran, 1995; Aman and AItaff, 2004). Copepods unlike other zooplanktons have a much wider adaptation to unfavorable climate (Reid and Williamson, 2010) and are also reported to be the most abundant members of the zooplankton population.They are food sources of aquatic organisms in the water ecosystem (Gannon and Stemberger, 1978; Gajbhiye and Desai 1981).

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The zooplankton which play a role of converting phytoplankton into food, suitable for fish and aquatic animals have acquired importance in fishery research. The plankton can also play an important role in indicating the presence or absence of certain species of fishes on in determining the population densities (Jayabhaya, 2009).

Zooplanktons are affected by environmental conditions and can rapidly respond to environmental change. They are good indicator of water quality because they are strongly affected by environmental conditions and due to their short life cycle, these communities often respond quickly to environmental change and water quality. Zooplanktons play an important role in indicating the eutrophication status and productivity of a freshwater body. Planktons not only increases fish production but also helps in bioremediation of heavy metals and other toxic material. Plankton can also as biomarker for water quality assessment for fish production (Pradhan *et al*., 2008). Distribution of zooplankton community depends on complex factor (change of climatic condition, physical and chemical parameter and vegetation cover) (Mikschi,1989). Diversity and abundance of zooplanktons are also indicators of the water ecosystem. Hlawga wildlife park is one of the important high diversity area in Myanmar. No or little investigation of the zooplankton diversity of freshwater ecosystem of Hlawga wildlife is conducted yet. Thus, the present study was conducted to know the zooplankton population in different sites of Hlawga wildlife park including seasonal aspects by the following aims and objectives;

- to record the occurrence of zooplankton species in the studied area
- to analyze the correlation of the distribution of zooplankton and weather parameters
- to assess the seasonal variation of zooplankton species

Materials and Methods

Study area and Study period

The lake of Hlawga Wildlife Park, Mingaladon Township, Yangon Regionwas chosen as study area. Hlawga Wildlife park was constituted in 1982 and is located between Longitude 96° 05' E to 96° 08' E and Latitude 17° 17' N to 17° 42' N, Approximately 35kmand are span over (1540 acres) north of Yangon. It is situated in the north of Mingaladon – Insein area, to the west of the Yangon- Pyay road and adjacent to the township of Taukkyan. Six study sites were chosen to assess the population structures. The study period lasted from June 2017 to Jan, 2019(Fig.1 and Plate 1).

Data collection

Water samples were monthly collected from six study sites of in the lake of Hlawga Wildlife Park. The collection was done between 8-10 am in the morning. Water sample from site III was taken first at 9:30 am. Then site IV, site I, site V, site II, site VI with the interval of 30 minutes. Plankton net with the mesh size of 100 μm was used to collect with the zooplankton. The mouth diameter of the net was 26 cm with 40 cm handle. The plankton samples were collected by filtering 60 liters of water through the plankton net. After collection, the plankton samples were filtered into the plastic bottles. A standard volume of 480ml were mixed and preserved in 4% formalin. The preserved plankton specimens were examined in under stereomicroscope. The water sample brought to the laboratory of Zoology Department, University of Yangon for further identification (Plate2).

Identification

Identification of the zooplankton species was made according to Davis (1955), Edmondson (1959)and Shiel (1995).

- "Lac Keys" dropping method (1935) , the following formula;

$$\text{Zooplankton / Liter} = \frac{N \times C}{Y} \times 10$$

N = Number of zooplankton counted in 0.1 ml. concentrate

C = Total volume of concentrate in ml

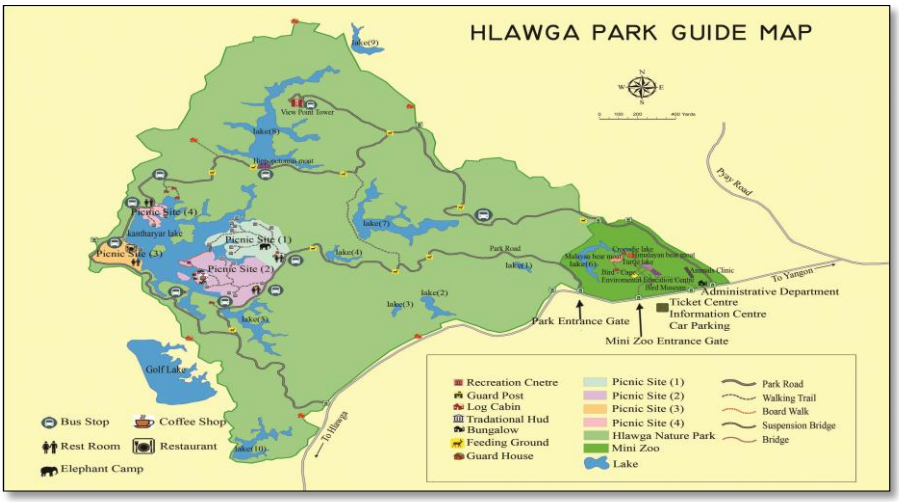
Y = Total volume of water filtered for sample in liters

Weather parameters

Monthly weather data of rainfall and humidity during the study period (2017-2018) were obtained from the Department of Meteorlogy and Hydrology, Mayangone Township in Yangon Region.

Data analysis

Pearson correlation coefficient was computed to analyze the relationship between abundance of the species and weather parameters and statistical analyses was made by Analysis of Variance (ANOVA) to determine significances of the species compositions. All analyses of data were conducted using Statistical Package for Social Science (SPSS) version 16 while graphics were performed by Excel program.



(**Source:** Trustee Office, Hlawga Wildlife park, 2017)

Figure 1 Map of Hlawga wildlife park showing study area

1.Site - I
Between east and north)

2. Site -II
(Between east and south)

3. Site-III
(Between south and west)

4.Site IV
(Between west and north)

5. Site V
(Between south and north)

6. Site VI
(Between south and north)

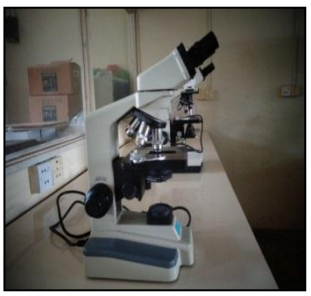
Plate1. Selected sampling sites of the study area

1.Boat used for water sample

2. Drifting water through plankton net

3. Plankton net

4. Equipment and apparatuses



5. Stereomicroscope

Plate2. Equipment and apparatuses used for collection and identification of the specimens

Results

A total numbers of 52 species of zooplanktons, under 23 families, seven orders, belonging four classes and two phylum of zooplanktonwere collected from the six different sites in the lake of Hlawga Wildlife park. Among them Phylum Rotifera comprised of Class-Monogononta and Digononta. Class-Monogononta was belonging to 30 species, 21 genera, 16 families of three orders. Class Digononta was belonging to three species, three genera, two families of only one order. And also Phylum Arthropoda comprised of class Branchiopoda (Cladocerans) and Maxillopoda (Copepods).Class Branchiopoda was belonging to eight species, five genera, three families of one order anf class Maxillopoda with 11 species, seven genera, two families of two orders (Table 1 and Plate 3,4 and 5).

In the study period, Class Monogononta covered58 % of the total harvest indicating the highest recorded and followed by Class Maxillopoda with21 % Class Branchiopoda with 15% and the lowest Class Digononta with 6% were recorded respectively (Fig.2).

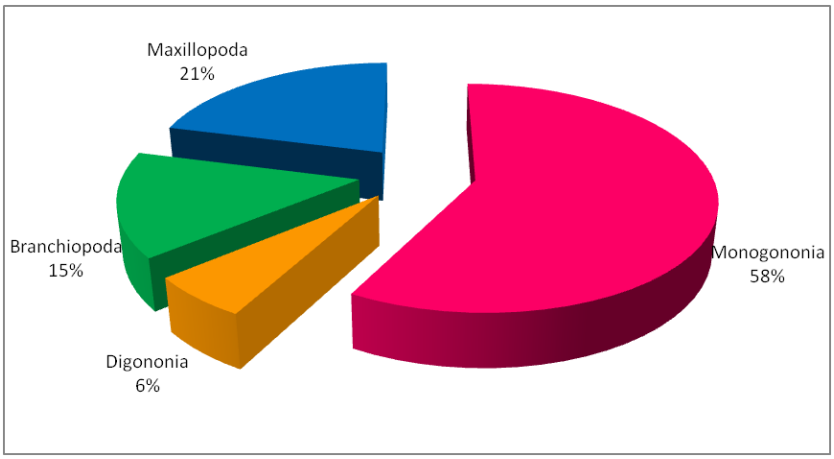


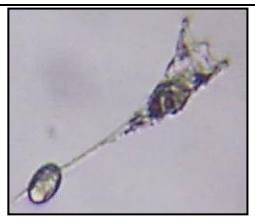
Figure2 Percentage of species occurrence among the class of recorded zooplanktons group in the study area

Table 1 Occurrence of zooplanktons in the study area

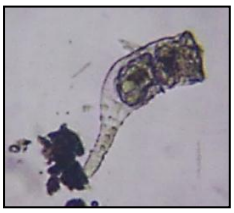
No	Phylum	Class	Order	Family	Species name
I	Rotifera	Monogononta	Collothecacea	Collothecidae	1.*Collotheca mutabilis* (Hudson, 1885)
			Flosculariaceae	Conochilidae	2. *Conochilus coenobasis* (Sudzuki, 1964)
				Filiniidae	3. *Filinia longiseta* (Ehrenber,1834)
					4. *Filinia terminalis* (Aacharias,1898)
				Flosculariidae	5. *Ptygura longicornis* (Davis, 1867)
					6. *Sinantheria socialis* (Linne,1758)
				Hexartharidae	7. *Hexarthra propinqua* (Bartos, 1947)
				Testudinellidae	8. *Pompolyx sulcata* (Pejler,1957c)
			Ploima	Asplanchnidae	9.*Asplanchna priodonta* (Gosse,1850)
				Brachionidae	10.*Anuraeopsis fissa* (Gosse, 1851)
					11.*Brachionus angularis* (Gosse, 1851)

No	Phylum	Class	Order	Family	Species name
					12.*Brachionus calyciflorus* (Pallas, 1766)
					13.*Brachinous caudatus* (Barrois & Daday,1894)
					14.*Brachionus falcatus* (Zacharias, 1898)
					15.*Brachionus plicatilis* (Muller, 1786)
					16.*Keratella cochlearis* (Carlin, 1943)
					17. *Keratella valga* (Ehrenberg, 1834)
					18. *Plationus patulus* (Ahlstrom, 1940)
				Colurellidae	19. *Colurella obtusa* (Gosse, 1886)
				Epiphanidae	20.*Mikrocodides chlaena* (Gosse, 1886)
					21.*Proalides tentaculatus* (Barrios & Daday,1894)
				Gastropodidae	22. Ascomorpha ovalis (Carlin, 1943)
				Lecanidae	23. *Lecane mira* (Myers, 1926)
				Mytilinidae	24. *Mytilina mucronata* (O.F.Muller,1773)
				Synchaetidae	25. *Polyarthra vulgaris* (Carlin, 1943)
			Ploima	Trichocercidae	26. *Trichocerca elongate* (Gosse,1886:E)
					27. *Trichocerca cylindrical* (Imhaf,1891)
					28. *Trichocerca similis* (Wierzejski,1893)
					29. *Trichocerca dixon nuttalli* (Carlin,1939)
				Trichotriidae	30. *Wolga spinifera* (Western, 1894)
		Digononta	Bdelloida	Adinetidae	31. *Embata hamate* (Pallas, 1736)
					32. *Adineta vaga* (Bartos, 1951)
				Philodinidae	33. *Rotaria neptunia* (Ehrenberg, 1832)
	Arthropoda	Branchiopoda	Cladocera	Daphnidae	34. *Daphnia pulex* (Richard,1896)
					35. *Ceriodaphnia cornuta* (G.O. Sars, 1885)
				Moinidae	36. *Moina brachiate* (Jurine,1820)
					37. *Moina macrocopa* (*Straus,1820*)
				Bosminidae	38. *Bosmina longirostris cornuta* (G.O. Sars, 1862)
					39. *Bosmina longirostris pellucida* (Stingelin, 1895)
					40. *Bosmina longirostris brevicornis* (Hellich, 1877)

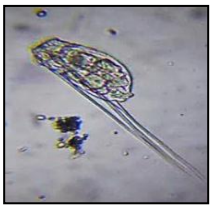
No	Phylum	Class	Order	Family	Species name
					41. *Bosminopsis deitersi* (Richard, 1895)
	Arthropoda	Maxillopoda	Cyclopoida	Cyclopidae	42. *Cyclops bicolor* (Sars,1863)
					43. C*yclops strennus* (Fischer,1851)
					44. *Cyclopoid nauplius* (Forbes,1882)
					45. *Eucyclops prionophorus* (Kiefer,1931)
					46. *Mesocyclops edax* (S.A.Forbes,1891)
					47. *Mesocyclops tenuis* (Marsh,1909)
					48. *Mesocyclops leuckarti* (Claus,1857)
			Calanoida	Diaptomidae	49. *Diaptomus sp*. (Westwood, 1836)
					50. *Neodiaptomus yangtsekiangensis* (Mashiko, 1951)
					51. *Calanoides acutus* (Giesbrecht,1902)
					52.*Calanoides carinatus* (kroyer,1849)
Total	2	4	7	23	52



1. *Collotheca mutabilis*



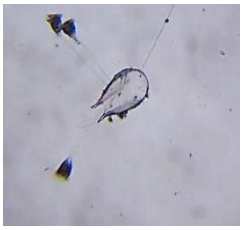
2.*Conochilus coenobasis*



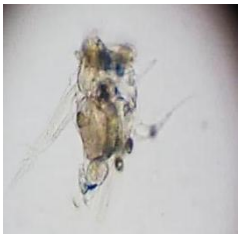
3.*Filinia Longiseta*

4.*Filinia terminalis*

5.*Ptygura longicornis*



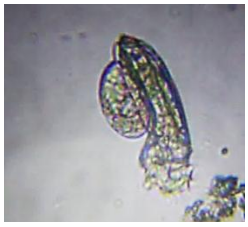
6.*Sinantherina socialis*



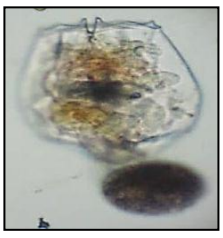
7.*Hexarthra propinqua*

8.*Pompolyx sulcata*

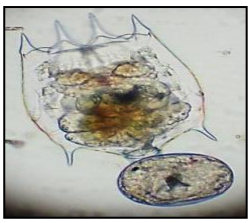
9. *Asplanchna priodonta*



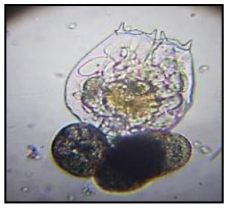
10.*Anuraeopsis fissa*



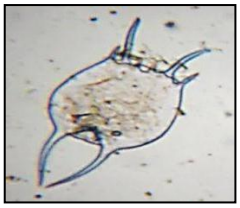
11.*Brachionus angularis*



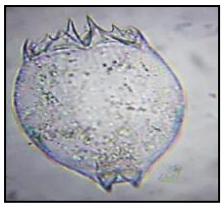
12. *Brachionus calyciflorus*



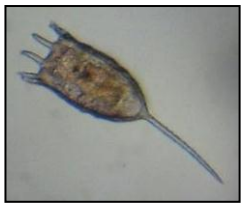
13.*Brachionus caudatus*



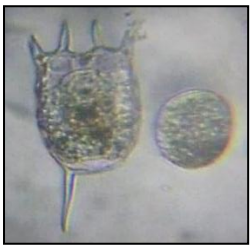
14. *Brachionus falcatus*



15. *Brachionus plicatilis*

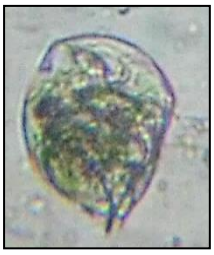


16.*Keratella cochlearis*



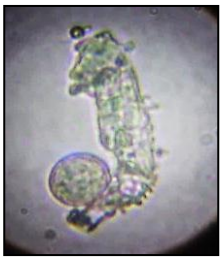
17. *Keratella valga*

18.*Plationus patulus*

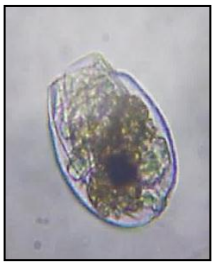


19. *Colurella obtuse*

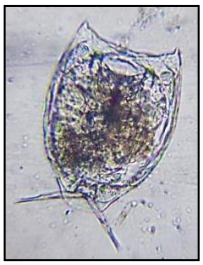
20.*Mikrocodides chlaena*



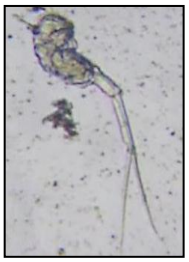
21.*Proalides tentaculatus*



22.*Ascomorpha ovalis*

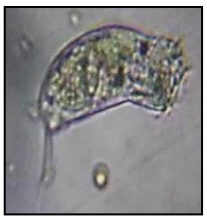


23. *Lecane mira*

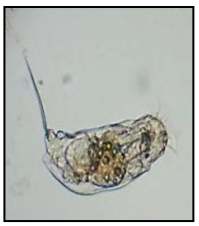


24.*Mytilina mucronata*

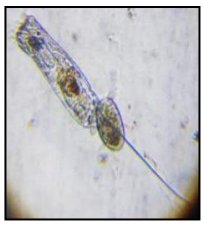
25. *Polyarthra vulgaris*



26. *Trichocerca dixon nuttalli*



27.*Trichocerca elongate*



28.*Trichocerca cylindrica*

29.*Trichocerca similis*

30.*Wolga spinifera*

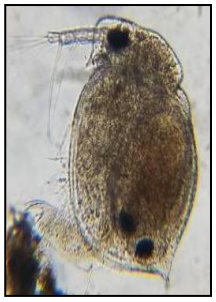
31.*Embata hamate*

32. *Adineta vaga*

33.*Rotaria neptunia*

Plate 3. Recorded rotifer species from the study area (x 40μm)

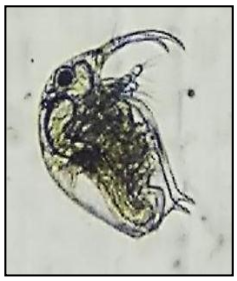

1.*Daphnia pulex*


2.*Ceriodaphnia cornuta*


3. *Moina brachiata*


4.*Moina macrocopa*


5. *Bosmina longirostris cornuta*


6.*Bosmina longirostris pellucida*


7. *Bosmina longirostris brevicornis*


8.*Bosminopsis deitersi*

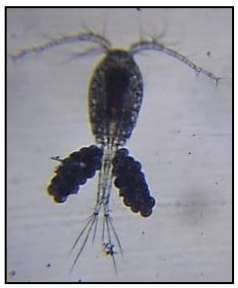
Plate 4. Recorded cladoceran species from the study area (x 40µm)


1.*Cyclops bicolor*


2.*Cyclops strennus*


3. *Cyclopoid nauplius*


4.Eucyclops prionophorus

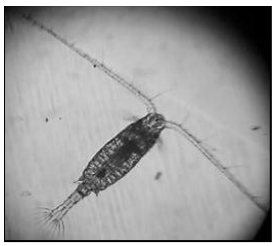

5.*Mesocyclops edax*


6. *Mesocyclops leuckarti*


7. *Mesocyclops tenuis*


8. Diaptomus sp


9.*Neodiaptomus yangtskiangensis*


10.*Calanoides acutus*


11.*Calanoides carinatus*

Plate 5. Recorded copepod species from the study area (x 40µm)

Population status of zooplankton

Maximmun population of rotifer was recorded in July 2017(n=1775) followed by June (n=1621) and minimum population in January 2018 (n=451) in the study period. Highest number of population of copepod was recorded in June 2017 (n=412) and lowest number of species in December 2017 (n=256) respectively. As well as, highest number of population of the cladoceran in January 2018 (n=329)and lowest number in May 2018(n=154) recorded respectively (Fig. 3).

Abundance of zooplankton species as related to weather parameters

No significant correlation was recorded between the abundance of all species and temperature. Positive correlation was recorded between the abundance of rotifer and humidity (r=0.591, p>0.05) and highly significant positive correlation with rainfall (r=0.9331, p>0.01). Positive correlation no significant was recorded between the abundance of Copepods with humidity (r=0.130, p >0.05) and rainfall (r=0.312, p>0.05). However, no significant negative correlation was recorded between the abundance of cladoceran with humidity (r=-0.073, p<0.05) and rainfall (r=-0.224, p<0.05) (Table 2and Fig. 4,5 and 6).

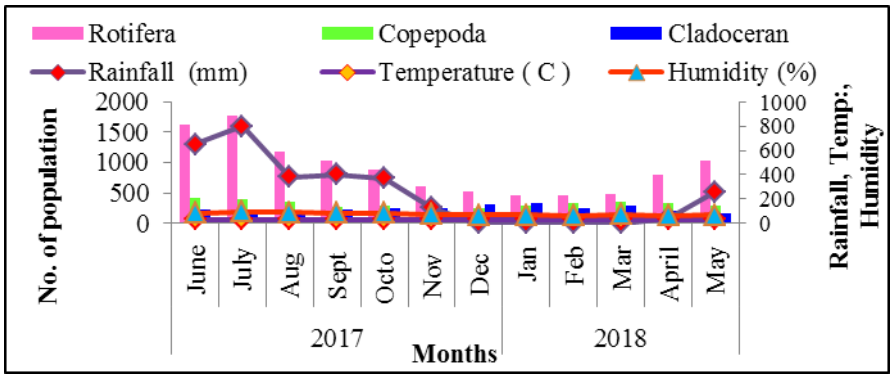


Figure 3 The relation of zooplankton population and weather parameters in all study sites

Table 2 Correlation of zooplankton variation and weather parameter in the study

Species	Temperature(ºC)		Humidity (%)		Rainfall (mm)	
	Mean	r value	Mean	r value	Mean	r value
Rotifer	30.7500	.144	76.5000	.591(*)	252.6667	.933(**)
Copepod	30.7500	.043	76.5000	.130	252.6667	.312
Cladoceran	30.7500	.013	76.5000	-.073	252.6667	-224

*Correlation is significant at the 0.05 level (2- tailed)
**Correlation is significant at the 0.01 level (2- tailed)

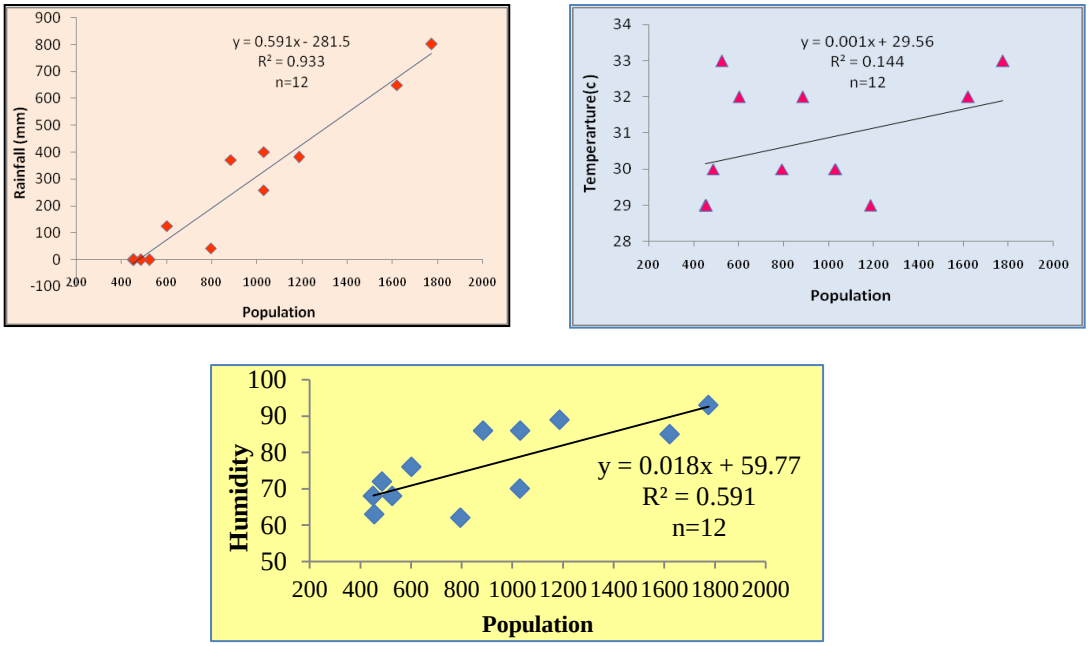


Figure 4 Correlation of rotifer population and weather parameters in study area

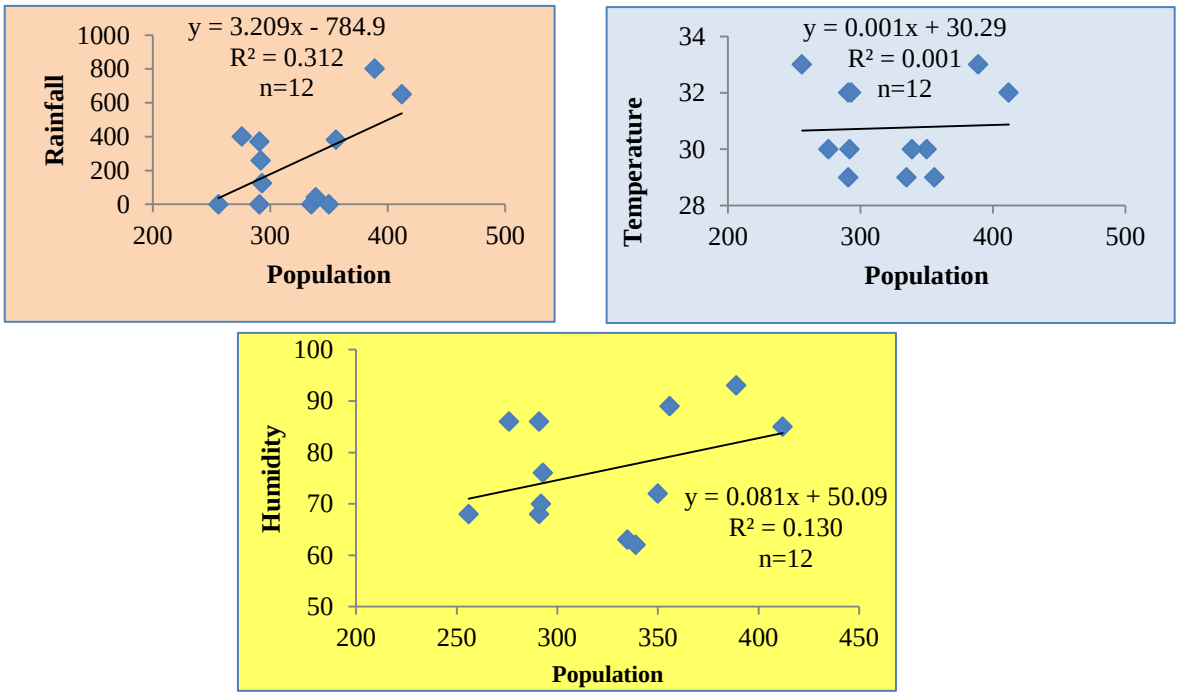


Figure 5 Correlation of copepod population and weather parameters in study area

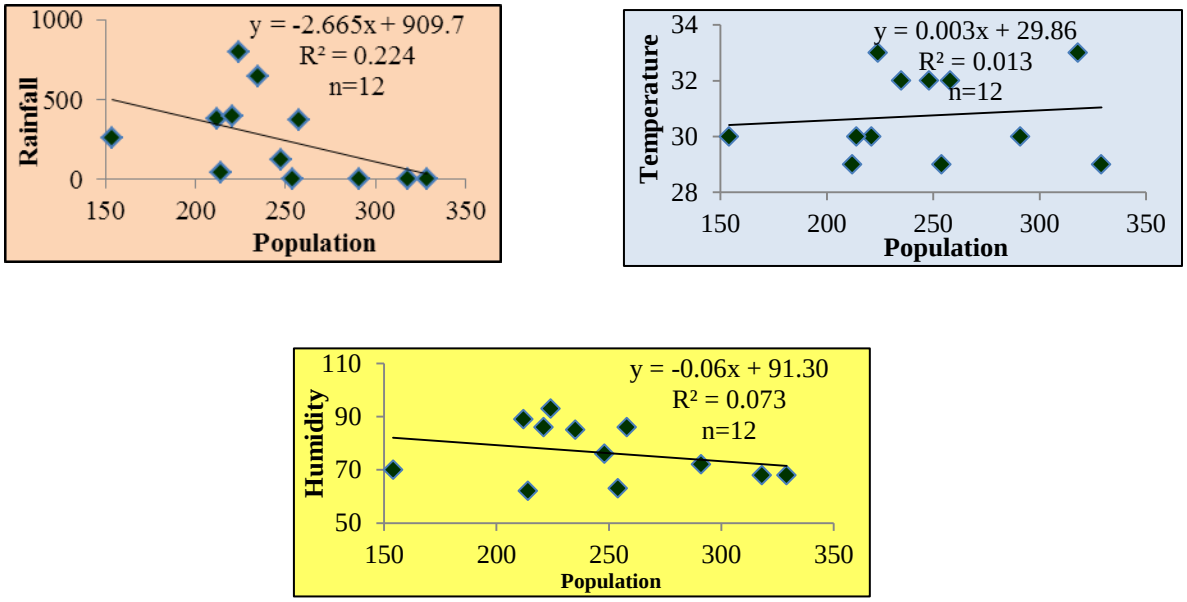


Figure 6 Correlation of cladoceran population and weather parameters in study area

Seasonal population of zooplankton

In the seasonal variation of rotifer, a highly variation was observed. The mean number of highly in the wet season was 1300 ±382 (50.43%) followed by hot season 770.3±272.83 (29.87%) and the lowest in cold season 508.5±71.22 (19.7%) in the study period. The population abundance of this species was significant among the seasons ($F=7.058, p<0.05$).Similar trend was observed in copepod, a highest in wet season 344.8±59.62 (35.68%) followed by hot season 327±30.80 (33.9%) and the lowest in cold season 293.8±32.32 (30.39 %) was observed. While, The population abundance of this species was no significant among the season ($F=2.058$, $p>0.05$).Seasonal population abundance of cladoceran recording as the highest in cold season 287.3±42.16 (38.99%), followed by 230±17.67 (31.25%) in wet season and the lowest 219.7±68.67 (29.76%) in hot seasons respectively. However, the population abundance of this species was no significant among the seasons ($F=2.016, p>0.5$) (Table.3 and Fig.7).

Table 3 Seasonal population of zooplankton in study area (Mean ± SD)

Species	Wet (Mean ±SD)	Cool (Mean ±SD)	Hot (Mean ±SD)
Rotifer	1300 ±382.58	508.5±71.22	770.3±272.83
Copepod	344.8±59.62	293.8±32.32	327±30.80
Cladoceran	230±17.67	287.3±42.16	219.7±68.67

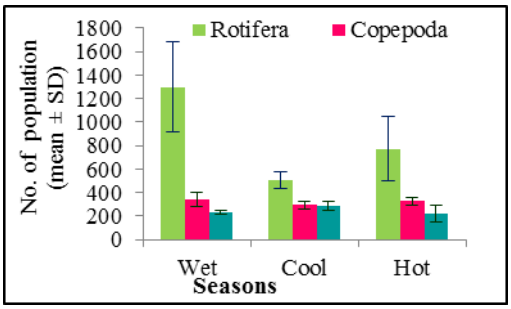


Figure7 Seasonal population of zooplankton in the study areas

Discussion

Zooplankton community structure in the lake of Hlawga Wildlife Park, Yangon Region was studied by monthly surveys in the study period. A total number of 52 species of zooplankton, 36 genera, 23 families, seven orders, four classes under two phyla were recently recorded. Especially, the family Brachionidae were recorded nine species formed the dominant and diversified genus among the rotifers in the study sites throughout the studied period.

Aung Kyaw Zaw (2012) also recorded that Brachionidae was the most diverse genus comprises nine species. The present recorded rotifers are more species rich and abundant, especially *Brachionus caudatus* are highest population density than the other recorded species. Lower species was *Collotheca mutabilis* from the rotifer group. Abundance of rotifer population was increased due to the low water temperature, flooding (causes high nutrients condition, food availability and hatching of egg) and the presence of diatoms (cyanobacteria blooms) (Gilbert, 1988).

Population of rotifer in Site V and VI were more abundant than other site. This may be due to the directly sunlight affect in quantitative changes of zooplankton. Highly significant positive correlation was recorded between the abundance of rotifer and rainfall while positive correlation, no significant was recorded in copepod. Negative correlation was recorded between the abundance of cladoceran and rainfall and humidity. The changes of weather parameter were affected positively; sometime negatively the abundance of zooplankton so this suggests that the zooplankton may be good indicator of the variation in the water quality of these lotic ecosystem. Seasonal abundance the highest numbers of rotifer population were observed in wet season followed by hot season. It may be due to environmental factors and habitats were suitable for rotifers to increase in wet season.

Rotifers population are very useful in indicating water quality particularly in pollution studies and then is less specialized feeding habits and high rate fecundity in wet season (Shadeck, 1983). During all season, cladoceran and copepods accounted of 15.38 % and 21.15 % of total abundance, respectively. According to (Karus, 2014) both groups are larger size and compared to rotifers which are smaller than 250μm. The large size of cladoceran and copepoda will decrease their abundance due to the fish predation. Therefore, the low composition of larger zooplankton size resulted in higher smaller species particularly rotifers.

Conclusion

The results of this present study revealed species occurrence and seasonal variation of zooplankton. A total number of 52 species of zooplankton, 36 genera, 23 families, seven order belonging to four class of zooplankton were recorded. Seasonal variation is highest in wet season and lowest in cold season. Peak in the family Branchionidae were recorded during in wet season. The largest number individuals were found in July and the lowest number in January. It could be concluded that availability of nutrients habitats and environmental condition of Hlawga wildlife lake is still in favourable for zooplankton species. More study will be needed to understand the structure and ecology of the zooplankton community in Hlawga lake.

Acknowledgements

We are greatly indebted to Dr. Thida Lay Thwe, Professor/Head, Department of Zoology, and Yangon University for her kind encouragement. We would like to specially thank to Dr. Aye Mi San, Professor, Department of Zoology, and Yangon University for her invaluable help in this work.

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REVALIDATING THE BIOMETRIC CHARACTERS OF NILE TILAPIA *OREOCHROMIS NILOTICUS* (LINNAEUS, 1758) IN MEIKTILA

Myo Myo*

Abstract

Nile tilapia *Oreochromis niloticus* was carried out to assign the classification of local population relation to their biometric parameters. A total of 265 samples were collected from five markets in Meiktila. Biometric characters of eleven meristic counts and seventeen morphometric traits were generated with Principal Component Analysis (PCA). The result shows the meristic characters of *O.niloticus* did not change with any increasing in relation to variable morphometric parameters. Analysis of variance (PCA) revealed the two components with 86.85 % (λ=14.76) at Taw Ma market, 86.43 % (λ=14.69) at Wun Zin market, 84.67 % (λ=14.39) at Pauk Chaung market, 83.44 % (λ=14.18) at Myo Thit market and three components with 80.66 % (λ=13.71) at Myo Ma Central market. Coefficient determination R^2 revealed variable significant relationships in total length and other body parts among population, however, with head length relationship showed low correlation in interorbital width (IOW) and mouth length (ML) in all markets. The most striking pattern of hybrid tilapia was found in Pauk Chaung market and Myo Thit market. These pilot surveys strongly suggest the local population of non-indigenous Nile tilapia *O.niloticus* with the existence of morphological differentiations among population. In addition, the pure population of tilapia species is urgently needed to maintain without hybridizing with other species in Meiktila Environs.

Keywords: Nile tilapia, meristic, morphometric, biometric, growth

Introduction

The origin of Nile tilapia (Cichlid) species is in East Africa (EA), which includes three economically important genera *Tilapia*, *Oreochromis*, and *Sarotherodon.* In aquaculture, the correct identification of species frequently requires based on the features of meristic counts employing traditional morphometric (TM) by using linear measurements of the fish anatomical parts of the body (Trewavas, 1983). Morphometric characters have been commonly used in fisheries biology as powerful tools which are helpful in easy and correct classification of fish species and their performance and morphological traits are used to estimate the economic output by fish farmers (Marengoni *et al.,* 2015). Recently, the genetic improvement of tilapias will be accelerated by using contemporary molecular techniques (Yue *et al*., 2016).

Tilapia species play a main role in supplement of protein resources that demand on the requirement of local population. Therefore, the original source of tilapia species must be maintained with different culture systems (either ponds or lakes or dams) as well as in natural habitat for long term sustainable development. The successful management of fisheries resource produces the good quality of fish shape that reflects on the interest of aquaculturists, researchers and consumers. In Myanmar, aquaculture is the second sectors of economic income so every species is necessary for sustainable development without changing their intrinsic (e.g. habitat heterogeneity) and extrinsic factors (e.g. dispersal capability, mating system and habitat preference).

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According to Myanmar Fisheries resources, tilapia species were introduced from Thai and Israel since 1975. Due to exploitation of breeding success, tilapia species are widely distributed throughout Myanmar, especially in rural areas. The Myanmar Aquaculture-Agriculture Survey (MAAS) has implemented in May 2016 to provide the national fish supply and study the technical and economic incomes. In order to correct identification of tilapia species in Myanmar, it is urgently needed to evaluate the non-indigenous species of Nile tilapia in Meiktila environs. The present study was conducted to confirm the species status of the genus *Oreochromis niloticus* in Meiktila, using diagnostic morphological characteristics among population.

Materials and methods

Study area and site

Meiktila lies between the 20° 53′ 29.96″N and 95° 56′ 18.51″E, at elevation 244 meters above sea level. Samples were collected from Pauk Chaung market, Myo Ma Central market, Wun Zin market, Taw Ma market, and Myo Thit market.

Source: Google 2019

Figure 1 Map showing sampling sites at Meiktila.

Study period

The present study was conducted from August 2018 to March 2019.

Sample collection and biometric characters

Fifty samples from Pauk Chaung market, 55 from Myo Ma Central market, 50 from Wun Zin market, 44 from Taw Ma market, and 66 from Myo Thit market were collected for studying their biometric characters. All collected specimens were brought to Laboratory, Department of Zoology for further analysis. Eleven meristic counts were recorded from each fish such as dorsal fin spine (DFs) and rays (DFr), pectoral fin (PF), pelvic fin spine (PcFs) and rays(PcFr), anal fin spine (AFs) and rays (AFr), caudal fin (CF), scale along lateral line (SLL), scale below lateral (SBLL), scale above lateral line (SALL), scale before dorsal fin (SBDF), branchiostegal rays (BR) and first lower gill rackers (FLGR), respectively. Digital photograph was taken on the left side of each specimen. Seventeen morphometric characters were measured from each labeling fish with designated landmark points (Plate 1). Morphological characters were measured with Image J (1.51j8) in nearest 0.01 cm.

Plate 1 Landmark points of Nile tilapia *Oreochromis niloticus* for morphometric measurements.

AB – total length (TL), AC – standard length (SL), AD – predorsal length (PrDL), DB – postdorsal length (PoDL), AE – head length (HL), AF – snout length (SnL), FG – eye diameter (ED), HI – dorsal fin length (DFL), JK – anal fin length (AFL), LM – pelvic fin length (PcFL), NO – pectoral fin length (PlFL), CB – caudal fin length (CFL), PQ – caudal peduncle length (CL), RS – caudal peduncle depth (CD) and TL – height of the body (HB).

Length-length relationship (LLR)

Twelve morphometric characters such as standard length (SL), predorsal length (PrDL), postdorsal length (PoDL), head length (HL), dorsal fin length (DFL), anal fin length (AFL), pelvic fin length (PcFL), pectoral fin length (PlFL), caudal fin length (CFL), caudal peduncle length (CL), caudal peduncle depth (CD) and height of the body (HB) were expressed as a percentage of the total length (TL). Four morphometric characters such as snout length (SnL), eye diameter (ED), interorbital width (IOW) and mouth length (ML) were also presented as a percentage of the head length (HL). According to Froese (2006), the growth performance of length-length relationship was determined as follows : $\mathrm{Log\,L} = \mathrm{Log\,a} + \mathrm{b\,Log\,TL}$, where TL is total length (mm) and L is length of body parts (mm).

Sample identification

The species of Nile tilapia *Oreochromis niloticus* were identified following Trewavas (1983), Eccles (1992), Skelton (1993) and Al-Faisal and Mutlak (2014).

Statistical analysis

Observed data were presented as descriptive statistics, correlation coefficient, and the morphometric measurements were used by the principal component analysis (PCA) generated the loading components using SPSS ver.21.

Results

The non-indigenous populations of 265 Nile tilapia *Oreochromis niloticus* from five markets in Meiktila were investigated for verification of their biometric characters. Comparative studies on eleven meristic counts of Nile tilapia; they were not largely changed among populations. The mean values of meristic counts were presented in Table 1. In which pectoral fin rays, pelvic fin spine and rays, and anal fin spies and rays of tilapia species were fixed.

Table 1 Descriptive statistics of meristic counts on Nile tilapia *Oreochromis niloticus* from five markets in Meiktila

Meristics	Pauk Chaung market (N=50)	Wun Zin market (N=50)	Myo Ma market (N=55)	Taw Ma market (N=44)	Myo Thit market (N=66)
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
DFs	16.62 ± 0.57	16.62 ± 0.49	16.73 ± 0.45	16.43 ± 0.55	15.68 ± 0.93
DFr	12.54 ± 0.76	12.72 ± 0.57	12.87 ± 0.51	12.27 ± 0.69	10.91 ± 0.96
AFs	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.41 ± 1.91	3.02 ± 0.21
AFr	9.80 ± 0.73	9.68 ± 0.55	10.02 ± 0.53	8.50 ± 1.81	8.73 ± 1.05
PF	12.80 ± 0.99	13.14 ± 0.57	13.33 ± 0.47	12.52 ± 1.81	12.82 ± 0.39
PcFs	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.09 ± 0.42	1.00 ± 0.00
PcFr	4.84 ± 0.37	5.00 ± 0.00	5.00 ± 0.00	5.20 ± 0.95	5.00 ± 0.00
CF	17.88 ± 0.75	17.68 ± 0.84	17.93 ± 0.38	17.64 ± 0.78	17.94 ± 0.35
SLL	36.80 ± 2.53	34.74 ± 1.26	36.07 ± 2.35	35.73 ± 1.34	36.00 ± 2.29
SALL	4.74 ± 0.48	6.28 ± 7.99	4.70 ± 0.40	4.34 ± 0.43	4.70 ± 0.40
SBLL	9.20 ± 1.37	6.74 ± 0.43	8.68 ± 0.75	7.77 ± 0.66	8.74 ± 0.79
SBDF	11.42 ± 1.50	9.44 ± 1.03	11.02 ± 1.15	11.30 ± 1.21	10.88 ± 1.48
BF	3.82 ± 0.48	3.68 ± 0.51	3.42 ± 0.50	3.05 ± 0.21	3.65 ± 0.48
FLGR	25.48 ± 2.61	24.50 ± 3.61	26.67 ± 3.17	28.20 ± 3.17	24.50 ± 2.34

Morphometric analysis by Principal Component Analysis (PCA)

In Pauk Chaung Market, the Principal Component Analysis explained 72.849 % (λ = 12.384) variability in Component (PC 1) and 11.824 % (λ = 2.010) in PC 2, together explained 84.673 % (λ = 14.394) of the variability indicating that the PCA was largely significant different among morphometric characters (Table. 2).

Table 2 Summary of total variance explained by Principal Component Analysis

Markets	Components	Eigenvalues	% Variances	Total %
Pauck Chaung	PC 1	12.384	72.849	
	PC 2	2.010	11.824	84.673
Wun Zin	PC 1	13.516	79.503	
	PC 2	1.177	6.923	86.426
Myo Ma Central	PC 1	11.238	66.105	
	PC 2	1.418	8.343	
	PC 3	1.056	6.214	80.662
Taw Ma	PC 1	13.278	78.106	
	PC 2	1.487	8.745	86.851
Myo Thit	PC 1	12.820	75.411	
	PC 2	1.364	8.026	83.437

The scree plot revealed the first two PCs had the most variables of characters from the data and the curve started flattened out at the third component indicating that its components were significant, while other components were not significant having with Eigenvalues under the red line $\lambda < 1$ (Fig. 2). PCA biplot showed the positive correlation on the clusters located around the PC 1. Although interorbital width and mouth length were positively correlated with each other, these two were negatively correlated with SnL, AFL, PrDL, HL, CFL, DFL, PcFL, CL, and SL (Fig. 3).

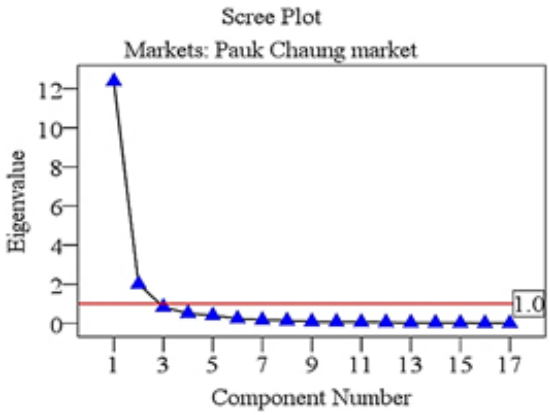

Figure 2 Scree plot on principal components of morphometric characters of *Oreochromis niloticus* at Pauk Chaung market

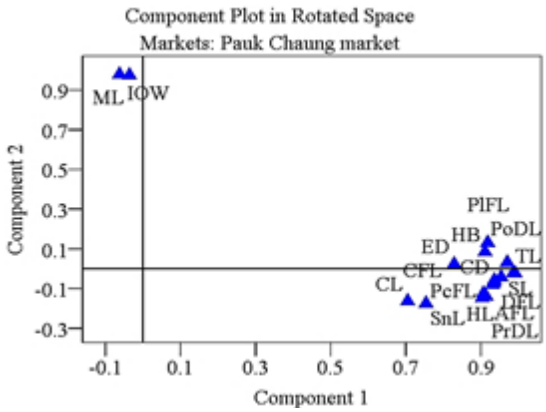

Figure 3 Component plot in rotated spaces from Pauk Chaung market

In Wun Zin market, PCA explained 79.503 % ($\lambda = 13.516$) variability in PC 1 and 6.923 % ($\lambda = 1.177$) in PC 2, together explained 86.426 % ($\lambda = 14.693$) variability (Table. 2). The scree plot revealed the first two PCs had the most variables while other components were not significant having with Eigenvalues under the red line $\lambda < 1$ (Fig. 4). The loading components were found on the right side of the top explained the positively correlated; however, the snout length (SnL) was no correlation with interorbial width (IOW), caudal fin length (CL) and dorsal fin length (DFL) (Fig. 5).

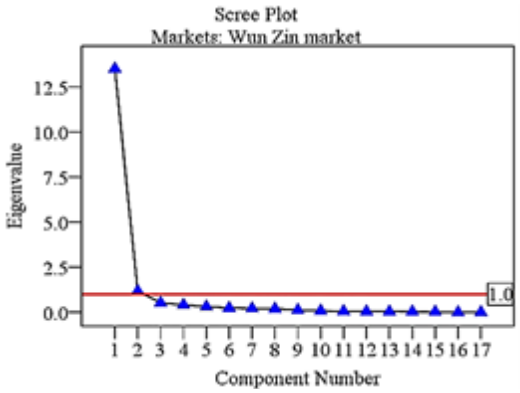

Figure 4 Scree plot of principal components of morphometric characters of *Oreochromis niloticus* at Wun Zin market

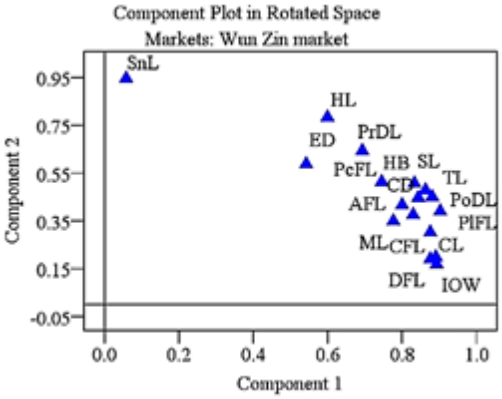

Figure 5 Component plot in rotated spaces from Wun Zin market

In Myo Ma Central market, PCA explained 66.105 % (λ = 11.238) variability in Component (PC 1), 8.343 % (λ = 1.418) in PC 2 and 6.214 % (λ = 1.056) in PC 3, with 80.662 % (λ = 13.712) of the total variability (Table. 2). The scree plot revealed the first three PCs had the most variables while other components were not significant having with Eigenvalues under the red line λ < 1 (Fig. 6). The most variation was found in PC 1 followed by PC 2 and PC 3 revealed the positively correlated on the loading plots PC 1 and PC 2. In addition, the snout length PC 3 was positively correlated with other factors (Fig. 7).

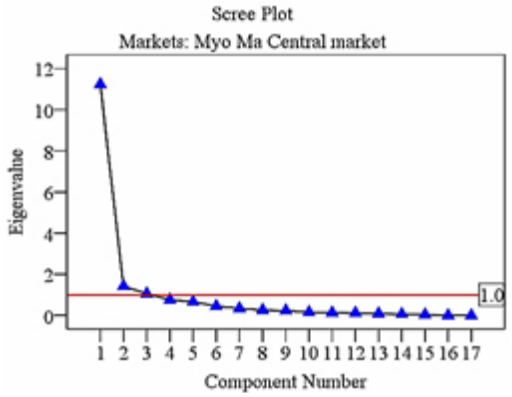

Figure 6 Scree plot of principal components of morphometric characters of *Oreochromis niloticus* at Myo Ma Central market

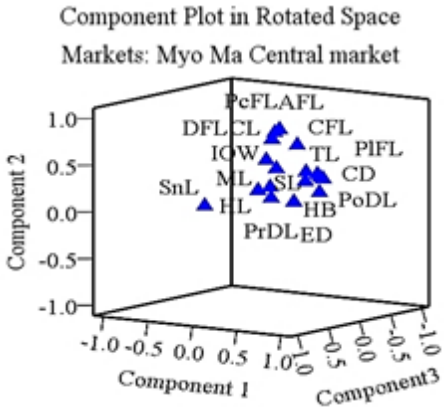

Figure 7 Component plot in rotated spaces from Myo Ma Central market

In Taw Ma market, PCA explained 78.106 % (λ = 13.278) of the variability in PC 1 and 8.745 % (λ = 1.487) in PC 2, together explained 86.851 % (λ = 14.765) variability indicating that the PCA was largely significant different among morphometric characters (Table. 2). The scree plot revealed the first two PCs had the most variables of characters from the data; however, the other components were not significant having with Eigenvalues under the red line λ < 1 (Fig. 8). The loading components were found on the plot 1 showed that the snout length was no correlated with mouth length; however, it was positively correlated with other factors. Four groups of closely correlated factors were (1) interorbital width and mouth length; (2) pelvic fin length, dorsal fin length, and pectoral fin length; (3) caudal depth, height of the body and total length; and (4) head length and predorsal length were found (Fig. 9).

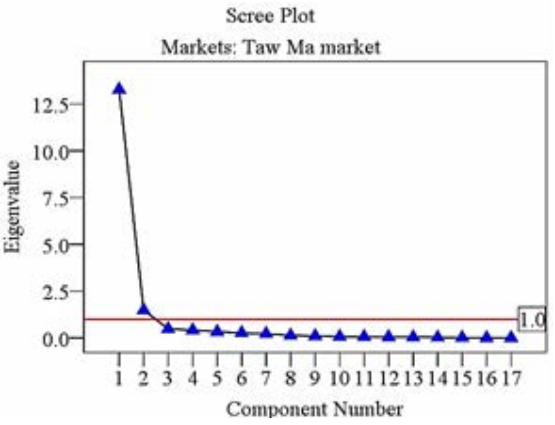

Figure 8 Scree plot of principal components of morphometric characters of *Oreochromis niloticus* at Taw Ma Market

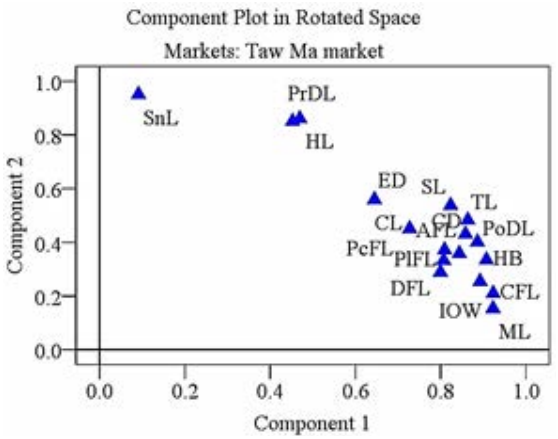

Figure 9 Component plot in rotated spaces from Taw Ma market

In Myo Thit market, PCA explained 75.411 % (λ = 12.820) variability in PC 1 and 8.026 % (λ = 1.364) in PC 2, together explained 83.437 % (λ = 14.184) variability indicating that the PCA was largely significant different among morphometric characters (Table. 2). The scree plot revealed the first two PCs had the most variables of characters from the data; however, the other components were not significant having with Eigenvalues under the red line λ < 1 (Fig. 10). The loading components were also found on the plot 1 showed that the snout length was not correlated with mouth length and interorbital width; however, other loading factors were positively correlated. The mouth length and interorbital width showed more positive correlation with other variables except with snout length (Fig. 11).

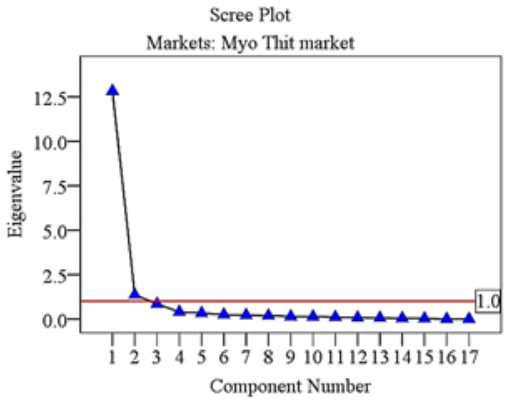

Figure 10 Scree plot of principal components of morphometric characters of *Oreochromis niloticus* at Myo Thit market

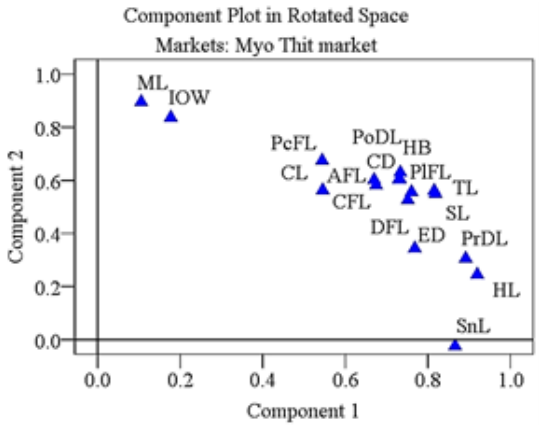

Figure 11 Component plot in rotated spaces from Myo Thit market

Percent relation of morphometric parameters

In order to better understanding the growth performance of Nile tilapia species in Meiktila, the percent relation of total length to 12 morphometric measurements and head length to 4 morphometric measurements were investigated. The mean percent relationship of total length to 12 morphometric measurements revealed positive allometric growth patterns (b > 1) with highly significant correlation coefficient as well as negative allometric growth patterns (b < 1 in length and b < 3 in weight) with low correlation growth performance. In addition, the characters on head length showed low correlation coefficient with negative allometric growth patterns (b < 1), except the relationship of snout length and head length (Table 3).

Diagnostic characters revealed

The most striking patterns of Nile tilapia were observed in Pauk Chaung market and Myo Thit market. They exhibited the inheritance characters of *Oreochromis aureus* (Plate 2) and *Oreochromis mossambicus* (Plate 3)

Plate 2. Representative caudal fin pattern of *Oreochromis* sp.

Plate 3. Representative caudal fin, dorsal fin and mouth patterns of *Oreochromis* sp.

Table 3 Percent relation of total length and head length to other body parts of Nile tilapia *Oreochromis niloticus*

Percentage	Pauk Chaung market (n = 50)			Wun Zin market (n = 50)			Myo Ma market (n = 55)			Taw Ma market (n= 44)			Myo Thit market (n = 66)		
	M	Y= a + bx	R^2	M	Y= a + bx	R^2	M	Y= a + bx	R^2	M	Y= a + bx	R^2	M	Y= a + bx	R^2
SL % TL	81.40	Y = - 0.26 + 1.01 x	0.99	80.69	Y = - 0.25 + 1.01 x	0.99	80.73	Y = - 0.25 + 1.01 x	0.99	79.59	Y = - 0.18 + 0.99 x	0.98	80.44	Y = - 0.39 + 1.03 x	0.99
PrDL % TL	24.70	Y = - 1.21 + 0.96 x	0.85	25.66	Y = - 0.69 + 0.86 x	0.86	24.88	Y = - 1.24 + 0.97 x	0.71	25.49	Y = 0.17 + 0.68 x	0.65	24.65	Y = - 1.78 + 1.08 x	0.82
PoDL % TL	56.57	Y = - 0.72 + 1.03 x	0.97	55.04	Y = - 0.95 + 1.07 x	0.98	55.80	Y = - 0.69 + 1.02 x	0.91	54.04	Y = - 1.26 + 1.13 x	0.97	55.79	Y = - 0.61 + 1.01 x	0.95
HL % TL	26.50	Y = - 1.30 + 0.99 x	0.79	27.13	Y = - 0.39 + 0.81 x	0.77	26.75	Y = - 1.68 + 1.07 x	0.69	26.93	Y = - 0.66 + 0.86 x	0.68	26.73	Y = - 1.77 + 1.09 x	0.73
DFL % TL	16.56	Y = - 2.92 + 1.22 x	0.86	15.58	Y = - 2.57 + 1.15 x	0.73	16.04	Y = - 3.57 + 1.35 x	0.61	17.56	Y = - 3.33 + 1.33 x	0.65	16.91	Y = - 2.71 + 1.19 x	0.80
AFL % TL	17.08	Y = - 2.42 + 1.13 x	0.83	16.96	Y = - 2.84 + 1.22 x	0.81	16.29	Y = - 2.34 + 1.11 x	0.50	16.75	Y = - 3.46 + 1.34 x	0.77	17.00	Y = - 2.43 + 1.13 x	0.76
PlFL % TL	31.74	Y = - 1.34 + 1.04 x	0.70	31.05	Y = - 1.84 + 1.14 x	0.78	31.37	Y = - 0.61 + 0.89 x	0.48	28.41	Y = - 1.57 + 1.06 x	0.70	32.15	Y = - 1.88 + 1.15 x	0.85
PcFL % TL	22.84	Y = - 2.63 + 1.23 x	0.81	24.13	Y = - 1.81 + 1.08 x	0.76	22.89	Y = - 1.13 + 0.93 x	0.49	21.75	Y = - 3.15 + 1.34 x	0.74	26.20	Y = - 1.52 + 1.04 x	0.66
CFL % TL	19.40	Y = - 1.73 + 1.02 x	0.86	19.55	Y = - 1.43 + 0.96 x	0.83	19.42	Y = - 1.76 + 1.03 x	0.74	20.50	Y = - 1.67 + 1.02 x	0.79	19.58	Y = - 0.94 + 0.86 x	0.79
CL % TL	8.21	Y = - 1.12 + 0.72 x	0.45	8.30	Y = - 2.32 + 0.96 x	0.79	8.37	Y = - 2.07 + 0.92 x	0.45	8.73	Y = - 2.66 + 1.05 x	0.71	8.05	Y = - 2.52 + 1.00 x	0.60
CD % TL	11.69	Y = - 2.08 + 0.99 x	0.92	11.24	Y = - 2.33 + 1.03 x	0.92	11.56	Y = - 1.60 + 0.89 x	0.76	11.77	Y = - 2.19 + 1.01 x	0.92	11.77	Y = - 2.00 + 0.97 x	0.89
HB % TL	36.14	Y = - 0.86 + 0.97 x	0.82	33.75	Y = - 1.13 + 1.01 x	0.93	34.89	Y = - 0.79 + 0.95 x	0.75	32.24	Y = - 1.82 + 1.14 x	0.90	35.56	Y = - 1.39 + 1.07 x	0.88
SnL % HL	19.55	Y = - 4.89 + 1.89 x	0.81	21.70	Y = - 2.08 + 1.16 x	0.54	18.16	Y = - 4.23 + 1.70 x	0.62	19.11	Y = - 2.49 + 1.24 x	0.69	17.45	Y = - 4.10 + 1.65 x	0.74
ED % HL	29.76	Y = 0.21 + 0.61 x	0.62	30.67	Y = - 0.23 + 0.73 x	0.64	31.46	Y = 0.07 + 0.66 x	0.47	30.91	Y = - 0.56 + 0.82 x	0.61	32.70	Y = - 0.18 + 0.74 x	0.70
IOW % HL	60.22	Y = 3.97 - 0.23 x	0.02	44.55	Y = - 0.53 + 0.92 x	0.43	42.45	Y = 0.39 + 0.65 x	0.32	40.97	Y = - 0.20 + 0.80 x	0.38	43.39	Y = 0.95 + 0.50 x	0.23
ML % HL	47.97	Y = 3.30 - 0.12 x	0.01	38.54	Y = - 0.70 + 0.93 x	0.56	34.32	Y = - 0.13 + 0.74 x	0.52	34.19	Y = - 0.50 + 0.83 x	0.29	32.83	Y = 1.14 + 0.37 x	0.19
Weight	53.22	Y = - 8.30 + 2.46 x	0.71	32.21	Y = - 9.15 + 2.63 x	0.85	36.53	Y = - 8.41 + 2.45 x	0.62	33.20	Y = - 10.93 + 2.97 x	0.92	38.32	Y = - 7.35 + 2.23 x	0.72

M = mean, SD = standard deviation, a = initial growth coefficient, b = estimated growth, R^2 = coefficient of determination

Discussion

The recorded samples of *Oreochromis niloticus* showed the mean percent total length was ranged from 79.59 mm in Taw Ma market to 81.40 mm in Pauk Chaung market and their mean weight were ranging from 32.21 g in Wun Zin market to 53.22 g in Pauk Chaung market. The meristic counts on Nile tilapia *Oreochromis niloticus* from five markets were not largely changed among them indicating that tilapia maintain their inheritance characters of genus *O.niloticus*.

However, the scree plot relative to biplot generated by Principal Component Analysis (PCA) using Pearson correlation exhibited the variable morphological characters among population. PCA components showed the more Eigenvalues lead to more variation among characters from the actual data. Among five markets, $\lambda = 13.516$ in Wun Zin was the highest in PC 1 and the lowest $\lambda = 1.056$ in PC 3 in Myo Ma Central market. The morphological variations among population revealed that similar degrees of variation were observed among population with 84.673 % in Pauk Chaung market, 86.426 % in Wun Zin market, 80.662 % in Myo Ma Central market, 86.851 % in Taw Ma market and 83.437 % in Myo Thit market, respectively.

Among 17 morphometric characters, the largely significant variation with low correlation in dorsal fin ($r^2 = 0.61$), anal fin length ($r^2 = 0.50$), pectoral fin ($r^2 = 0.48$), pelvic fin ($r^2 = 0.49$), caudal peduncle length ($r^2 = 0.45$), eye diameter ($r^2 = 0.47$), interorbital width ($r^2 = 0.32$), and mouth length ($r^2 = 0.52$) found especially in Myo Ma Central market compared with the other four markets. In addition, the percent relation of head length showed low correlation of head parts in five markets. These results are strongly recommended to Kosi *et al .,* (2014) who reported that the growth of different morphological body parts of the fish in relation to its least growth changes in those parameters over the fish size.

In this study, the significant differences of morphological traits were observed among the population with either dorsal fin insertion was similar or different with insertion of pectoral and pelvic fins, the distance between the insertion point of pectoral fin and pelvic, the position of insertion point of anal fin, the end of the dorsal fin and anal fin, the position of the eye on the angle of anterior end of mouth and insertion point of dorsal spine, and most of the short snout length found were long snout length relative to head length, respectively. Moreover, the percent of growth performance on length-length relationship showed positive and negative allometric growth patterns ($b < 1$ and $b > 1$) observed among intra-population and inter-population. In addition, growth development of coefficient determination R^2 values indicated the strong linear relationship among the length-length relationships. It was concluded that their growth patterns increase with temporal lines instead of linear relationship.

Moreover, the relation of length-weight relationships of tilapia population showed negative allometric growth ($b < 3$) indicating that their body compositions with less fat and thin. These results are strongly recommended the highest variation among population may be due to the availability of food requirements and the physical condition of water quality. However, these morphological variations are depending on the environmental condition rather than the genetic control (Ikpeme *et al.* 2017). Furthermore, these results are correspondence with Khallaf *et al.*, (2003) that the unfavorable environmental conditions such as stress, sex, season, availability of feeds and other water quality parameter impact on morphological variations.

Furthermore, the striking patterns of mix characters revealed in some tilapia species found in Myo Thit and Pauk Chaung markets due to the hybridization set up in closely related species. Most of the characters such as black oblique bands on dorsal fin rays and mixed stripe bands on caudal fin must be inheritance from *Oreochromis aureus*. Some fishes exhibited the significant characters of mouth shape, caudal fin and deep notched between dorsal fin spines and rays inherited from *O. mossambicus* and *O.aureus.* These results are consistent with the hybrids had indistinct or incomplete caudal fin barring; red hybrids have been developed to combine different traits of different tilapia species, and mossambicus lateral spots, partly caudal fin barred and iris yellowish and low dorsal fin (Fulton and Hall, 2014; Hill, 2017).

To sum up, the data among five populations from different markets have been clearly shown the real characteristics of remarkable patterns relation to their growth performance which would be very useful for stock management of fishery products as well as the scientific researchers.

Conclusion

The meristic analysis revealed more constant than biometric parameters. The Principal Component Analysis (PCA) generated the significant variable morphometric characters relation to their different population from five markets. The percent relation of length-length relationship to composition of body parts of tilapia species showed the different growth performance. In addition, the mixed characters of *Oreochromis* sp. indicated the hybridizing setting up in the local population. This information may provide the stock management of aquaculture resource concerning with the requirements of local fishermen.

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NUTRITIONAL VALUES OF SOME SELECTED SMALL INDIGENOUS FISH SPECIES (SIFS) IN BAWLE KYUN, HTANTABIN TOWNSHIP, YANGON REGION

Htike Htike Lin[1] Kalayar Win Maung[2] and Thida Aung[3]

Abstract

The present study was aimed to investigate the nutritional values of small indigenous fish species. The study was conducted at Nethamein village (17°2´N and 95°49´E) at Bawle Kyun, in Htantabin Township, Yangon Region. The study period lasted from January to December 2018. A total of thirteen small indigenous species were selected for nutritional analysis. The analysis showed the moisture content of studied species ranged from 66.28% (*Anabas testudineus*) to 82.9% (*Heteropneustes fossilis)*. The ash content was recorded from 1.13% (*H. fossilis*) to 10.41% (*A. testudineus*). The protein content of SIS in the present study was examined between 14.16% (*Trichogaster pectoralis*) and 18.52% (*A. testudineus*). Regarding the fat content of SIFS was ranged from 0.05% (*Notopterus notopterus*) to 4.92% (*Osterobrama belengeri*). The fiber content was ranged from 0-0.08% whereas carbohydrate was ranged from 0-1.15%. The gross energy content in fresh matter basis of small indigenous fish species in the present study was ranged between 66 kcal/ 100g (*H. fossilis)* and 94kcal/100g (*Amblypharyngodon mola*). The small indigenous fish species can play a significant role to fulfill the nutrient demand of poorer sections of people of the country. It may be concluded that the small indigenous fish species make a choice based on that information from a consumer point of view.

Introduction

Fish is one of the most important sources of animal protein and has been widely accepted as a good source of protein and other elements for the maintenance of healthy body (Andrew, 2001). Fish is one of the main food constituents in our diet as it contains essential fatty acids, amino acids and some of the principal vitamins and minerals in sufficient amounts for healthy living (Borgstrom, 1961).

The small indigenous species of fishes are generally considered which grow to a length of about 25cm, *i.e.,* 9 inches at maturity (Felts *et al.,* 1996, Hossain *et al.,* 1999). Small indigenous freshwater fish species (SIFFS) are defined as fishes which grow to the size of 25-30 cm in mature or adult stage of their life cycle. Small indigenous fish species (SIFS) are found in all types of natural waterbodies. They inhabit in rivers and tributaries, flood-plains, ponds and tanks, lakes, streams, lowland areas, wetlands and paddy fields (NACA, National Association for Campus Activities, 2011). A large diversity of small indigenous fish species are found in freshwater systems. These small indigenous fish species form a major component of food consumed by family, especially those living closer to freshwater resources (ICSF, International Collective in Support of Fishworkers, 2010).

The SIFS are rich in terms of proteins, micronutrients, vitamins and minerals. Therefore, small indigenous fishes are important for poor and lower income groups in terms of nutrition. The rural poor prefer to eat SIS instead of cultured carps because they can afford to buy a small amount at a time, and it is easier for them to distribute the fish among their family members.

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Fish, especially SIS, are a rich animal-source food of multiple, essential, highly bioavailable nutrients. They are significant in respect of their taste, availability, lower market price and nutritional value (Rajts *et al*., 1997).

The body composition is used as indicator to assess the nutritional status and condition of fish. Fish proximate body composition is of great interest in aquaculture because it affects fish appetite, growth and the efficiency of food utilization. Proximate body composition also affects other aspects of fish biology and ecology, including reproduction, survival, and energy value to predator (Breck, 2014). Body composition is a good indicator of the physiology condition of a fish but it is relatively time consuming to measure. Protein, fat and water content of fish is important to consumers, scientists and manufacturer for nutritional value, seasonal variations and considerations regarding processing (Murray and Burt, 2001).

Among the fishing communities, small fish occupy an important position as a popular food item. Thus, considering the importance of the small indigenous fish, the present study was undertaken to assess the nutritional values of some selected small fish available in Bawle Kyun, Htantabin Township. The objectives of the present study are:

- to examine the nutritional values of selected fish species and
- to compare the nutritional values among the species.

Materials and Methods

Study area and study sites

Htantabin Township is located in Northern District of Yangon Region, is bounded by Hlaing River in the east and Bawle River in the west. Kokkowa River flows east to west and divides the area into two parts: Bawle Kyun in the north and Tetthit Kyun in the south. The present study was conducted at Nethamein village (17°2´N and 95°49´E) in Bawle Kyun.

Study period

The study period lasted from January to December 2018.

Specimen collection

Based on their length of the individual, small indigenous species of the present study were considered as minimum 4cm. The most commonly found 13 small indigenous were sorted out in the collection area with the help of fishermen or fish traders (Plate 1).

Identification and classification

Identification and classification of the studied species was based on Jayaram (1999) and Fish Base website (http://www.fishbase.org).

Sample preparation

The fresh specimen (about 150g/species) for each species were separately packed, labeled and stored in the refrigerator for further nutritional analysis.

Notopterus notopterus (Nga-phe)

Amblypharyngodon mola (Nga-be-phyu)

Osteobrama belangeri (Nga-phe-oung)

Puntius chola (Nga-khone-ma)

Mystus pulcher (Nga-zin-yine)

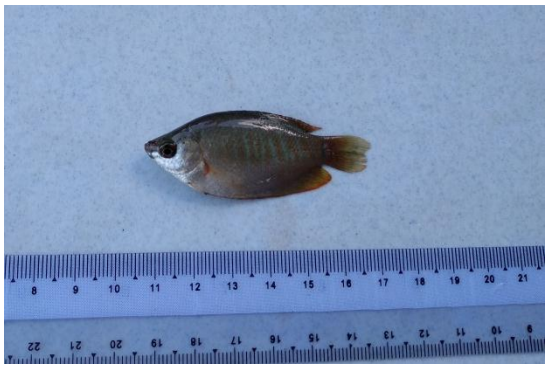
Ompok bimaculatus (Nga-nu-than)

Clarias batrachus (Nga-khu)

Heteropneustes fossilis (Nga-gyee)

Glossogobius giuris (Kat-tha-boe)

Anabas testudineus (Nga-pyay-ma)



Trichogaster labiosa (Nga-phyin-tha-let)

Channa punctatus (Nga- panaw)

Macrognathus aral (Nga-mway-doe)

Plate 1. The studied small indigenous fish species

Data analysis

The samples were analysed by AOAC-2000 test method for nutritional value in percentage (moisture, protein, ash and fat, fiber, carbohydrate) and energy values at Research and Innovation Analysis Department in Yangon.

Results

Nutritional Values analysis

A total of 13 small indigenous species were selected for nutritional analysis during the study period (Fig. 1 and Table 1).

Notopterus notopterus

The nutritional values of *Notopterus notopterus* was observed as moisture (81.21%), ash (1.22%), protein (17.10%), fat (0.05%), fiber (0.04%), carbohydrate (0.38%) and energy value (71kcal/100g).

Amblypharyngodon mola

The nutritional values of *Amblypharyngodon mola* was examined as moisture (76.3%), ash (2.61%), protein (16.82%), fat (2.98%), fiber (0.00%), carbohydrate (0.00%) and energy value (94kcal/100g).

Osteobrama belangeri

The nutritional values of *Osteobrama belangeri* was analysed as moisture (72.69%), ash (4.12%), protein (17.06%), fat (4.92%), fiber (0.03%), carbohydrate (0.02%) and energy value (85kcal/100g).

Puntius chola

The nutritional values of *Puntius chola* was observed as moisture (79.28%), ash (1.79%), protein (15.27%), fat (3.35%), fiber (0.00%), carbohydrate (0.00%) and energy value (91kcal/100g).

Mystus pulcher

The nutritional values of *Mystus pulcher* was analysed as moisture (80.20%), ash (2.23%), protein (16.04%), fat (1.50%), fiber (0.05%), carbohydrate (0.00%) and energy value (82kcal/100g).

Ompok bimaculatus

The nutritional values of *Ompok bimaculatus* was examined as moisture (79.69%), ash (3.24%), protein (15.59%), fat (1.61%), fiber (0.02%), carbohydrate (0.01%) and energy value (71kcal/100g).

Clarias batrachus

The nutritional values of *Clarias batrachus* was analysed as moisture (81.11%), ash (1.63%), protein (15.79%), fat (0.37%), fiber (0.06%), carbohydrate (1.04%) and energy value (72kcal/100g).

Heteropneustes fossilis

The nutritional values of *Mystus pulcher* was observed as moisture (82.90%), ash (1.13%), protein (14.59%), fat (0.24%), fiber (0.01%), carbohydrate (1.15%) and energy value (66kcal/100g).

Glossogobius giuris

The nutritional values of *Glossogobius giuris* were analysed to be moisture (80.85%), ash (1.56%), protein (16.89%), fat (0.09%), fiber (0.03%), carbohydrate (1.04%) and energy value (73kcal/100g).

Anabas testudineus

The nutritional values of *Anabas testudineus* was examined as moisture (66.28%), ash (10.41%), protein (18.52%), fat (3.41%), fiber (0.01%), carbohydrate (1.00%) and energy value (90kcal/100g).

Trichogaster labiosa

The nutritional values of *Trichogaster labiosa* was observed as moisture (79.53%), ash (3.41%), protein (14.16%), fat (2.71%), fiber (0.00%), carbohydrate (0.00%) and energy value (81kcal/100g).

Channa punctatus

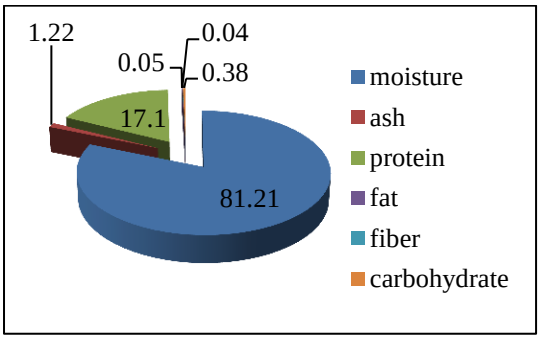
The nutritional values of *Channa punctatus* was analysed as moisture (79.91%), ash (1.53%), protein (17.19%), fat (0.46%), fiber (0.08%), carbohydrate (0.83%) and energy value (77kcal/100g).

Macrognathus aral

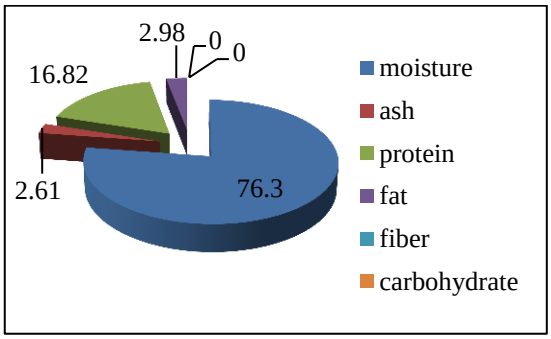
The nutritional values of *Macrognathus aral* was examined as moisture (79.28%), ash (2.16%), protein (15.88%), fat (1.72%), fiber (0.03%), carbohydrate (0.93%) and energy value (86kcal/100g).

Table 1 Nutritional values of small indigenous fish species in the study sites

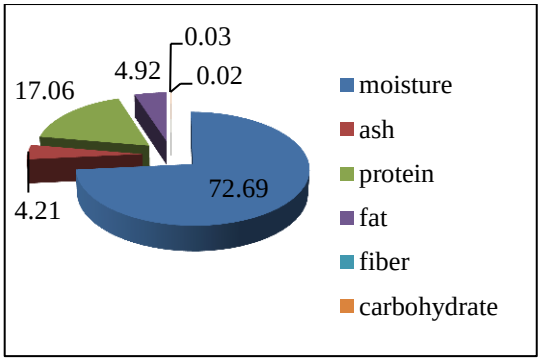
Fish species	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	Carbohydrate (%)	Energy value (kcal/100g)
N. notopterus	81.21	1.22	17.1	0.05	0.04	0.38	71
A. mola	76.3	2.61	16.82	2.98	0	0	94
O. belangeri	72.69	4.21	17.06	4.92	0.03	0.02	85
P. chola	79.28	1.79	15.27	3.35	0	0	91
M. pulcher	80.2	2.2	16.04	1.5	0.05	0	82
O. bimaculatus	79.6	3.2	15.5	1.61	0.02	0.01	71
C. batrachus	81.11	1.63	15.79	0.37	0.06	1.04	72
H. fossilis	82.9	1.13	14.57	0.24	0.01	1.15	66
G. giuris	80.85	1.56	16.89	0.09	0.03	0.58	73
A. testudineus	66.28	10.41	18.52	3.41	0.01	1	90
T. labiosa	79.53	3.41	14.16	2.71	0	0	81
C. punctatus	79.91	1.53	17.19	0.46	0.08	0.83	77
M. aral	79.28	2.16	15.88	1.72	0.03	0.93	86



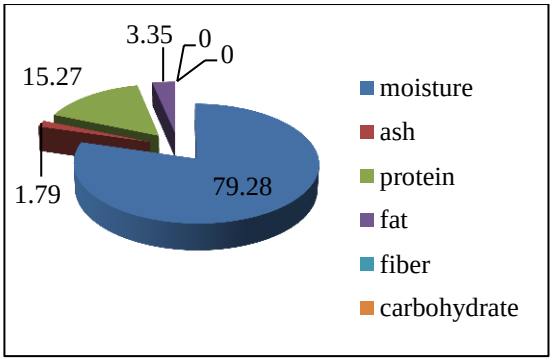
Notopterus notopterus (Nga-phe)



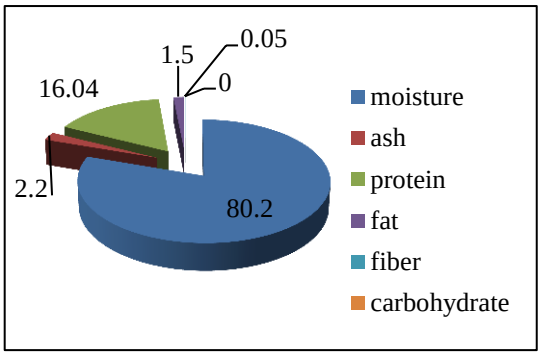
Amblypharyngodon mola (Nga-be-phyu)



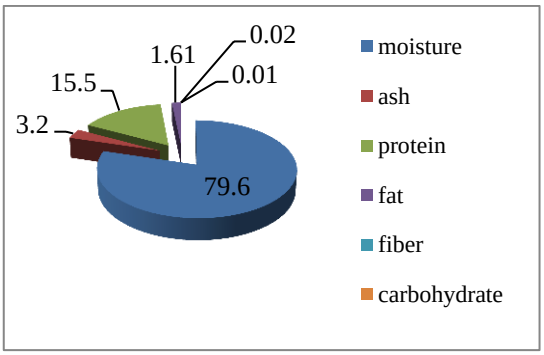
Osteobrama belangeri (Nga-phe-oung)



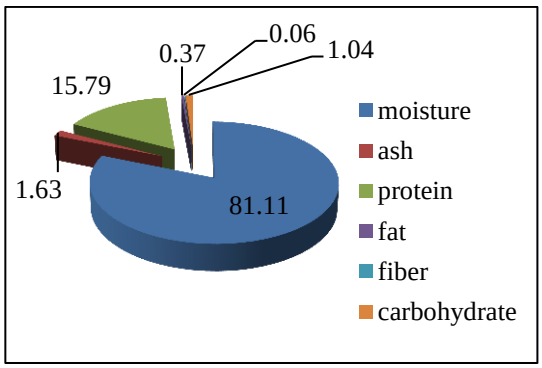
Puntius chola (Nga-khone-ma)



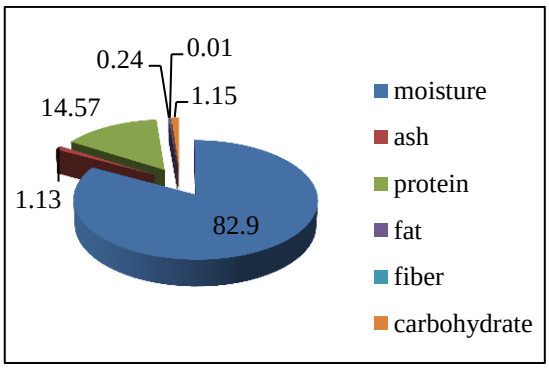
Mystus pulcher (Nga-zin-yine)



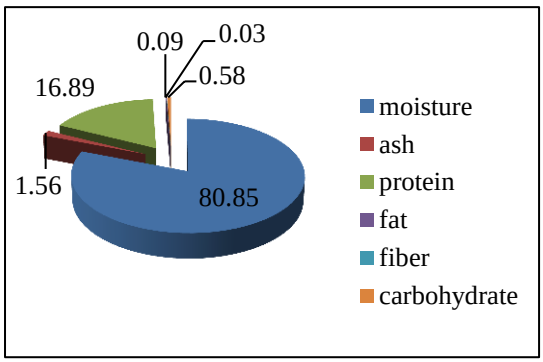
Ompok bimaculatus (Nga-nu-than)



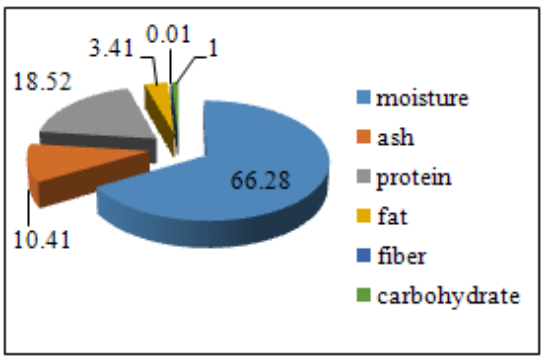
Clarias batrachus (Nga-khu)



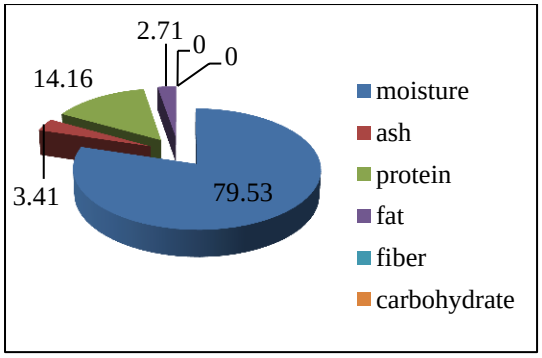
Heteropneustes fossilis (Nga-gyee)



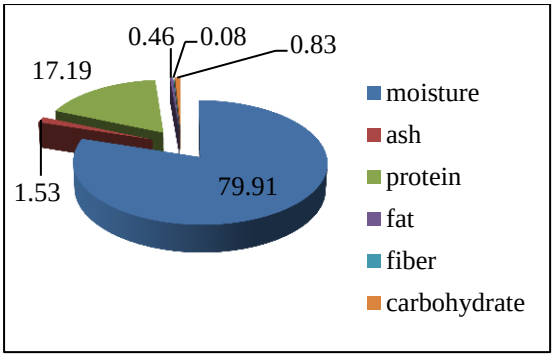
Glossogobius giuris (Kat-tha-boe)



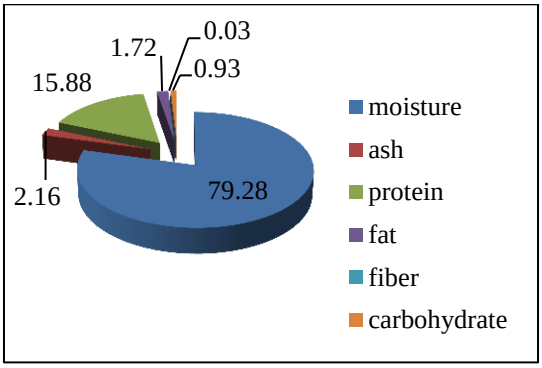
Anabas testudineus (Nga-pyay-ma)



Trichogaster labiosa (Nga-phyin-tha-let)



Channa punctatus (Nga- panaw)



Macrognathus aral (Nga-mway-doe)

Figure 1 Nutritional values analysed in the studied fish species

Variation of nutritional values among the species

The variation of moisture contents among the species ranged from 66.28% (*A. testudineus*) to 82.9% (*H. fossilis*) (Fig 1 and 2, Table 1). The variation of ash contents among the studied fish species ranged from 1.13% (*H. fossilis*) to 10.41% (*A. testudineus*) (Fig 1 and 3, Table 1). The variation of protein contents among the studied fish species ranged from 14.16% (*T. labiosa*) to 18.52% (*A. testudineus*) (Fig 1 and 4, Table 1). The variation of fat contents among the studied fish species ranged from 0.05% (*N. notopterus*) to 4.92% (*O. belangeri*) (Fig 1 and 5, Table 1). Highest crude fiber content was found in *C. puntatus* (0.08%) and no fiber content was found in *A. mola*, *P. chola* and *T. labiosa* (Fig 1 and 6, Table 1). Highest carbohydrate content was found in *H. fossilis* (1.15%) and no carbohydrate was found in *A. mola*, *P. chola*, *M. pulcher* and *T. labiosa* (Fig 1 and 7, Table 1). The variation of energy value among the studied fish species ranged from 66kcal/100g (*H. fossilis*) to 94kcal/100g (*A. mola*) (Fig 1 and 8, Table 1).

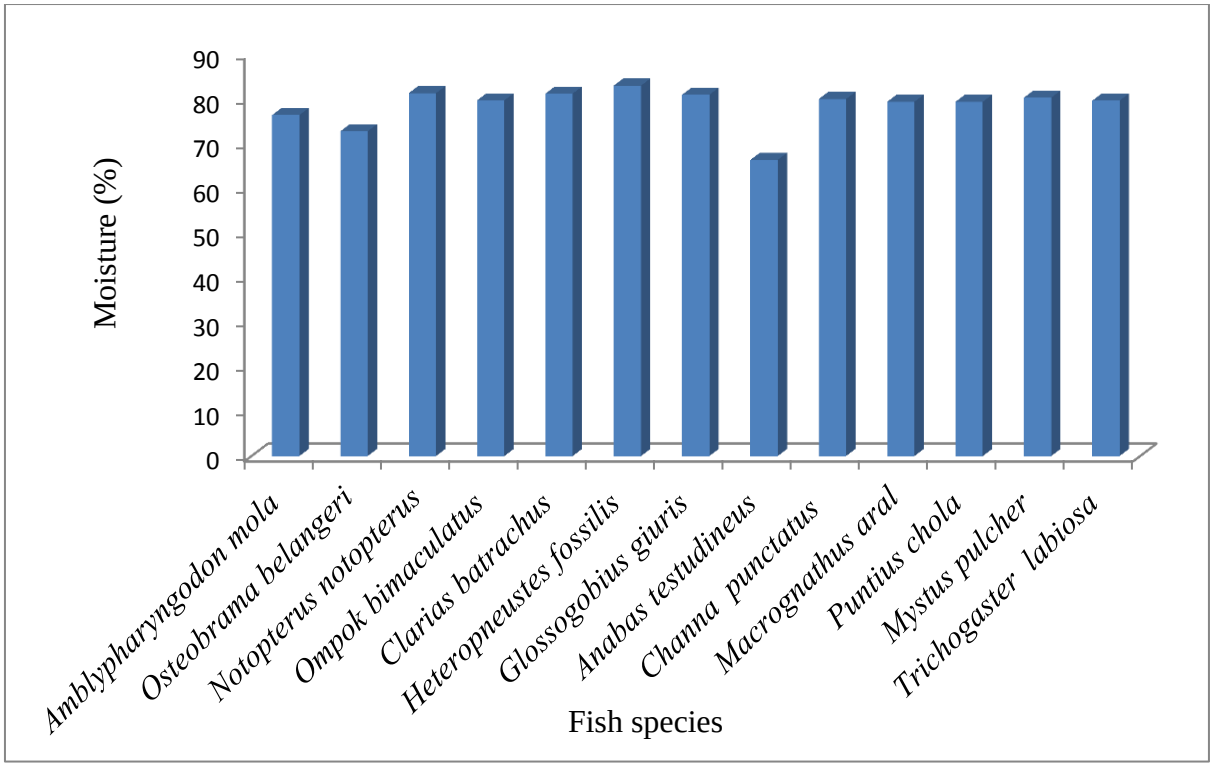


Figure 2 The variation of moisture contents among the species

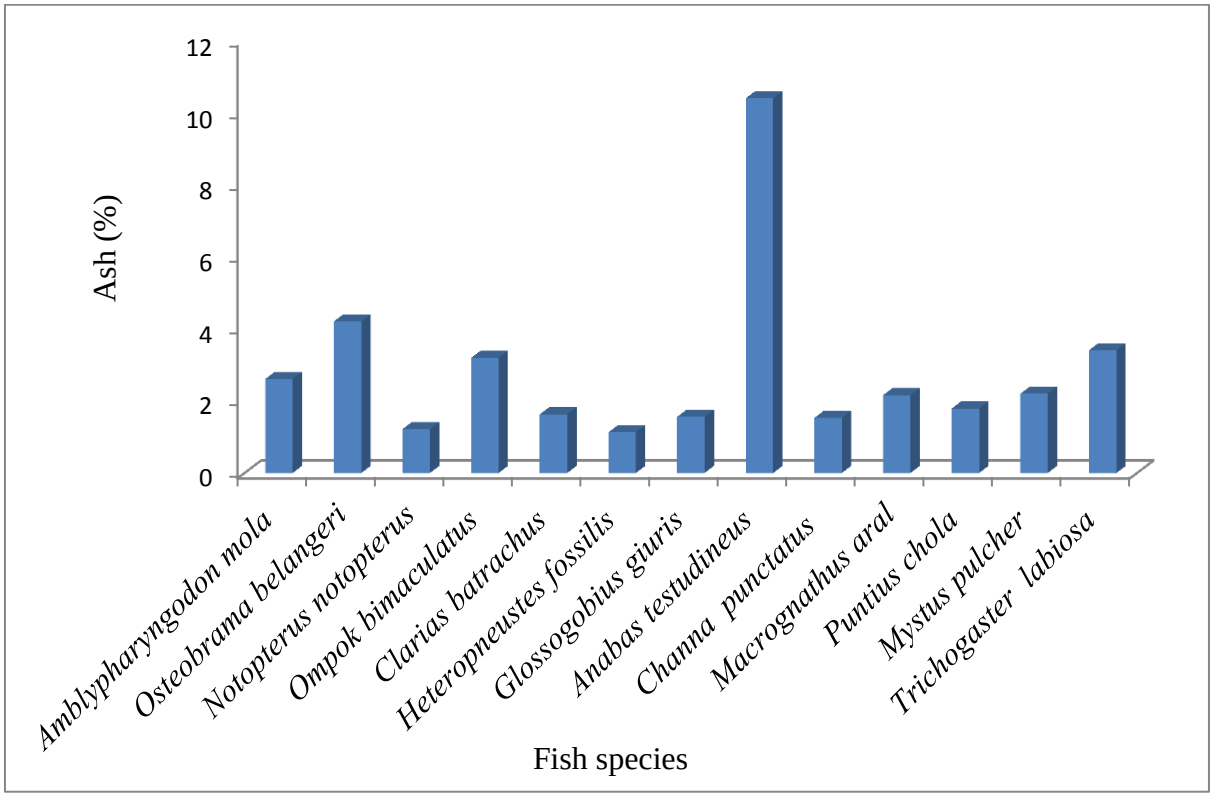


Figure 3 The variation of ash contents among the species

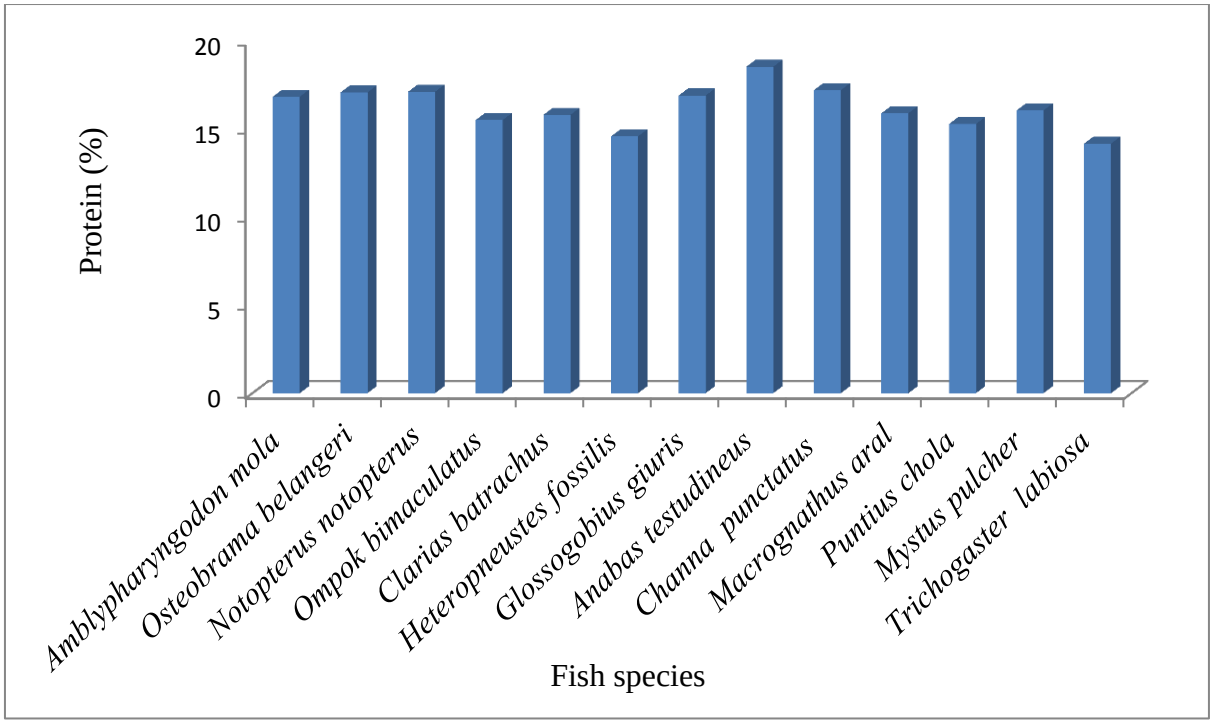


Figure 4 The variation of protein contents among the species

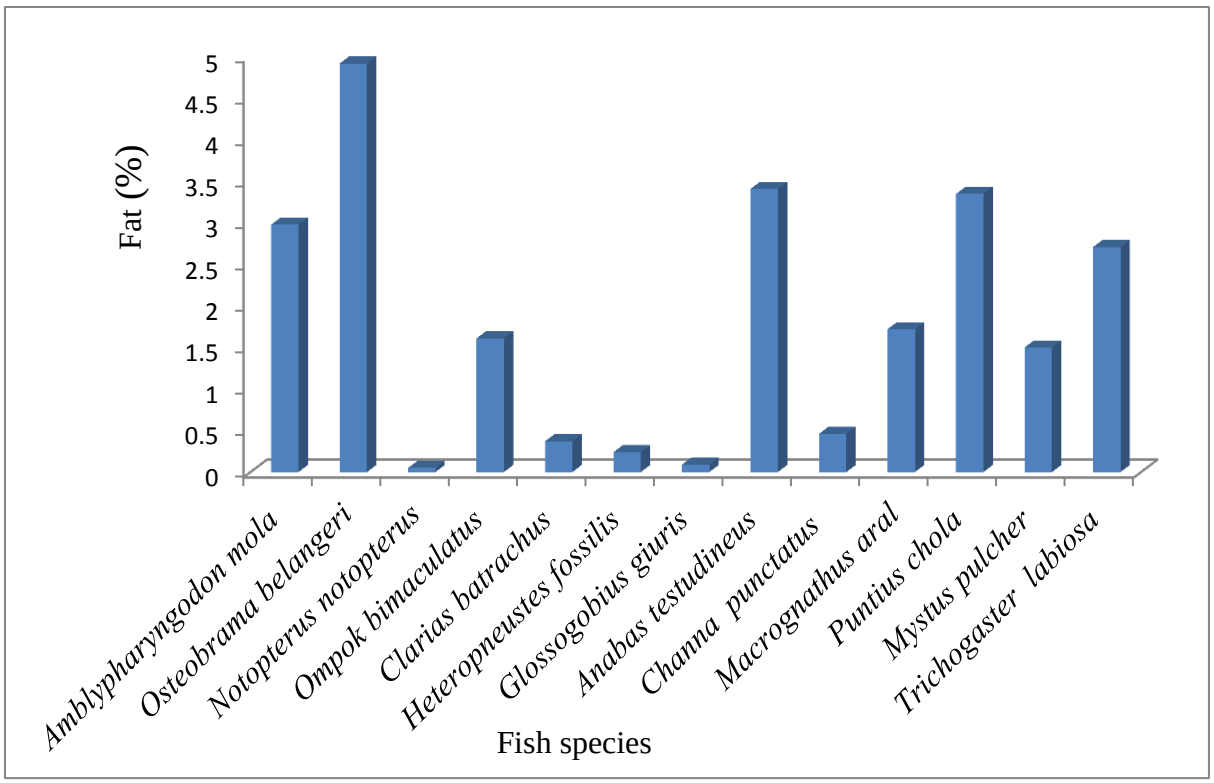


Figure 5 The variation of fat contents among the species

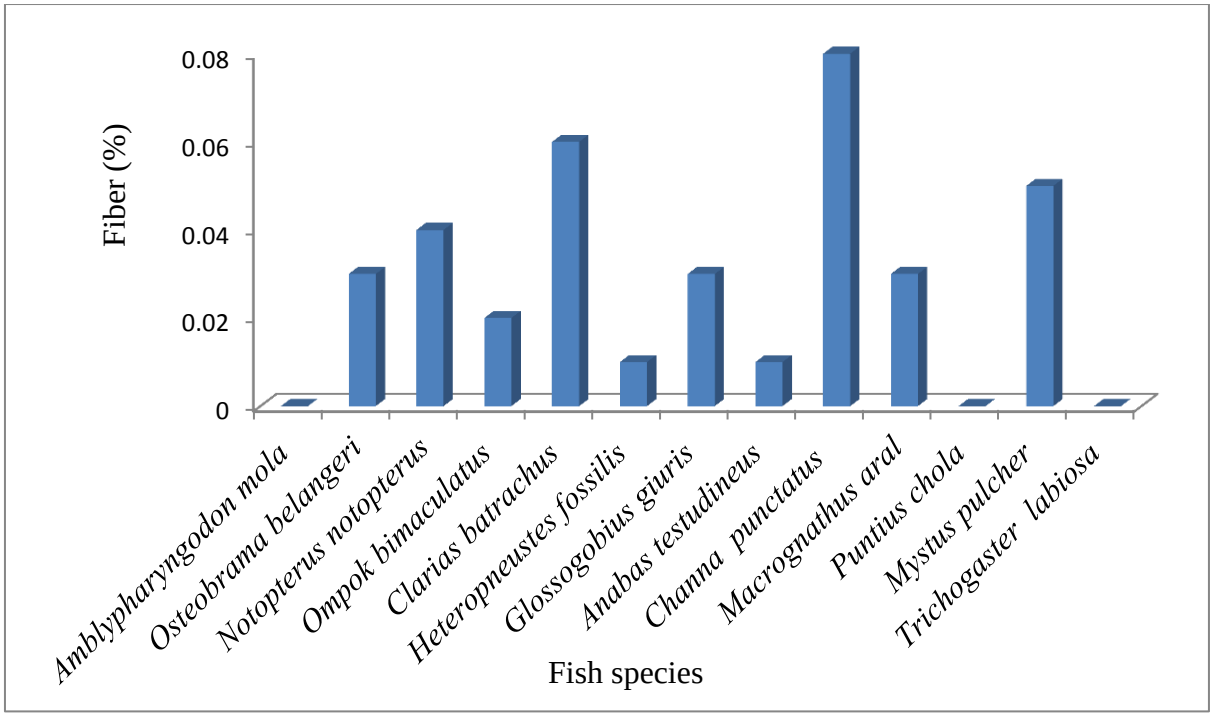


Figure 6 The variation of fiber contents among the species

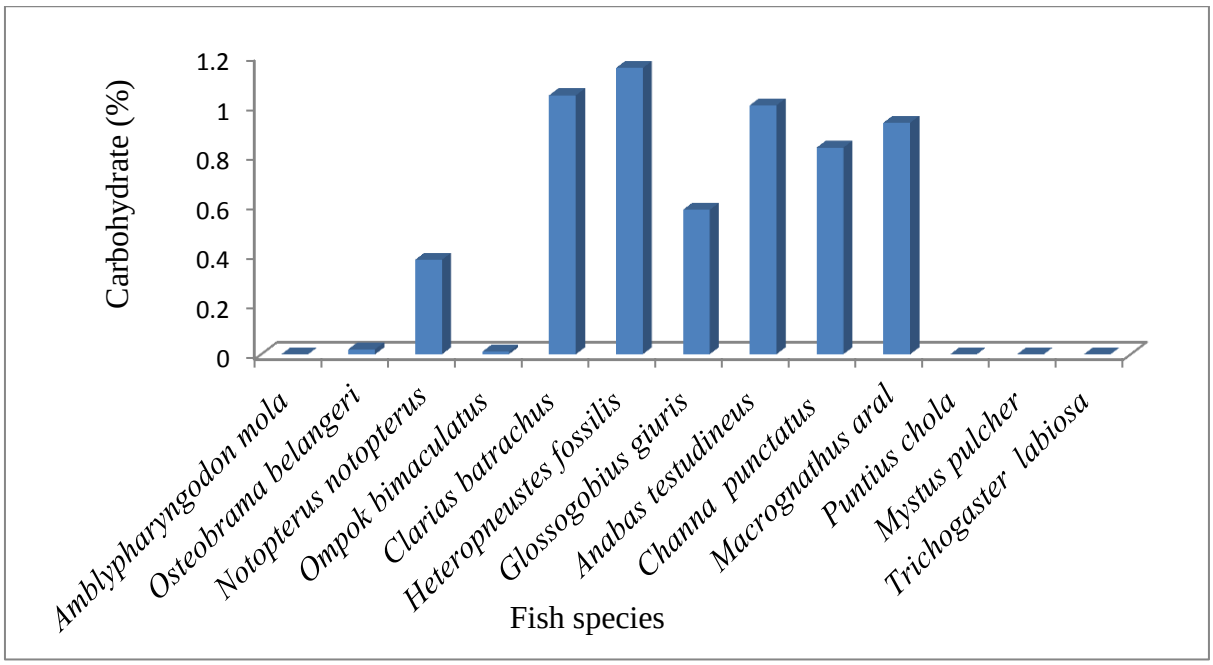


Figure 7 The variation of carbohydrate contents among the species

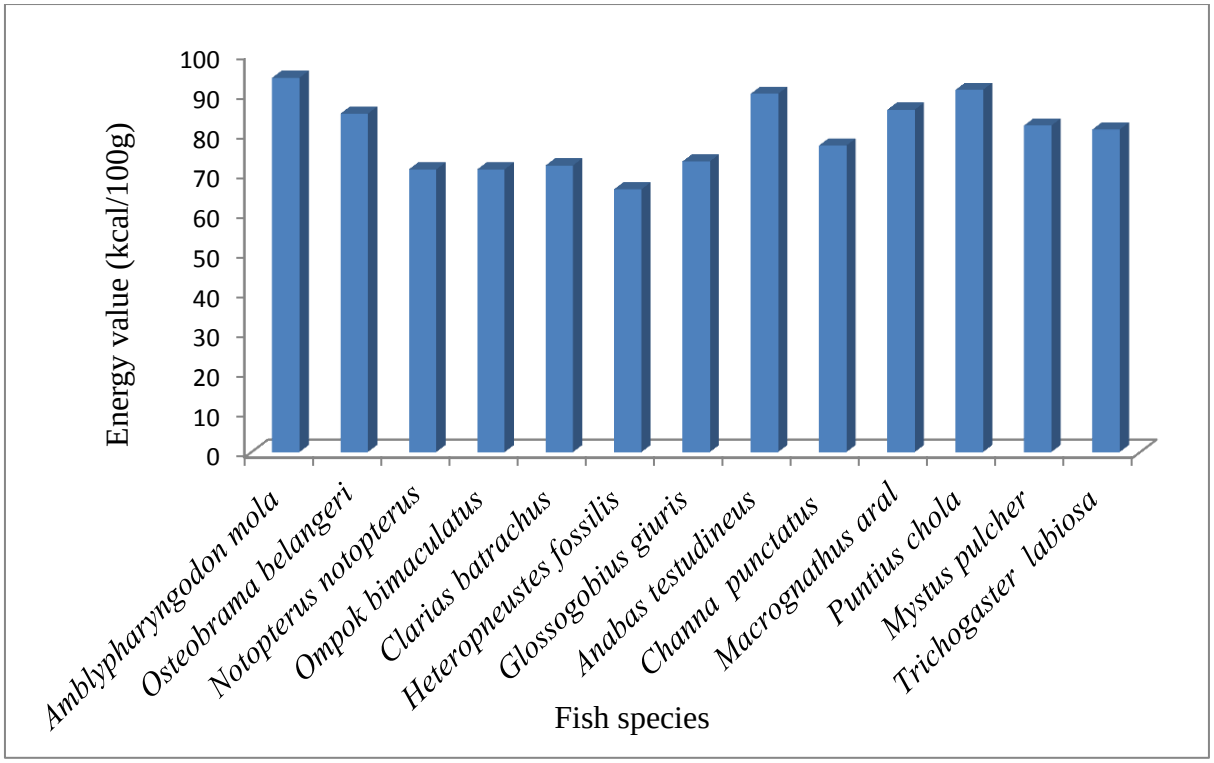


Figure 8 The variation of energy value contents among the species

Discussion

Small indigenous fish species of freshwater origin are not only a source of vital protein to the rural poor but also of micro-nutrients such as calcium, zinc, iron and fatty acids (Roos *et al.*, 2007). Considering the importance of the small indigenous fish species, this study is undertaken to assess the nutritional value of SIFS from the view of public health.

Proximate analysis is in term of nutritional values analysis a partitioning of compounds in a feed into six categories based on the chemical properties of the compounds. The six categories are: (1) moisture (2) ash (3) crude protein (or Kjeldahl protein) (4) crude lipid (5) crude fiber and (6) nitrogen-free extracts (digestible carbohydrates) (Analytical Techniques in Aquaculture Research, 2016).

The percentage of water is good indicator of its relative contents of energy, proteins and lipids. The lower percentage of water showed greater the lipids and protein contents and higher the energy density of the fish (Dempson *et al.*, 2004). The moisture content in the present study ranged between 66.28-82.9%. The moisture contents of SIFS recorded by Hossain *et al.* (1999) as 71.00-81.94%. The moisture contents of fishes in the present study slightly different than those that obtained by Hossain *et al.* (1999) and in culture fishes that obtained by Ali *et al.* (2006) with (66.75- 71.06%) and Bogard *et al.* (2015) with (65.5-84.19).

Concerning the ash content, the fish species in present finding was recorded between 1.13-10.41%. The ash content of fishes was recorded as 2.25-5.22% (Hossain *et al.*, 1999). The ash content of culture fishes was recorded as 1.11-7.02% (Ali *et al.*, 2006) and 0.9-1.5% (Bogard *et al.*, 2015). So this present result unbalanced with the previous data may be due to different feeding habits.

Proteins are not only necessary for hormonal and enzyme development (Wilsom, 1986), but are also an important source of energy (Halver and Hardy, 2002). The protein content of SIFS in the present study examined between 14.16-18.52%. The result showed little variations with Hossain *et al.*, (1999) who obtained the protein content range between 13.14-22.28% and Bogard *et al*. (2015) who recorded in culture fishes from 14.9-18.9%. Thus the present findings lie within the normal range.

Lipids are regarded as one of the most important food reserve contributing to the condition and this has led to the use of fat indices as a measure of relationship between percent water and percent fat (Sinclair and Duncan, 1972). It also provides much of energy and the essential body fatty acid (Gatlin, 2010). Regarding the fat content of SIFS in the present data ranged between 0.05 to 4.92%. The fat contents of indigenous fishes were recorded as 1.87-9.55% (Hossain *et al.*, 1999) and larger fish showed 0.31-8.2% (Ali *et al.*, 2006) and 0.7-17.7% (Bogard *et al*., 2015). It is considered that the variation of the lipid concentration is due to the different fish species examined.

In the present study, the fiber content ranged from 0-0.08% and carbohydrate showed 0-1.15%. The main body constituents of the fish include water, lipid, ash and protein. Carbohydrates and non-protein compounds are present in negligible amount and are usually ignored for routine analysis (Cui and Wootton, 1988).

The gross energy content in fresh matter basis of small indigenous fish species in the present study ranged between 66-94kcal/100g. The gross energy content of small indigenous fishes in Bangladesh was more or less similar to that reported by Hossain *et al.* (1999) who recorded as 97-169kcal/100g and and in culture large fish with 64-221kcal/100g by Bogard *et al*., (2015). Thus the energy values in SIS showed similar with that of larger culture fishes.

Conclusion

The small indigenous fish species can play a significant role to fulfill the nutrient demand of poorer sections of people of the country. It may be concluded that the small indigenous fish species make a choice based on that information from a consumer point of view.

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I extend our profound gratitude to Rector Dr. Tin Maung Tun, West Yangon University for giving us the opportunity to present this research paper. I am grateful to Pro-Rectors, Dr. Aye Aye Khaing and Dr. Soe Soe Aye, West Yangon University for their interest and encouragement. The invaluable guidance and helpful advice of Professor Dr. Thida Oo, Head of Zoology Department, West Yangon University is gratefully acknowledged. I also thanks goes to Dr. Kalayar Win Maung, Lecturer from University of Yangon and Dr. Thida Aung, Lecturer from Myitkyina University, for their guidance of this manuscript. Finally I also thanks goes to fishermen from Nethamein village, in Htantabin Township, Yangon Region.

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STUDY OF PARASITIC INFECTION IN *PIARACTUS BRACHYPOMUS* (CUVIER, 1817) IN LAY DAUNG KAN FISH FARM OF YANGON REGION

Yan Naung Tun[1], Myint Myint Win[2], Hnin Hnin Htay[3], Kay Lwin Tun[4]

Abstract

Freshwater fish culture is a major source of aquaculture production in Myanmar. In 2015-2016, the production of freshwater fish at 2.59 million MT (46% of total freshwater fish production). Examination of parasitic infection in freshwater as well as in marine fishes in Myanmar is still required to improve Myanmar aquaculture system. The present study was carried out to isolate and identify of different parasites and prevalence and mean intensity of infection from *Piaractus brachypomus*. A total of 240 fishes were observed in between August 2017 to July 2018 from Lay Daung Kan fish farm. Skin, gills, eyes, brain, heart, liver, gallbladder, muscles, intestine and kidneys were examined for infection. The isolated parasites were *Myxobolus* sp.1 and *Myxobolus* sp.2 were found in gills and gallbladder, *Trichodina heterodentata* in the gills and skin, *Ichthyophthirius multifiliis* in the skin and *Mymarothecium viatorum* and *Mymarothecium boegeri* in the gills respectively. Highest prevalence of infection was recorded in Monogenea parasites during the study period.

Keywords: *Piaractus brachypomus*, Parasitic, Platyhelminthes, Myxozoa, Cilophora, Prevalence

Introduction

Myanmar is the second largest country in Southeast Asia, with a land area of 676,577 square kilometers (km^2). Abundant natural resources in fresh- and brackish water fisheries contribute significantly to its food security (FAO, 2012). Myanmar had the highest number of fishers and fish farmers in the Southeast Asian region in 2014 (SEFDEC, 2012). Freshwater fish culture is a major source of the total aquaculture production (including mariculture) in Myanmar. Fishery products serve as major source of animal protein for the local population that largely consumes rice and fish in their daily meals. With population of 51.5 million in 2016, the country's average fish consumption was 68 kg/person/year (DoF, 2016).

The Red bellied pacu *Piaractus brachypomus*, also known as pirapitinga, is a native fish from the Amazon and Orinoco rivers and can reach up to 20 kg of weight (Alcantara *et al*., 1990). This species is used for fish farming, especially in Brazil, is valued for its meat and has a fast growth performance (Fresneda *et al*., 2004; MPA, 2013). This species also has economic importance for aquaculture in other countries in South America (Colombia, Peru, and Venezuela) and in Asia (China, Myanmar, Thailand and Vietnam) (Flores Nava, 2007; Honglang, 2007 and Lin *et al*., 2015). The demand of pacu fish significantly increased since 2010 because it is an almost boneless species, which is favoured by human consumers (FAO, 2013).

Currently, fish farmers in Myanmar do not maximize their productivity by enhancing the natural productivity of their ponds. Intensive systems in Myanmar are limited to a small number of specialized marine farms producing finfish and White Leg shrimp (*Penaeus vannamei*), and a few farms produce Striped catfish and Red-bellied pacu intensively (Belton *et al*., 2017b).

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Fish naturally carries a variety of pathogenic bacteria, fungi and parasites. Healthy fish with healthy immune systems should be able to fight off these ever-present disease organisms but unhealthy fish may fall victim. Fish can be affected by a wide range of infectious and non-infectious diseases. Infectious diseases are contagious and caused by parasites, bacteria, viruses or fungi. Non-infectious diseases can be infected environmental, nutritional or genetic (Floyd, 1997).

The production from culture systems is hampered by the infection of various fish parasites. Parasites and diseases are the most serious limiting factors in culture farms (FAO, 2004). Fishes are usually cultured in high density in restricted waterbodies, where pathogens can easily be transmitted between individuals (Woo *et al*., 2011). Besides direct losses caused by mortality, parasites may have considerable impact on growth, behavior of fish and their resistance to other stressing factors (Floyd, 1997). Protozoan and monogenean ectoparasites are common in juvenile carp in nursery ponds. High mortality rates caused by myxosporidian infections in the gills have raised serious concern among fish farmers (Awal *et al*., 2001).

The number of fish parasitologists in fishery and aquaculture sectors of the country is small. Most of the studies were emphasized on wild populations or single fish species from aquaculture farms. Examination of parasitic infections in freshwater as well as in marine fishes in Myanmar is still required to improve production local aquaculture system.

The present study was undertaken to collect, identify and record the occurrence of parasites in *Piaractus brachypomus*. Prevalence and mean intensity of infections were also reported.

Materials and methods

Sample collection and preparation of aquaria

Fish were produced using the induced breeding method and breeders were cultured separately in 1436.67 m^2 pond very extensively. Approximately 240 fishes were collected to examine parasitic infections in between August 2017 to July 2018 from Lay Daung Kan Fish Farm, Yangon Township. (Fig.1). The sampling fishes were transported to the Laboratory of Aquatic Bioscience, Department of Zoology, University of Yangon alive in plastic bags filled with pond water enriched with oxygen. On arrival, they were kept separately in fiber tanks with the supply of aeration. One day prior to the arrival of the fish, aquaria were thoroughly cleaned, filled with water and aerated. Some fish were immediately dissected to examine their parasite load and incidence, and the remainders were kept in aquaria for five days for subsequent studies. All diagnostic symptoms were carefully recorded for each individual fish.

Examination and identification of Monogenea parasites

For the collection of live gill parasites, live fishes were sacrificed after anaesthetization and the gills were removed immediately. Each gill arch was separately cut and placed in a Petri dish containing saline water. Alive monogeneans were dislodged from gill arches by gentle scraping and collected under a dissecting microscope. The alive monogeneans were placed in cavity blocks filled with physiological saline solution (0.9% NaCl) and transferred to a clean glass slide containing one drop of physiological saline solution. The parasite on the slide was then covered with a coverslip. Prepared live specimens were examined under a compound

microscope to study their internal soft organs such as the reproductive and digestive organs. According to Yamaguti (1963) and Brain (2004), identification of the monogeneans was based mainly on the sclerotized hard parts of the haptor, supporting bar, marginal hooks and copulatory organs.

Examination and identification of protozoan parasites

Mucous scrapped from fins, skin and gills removed from the branchial cavity were placed in a Petridish for microscopic examination. The body of the host was then opened and internal organs, viz., eye, brain, gills, heart, swim-bladder, liver, gall-bladder, muscles, fins, mucus, intestine and kidney were removed and transferred into Petridishes. Tissues were placed on a glass slide, physiological saline solution (0.9% NaCl solution) was added, and a cover slip was placed over the specimen prior to subsequent examination by light microscope. In order to prepare permanent slides, tissues were stamped on the slide and left for a few minutes to dry. Air-dried smears were stained with Giemsa after fixing in absolute methanol, they were then cleaned with distilled water, dipped in xylene and mounted permanently with D.P.X mounted. Identification of protozoan parasites was done following the description and figure of Lom and Dykova (2006).

Data analyses for parasites

Parasite infestation was quantified according to Bush, (1990) and Margolis *et al*., (1982).

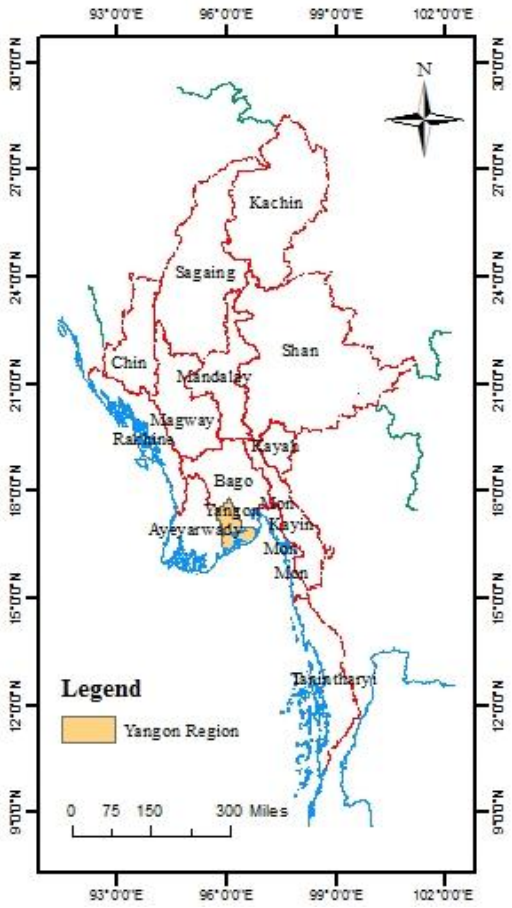
$$\text{Prevalence (\%)} = \frac{\text{Number of infected host} \times 100}{\text{Total number of host examined}}$$

While the Mean Intensity (MI) of monogenean parasites is given by the total number of parasites of a given species divided by the number of fishes infested with that species.

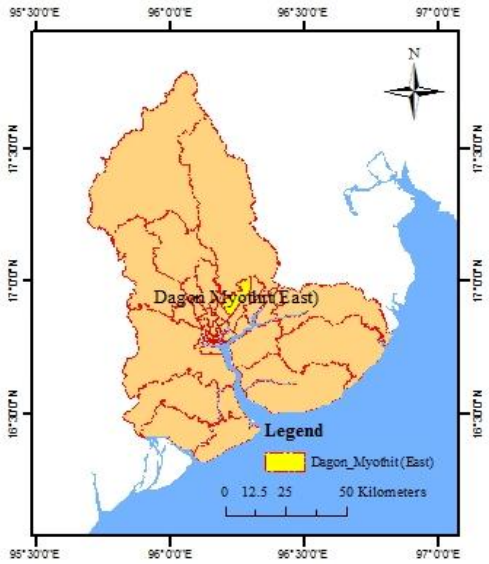
$$\text{Mean intensity} = \frac{\text{Total number of parasites}}{\text{Total number of infected fish}}$$

Intensity of infection was categorized into five stages for protozoan parasites according to Bachere *et al*., (1982) and Culloty *et al*., (1999).

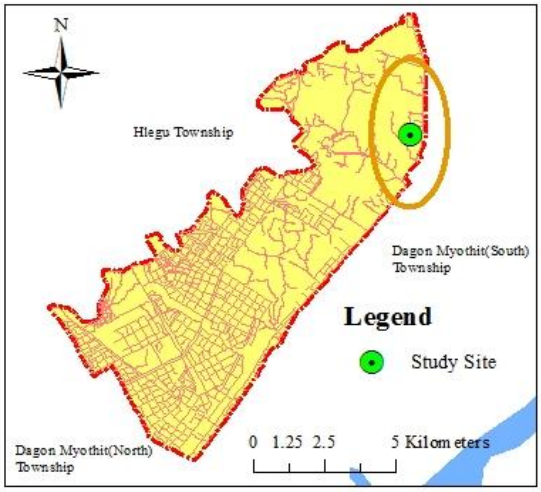
- Stage (I): 1 – 20 parasites observed within 3-min of screening under 40X magnification.
- Stage (II): 21– 40 parasites observed within 3-min of screening under 40X magnification.
- Stage (III): 41– 60 parasites observed within 3-min of screening under 40X magnification.
- Stage (IV): 1 – 10 parasites in all fields of vision observed immediately in screening under 40X magnification.



A. Yangon Division



B. Dagon Myothit (East)



C. Lay Daung Kan Fish Farm

Source: Geography Department, Institute of Education

Figure 1 Map showing location of Lay Daung Kan Fish Farm in Dagon (East) Township, Yangon Region

Results

1.1 Parasitic infection in *Piaractus brachypomus*

During the study period six species of parasites belonging to three phyla were detected in the gills and skin of *Piaractus brachypomus*, namely *Myxobolus* sp.1, *Myxobolus* sp.2, *Trichodina heterodentata*, *Ichthyophthirius multifillis*, *Mymarothecium viatorum* and *Mymarothecium boegeri* (the latter both fungi: Ascomycota) (Table 1).

1.1.1 Taxonomy of the recorded parasite taxa

Systematic positions of the two observed species of the phylum Cnidaria in *Piaractus brachypomus* are:

Phylum - Cnidaria Hatschek, 1888
Class - Myxozoa Grasse, 1970
Order - Bivalvulida Shulman, 1959
Family - Myxobolidae Thelohan, 1892
Genus - *Myxobolus* Butschli, 1882
Species - *Myxobolus* sp.1
Species - *Myxobolus* sp.2

Systematic positions of two observed species of the phylum Cilophora in *Piaractus brachypomus* are:

Phylum - Cilophora Doflein, 1901
Class - Oligohymenophorea de Puytorac *et al*., 1974
Order - Mobilina Kahl, 1933
Family - Trichodinidae Raabe, 1959
Genus - *Trichodina* Ehrenberg, 1838
Species - *Trichodina heterodentata* Duncan, 1977
Class - Oligohymenophorea de Puytorac *et al*., 1974
Order - Hymenostomatida Delage & Hérouard 1896
Family - Ichthyophthiriidae Brown, 1951
Genus - *Ichthyophthirius* Fouquet, 1876
Species - *Ichthyophthirius multifiliis* Fouquet, 1876

Systematic positions of the two observed species of the phylum Platyhelminthes in *Piaractus brachypomus* are:

Phylum - Platyhelminthes Claus, 1887
Class - Monogenea Van Beneden, 1858 and Bychowsky, 1973
Order - Dactylogyridae Bychowsky, 1937
Family - Ancyrocephalidae Bychowsky, 1937
Genus - *Mymarothecium* Kritsky Boeger et Jegu, 1996
Species - *Mymarothecium viatorum* Boeger, 2002
Species - *Mymarothecium boegeri* Boeger, 2002

1.1.2 Morphological description of Cnidaria in *Piaractus brachypomus*

Myobolus sp. 1 (Plate1A)

Host	-	*Piaractus brachypomus* (Cuvier, 1817)
Locality	-	Lay Daung Kan fish farm, Dagon (East),Yangon.
Site of infection	-	Spores were isolated from the gills.

Prevalence of infection (based on the seasonal data): 55% (134/240 investigated host individuals)

Characteristics of the spore:

Immature spores are round to ovoid in shape. Two polar capsules are pear shaped. Sporoplasm has two nuclei. Mature spores are ovoidal to rounded in shape in front view. Both the anterior and posterior ends are blunt. The two polar capsules are equal in shape. The capsules are pear shaped with anterior tip and blunt posterior end. Polar filaments were sometimes extruding outside the polar capsules and spores. The polar filaments were invisible and impossible to count the number of coil. Sporoplasm agranular, homogenous and occupying whole of the extracapsular space behind the polar capsules. The measurement of the spores was as follows;

Length of spore	= 7.5µm ± 0.3µm (n=10)
Width of spore	= 5µm ± 0.5µm (n=10)
Length of polar capsule	= 5µm (n=10)
Width of polar capsule	= 2.5µm ± 0.8µm (n=10)

Myobolus sp. 2 (Plate 1B)

Host	-	*Piaractus brachypomus* (Cuvier, 1817)
Locality	-	Lay Daung Kan fish farm, Dagon (East), Yangon.
Site of infection	-	Spores were found in gills

Prevalence of infection (based on the seasonal data): 35% (83/240 investigated host individuals)

Characteristics of the spore:

The spores were circle-shaped in front view. Shell valves thick, smooth and symmetrical. No parietal folds were present. Polar capsules two, unequal, oval to spherical in shape and placed anteriorly in the spore body cavity. The large polar capsules were pumpkin seed-like in shape and the smaller one was tear-shaped. The polar filaments were invisible and impossible to count the number of coil. The measurements of the spores were as follows;

Length of spore	= 51µm ± 3.2µm (n=10)
Width of spore	= 38µm ± 4.2µm (n=10)
Length of right polar capsule	= 34µm ± 5.2µm (n=10)
Width of right polar capsule	= 24µm ± 5.1µm (n=10)
Length of left polar capsule	= 14µm ± 1.5µm (n=10)
Width of left polar capsule	= 10µm ± 0.2µm (n=10)

1.1.3 Morphological description of Cilophora in *Piaractus brachypomus*

Trichodina heterodentata (Plate 1C)

Host	-	*Piaractus brachypomus* (Cuvier, 1817)
Locality	-	Lay Daung Kan fish farm, Dagon (East), Yangon
Site of infection	-	Parasites were isolated from the skin and gills.

Prevalence of infection (based on the seasonal data): 85% (205/240 investigated host individuals)

Characteristics of the taxon:

Trichodinid of medium size with a disc-shaped. They have a broad sickle-shaped blade that fit into the quadrant delimited. The distal surface of the blade form a shallow curve, parallel to the border membrane. The tangential point is sharp in the majority of the individuals, or slightly rounded in the others; it is located slightly below or at the same level as the distal tip of the distal blade margin. The blade is prominent. The blade shows moderate fit with the central part. The central part is thick and triangular shape. The rays are moderately thick and straight, and their tips are generally sharp. The measurements of the parasites were as follows;

Diameter of adhesive disc (da)	= 45µm ± 4.2µm (n=10)
Diameter of denticulate ring (dd)	= 15.2µm ± 2.5µm (n=10)
Diameter of clear area (dc)	= 12.5µm ± 1.4µm (n=10)
Number of denticles	= 20 ± 1.5 (n=10)

Ichthyophthirius multifiliis (Plate 1D)

Host	-	*Labeo rohita* (Hamilton, 1822) and *Piaractus brachypomus* (Cuvier, 1817)
Locality	-	Lay Daung Kan fish farm, Dagon (East), Yangon
Site of infection	-	White spot were found on the skin, gill filaments and gill arch.

Prevalence of infection (based on the seasonal data): 52% (125/240 investigated host individuals)

Characteristic of the spores:

Ichthophthirius multifiliis shows macronucleus surrounded by a thin rounded, transparent jelly mass changing its shape by a thin rounded. The mature parasites secrete a cyst around themselves which is generally spherical or ovoid in shape although considerable variation in shape has been observed. The whole body of *Ichthyophthirius multifiliis* bears a large number of cilia. It has a tubular mouth, several vacuoles and a large horse-shoe-shaped nucleus.

Length of spore	= 20.5µm ± 4.5µm (n = 10)
Width of spore	= 26.2µm ± 6.5µm (n = 10)

1.1.4 Morphological description of Platyhelminthes in *Piaractus brachypomus*

Mymarothecium viatorum (Plate 2A)

Host	-	*Piaractus brachypomus* (Cuvier, 1817)
Locality	-	Lay Daung Kan fish farm, Dagon (East),Yangon
Site of infection	-	Gills
Number of specimen measured	-	10

Prevalence of infection (based on the seasonal data): 100% (240/240 investigated host individuals)

Characteristics:

Body - Body elongate, 716 µm ± 78.3 µm long, greatest width 96µm ± 7.1µm. Tegument smooth. Cephalic lobes well developed, head organs present. Eyes 4, posterior pair slightly larger than anterior pair; accessory granules few or absent. Pharynx bulbous. Male copulatory organ 12.5µm ± 1.9µm long, a sinuous tube, tapering distally, base skirt like. Accessory piece, 14.3µm ± 0.9µm long. Testis ovate, single prostatic reservoir, seminal vesicle fusiform.

Haptor - Ventral Haptor 30µm ±4.8 µm long, with elongate superficial root, evenly curved shaft, point; 4µm ± 1.8µm wide. Dorsal Haptor 32µm ± 2.1µm long, with coparatively shorter superficial root often bent, articulates to respective extremities of dorsal bar, curved shaft, elongate point; base 11.8µm ± 0.6µm wide. Ventral bar 30µm ± 4.8µm long, broadly Vshaped, with expanded ends, posteromedial process. Dorsal bar 33µm ± 4.1µm long, broadly V-shaped with slightly expanded ends, short posteromedian process. Hooks 14, similar in shape; each with delicate point, protruding thumb. Their total length is 8.7 µm ±2.9 µm.

***Mymarothecium boegeri* (Plate 2B)**

Host	-	*Piaractus brachypomus* (Cuvier, 1817)
Locality	-	Lay Daung Kan fish farm, Dagon (East), Yangon
Site of infection	-	Gills
Number of specimen measured	-	10

Prevalence of infection (based on the seasonal data): 15% (35/240 investigated host individuals)

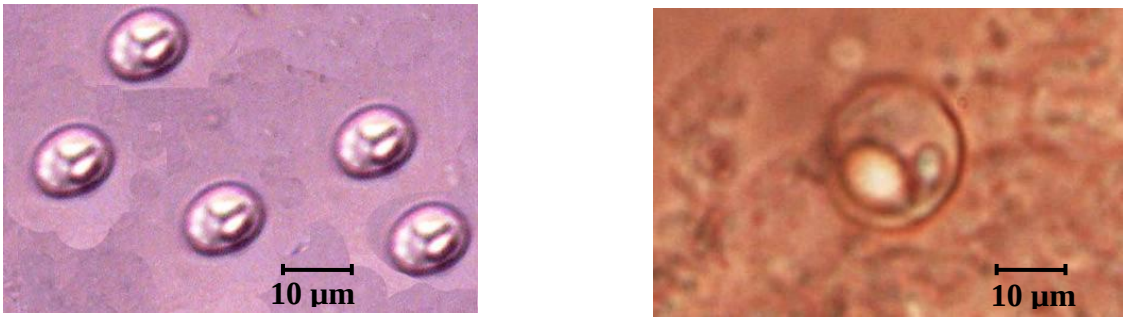
Characteristics:

Body - Body 730µm ± 48.3µm long by 88µm ± 10.3µm wide at level of ovary. Tegument smooth or presenting scaled annulations. Cephalic lobes developed. Two pairs of eyes; posterior pair larger and separated from anterior pair. Pharynx spherical to ovate. Copulatory organ comprising a narrow tube. Accessory piece bifurcated at base, measuring 12µm ±1.7µm, ring-shaped sub-terminal flap and hook-shaped process. Testis subovate, 10µm ± 0.2µm, seminal vesicle elongate; germarium elongate. Vaginal aperture dextroventral. Vitelline follicles in two bilateral fields of trunk, extending from pharynx to level of haptor.

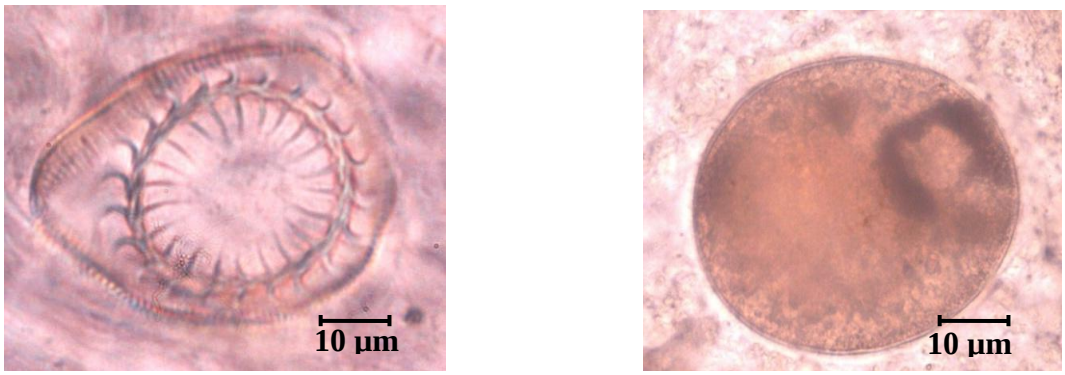
Haptor - Haptors similar; ventral haptor 12µm ± 1.5µm long, dorsal haptor 10.8 µm ±0.2 µm long; each having well-developed superficial root with slight depressions, deep root comparatively smaller, curved shaft, elongate point. Ventral bar V-shaped, 29.2µm ± 7.9µm long, with short posteromedial process. Dorsal bar broadly U-shaped, 21.7µm ± 10.4µm long. Haptor with 7 pairs of marginal hooks. Their total length is 7.5µm ±1.5µm.

Table 1 List of parasites recovered and their site of infection

Parasite		**Host**	**Site of infection**
Cnidaria	*Myxobolus* sp.1	*Piaractus brachypomus*	Gills
	Myxobolus sp.2		Gills
Cilophora	*Trichodina heterodentata*		Gills and skin
	Ichthyophthirius Multifiliis		Skin
Platyhelminthes	*Mymarothecium Viatorum*		Gills
	Mymarothecium Boegeri		



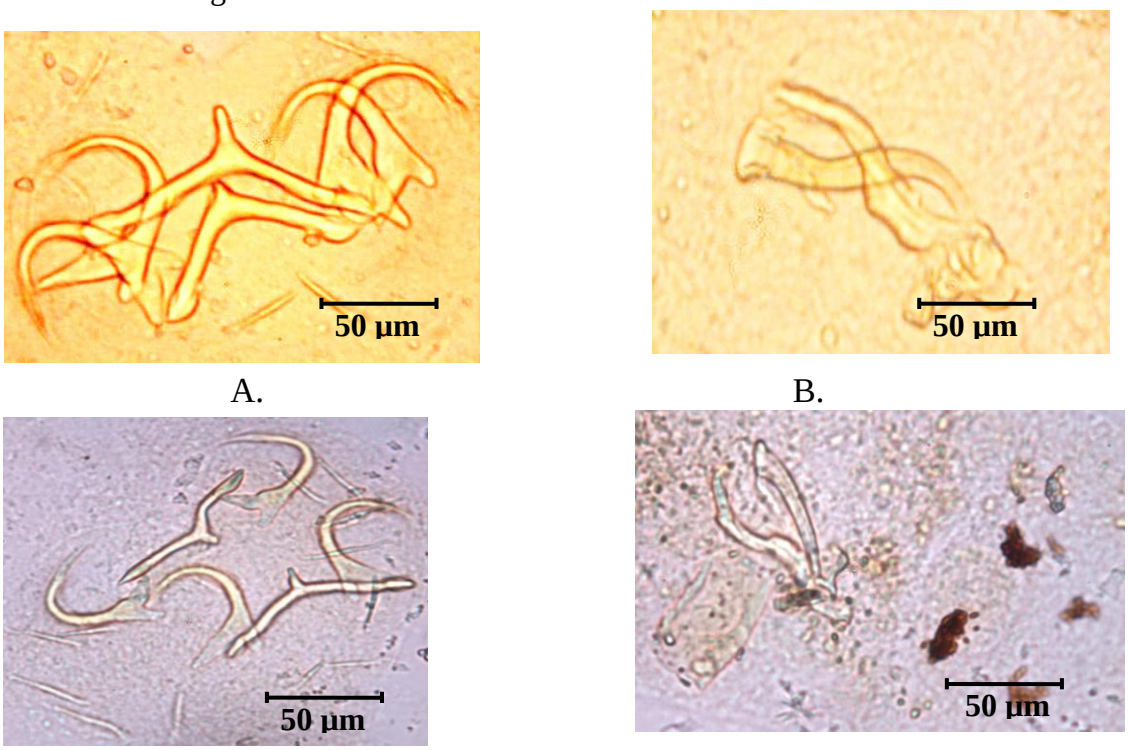
A. B.



C. D.

Plate 1: Recorded protozoan parasite from *Piaractus brachypomus* during the study period.

A. *Myxobolus* sp.1 recorded from the gills, B. *Myxobolus* sp.2 recorded from the gills, C. *Trichodina heterodentata* recorded from the gills and skin and D. *Ichthyophthirius multifiliis* recorded from the gills and skin



A. B.

C. D.

Plate 2: Recorded Monogenea parasite from *Piaractus brachypomus* during the study period.

A. Sclearotised parts of *Mymarothecium viatorum*, B. Copulatory organ of *Mymarothecium viatorum*, C. Sclearotised parts of *Mymarothecium boegeri* and D. Copulatory organ of *Mymarothecium boegeri*

1.2 Prevalence and mean intensity of parasaite infestations of the phylum Cnidaria in *Piaractus brachypomus*

Figure (2) shows two species of Cnidaria recorded in *Piaractus brachypomus.* Infection started when fish were five months old.

Myxobolus sp.1 was found in the whole study period except from August and September, 2017 and July, 2018. Low prevalence of infection was detected during the study period. Prevalence of infestation was ranging from 15% to 55%. The highest prevalence, 55% was recorded in May, 2018 while the lowest one, 15% was found in June, 2018. Low mean intensity of infection ranged from 1.2 to 2. Intensity decreased slightly from October, 2017 to January, 2018 while it was increased in March, 2018.

Prevalence of infection of *Myxobolus* sp.2 from the gills of *Piaractus brachypomus* during study period was 35%. The highest prevalence found in the month of October, 2017 (35%) and lowest in June, 2018 and July, 2018 (10%). No infection was recorded from December, 2017 to April, 2018.

Monthly intensity of infestation was described in Fig. 3. High mean intensity of 1.4 was recorded in October, 2017 and while it was decreased around about 1.2 in November, 2017.

1.3 Prevalence and mean intensity of parasite infestations of the phylum Cilophora in *Piaractus brachypomus*

The highest prevalence of infestation was recorded in *Trichodina heterodentata*. Prevalence of infestation fluctuated monthly ranging from 50% to 85%. The highest prevalence was recorded in October, 2017 and the lowest prevalence was recorded in March, 2018 (Fig. 4). Monthly intensity of *Trichodina heterodentata* infestation was described in (Fig. 5). High mean intensity 2.5 was recorded in April 2018. Mostly, mean intensity of *Trichodina* sp. range was 1.2 during study period.

Ichthyophthirius multifiliis was recorded only three times in December, 2017, January and February, 2018 with the prevalence of infection of 30%, 45% and 20% respectively. The intensity of infection ranged from 1 to 1.5. Except from theses three months, *Ichthyophthirius multifiliis* was not recorded in the studied area.

1.4 Prevalence and mean intensity of parasite infestations of the phylum Platyhelminthes in *Piaractus brachypomus*

Figure (6) shows prevalence of infestation of *Mymarothecium* spp. from gills of *Piaractus brachypomus* during the study period. *Mymarothecium viatorum* was found in gills of *Piaractus brachypomus* in October, 2007 when the fish was five months old. Prevalence of infection of *Mymarothecium viatorum* range from 25% to 85%. Prevalence of infecting did not change very much during study period (until April, 2018). Prevalence of infestation was sharply increased with high prevalence of 85% was recorded in May, 2018 (Fig. 6). Monthly intensity of *Mymarothecium viatorum* infestation was described in (Fig. 7). Mean intensity ranged from 1to 1.5.

Prevalence of infection of *Mymarothecium boegeri* was described in Fig. 6. Prevalence of infection was very low when it was compared with *Mymarothecium bogeri*. It was recorded only four months among study period. The highest prevalence range was 15% in *Mymarothecium boegeri*. Mean intensity of infestation was described in Fig. 7. Mean intensity of infection ranged from 1.5 to 3.2. Intensity slightly increased from November, 2017 to June, 2018. The highest intensity of infection 3.2 was recorded in June, 2018.

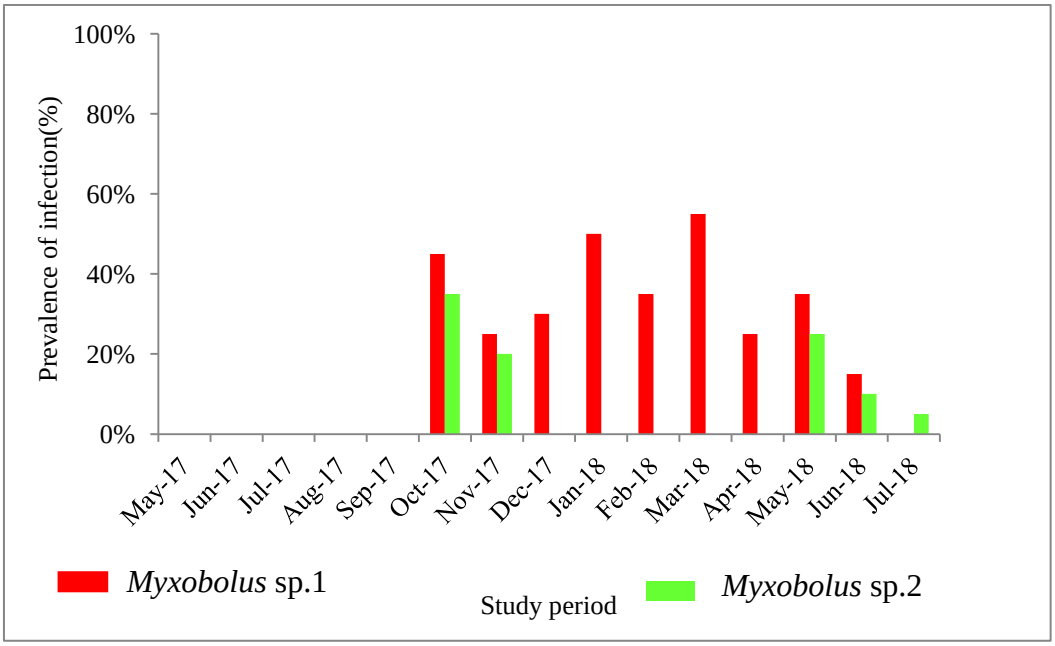


Figure 2 Prevalence of Cnidaria parasites in *Piaractus brachypomus* during the study period

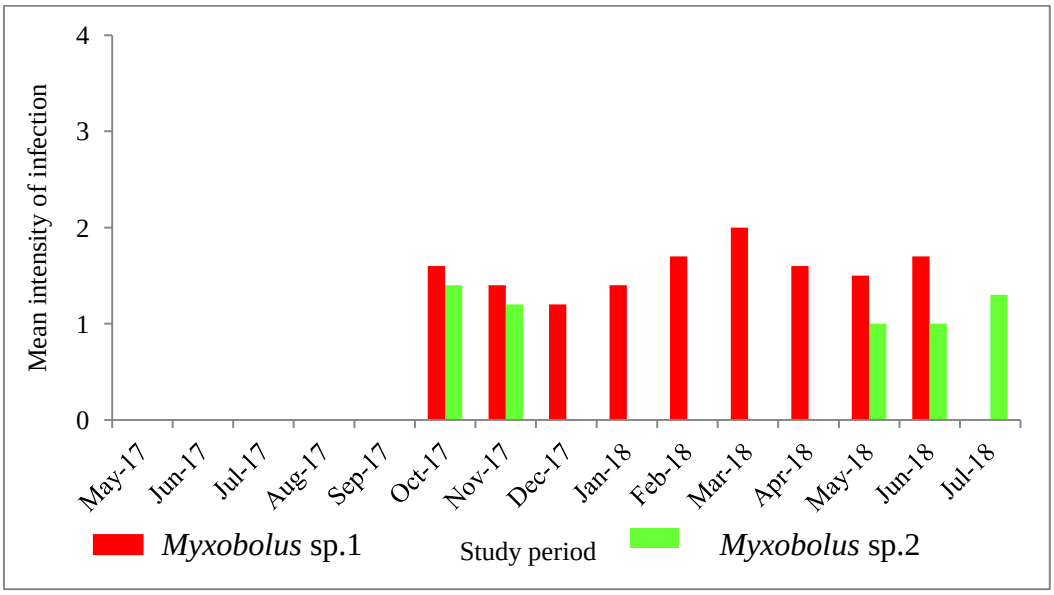


Figure 3 Mean intensity of Cnidaria parasites in *Piaractus brachypomus* during the study period

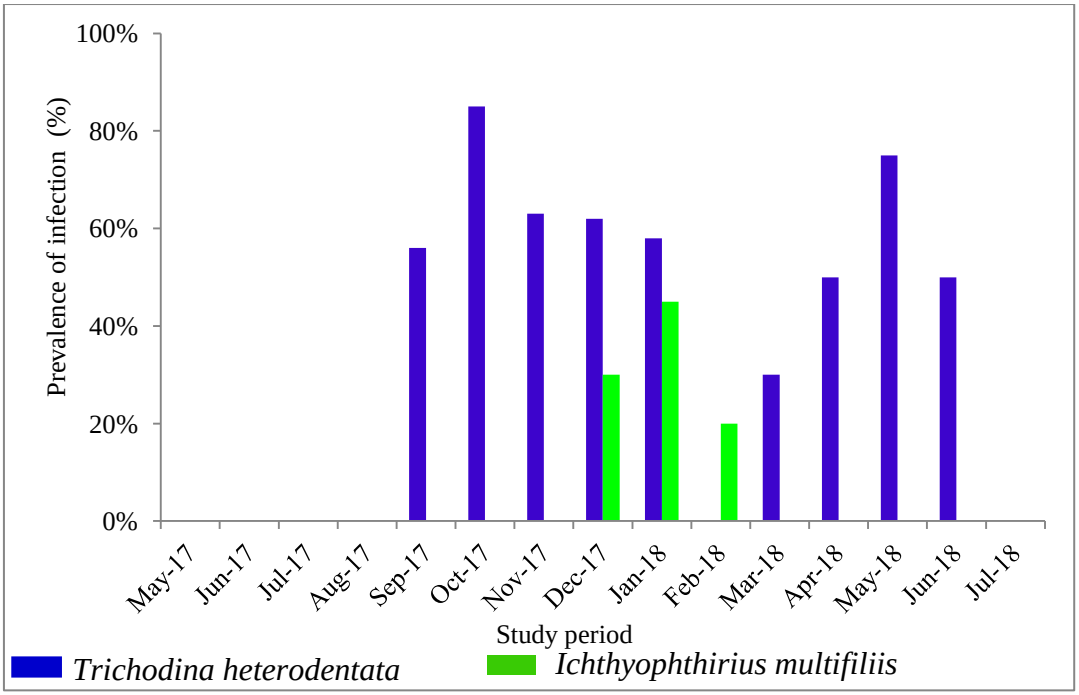


Figure 4 Prevalence of Cilophora parasites in *Piaractus brachypomus* during the study period

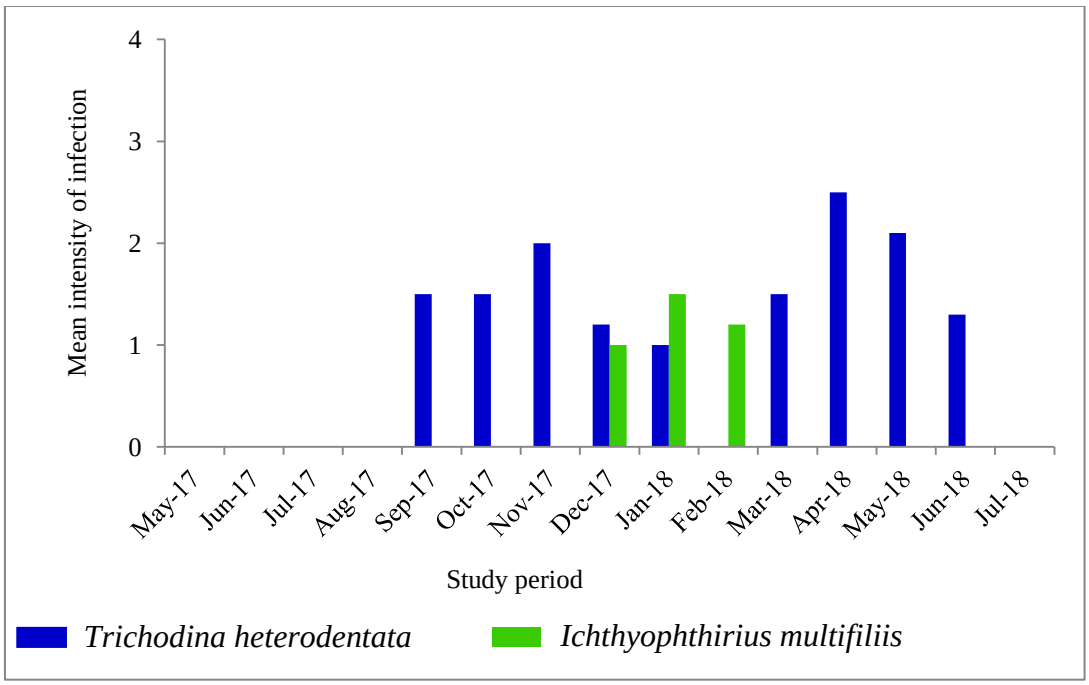


Figure 5 Mean intensity of Cilophora parasites in *Piaractus brachypomus* during the study period

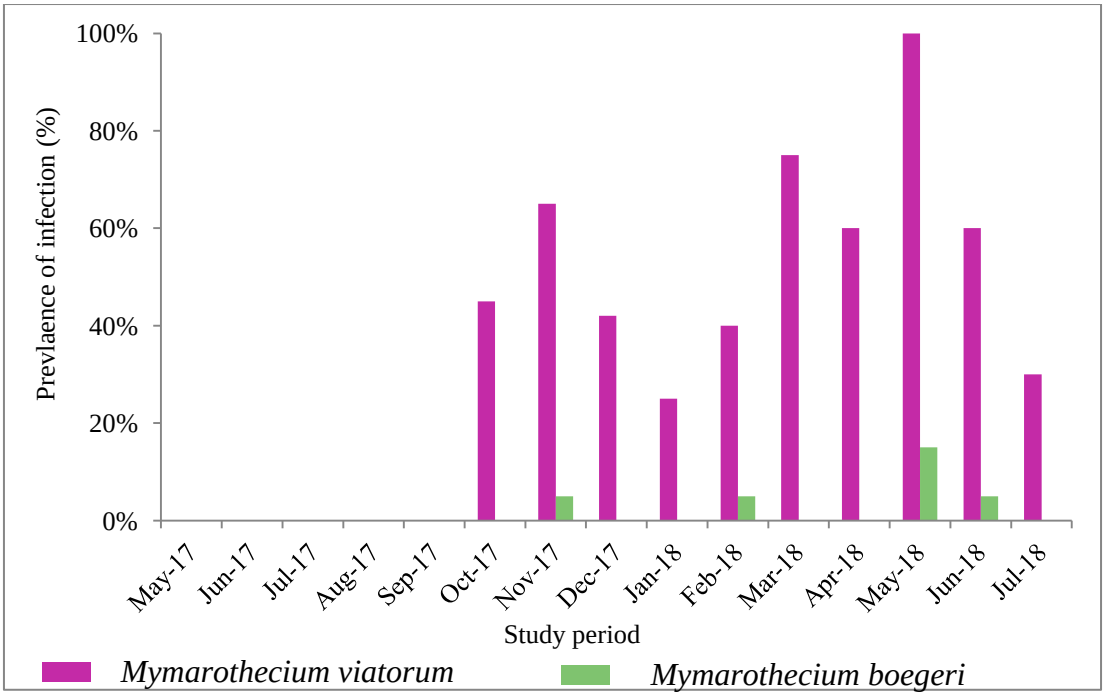


Figure 6 Prevalence of Monogenea parasites in *Piaractus brachypomus* during the study period

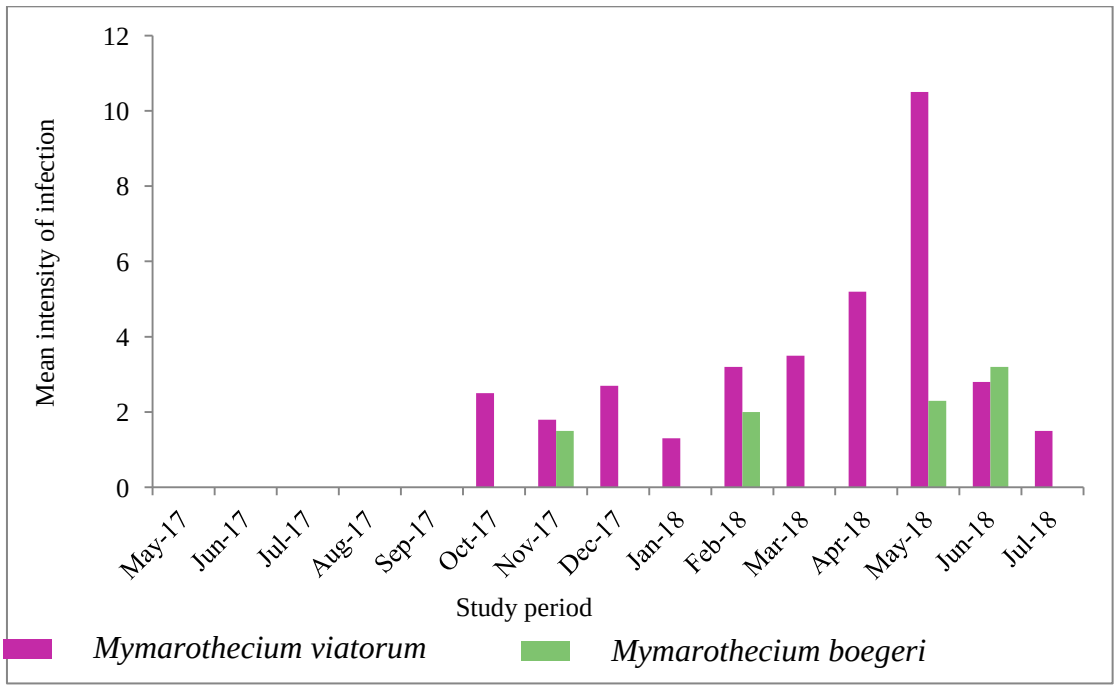


Figure 7 Mean intensity of Monogenea parasites in *Piaractus brachypomus* during the study period

Discussion

The present work was conducted to examine the occurrence of parasitic infection in *Piaractus brachypomus* in Myanmar aquaculture. *Piarctus brachypomus* was infested with six parasite taxa, namely *Myxobolus* sp.1 and 2, *Trichodina heterodentata, Ichthyophthirius multifillis, Mymarothecium viatorum* and *M. boegeri.*

Within the protozoan phyla, the most destructive pathogens causing large scale mortalities in natural and culture conditions are undeniably the myxosporeans of the phylum Myxozoa (Schulmann, 1966). Egusa (1991) observed that the myxosporeans parasitize on the different tissues and organs of the host fishes and hamper the growth as well as cause deformities and bizarre external appearances leading to commercial rejection of the infected fishes.

Myxobolus sp. 1 recorded in the present study is very similar in shape and dimension reported by Moe Kyi Han (2006) and Su Su Mon (2014) from *L. rohita*. The shape of *Myxobolus* sp. 2 was nearly similar to the reported by Basu and Haldar (2003) from Punjab wetlands (India) that infected in the gills of *Labeo bata*, but the spore dimension and the size of the polar capsule were quite different, although the length and width of the spore did not differ from those species.

In Myxozoa, high prevalence of infections were found for the first four months but it decreased after five months of culture period. Myxozoa is a spore formation parasite and they produce spore in the infected tissue and then the tissue will be burst out and fish will be recover from infection (Yokoyama *et al*., 2008, Yanagita *et al*., 2010). Therefore, infection was high in the beginning of sample period and it decreased after five months of culture period. Due to their low intensity of infection, impact of parasite in culture fish is assumed as low. However, secondary infection such as bacteria and fungus from infected skin/ gills should be considered.

Parasitic infestation of cultured fish in tropical and subtropical countries represents a serious problem for aquaculture due to severe economical losses either as directly or indirectly (Roberts, 1978).

Trichodina sp. was isolated from examined fish species within the present study. In Myanmar, *Trichodina* spp were so far recorded in *Carassius auratus*, *Cyprinus carpio, Labeo rohita* and *Pangasianodon hypophthalmus* (Thi Thi Thaw, 2007; Pa Pa Win, 2008; Khin May That, 2009 and Su Su Mon 2014). The genus *Trichodina* is the largest within the family Trichodinidae (Raabe, 1959). *Trichodina* spp. are most famous and best known as ectoparasites of skin, fin and gill of fish hosts (Hoffman, 1998). They are typically reported from aquaculture farms and also from natural water resources (Lom and Dykova, 1992).

Although *Trichodina heterodentata* was recorded within the present study, the intensity of infection was very low (range from 1 -2). The impact of *Trichodina* spp. in the culture fish species is therefore considered to be low. However, since poor water qualities enhance the

reproduction and biomass of *Trichodina* spp., trichodinids become a problem in aquaculture if farmers do not maintain a high water quality. In order to keeping high water qualities, feed residues and cleaning of the pool is mandatory to control *Trichodina* spp. outbreaks (Arthur and Lorn, 1984; Ogut *et al*, 2005). Contrary to Lom (1962), who reported that the parasite occur only on the skin in the fresh water, and on the gills in the marine forms. On the other hand, the present findings confirm those of Snieszko and Axelrod (1971) in which they mentioned that *Trichodina* spp. occur on the gills and skin in numbers that could obscure the normal structure of the epithelium.

Ichthyophthirius multifiliis, causative agent of the famous white spot disease, was isolated from *Piaractus brachypomus* within the present study. In Myanmar, *Ichthyophthirius multifiliis* was recorded in ornamental fish species (Thi Thi Thaw, 2007 and Phyo Ma Ma Lin, 2014). However hitherto it was not reported from aquaculture fish species. *I. multifiliis* is a histophagous ciliate and fatal to its host (Lom and Dykova, 1992). It is known as a causative agent of the so called 'Ich' disease of tropical aquarium fishes and is now causing a serious problem in the ornamental fish culture industry world wide (Ponpornpisit *et al*, 2009).

I. multifiliis can infect all freshwater fish species, especially when the water quality is poor, the weather is cool and dark and the fish are immunologically depressed (Lom and Dykova, 1992 and Gratzek, 1993). It may cause even severe problems in tropical fish farms in Myanmar in the future if an appropriate control method is not applied.

Monogenea species were reported from various authors in Myanmar, e.g. by Thi Thi Thaw, 2007, Khin May Thet, 2009, Myint Myint Win, 2012 and Su Su Mon, 2014. Seven *Dactylogyrus* species from *Channa striatus, Anabas testudinus* and *Clarias batrichus* were reported by Kyaw Thu Win (2014) in Myanmar. 14 *Dactylogyrus* species are recorded from 20 freshwater fishes in Sittaung river (Myint Myint Win, 2012). Ogawa (2006) noted that monogeneans parasites are very common in freshwater fish in culture ponds. It is assumed that Monogenea are dispersal in culture ponds in Myanmar.

High prevalences of monogenean infection are reported within the present study. Monogeneans are naturally found in rivers and streams as well as in lakes but prevalence of infection is naturally not high (Khin Mi Mi Oo, 2009; Myint Myint Win, 2012). Based on the life cycle and reproductive development of monogenean parasites, it is assumed that because they are hermaphrodites, they can cross-fertilize themselves to reproduce easily (Cecchini *et al*., 1998).

It is more easy to find, invade and settle on host fishes in captive conditions than in natural water bodies. Therefore, high prevalences of infection in the sampled fish was recorded in the present study because all fishes were collected from relatively dense culture farms compare to natural environments. *Mymarothecium viatorum and M. boegeri* from *Piaractus brachypomus*

have not been reported yet in Myanmar. These species therefore represent new locality records for Myanmar.

Monogeneans are represented by a great variety of mainly fish parasitic taxa, inhabiting both freshwater and sea water environments (Harris, 1985). Monogenean infection in the gills of carp fry induces severe hyperplasia of the gill filament epithelium (Myint Myint Win, 2012). Extreme proliferation of the respiratory epithelium of the gills interferes with respiratory function. Bashirullah (1973) reported that the degree of parasitism was obviously related to the age of the host fishes. The author also reported that the abundance of Monogenea in old fish pond was higher comparted to new ponds leading to higher prevalence in older fish.

Conclusion

The data obtained from the present study are informative and useful for the expanding fish culture and its management in Myanmar. Six species of parasites were recorded in *Piaractus brachypomus*. Highest prevalence of infection was found in Monogenean parasites while the lowest one is found in Cilophora parasites. Intensity of infections of in all parasite species were low. Impact of parasitic infection in study area is assumed as low, however, secondary infection from the damage of infected skin and gills should be considered. The findings will not only be applicable for locally cultivated freshwater fish, but will also provide a frame work for further basic and applied research in the underrepresented field of fish parasitology in Myanmar.

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HEAVY METALS ANALYSIS OF SOME FISHES IN AYEYARWADY RIVER SEGMENT OF SALAY ENVIRONS

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Abstract

A total of 15 fish species which included two surface dwellers, seven mid-water dwellers and six bottom dwellers were collected from Ayeyarwady River segment of Salay environs to analyze elements content in muscle tissue. Study period lasted from January 2014 to December 2014. The concentration of toxic metals (cadmium, lead and arsenic) in muscle of fish specimens and aquatic environs of study area were analyzed three replicates by Flame Atomic Absorption Spectrometer (FAAS) (Perkin Elmer AAanalyst 800 and Winlab-32 software) in Universities' Research Centre (URC). Seasonal variations of test results were compared with WHO/FAO maximum permissible limits (MPL). In this study, the effects of the seasons on elements accumulation in muscle of fishes were determined. The concentrations of toxic metals in fishes of study area were higher than maximum permissible limits except from rainy season. The concentrations of cadmium and arsenic of water in hot and rainy seasons were higher in the study environs. The concentrations of toxic metal in sediment were lower than the maximum permissible limits (MPL) in all seasons.

Keyword: Toxic Metals, Fish Muscle, Water, Sediment

Introduction

Pollution of the aquatic environments is one of the serious environmental problems in the World (Azizullah *et al*., 2011). Among the several pollutants, heavy metals are known the most usual environmental pollutants. Owing to bioaccumulation in the food chain, long persistence and their toxicity, they are very harmful for the environment (Papagiannis *et al*., 2004). Heavy metals diffuse to aquatic environment from different natural and anthropogenic sources like industrial effluents, agricultural runoffs, burning of fossil fuels, geological structure, mining activities and atmospheric deposition (Papagiannis *et al*., 2004).

The United Nations Environment Programme's World Conservation Monitoring Centre (UNEPWC) lists the Ayeyarwady as one of the world's top thirty high priority river basins due to both its support of high biodiversity and high vulnerability to future pressures.

Fish species are widely used to biologically monitor variations in environmental levels of anthropogenic pollutants (Amundsen *et al*., 1997). Fish are often at the top of the aquatic food chain and may concentrate large amounts of some metals from the water (Farkas *et al*., 2002). In fish toxic effects of heavy metals may influence physiological functions, individual growth rates, reproduction and mortality (Zyadah, 1999). The concentration of heavy metals in tissues are the result of uptake and release processes with characteristics kinetics for the elements and their biological halftime, influence by the age and size of individuals, the feeding habits of the species, their life cycle and life history, and also the seasons (Romeo *et al*., 1999). Heavy metals may enter fish bodies in three possible ways; through the body surface, the gill or the digestive tract (Chi *et al*., 2007). Food may also be an important source for heavy metal accumulation (Dallinger *et al*., 1987).

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Heavy metals have in common relatively high density that causes them to be toxic or poisonous even at low concentrations. Some metals like copper, iron and zinc are essential for metabolism. For normal metabolism, the essential metals must be taken up from water or food, but excessive intake of the essential metals can produce toxic effects (Yousafzai, 2004).

Once metals are accumulated by an aquatic organism, they can be transferred through the upper levels of the food chain. Carnivores at top of the food chain including humans, obtain most of their metal burden from the aquatic ecosystem by way of their food, especially where fish are present so there exists the potential for considerable biomagnifications (Cumbie, 1975; Mance, 1987).

Fish cover a wide range of trophic levels and are an important link of aquatic food chains with human populations (Costa and Kehrig, 2002). These two main features made fish highly suitable for toxic and essential metals contamination studies and monitoring programmes.

Due to these reasons, present study has been conducted with the following objectives; to determine seasonal variation of cadmium, lead and arsenic concentrations in muscle tissue of fish species related to different feeding habits and habitats types and to examine the toxic metals concentrations in water and sediment of study area.

Materials and Methods

Study area

Salay Township, Magway Region of Ayeyarwady River, situated at 20° 42′ N to 20° 51.30′ N and 94° 14′ E to 97° 47.51′ E, was chosen as the area of study. Fish, water and sediment samples were collected from this area and metal content of each sample was determined (Fig. 1).

Study period

Study period lasted from January, 2014 to December, 2014.

Collection and preparation of fish specimens

From the chosen study sites, 24, 27 and 60 specimens of 15 fish species in hot, rainy and cold seasons respectively were collected from local fishermen. Recorded species were categorized as surface dweller, mid-water dweller and bottom dweller according to Fish Base (2011). Feeding habits of recorded fish species were designated in accordance with Talwar and Jhingran (1991). From the collected species, five species of each feeding habit were selected for determination of essential metals. Collected specimens were washed by tap water until the contamination on the body surface was runoff. Total length (cm) and body weight (g) of specimens were measured. After that, the specimens were decapitated, scaled and gutted with a clean stainless steel knife. The metal contents in the dorsal muscle (filet) of each species were analyzed to determine their suitability for human consumption.

Sample Preparation

Digestion of the muscle samples was conducted according to the dry method (Plate 1). Muscle samples were dried to a constant weight in an oven and dried samples were weighted and stored in airtight containers. Five grams each, of the dried muscle samples was placed into a crucible and transferred to a furnace (Model-L3383), in which temperature was slowly raised to 500°C over 2 hours. Samples were allowed to ash overnight. Once removed, samples were

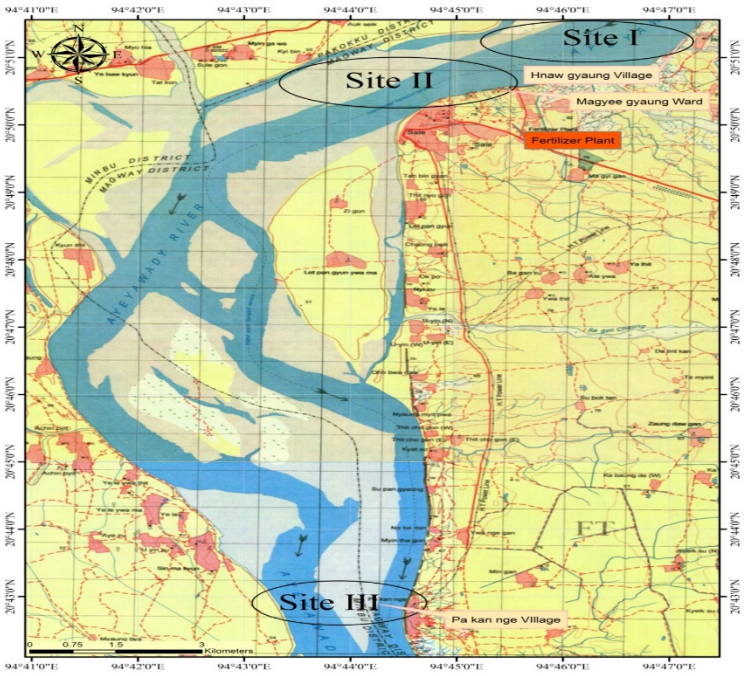
allowed to cool in room temperature and 5 mL of nitric acid were added and followed by addition of 10mL hydrochloric acid. The digestion was transferred to furnace and the temperature was raised slowly to 450° C and hold at this temperature for 1 hour. The crucible was removed, cooled and 50mL deionized water was added and transferred to volumetric flask.

The sediment samples were sun dried, grounded and sieved with 200 mm sieve to obtain a find powder. A quantity 1.0 g of dried sediment sample in a crucible was placed in a furnace at 200°-250° C for 30 min, and then ashed for 4 hours at 480° C. Then the sample was removed from the furnace, cooled and 2mL of nitric acid was added. The preparation was evaporated to dryness on a sand bath. Subsequently, 2 mL of concentrated HCl was added and transferred to furnace in which the temperature was raised slowly to 450° C and hold at this temperature for 1 hour. The crucible was then removed, cooled and 50mL of deionized water was added. The solution was filtered through Whatman No-42 filter paper and 0.45µm Millipore filter paper (Issac and Kerber, 1971).

For water, each sample was filtered through a 0.45 micron Whatman filter.

Chemical Analysis

The concentration of three elements (cadmium, lead and arsenic) in muscle tissue samples of the fish specimens as well as in sediment and water samples were analyzed in tri-replicates by Flame Atomic Absorption Spectrometer (FAAS) (Perkin Elmer AAanalyst 800 and Winlab-32 software) in the Universities' Research Centre (URC) at University of Yangon. Seasonal variations of test results were compared with WHO/FAO maximum permissible limits. TEC (threshold effect concentration), MEC (midpoint effect concentration), and PEC (probable effect concentration) were also determined for toxic metal concentrations of sediment samples according to MacDonald *et al.*, (2000).



Source: Universal Transverse Mercator (UTM) Map Sheets, 2004

Figure 1 Map of the study area

(A). Ashing samples in furnace

(B). Filtration of samples

(C). Samples ready for AAS

(D). Analysis by AAS

Plate 1. Apparatus used in sample analysis

Results

A total of 15 fish species which included two surface dwellers, seven mid-water dwellers and six bottom dwellers were collected from Ayeyarwady River segment of Salay Township (Table 1).

Base on literature, a total of 15 fish species which included five species of herbivores, five species of carnivores, and five species of omnivores were collected from this study area. The total length and weight of study species in three seasons were recorded in Table 2 and Table 3.

Cadmium concentrations of *Macrognathus zebrinus* (2.151 mg/L) and *Mastacembelus dayi* (0.265 mg/L) in hot season, and *Salmostoma sardinella* (6.646 mg/L) and *Macrognathus zebrinus* (1.126 mg/L) in cold season were found to be higher than those of the maximum permissible limit 0.2 mg/L. Above mentioned three species were recognized as omnivorous mid-water dwellers. Under limited concentrations of cadmium in all study fish species were observed in rainy season (Table 4 and Fig. 2).

Lead concentrations of *Mastacembelus dayi* (2.076 mg/L) in hot season and those of eight species (*Tenualosa ilisha* (1.388 mg/L), *Labeo rohita* (5.437 mg/L), *Mystus cavasius* (7.252 mg/L), *Oreochromis mossambicus* (2.961 mg/L), *Rhinomugil corsula* (4.342 mg/L), *Channa punctatus* (2.496 mg/L), *Macrognathus zebrinus* (3.835 mg/L) and *Mastacembelus dayi* (2.869 mg/L)) in cold seasons were found to be higher than recommended highest standard 1 mg/L (Table 5 and Fig. 3).

Arsenic concentrations of all studied fish species in all seasons were found to be higher than those of the maximum permissible limits 0.26 mg/L (Table 6 and Fig. 4).

Cadmium (0.056 mg/L in hot and 0.03 mg/L in rainy) and arsenic (2.158 mg/L in hot and 2.034 mg/L in rainy) of water in hot and rainy seasons were higher than the MPL (Table 7 and

Fig. 5). The concentrations of cadmium, lead and arsenic of sediment in three seasons were observed to be lower than those of MPL (Table 8 and Fig. 6).

Table 1 Habitats of studied fish speies

Sr.No.	Species	Habitats		
		Surface	Mid	Bottom
1	*Notopterus notopterus*			√
2	*Tenualosa ilisha*			√
3	*Cirrhinus mrigala*		√	
4	*Labeo boga*		√	
5	*Labeo calbasu*			√
6	*Labeo rohita*		√	
7	*Salmostoma sardinella*		√	
8	*Separata aor*			√
9	*Mystus cavasius*			√
10	*Eutropiichthys vacha*	√		
11	*Oreochromis mossambicus*		√	
12	*Rhinomugil corsula*	√		
13	*Channa punctatus*			√
14	*Macrognathus zebrinus*		√	
15	*Mastacembelus dayi*		√	
	Total	2	7	6

Table 2 Mean total length (cm) of fish species selected to test metal concentration

Sr. No.	Species	No.	Hot	Rainy	Cold
Herbivore	*Cirrhinus mrigala*	5	25±3.08	51.8 ± 4.82	29.4 ± 17.31
	Labeo boga	5	13.8±1.15	16.8 ± 1.64	15±3.32
	Labeo calbasu	5	18.5±3.20	12 ± 6.71	10.7 + 1.48
	Labeo rohita	3	27.33±4.04	25.67±25.42	13 ± 1.73
	Oreochromis mossambicus	6	13.83±7.9	14.42±1.69	10.17 ± 0.41
Carnivore	*Notopterus notopterus*	5	22.6 ± 4.81	14.42±1.69	25.2 ± 4.71
	Separata aor	5	22.4 ±1.95	22.6±2.19	32.2 ± 2.77
	Mystus cavasius	7	16.27 ± 3.11	12 ± 1	12.71±4.86
	Eutropiichthys vacha	5	18.8 ± 0.91	13.8±0.45	15.2 ± 1.15
	Channa punctatus	5	16.2 ±1.64	18.4 ±5.37	17.8 ± 6.53
Omnivore	*Tenualosa ilisha*	5	21.4 ± 4.98	22.2 + 3.56	17.8 ± 6.53
	Salmostoma sardinella	35	9.63 ± 1.37	9.64 + 1.04	9.14 ± 0.85
	Rhinomugil corsula	10	11.7 ± 0.79	12.5 + 2.88	13.1±1.71
	Macrognathus zebrinus	5	28 ± 5.43	25.6 + 10.06	20.3±6.99
	Mastacembelus dayi	5	25.5 ± 5.45	18.6 + 5.37	26.2 ±6.26

Table 3 Mean body weight (g) of fish species selected to test metal concentration

Sr. No.	Species	No.	Hot	Rainy	Cold
Herbivore	*Cirrhinus mrigala*	5	211.6±82.30	1674 ± 98.7	147±51.19
	Labeo boga	5	99±60.77	49 ± 12.94	41 ± 15.68
	Labeo calbasu	5	83±42.22	36 ± 58.14	9 ± 1.22
	Labeo rohita	3	306.67±120.99	34 ± 3.54	35 + 34.64
	Oreochromis mossambicus	6	64.17±9.70	60±23.66	26 ± 0.63
Carnivore	*Notopterus notopterus*	5	92 ± 55.18	38 ± 2.74	127 ± 62.79
	Separata aor	5	80.2 ± 22.80	83 ± 25.88	199.6 ±25.51
	Mystus cavasius	7	35 ± 19.79	14.86±1.68	28.43±9.13
	Eutropiichthys vacha	5	40 ± 6.12	22.4 ± 0.89	25.8±1.48
	Channa punctatus	5	48.6 ± 7.99	70 ± 53.62	77.6 ± 57.72
Omnivore	*Tenualosa ilisha*	5	23 ± 4.30	109 ± 56.61	119 ± 64.36
	Salmostoma sardinella	35	3 ± 0.33	3.72 ± 0.18	3.36 ±0.13
	Rhinomugil corsula	10	16.5 ± 1.41	28.5±9.56	30.6±.64
	Macrognathus zebrinus	5	65 ± 20	50 ± 42.72	22.4±1.33
	Mastacembelus dayi	5	42 ± 17. 89	23 ± 9.75	40±12.73

Table 4 Seasonal variation of cadmium concentration (mg/L) in different fish species

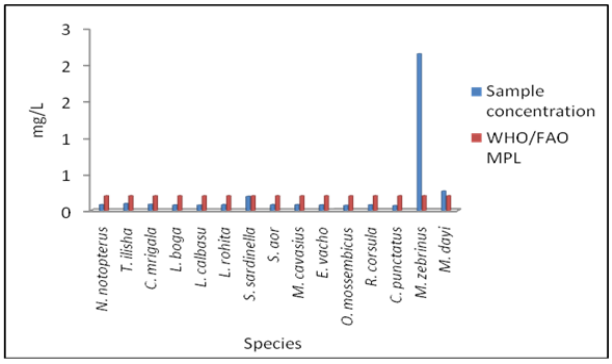
Sr.No.	Species	Concentration (mg/L)			WHO/FAO MPL
		Hot	Rainy	Cold	
1	*Notopterus notopterus*	0.078	0.040	0.073	0.2
2	*Tenualosa ilisha*	0.094	0.047	0.149	0.2
3	*Cirrhinus mrigala*	0.083	0.037	0.060	0.2
4	*Labeo bogo*	0.074	0.036	0.201	0.2
5	*Labeo calbasu*	0.071	0.030	0.067	0.2
6	*Labeo rohita*	0.079	0.049	0.135	0.2
7	*Salmostoma sardinella*	0.192	0.035	6.646	0.2
8	*Separata aor*	0.078	0.044	0.051	0.2
9	*Mystus cavasius*	0.078	0.050	0.078	0.2
10	*Eutropiichthys vacha*	0.075	0.049	0.036	0.2
11	*Oreochromis mossambicus*	0.068	0.038	0.033	0.2
12	*Rhinomugil corsula*	0.075	0.040	0.140	0.2
13	*Channa punctatus*	0.065	0.033	0.048	0.2
14	*Macrognathus zebrinus*	2.151	0.028	1.126	0.2
15	*Mastacembelus dayi*	0.265	0.026	0.141	0.2

Table 5 Seasonal variation of lead concentration (mg/L) in different fish species

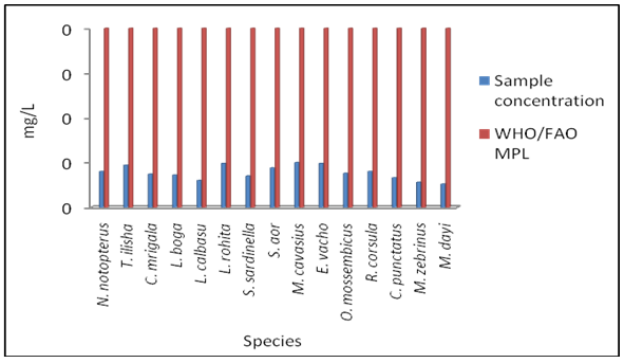
Sr.No.	Species	Concentration (mg/L)			WHO/FAO MPL
		Hot	Rainy	Cold	
1	*Notopterus notopterus*	0.028	−0.233	0.066	1
2	*Tenualosa ilisha*	0.07	−0.261	1.388	1
3	*Cirrhinus mrigala*	0.042	−0.167	0.656	1
4	*Labeo bogo*	0.102	−0.302	−0.081	1
5	*Labeo calbasu*	−0.102	−0.318	−0.070	1
6	*Labeo rohita*	−0.024	−0.222	5.437	1
7	*Salmostoma sardinella*	0.28	−0.264	−0.128	1
8	*Separata aor*	−0.062	−0.270	0.659	1
9	*Mystus cavasius*	−0.024	−0.265	7.252	1
10	*Eutropiichthys vacha*	−0.057	−0.241	0.275	1
11	*Oreochromis mossambicus*	−0.077	−0.298	2.961	1
12	*Rhinomugil corsula*	−0.072	−0.293	4.342	1
13	*Channa punctatus*	−0.102	−0.307	2.496	1
14	*Macrognathus zebrinus*	0.664	−0.325	3.835	1
15	*Mastacembelus dayi*	2.076	−0.292	2.869	1

Table 6 Seasonal variation of arsenic concentration (mg/L) in different fish species

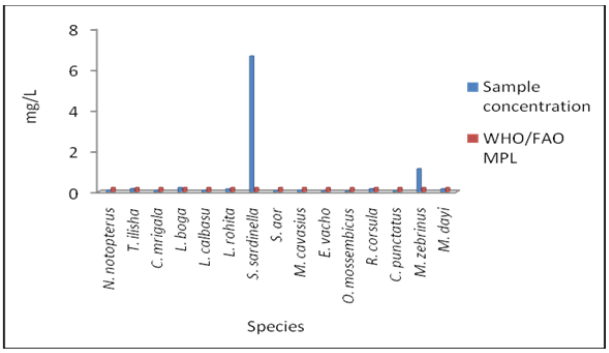
Sr.No.	Species	Concentration (mg/L)			WHO/FAO MPL
		Hot	Rainy	Cold	
1	*Notopterus notopterus*	1.446	1.221	2.274	0.26
2	*Tenualosa ilisha*	1.047	0.973	1.947	0.26
3	*Cirrhinus mrigala*	1.858	2.651	3.411	0.26
4	*Labeo bogo*	1.744	2.352	2.021	0.26
5	*Labeo calbasu*	2.830	1.941	2.772	0.26
6	*Labeo rohita*	2.476	1.466	2.148	0.26
7	*Salmostoma sardinella*	4.872	2.923	4.068	0.26
8	*Separata aor*	2.741	2.231	2.089	0.26
9	*Mystus cavasius*	1.274	2.645	2.113	0.26
10	*Eutropiichthys vacha*	2.350	3.016	2.553	0.26
11	*Oreochromis mossambicus*	2.206	2.639	1.379	0.26
12	*Rhinomugil corsula*	3.659	2.748	2.556	0.26
13	*Channa punctatus*	3.192	2.523	1.867	0.26
14	*Macrognathus zebrinus*	3.289	1.875	4.284	0.26
15	*Mastacembelus dayi*	2.829	3.393	2.144	0.26



(A) Hot season

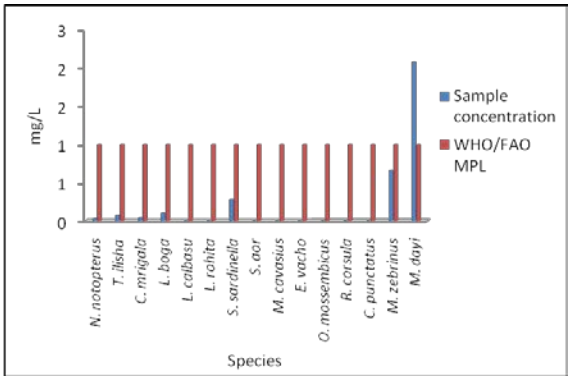


(B) Rainy season

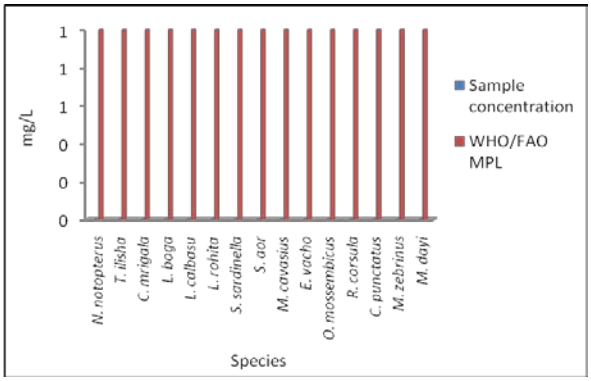


(C) Cold season

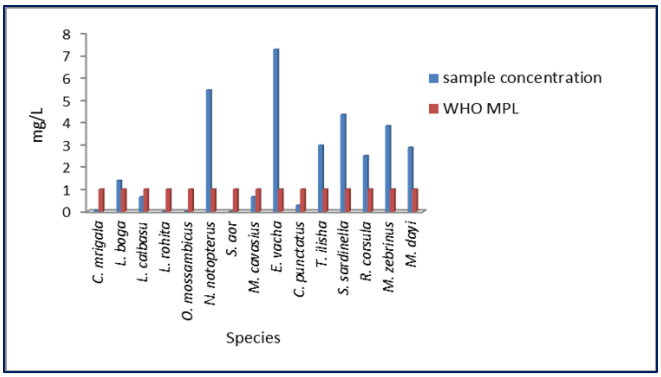
Figure 2 Seasonal variation of cadmium concentration in different fish species



(A) Hot season

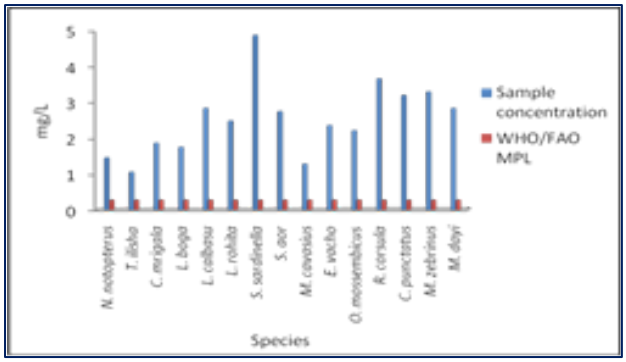


(B) Rainy season

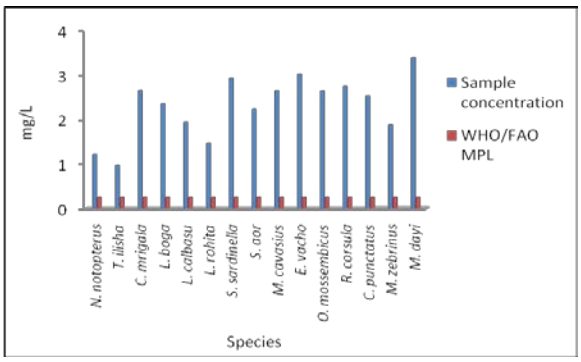


(C) Cold season

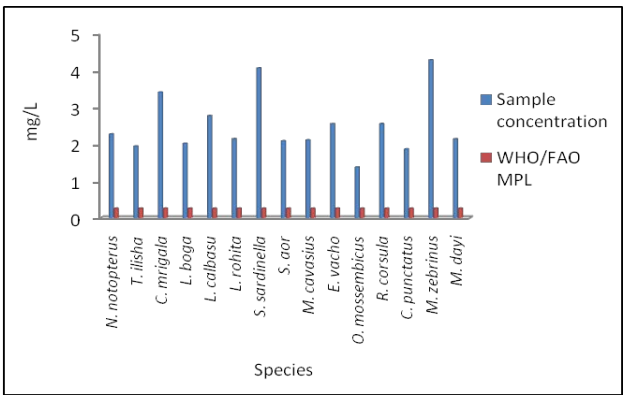
Figure 3 Seasonal variation of lead concentration in different fish species



(A) Hot season



(B) Rainy season



(C) Cold season

Figure 4 Seasonal variation of arsenic concentration in different fish species

Table 7 Seasonal variation of elements (mg/g) in water of study area

Particular	Elements	Concentration (mg/L)			WHO/FAO MPL
		Hot	Rainy	Cold	
Water	Cadmium	0.056	0.03	0.004	0.01
	Lead	−0.228	−0.330	−0.371	0.05
	Arsenic	2.158	2.034	−0.731	0.01

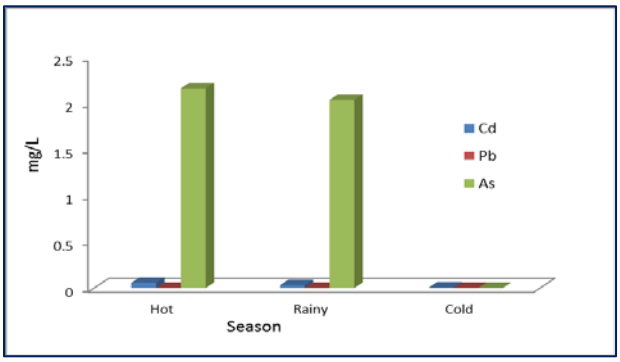


Figure 5 Seasonal variation of elements in water of study area

Table 8 Seasonal variation of elements (mg/g) in sediment of study area

Particular	Element	Concentration (mg/g)			MPL		
		Hot	Rainy	Cold	TEC	MEC	PEC
Sediment	Cadmium	0.063	0.063	0.015	0.99	3	5
	Lead	0.117	−0.229	−0.236	36	83	130
	Arsenic	2.319	2.278	2.264	9.8	21.4	33

TEC = Threshold effect concentration, MEC = Midpoint effect concentration
PEC = Portable effect concentration, MPL = maximum permissible limit

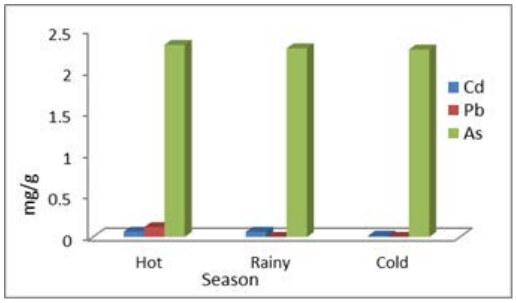


Figure 6 Seasonal variation of elements (mg/g) in sediment of study area

Discussion

A total of 15 fish species of different feeding habits which included two surface dwellers, seven mid-water dwellers and six bottom dwellers were collected from study area to analyze toxic elements content in muscle tissues.

Fish are known to be an important exposure pathway of metals to human and considered as one of the most indicative factors in freshwater ecosystems for the estimation of trace metals pollution (Rashed, 2001). There are five potential routes for a pollutant to enter a fish: food, non-food particles, gills, oral consumption of water and the skin (Ayandiran *et al*., 2009). Knowledge of element concentrations in fish is important for both human consumption and natural management.

Aye Aye Mu (2011), As concentration in muscle tissue of *Lates calcarifer* were high in hot > rainy > cold season.

Khin Myint Mar (2011) detected that Pb and Cd concentrations in different feeding type of fish were found lower the recommended limit of WHO/FAO in Gaw Wein Landing Sites, Ayeyarwady River Segment, Mandalay.

Damodharan *et al*., (2013) observed that Cd concentration in the muscle tissue of *O. mossambicus* showed little variable in dry and wet season.

In the present study, cadmium concentrations in three species (*Macrognathus zebrinus, Mastacembelus dayi* and *Salmostoma sardinella*) of omnivorous mid-water dwellers were observed to be higher in hot and cold season. WHO (2007) stated that cadmium exposures are associated with kidney and bone damage. Cadmium has also been identified as a potential human carcinogen, causing lung cancer.

In the present study, lead concentrations in one species at hot season and eight species in cold season were higher. Most of higher lead concentrated species were bottom and mid-water dwellers and they were also carnivores and omnivores. Lead is toxic metal and non-essential element for human body as it causes a rise in blood pressure, kidney damage and miscarriage (Kiran *et al*., 2011). In the present study, the overall mean values for arsenic recorded in the all sample fish species in all seasons were higher than the maximum permissible limit. Toxic effects appear when arsenic is ingested in excess for long periods, resulting in cancer, cutaneous malignancies, etc. Toxic metals are very harmful because of their potential to accumulate in different body parts.

In present study, cadmium and arsenic concentration of water in hot and rainy seasons were higher in study environs. The concentrations of toxic metal in sediment were lower than the maximum permissible limits in all seasons. Forstner and Wittmann (1981) reported that aquatic organisms such as fish are capable of accumulating metals also in their living cells to concentrations much higher than those present in water, sediments and micro flora in their environment. Metal content of fish increases with the increment of the metal level in water, sediment and food organism (Arvind, 2002). The present of metals in river, lake or any aquatic environment can change both aquatic species diversity and ecosystem due to their toxicity and accumulative behavior (Heath, 1987).

In this study, the effects of the seasons on elements accumulation in muscle of fishes were determined. From above mentioned results, it is clear that the concentration of toxic metals of fishes in study environs is high. A number of serious health problems may develop as a result of excessive uptake of dietary heavy metals in human body. Furthermore, the consumption of heavy metal-contaminated food can seriously deplete some essential nutrients in the body (Rafiqul Islam *et al*., 2013).

Conclusion

The result of this research based on toxic elements concentration of 15 fish species of different feeding habits and habitats, water and sediment of Ayeyarwady river segment of Salay environs. In the present study, toxic metal concentrations of all studied fish species were lower than the maximum permissible limits except the lead in cold season and arsenic in all seasons. Toxic metal concentrations of water were found lower than the maximum permissible limits except the arsenic in hot and rainy season and sediment were also found to be lower than the maximum permissible limits. Based on the results, it could be concluded that the study fish species were not suitable for human health.

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AN INVESTIGATION ON BREEDING BIOLOGY OF FEMALE CLIMBING PERCH, *ANABAS TESTUDINEUS*(BLOCH, 1792) FROM THANATPIN CREEK, BAGO REGION

Thi Thi Han,[1] Cho Mie Aung,[2] Khin Thuzar Win[3]

Abstract

An investigation was conducted to observe the breeding biology of climbing perch (*Anabas testudineus*), locally known as Nga-pyae-ma. Monthly samples were collected from Thanatpin creek which situated south from Thanatpin Township, Bago Region through the period from January to December 2018. The highest GSI value for female was 15.74±2.08% in July that indicates the peak spawning season. Size at first maturity for female was 17.2 cm in total length and fecundity was range from 7033 to 93258. Specimens in this study were found to have mature gonads in the dry and wet season months, indicating no seasonality in breeding patterns. Growth ovarian stages i.e. maturity stage III, IV and V occurred from April to September, indicating that mature females were actively spawning throughout these months. The relationship between total length (TL) and bodyweight (BW), fecundity and total length (TL), fecundity and body weight (BW), fecundity and ovary weight (OW) were linear. The present study provides the first information about the breeding biology of female *Anabas testudineus* because it potentially indicates their current population condition in the study area.

Keywords: Climbing perch, maturity stages of ovary, GSI, size at first maturity, fecundity, peak spawning season

Introduction

Anabas testudineus(climbing perch) is a small sized food fish, inhabits both freshwater and brackish water and found in most tropical or subtropical area including India, Pakistan, Bangladesh, Nepal, China, Myanmar, Thailand, Cambodia, Philippines, Indonesia, Singapore and Sri Lanka (Mirsa, 1962). It is one of air breathing fishes in Myanmar forming an economically important group of fishes. It is indigenous fish of Myanmar highly favoured by the consumersdue to its high nutrition value as well as for great taste and flavor.It has been kept under Data Deficient-ver 3.1 category of IUCN Red List of Threatened Species (IUCN, 2014). To utilize and manage this species wisely in culture system understanding of breeding biology is very essential. Studies on the breeding biology of any fish is essential for evaluating the commercial potentialities of its stock, life history, cultural practice and actual management of small indigenous fishes (Doha and Hye, 1970).This fish is highly esteemed for its highly nourishing quality and prolonged freshness out of water. This species inhabits in all types of freshwater and also survive in brackish water. It also inhabits standing and sluggish waters of lakes, flood plains, canals and ponds.There are few reports on the breeding biology of *A.testudineus* in Myanmar. It will help to produce high quality seeds in the hatcheries for supporting the sustainable aquaculture production in Myanmar. The present study was to assess the breeding biology such as maturity stages of ovary, size at first maturity, GSI, fecundity and biometric indices, and peak breeding season of climbing perch, *A.testudineus* from the study area.

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Materials and methods

Sample collections

Samples of studied species were monthly collected from Thanatpin creek which situated south from Thanatpin Township, Bago Region (during January to December 2018).This creek is about 17 milesfrom north to south miles and connected with Bago-Sittaung canal and heavily used by fishermen and farmers. Samples were caught by the local fisherman using three fishing gears such as cast net or kun (let-pyit-kun), lift net or portable lift net (yagwin) and stow net (tiger mouth) in the study site.

Study period

The study period lasted from January to December, 2018.

Morphometry

Total length of each fish was measured using a centimeter scale and the body weight was determined by an electronic balance. Size, color and appearance of the ovary were recorded. The sample size varies depending on the abundance of fish catch. Sample sizes ranging from 13 to 22.00 cm were collected for female *A.testudineus*.

Gonadosomatic index (GSI) and Hepatosomatic index (HSI)

$$GSI = \frac{\text{Gonad Weight}}{\text{Body Weight}} \text{ x } 100 \text{(Alam and Pathak, 2010)}$$

$$HSI = \frac{\text{Liver Weight}}{\text{Body Weight}} \text{ x } 100 \text{ (Cek and Yilmaz, 2009)}$$

The condition factor (K) was calculated according to Pauly (1983).

$$K\ (\%) = \frac{\text{Liver Weight}}{\text{Length}^3} \text{ x } 100$$

where K = condition factor; W = weight in gram; L = length in cm

Fecundity

$$N = \frac{Wt}{Ws} \text{ x Ns (Bagenal, 1978)}$$

Wt = Total weight of ovaries; Ws = Weight of subsample;

Ns = Number of oocytes in the subsample

Size at first maturity

Size at first maturity, which is defined as the total length (cm) at where 50% of the sample in maturity stage III, were analyzed using cumulative percentage (Murua*et al*., 2003). The fish were grouped 1 cm size classes and the percentage mature in each size class was calculated.

Linear relationship and correlation coefficient (r) between fecundity and biometric indices were analyses in Microsoft Excel, 2007.Histological sections were assessed for the morphological characteristics of the eggs. The spawning periodwas determined by monthly

evaluation of the gonadosomatic index and percentage of maturity stages of each of the female fish samples. Gonadal stages of females will be examined macroscopically and microscopically classified to Jacob (2005) and Agarwal (1980). The estimated fecundity was computed from the number of mature and ripe oocytes (Lagler, 1971) that were counted.The preserved specimens were identified by Jayaram (1981), Talwar and Jhingran (1991).A test with a $p<0.05$ indicates a significant statistical analysis.

Results

Length-Weight Relation (LWR) and condition factors

Monthly variations in mean relative condition factor and other body parameters for female *A. testudineus* were presented in Table 1. The regression equation for the length-weight relationship of females was calculated as follows:

$$\text{Log } Y = 3.017 \text{ Log } X - 1.721 \quad (p < 0.05)$$

Where $R^2 = 0.836$, $r = 0.911$, Y =body weight, X =body length

The values of coefficient 'b' for length-weight relationship was 3.017 for female (N =236) (Fig. 1).

Gonadosomatic index (GSI) and hepatosomatic index (HSI)

Monthly mean variations of gonadosomatic index (GSI) and hepatosomatic index (HSI) were presented in Table 1 and Fig. 2. The GSI was recorded highest in the month of July which may suggest the peak breeding season and then it gradually decreased and attained its lower value in December for female. The present study showed that a high hepatic activity in females during November while low hepatic activity in August during the study period.

Maturity stages of ovary

In the present study, the ovaries were categorized into five maturity stages and monthly percentage occurrences of gonadal stages in female *A. testudineus* were presented inTable 2 and Fig.3. The seasonality of the reproductive stages of female *A. testudineus* using the GSI, fecundity data and monthly proportion of gonadal maturity stages of female *A. testudineus* was predicted as shown in Table 3. Microscopic observation of ovary of female was shown in plate1.

Fecundity estimation (F)

In the present study, the eggs from 125 matured females were analyzed for fecundity. The mean fecundity was found to be maximum (70593±12583) eggs with a fish having mean total length 18.99 cm and mean body weight 129.16 g with mean ovary weight 20.30 g in July. While the minimum mean fecundity (8946±2705) eggs was recorded with a fish of mean total length 16.35 cm and body weight 101.10 g with mean gonad weight 1.44 g in March. High fecundity was observed in July (40.73%) and low in March (0.34%) (Table 4).

Relationship between fecundity and total body length

The scattered diagram of log of body length and log of fecundity suggested that there is a linear relationship between the two variables (Table4 and Fig. 4).

$$\text{Log } Y = 4.986 \text{ Log } X - 1.631 \quad (p < 0.05)$$

Where $R^2 = 0.431$, $r = 0.688$ Y = fecundity, X = body length

The fecundity was correlated with the body length and comprised a correlation coefficient of 0.431, which means approximately 43% of changes in fish fecundity was explained by fish body length.

Relationship between fecundity and body weight

The scattered diagram of log of body weight and log of fecundity suggested that there is a linear relationship between the two variables (Table4 and Fig. 5)

$\text{Log } Y = 2.191 \text{ Log } X + 0.129 \ (p < 0.05)$

Where $R^2 = 0.518$, $r = 0.755$, Y = fecundity, X = body weight

The fecundity was correlated with the body weight and comprised a correlation coefficient of 0.518, which means approximately 52% of changes in fish fecundity was explained by fish body weight.

Relationship between fecundity and ovary weight

The scattered diagram of log of ovary weight and log of fecundity suggested that there is a linear relationship between the two variables (Table4 and Fig. 6)

$\text{Log } Y = 0.782 \text{ Log } X + 3.782 \ (p < 0.05)$

Where $R^2 = 0.943$, $r = 0.983$, Y = fecundity, X = ovary weight

The coefficient of correlation between the numbers of eggs with ovary weight was positive as the value of R^2 was 0.943. This implied 94% changes in fecundity could be explained by gonadal weight.

Size at first maturity

Sexual maturity was attained in female *A. testudineus* at highly variable sizes. In the present study, the smallest mature females matured at 14.50 cm and the highest mature females were observed at 17.30 cm and the size at first maturity i.e. 50% mature females were approximately 16.22 cm. (Table 5, Fig. 7).

Table 1 Monthly variations in body parameters, condition factor and gonadosomatic index of *A. testudineus*

Months	N	Average length (cm)	Range	Average weight (g)	Range	GSI (%)	HSI (%)	K (%)
January, 2018	11	16.52±0.64	14.50-17.50	97.51±9.69	81.65-114.64	0.50±0.37	1.15±0.38	2.16±0.08
February, 2018	17	16.65±0.66	15.50-17.50	99.94±17.16	59.19-120.67	0.35±0.10	1.25±0.36	2.14±0.17
March, 2018	16	15.98±0.73	15.00-18.00	80.87±22.84	54.45-135.45	0.54±0.37	1.55±0.37	1.94±0.27
April, 2018	27	17.74±1.52	16.30-22.00	100.42±24.16	66.19-150.89	6.54±2.96	0.85±0.23	1.78±0.14
May, 2018	23	16.55±1.08	15.00-19.50	90.09±15.74	66.33-110.16	9.26±4.47	0.87±0.16	1.98±0.16
June, 2018	26	16.31±0.53	15.00-17.50	100.09±10.82	84.38-122.72	11.76±5.98	0.86±0.19	2.31±0.22
July, 2018	30	18.97±0.99	16.50-20.50	129.16±18.14	101.89-172.39	15.74±2.08	0.76±0.22	1.89±0.19
August, 2018	6	18.08±1.32	16.50-19.50	120.91±17.44	88.11-134.6	14.74±3.40	0.69±0.31	2.06±0.35
September,2018	20	15.87±0.58	14.50-17.00	82.56±14.10	58.92-98.82	4.14±3.82	0.99±0.61	2.05±0.23
October, 2018	19	16.46±1.24	14.00-19.00	99.90±35.79	40.36-184.4	0.37±0.11	1.41±0.55	2.25±0.30
November, 2018	20	15.78±0.77	14.00-17.00	82.43±15.19	55.87-112.78	0.18±0.07	2.00±0.37	2.08±0.15
December,2018	21	14.00±0.81	13.00-16.50	49.26±11.98	38.12-95.56	0.28±0.14	1.81±0.50	1.77±0.12

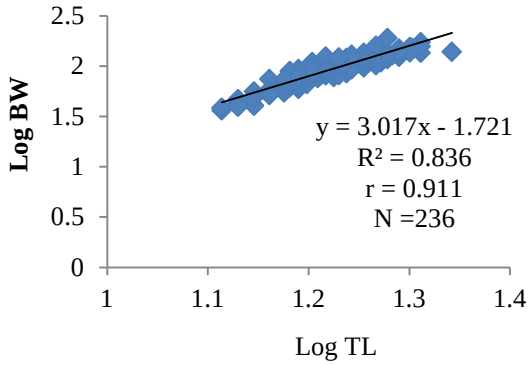


Figure 1 Length-weight relationship of female *Anabas testudineus*

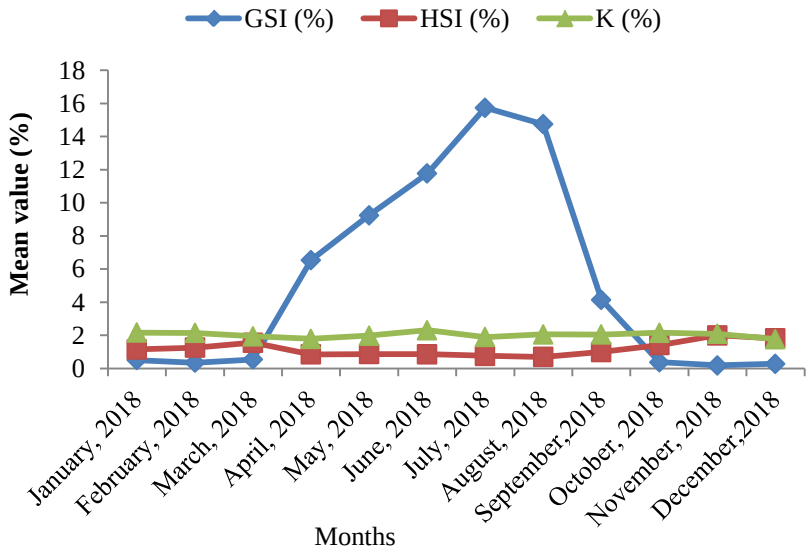


Figure 2 Monthly fluctuation of mean gonadosomatic index, hepatosomatic index and condition factor of female *A. testudineus*

Table 2 Monthly percentage occurrences of gonadal stages in female *A. testudineus*

No.	Month	Sample size	Stage I Immature)	Stage II (maturing)	Stage III (mature)	Stage IV (Ripe)	Stage V (Spent)
1	January	11	55	36	0	9	0
2	February	17	47	47	0	0	6
3	March	16	31	56	13	0	0
4	April	23	0	0	57	43	0
5	May	27	0	0	93	7	0
6	June	26	0	0	81	19	0
7	July	30	0	0	70	30	0
8	August	6	0	0	33	67	0
9	September	20	10	5	40	10	35
10	October	19	50	22	0	0	28
11	November	20	5	0	0	0	95
12	December	21	19	76	0	0	5

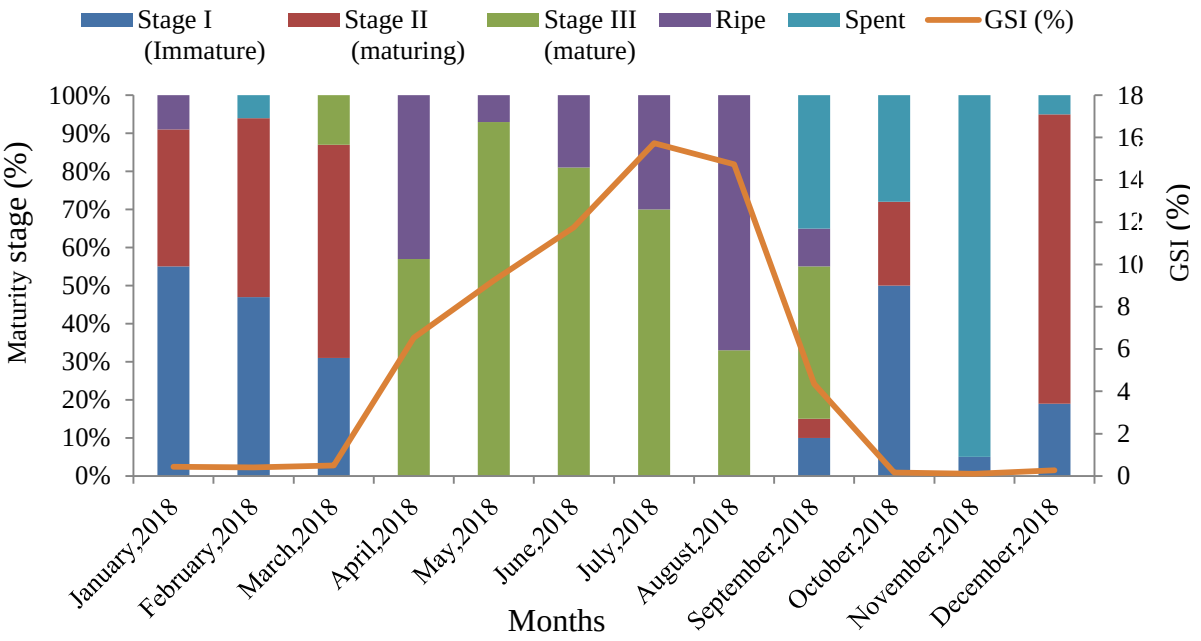


Figure 3 The proportion of gonad stages and GSI of female *A.testudineus* at sampling period

Table 3 Seasonality of reproductive stages based on some aspects of reproductive biology of *A. testudineus*

Period	Months
Maturation period (immature and maturing)	Jan-March and December
Spawning season (mature and ripe)	April - September
Breeding season (spent)	September-November

Table 4: .Monthly variation of mean absolute fecundity with weight and length for gravid female *A.testudineus*

Period	Sample size(n)	Body weight(g)		Total length(cm)		Ovary weight(g)		Fecundity	
		Mean±SD	Range	Mean ± SD	Range	Mean±SD	Range	Mean±SD	(%)
January, 2018	1	114.64	_	17.5	_	1.84	_	11068	0.21
March, 2018	2	101.1 ±2.81	99.11-103.08	16.35± 0.21	16.2-16.5	1.44±0.34	7033-10858	8946±2705	0.34
April, 2018	23	100.42±24.16	66.19-150.89	17.74±1.52	15.8-22	6.96±4.74	10717-70678	25766±14064	11.40
May, 2018	27	90.09±15.74	61.27-123.79	16.5 ±1.08	15-19.5	8.66±5.04	8455- 55872	28793±14698	14.95
June, 2018	26	100.09±10.82	84.38-122.72	16.31±0.53	15.2-17.5	11.85±6.23	12521-72948	42517±21410	21.26
July, 2018	30	129.16±18.14	101.89-172.39	18.99±0.99	16.5-20.5	20.30±3.60	49863-93258	70593±12583*	40.73
August, 2018	6	120.91±17.44	88.11-134.6	18.08±1.32	16.5-19.5	18.07±5.89	29872-89854	60951±23399	7.03
September,2018	10	85.74 ±8.93	72.63-98.82	15.76±0.56	14.5-16.3	6.28±2.76	7128-28455	21106±6005	4.06
Total	**125**								

Maximum*

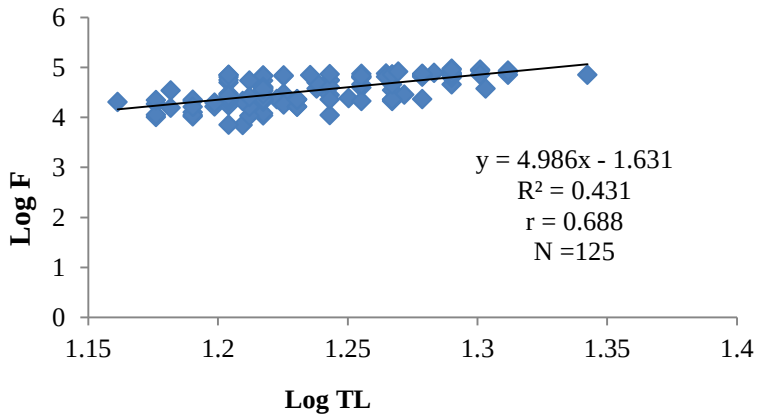


Figure 4 Relationship between fecundity and total length in female *A.testudineus*

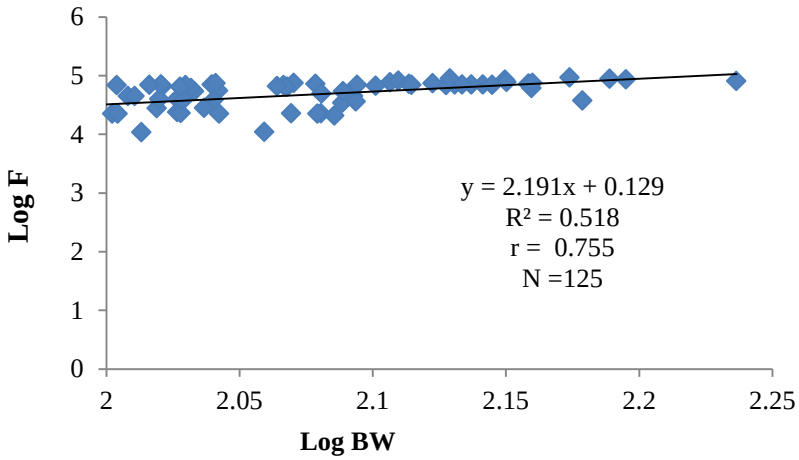


Figure 5 Relationship between fecundity and body weight in female *A. testudineus*

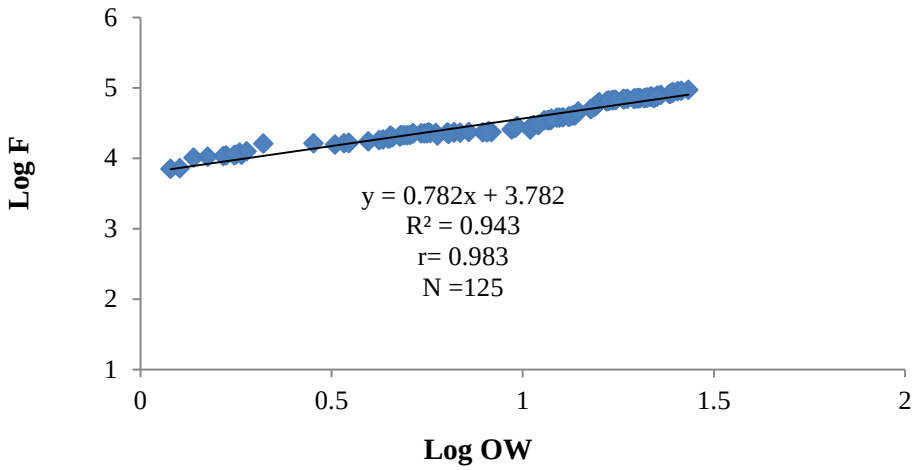


Figure 6 Relationship between fecundity and ovary weight in female *A. testudineus*

Table 5 Size at first sexual maturity of observed female *A. testudineus*

Months	Sample size (n)	Size range of mature female fish (cm)	Minimum size of mature female fish (cm)
March, 2018	2	16.20-16.50	16.20
April, 2018	25	15.00-19.50	15.00
May, 2018	13	15.80-22.00	15.80
June, 2018	21	15.50-17.00	15.50
July, 2018	21	17.30-20.50	17.30
August, 2018	2	16.50-19.00	16.50
September, 2018	8	14.50-16.30	14.50

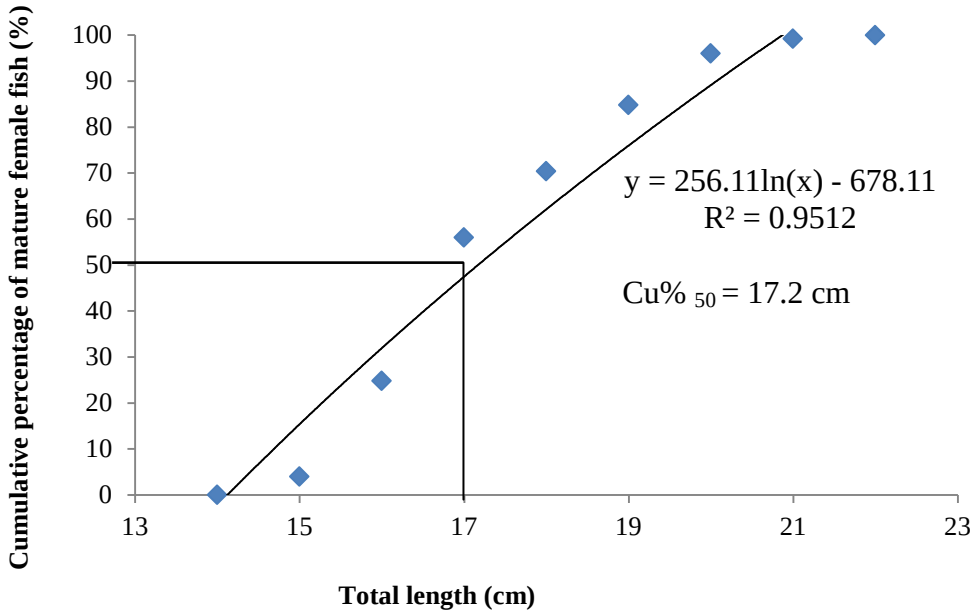
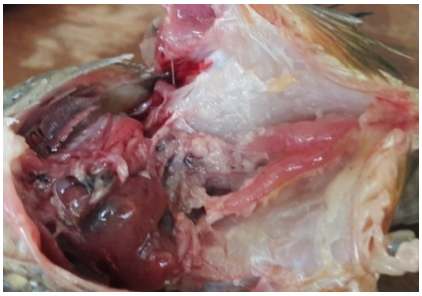
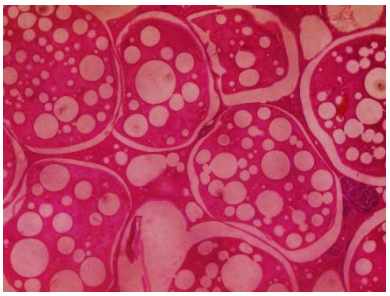


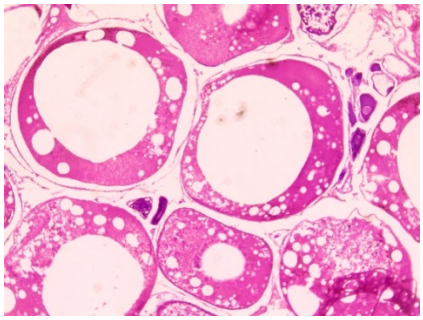
Figure 7 Size at first maturity for female *A. testudineus*



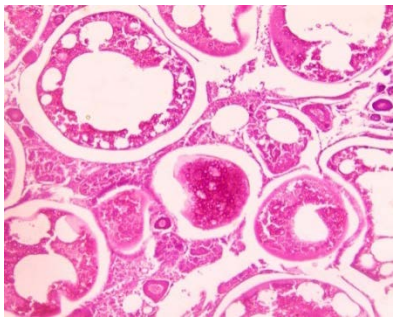
A. The ovary in the coelomic cavity



B. Maturing stage of female fish



C. Mature stage of female fish



D. Ripe stage of female fish

Plate 1: The reproductive stages of female *Anabas testudineus*

Discussion

The maximum size of this female fish reported by (Rahman, 2012) in Assam, India was 13.40 cm, but the largest female specimen observed in this study was 22.00 cm. The growth performance in female was found high since the correlation coefficient 'r' exhibits high degree of positive allometric correlation between the length-weight relationship. The positive allometric growth can be due to higher proficiency in feeding (Saikia *et. al.,* 2011) and better condition for survival for the species (Gohain and Goswami, 2013).Degree of variation of exponential value of length-weight relationship indicated by 'b' value in female *A. testudineus* was 3.0. The value of exponent 'b' is found to be in normal range between 2.5 and 4.0 as suggested by Hile (1936) and Martin (1949).Variation in 'b' value can be attributed due to feeding (Le-Cren, 1951) and sex (Hile and Jobes, 1940), development stages of gonads, specially the ovary affect the weight (Weathely, 1972) and state of maturity (Frost, 1945). In the present study, 'b' remains constant at 3.0 showing isometric growth pattern in an ideal environment (Gohain and Goswami, 2013).

Condition, fatness or well-being of fish expressed by 'K' factor, is an index to monitor feeding intensity and growth rate (Oni *et al*., 1983) is based on hypothesis that heavier fish for given length are in better condition (Bagenal and Tesch, 1978). Fish with high value of 'K' are heavy for its length, while with low 'K' are lighter. 'K' value greater than 1 indicates better condition of fish (Le-Cren, 1951).The present study showed that monthly variations of condition factor were all resulted above one indicating the food supply of studied species were stable and good in the study area.

The GSI indicates gonadal development and maturity of fishincreases with the maturation of fish declining thereafter (Parameswam *et al*., 1974). Bernal *et. al*. (2015) reported GSI of

female climbing perch peaked in May during the onset of the wet season (May to November) in the Candaba wetland, Philippines. During the study period, the GSI of female peaked in June, July and August, and the spawning season was from April to September. The present study agreed with the study of Morioka *et al.,* (2009) which they also mentioned this species has extended breeding period following the peak between April to August. The GSI and HSI value in the present study were found to be inversely related with each other which reveal liberation of energy from liver into the ovary.

The GSI values for the 236 female fish ranged from 0.05-9.19% with a mean of 5.84%. This indicated that *A. testudineus* on the average used 5.84% of its body weight for egg production. Nikolsky (1963) said that the ovaries weight was around 15% from the body weight but Scott (1947) reported the values between 15%and 30%.Maturity stage III, IV and V occurred from April to September, indicating that mature females were actively spawning throughout these months. The months with the highest peaks in the GSI may be indicated of fish species respective spawning periods.

The peak fecundity observed for *A. testudineus* during the months from April to September suggested the possible spawning season of the fish species. In this study period, the correlation between fecundity and ovary weight was more correlated than that of total length and fish weight. It suggested that fecundity generally was increased in direct proportional to ovary weight. Variation in fecundity may be due to environmental conditions and food availability in the study area. This variation was also reported by some previous study of other fish (Doha and Hye, 1970).

Size at first maturity i.e. 50% of matured female *A.testudineus* was approximately 17.2 cm in total length. Few researchers result on length at first maturity is different from that of the researches done in India. Most likely genetic variation and environmental influences played a major role on the changes (Chanchal *et. al*., 1978).

Conclusion

Specimens in this study were found to have mature gonads in the dry and wet season months, indicating no seasonality in breeding patterns. The periods predicted to be the spawning, maturation, and breeding season coincide with the GSI data. The months of September to December that showed decrease in the AF values, GSI values and occurrence of gonad stage V (spent) may possibly indicate the maturation-to breeding season. Catching female *A. testudineus* with a minimum size limit to allow them time to reach maturity and produce eggs in order to substain their population and prohibition on fishing the fish in the spawning season should be instituted in this area. The information on the reproductive biology of this species is important because it potentially indicates their current population condition in the study area.

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ARTIFICIAL PROPAGATION OF SEABASS, *LATES CALCARIFER* (BLOCH, 1790) IN MYEIK ARCHIPELAGO

Yu Yu Htwe[1], Thida Ei [2], Kalayar Win Maung [3]

Abstract

Artificial propagation of fish is an alternative to meet increasing demand for food fish. Seabass (*Lates calcarifer*) is one of the commercially important species in Myanmar. It is fast growing and euryhaline. This fact is seen as a valuable attribute for species enabling its adoption for ponds and cages culture under marine and brackish water environment. This study was conducted to assess the breeder management for artificial propagation of seabass. The study period was from September 2017 to December 2018 in Myeik Archipelago. The breeders were reared for two months before injection to be healthy fish. The male and female spawners were selected and injected hormone which was calculated to males and females, and their body weight. Induced breeding of study species was studied and number of fertilized eggs, per cent of mortality rate and survival rate were observed seasonally. The hatching rate and survival rate of study fish were highest in hot season. Larvae rearing and different diets on larvae were observed. The effects of weather and water parameters of the study area were collected.

Keywords : Artificial propagation, Seabass, *Lates calcarifer*, spawners, pond and cage culture, larvae rearing, embryonic developmental stages.

Introduction

The seabass (*Lates calcarifer*) is one of the species with a high potential for cultivation. It is fast growing and euryhaline. This fact is seen as a valuable attribute for a species enabling its adoption for pond and cage culture under marine, brackish, and freshwater environments. *Lates calcarifer* (Bloch, 1790) is one of the commercial important species in Myanmar. It is one from nine *Lates* species (the others mostly in fresh waters in Africa) of the Latidae (Parazo *et al*., 1998). The fish comes under a diverse group of common names “Barramundi” in Australia, “Giant sea perch” in Papua New Guinea, “sea bass” in South-east Asia and “Ka-kadit” in juvenile and male phase and Ka-tha-baung in Myanmar (Tin Tin Aye,2004).

Artificial propagation of sea bass was first achieved in Thailand during 1971 by stripping the ripe spawners collected from natural spawning grounds. Wongsomnuk and Maneewongsa (1974) successfully induced cultured broodstock to spawn in captivity by hormone stimulation. Captive broodstock of sea bass were successfully induced to spawn naturally using environmental stimulation (Kungvankij, 1981). Thailand is the most advanced country in the production of sea bass seed from spawners collected from the wild and induced to breed since 1973. Thailand is presently producing more than 100 million seeds annually. The popularity and demand for this species made it an obvious choice for the development of aquaculture technology. Techniques for the culture of barramundi were developed in Thailand in the early 1970s (Wongsomnuk and Manevonk, 1974) and considerable progress in aquaculture techniques for the species has been achieved since that time.

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The growth of barramundi was investigated by tagging and scale reading in northern Australia (Davis and Kirkwood 1984), by scale reading in the sexually precocious population of the northeast Gulf of Carpentaria (Davis 1984a). In present study, sea bass are cultured in cage and tested by tagging with biomark during the study period.

Lates calcarifer, known as sea bass in Asia and barramundi in Australia, is a euryhaline member of the family Centropomidae. It inhabits freshwater, brackish and marine habitats including streams, lakes, estuaries and coastal waters. The newly-hatched larvae are distributed along the coastline of brackishwater estuaries while 1cm size larvae can be found in freshwater bodies (Bhatia and Kungvankij, 1971). Under natural condition, sea bass grows in fresh water and migrates to more saline water for spawning. The sea bass (*Lates calcarifer*) is one of the species with a high potential for cultivation. Seabass spawn naturally in captivity (Toledo et al. 1991). Alternatively, they can be induced to spawn by hormonal or environmental manipulations (Kungvankij 1987, Garcia 1989a, b). This widely developed in South East Asia, sea bass *(Lates calcarifer)* are popular marine food fish of high market value and culture of sea bass has been successful in coastal regions in Myanmar.

Based on size and weight of larvae, the stocking density of 30 ind/l is the most appropriate in mass producing sea bass fry (Juario and Duray 1985). Since the seabass is cannibalistic, the larger ones eating up the smaller, it is essential to grade them from the fry stage onwards into different size groups. Stocking the same size of larvae and fry will reduce the rate of cannibalism, the survival rate will be increased and the growth rate of the fish could also be faster and more uniform in size (Chantarasri *et al*., 1989). At about 2-3 months old stage they are ready for stocking in large meshed grow-out net cages and ponds for commercial culture.

The objectives of the present study were as follows:

- to assess the breeder management for pre-spawning of seabass
- to conduct the induced breeding procedure for seabass
- to find out the seasonal variation of fertility rate, hatching rate and survival rate of seabass
- to identify the larvae rearing of seabass

Materials and Methods

Study period and study sites

The study period lasted from September 2017 to December 2018. The investigation on *Lates calcarifer* was conducted at Sarr kyun (Sarr insand) located in (12° 33´ 1.98” N, 98° 28´ 40.33”E), and Ye myit kyi village (12° 33´ 27.0” N, 98° 19´ 34.13”E) in Myeik Township, Taninthayi Region (Fig. 1)

Food and feeding the selected broodstock

The breeders fed twice daily with fresh fish and squid at the rate of 1 per cent of body weight for 1-2 time a week. Moreover, vitamin A, B, C and E were supplemented once daily alternately (by putting inside fresh fish). It is fed twice daily with 5 per cent of body weight for pellet diet to old breeding stock.

Selection of spawners

The breeders were stocked for two months before injection. It comes from both wild type and cultured fish. They were selected as broodstock size– female 3 kg up, male < 4 kg (between 2yr and 7yr). They must be healthy and active fish. Their fins and scales must be complete for sign of health. They were free from disease and parasite and also free from injury.

Tagging and preparation for injection

The breeders were marked tagging by biomass to calculate the dose for injection. Ten females and five males of broodstock were injected with single dose of 1 cc hormone per 1 kg fish. Suprefact and Dextrose ratio of (1:2) for female and ratio of (1:1) for male were injected. According to the spawning behavior of species, the breeders were injected at 11:00 in the morning, and then released injected-fishes back into spawning tank. Begin releasing mucus before spawning (after 30:30 hours of injection). Spawning was occurred at 32:30 hours after injection.

Procedures of seed production

After hormone-inducing, the brood fish was transferred into the spawning tank. The water overflowing method was used for collecting eggs from a spawning tank. The flowing water carries the eggs into the eggs collecting tank from which it transfers into the hatching tanks by using (200µ) fine netting. The unfertilized eggs sinking to the bottom were discarded before placing in the larvae rearing tanks. The fertilized eggs were stocked in the incubation tanks until hatching about 12-15 hrs.

Egg collection and incubation

The fertilized eggs were collected by fine cloth and 50µ hand net by using air lift method. It is used sampling method; density of egg, estimate egg number per litre for egg counting. The fertilized eggs were stock into hatching tanks that is concrete tank by using incubate equipments. The optimum water quality is monitored with five parameters. The eggs density was placed by about 1,000,000 in one tank (100 eggs/liter). The tanks were given gently aeration to prevent the eggs from settle on the bottom.

Data analysis

$$\text{Hatching rate} = \frac{\text{Total fertilized eggs – unfertilized eggs}}{\text{Total number of hatched eggs}} \times 100$$

$$\text{Survival rate} = \frac{\text{Total number of spawn}}{\text{Total number of spawn stocked}} \times 100$$

(**Source:** Department of Geography, Myeik University)

Figure 1 Map of study site

Spawning tank and egg collection tank

Hatching tanks

Larvae rearing tank

Plate 1. Hatchery facilities

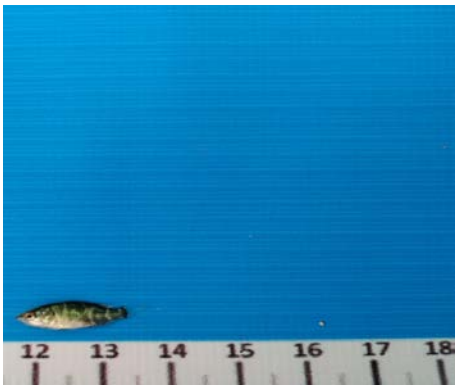
Step1. Carrying with hand net

Step 2. Tagging with biomark

Step 3. Injection of hormone

Step 4. Releasing injected fish into the spawning tank

Plate 2. Steps of inducing hormone to seabass

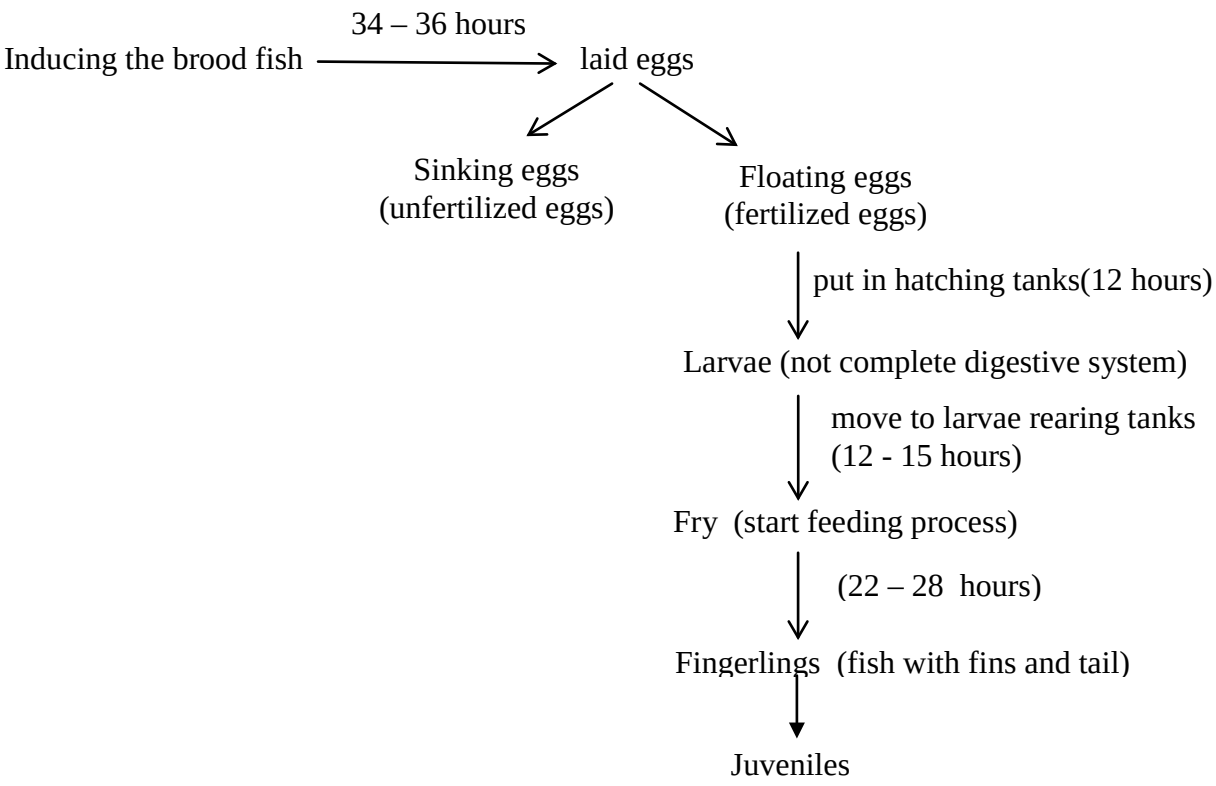


Length measuring

Weight measuring

Plate 3. Measuring size of seabass in larvae rearing tank

Larvae rearing procedure



Results

Systematic position of study species

The systematic positions of *Lates calcarifer* was followed after

Kingdom	-	Animalia
Phylum	-	Chordata
Class	-	Actinopterygii
Order	-	Perciformes
Family	-	Latidae
Genus	-	*Lates*
Species	-	*L.calcarifer* (Bloch, 1790)
Common name	-	Barramundi / Seabass
Local name	-	Ka kadit (Ka tha baung)

Plate 4. Male and female Seabass

Sex ratio and size of breeder

The sex ratio of male and female breeders were 1 : 2, some breeders were wild and some had been injected previous time. The size of female were 62.5 cm to 77.5 cm in length and 3.5 kg to 7 kg weight and male were 55 cm to 67.5 cm length and 2.8 kg to 3.5 kg weight (Table 1, 2 and 3).

Table 1 Measuring and tagging to Seabass for injection in hot season

No.	Date	Tag Number	Sex (M/F)	Length (cm)	Weight (kg)	Dose	Induced Time (pm)
1	29/4/18	wild stock	F	65	4.5	5 cc	10:18
2	29/4/18	2051374*	F	70	5.5	5 cc	10:20
3	29/4/18	wild stock	F	70	5.5	6 cc	10:22
4	29/4/18	wild stock	F	67.5	5	5.5 cc	10:25
5	29/4/18	wild stock	F	75	6	6.5 cc	10:28
6	29/4/18	wild stock	F	70	4	4.5 cc	10:30
7	29/4/18	wild stock	F	75	6	6.5 cc	10:31
8	29/4/18	wild stock	F	67.5	5	5.5 cc	10:33
9	29/4/18	wild stock	F	60	4	4.5 cc	10:24
10	29/4/18	1916542*	F	67.5	5.5	5 cc	10:39
11	29/4/18	wild stock	M	65	3	3.5 cc	10:27
12	29/4/18	wild stock	M	62.5	3	3.5 cc	10:35
13	29/4/18	wild stock	M	67.5	3.5	4 cc	10:37
14	29/4/18	wild stock	M	67.5	3	3.5 cc	10:42
15	29/4/18	wild stock	M	62.5	3	3.5 cc	10:45

Table 2 Measuring and tagging to Seabass for injection in wet season

No.	Date	Tag Number	Sex (M/F)	Length (cm)	Weight (kg)	Dose	Induced Time (pm)
1	30/6/18	1299817*	F	67	5.5	5cc	10:00
2	30/6/18	wild stock	F	70	5.5	6cc	10:12
3	30/6/18	wild stock	F	67.5	5	5.5cc	10:04
4	30/6/18	1916542985*	F	75	6	5.5cc	10:16
5	30/6/18	2049387985*	F	67.7	6	5.5	10:22
6	30/6/18	2025977*	F	70	7.5	7cc	10:07
7	30/6/18	2043341*	F	65	5	4.5	10:20
8	30/6/18	2037659*	F	62.5	4.5	4	10:15
9	30/6/18	1802957*	F	65	5	4.5	10:32
10	30/6/18	1762213*	F	65	4.5	4cc	10:35
11	30/6/18	wild stock	M	67	3.5	4cc	10:10
12	30/6/18	wild stock	M	70	4	4.5cc	10:23
13	30/6/18	wild stock	M	62.5	3	3.5cc	10:25
14	30/6/18	wild stock	M	67.5	3.5	4cc	10:28
15	30/6/18	wild stock	M	67	3	3.5cc	10:38

Table 3 Measuring and tagging to Seabass for injection in cold season

No.	Date	Tag Number	Sex (M/F)	Length (cm)	Weight (kg)	Dose	Induced Time (pm)
1	30/12/18	02018978*	F	70	5.5	5 cc	11:00
2	30/12/18	wild stock	F	62.5	3.5	4 cc	11:13
3	30/12/18	wild stock	F	62.5	4	4.5 cc	11:18
4	30/12/18	wild stock	F	65	4.5	5 cc	11:21
5	30/12/18	wild stock	F	67.5	3.5	4 cc	11:24
6	30/12/18	wild stock	F	62.5	4	4.5 cc	11:25
7	30/12/18	wild stock	F	75	6	6.5 cc	11:27
8	30/12/18	01811216*	F	75	6	5.5 cc	11:28
9	30/12/18	wild stock	F	77.5	7	7.5 cc	11:30
10	30/12/18	02048854*	F	70	7	6.5 cc	11:34
11	30/12/18	wild stock	M	67.5	3	3.5 cc	11:02
12	30/12/18	wild stock	M	60	2.8	3 cc	11:04
13	30/12/18	wild stock	M	60	2.9	3 cc	11:08
14	30/12/18	wild stock	M	65	3	3.5cc	11:10
15	30/12/18	01934984*	M	55	3.5	3 cc	11:11

Seasonal fertility rate, hatching rate and survival rate of Seabass

Total number of eggs were collected differently in seasonally, during the hot season the total number of eggs are highest amount and lowest in wet season. The fertility rate was highest in hot season but lowest in wet season. The hatching rate was highest in hot season but lowest in cold season (Table 4). The survival rate of Seabass was different; highest survival rate (63 per cent) was found in hot season with salinity was 29 to 30 ppt and water temperature was 28 – 32 °C, and the lowest (30 per cent) was observed in cold season with salinity was 28 to 30 ppt and water temperature was 28 – 30 °C (Table 5 and Table 7).

Table 4 Seasonal variation of fertility rate of Seabass

Season	Total no. of eggs	No. of fertilized eggs	No. of unfertilized eggs	Fertility rate (%)	No. of hatched eggs	Hatching rate (%)
Hot	16,000,000	14,000,000	2,000,000	87.5	13,600,000	97.1
Wet	9,000,000	6,250,000	2,750,000	69.4	5,000,000	80
Cold	15,000,000	12,000,000	3,000,000	80	9,500,000	79.2

Table 5: Seasonal variation of survival rate of Seabass

	Larval age	**Survival rate %**	**Number**	**Salinity**	**Temp (C)**
Rearing (Hot Season)	Day 1	100	13,600,000	29-30	28-32
	Day 30	91	12,404,000	29-30	28-32
	Day 60	76	9,358,000	29-30	28-32
	Day 90	65	6,080,000	29-30	28-32
	Day 100	63	3,850,000	29-30	28-32
Rearing (Wet Season)	Larval age	Survival rate %	Number	Salinity	Temp (C)
	Day 1	100	5,000,000	23-25	25-28
	Day 30	83	4,130,000	23-25	25-28
	Day 60	24	975,000	23-25	25-28
	Day 90	61	590,000	23-25	25-28
	Day 100	43	253,000	29-30	28-32
Rearing (Cold Season)	Larval age	Survival rate %	Number	Salinity	Temp (C)
	Day 1	100	9,500,000	28-30	28-30
	Day 30	84	7,950,000	28-30	28-30
	Day 60	74	5,875,000	28-30	28-30
	Day 90	61	3,560,000	28-30	28-30
	Day 100	30	1,050,000	28-30	28-30

Age and amount of supplementary food for Seabass larvae

The larvae were supplied live food (such as rotifer and artemia) until they reached the age of 14days and then supplied the artificial pellet with respective size and weight (Table 6).

Growth rate of Seabass larvae

Larvae were metamorphosed from fry to fingerlings after one month with the weight of 8.3 gm and length 9 cm, in two month larvae were not much increase growth rate of 8.76 gm to 9 cm. After two and half month, the larvae reached 11 cm to 14. 78 gm which stage was fingerling (Fig 2, Table 8).

Table 6 Age and amount of food supply to Seabass larvae

Age	**Food supply**	**Amount of food gm / thousand**
2 - 4 days	Rotifers	12
5 - 9 days	Rotifers	12
10 - 13 days	Rotifers	24
	Artemia	0.4
14 - 16 days	Artemia	40
	Inve ½ + ⅔	40
17 – 22	Artemia	67
	Pellet (size -Inve ⅔ + ⅗)	67
23-31 days	Pellet (size - Inve 3/5 + 5/8)	67g
1½ months	Pellet (size -Inve 5/8+ G8)	16g
2 months	Pellet (size -Inve G8+G12)	16g
2½ months	Pellet (size -Inve G12+ 1042)	125g

Table 7 Water parameter in larvae rearing tank

Parameters	Range of value (standard) by FAO	Range of value (experiment)
Water temperature	26 – 32 °C	23 - 32 °C
Salinity	10 – 30 ppt	29 - 32 ppt
pH	7.5 – 8.5	6.8 – 8
Dissolved oxygen	4 – 9 mg/ l	6 – 9 mg / l
Ammonia(NH3)	Less than 1 ppm	0.3 – 0.5 ppm

Table 8 Grading of fish by length in larvae rearing tank (n = 100)

Weight(gm) / Length(cm)	1st Grading	2nd Grading	3rd Grading	4th Grading	5th Grading
1.0 - 2.0	0.07				
2.01 - 3.0	0.42	0.45			
3.01 - 4.0	0.93	0.78	0.77		
4.01 - 5.0	1.62	1.52	2.08	1.47	1.67
5.01 - 6.0		2.39	2.69	2.24	2.61
6.01 - 7.0		3.1	3.96	4.45	3.95
7.01 - 8.0		6.29	6.58	5.46	6.32
8.01 - 9.0		7.97	8.31	8.76	8.75
9.01 - 10.0					11.91
10.01 - 11.0					14.78

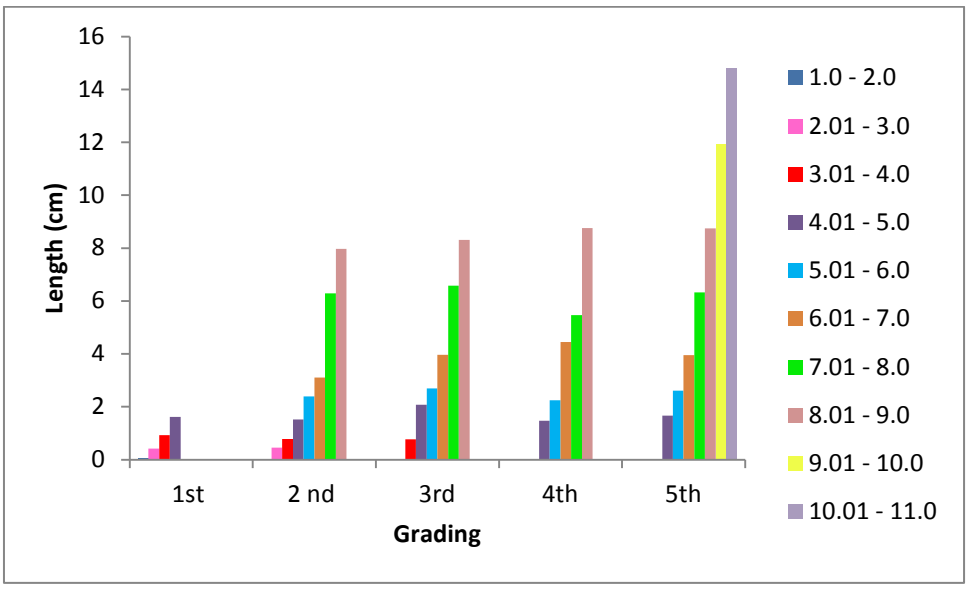


Figure 2 Larvae size grading of Seabass

Discussion

Grace (1985) described that *Lates calcarifer* was one of the important cultured fish as a commercial and subsistence food fish and its relatively high market value, it had become an attractive commodity of both large and small-scale aquaculture enterprises. Sea bass is farmed with high production in Southeast Asia, generally from small coastal cage farms and large-scale sea bass farms in Australia. In Myanmar, sea bass are successful in induced breeding and also cultured in cage for its commercial value.

Adult seabass (3 – 5 kg) migrated towards the mouth of the river from inland water into the sea where the salinity ranges between 30 – 32 ppt for gonadal maturation and subsequent spawning (Grace, 1985). Sources of adult seabass spawners could be collected from wild-caught adults and from cages or ponds about 2 – 6 years old species averaging in weight from 2 – 8 kg (Ramon Y. Tangon, 2016). In present study, 2 – 7 kg breeders were selected for induced breeding and optimum salinity range is between 28 – 32 ppt, the previous study is in agreement with previous authors Grace Mathew, 1985 and Ramon Y. Tangon, 2016). Captive broodstock of sea bass were also successfully induced to spawn naturally using environmental stimulation (Kungvankij 1981).

Good broodstock management and proper nutrition are essential for full maturity to ensure sustainable supply of quality eggs and fry for stock enhancement (Reyes, 2014). In present study, breeders were well feed and care for two months before the induced breeding, the present study is in agreement with Reyes, 2014.

The hatching were occurred approximately 14hrs after fertilization at 28°C and 32-33 ppt according to Parazo *et al*., 1998). MacKinnon et al. found barramindi spawned in a northern Gulf of Carpentaria estuary when water temperatures ranged from 27°C to 33°C and salinities from 28 to 34 ppt. Salinities in the range necessary for barramindi egg fertilization in Thailand 28-32 ppt (MacKinnon *et al*., 1984). Newly hatched *nauplii* incubate at a density of (1g cysts/l) in plastic hatching vessels filled with clean filtered seawater and provided with vigorous aeration. The cysts will hatch normally within 24 h (Parazo *et al*., 1998).

In one month, the spawn metamorphose into the fry and fingerling stages was completed in one month, which has the appearance very similar to the parent fish. The length measured 1.5-2.0 cm (Tookwinas, 1987) which was coincide with the present finding of 28 days and 0.07 – 1.58 cm. Ergun Buke (2002) found that the hatching rates were 80% to 90% and the survival rates were 40% to 65% in Turkey, Europe. In the present study, 97% of the hatching rate and 63% of the survival rate was found in hot season, 80 % of the hatching rate and 43 % of survival rate and then 79.2 % of hatching rate and 80 % of survival rate were observed, so the present study was agreement with above authors.

As the grading result, sea bass larvae were better growth rate in larvae rearing tank during the hot season. The present finding was agreement with Charles *et al*., (2003), he said that the effects of temperature and salinity are significantly different in weight of juvenile black sea bass.

Summary

The seabass breeders were stocked for two months at Sarr kyun (salt island) before injection and 1: 2 ratio of male and female breeders were injected for artificial propagation at Ye Myint Kyi village in Myeik Township, Taninthayi region deported from September 2017 to

December 2018. The fertilized eggs, hatched eggs and survival fry were observed with estimate counting method. The hatching rate and survival rate were highest in hot season and lowest in cold season.

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