JOURNAL OF THE MYANMAR ACADEMY OF ARTS AND SCIENCE

Mathematics, Computer Science and Zoology

Vol. XVII, No.3, August 2019

Myanmar Academy of Arts and Science

Journal of the Myanmar Academy of Arts and Science

Vol. XVII, No.3

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NUMBER OF VERTICES OF DEGREE THREE IN SPANNING 3-TREES IN SQUARE GRAPHS*

Win Min Aye[1], Liming Xiong[2], Tao Tian[3]

Abstract

In this paper, we show that the square graph of a tree T has a spanning tree of maximum degree at most three and with at most $\max\left\{0, \sum_{x\in W_3(T)} (t_T(x)-2)-2\right\}$ vertices of degree three, where $W_3(T)=\{x\in V(T):$ there are at least three edge-disjoint paths of length at least two that start $x\}$ and $t_T(x)$ is the number of edge-disjoint paths with length at least two that start at a vertex *x*.

Keywords: Square graph; 3-tree; spanning tree.

Introduction

For graph-theoretic notation not explained in this paper, we refer the reader to J. A. Bondy and U. S. R. Murty, 2008. We consider only simple graph in this paper. Let $G=(V,E)$ be a graph with vertex set *V* and edge set *E*. A *k*-tree is a tree with the maximum degree at most *k*. A graph is called *hamiltonian* (*traceable*, respectively) if it has a *spanning cycle* (*path*, respectively). Thus a graph is traceable if and only if it has a spanning 2-tree. Therefore, the minimum number of vertices of degree three in a spanning 3-tree *F* of a graph G shows how closed to be traceable the graph G is.

The classic condition for a graph to be traceable is the minimum degree condition, see O. Ore, 1960. It has been extended to consider whether a graph has a spanning *k*-tree, see S. Win, 1979, in references. It has also been extended to the condition for the existence of a spanning tree with at most *k* leaves, see H. J. Broersma and H. Tuinstra, 1998. H. J. Broersma and H. Tuinstra gave more structures of the graphs satisfying the condition given by S. Win, 1979; M. Aung and A. Kyaw, 1998, considered the maximum *k*-tree. V. Neumann-Lara and E. Rivera-Compo, 1991, gave an independence number condition for a graph to have a spanning *k*-tree with bounded number

1. Dr, Professor, Department of Mathematics, Kalay University.
2. Dr, Professor, School of Mathematics and Statistics, Beijing Institute of Technology.
3. Ph.D Candidate, School of Mathematics and Statistics, Beijing Institute of Technology.

* Best Paper Award Winning Paper in Mathematic, (2018)

of vertices with degree *k*, for $k \geq 4$. M. Tsugaki, 2009, gave a similar condition for $k = 3$.

The *square graph* of a graph *G*, denoted by G^2, is the graph with $V(G^2) = V(G)$ in which two vertices are adjacent in G^2 if their distance in *G* is at most two. Thus $G \subseteq G^2$. H . Fleischner, 1974, proved that the square graph of a 2-connected graph is hamiltonian, which was extended by G. Hendry and W. Vogler, 1985. Y. Caro, I. Krasikov and Y. Roditty, 1991, showed that the square graph of a connected graph has a spanning 3-tree.

Motivated by the results given above and by the observation that the minimum number of vertices of degree three in a spanning 3-tree *F* of a graph G^2 may measure how closed to be traceable the graph G^2 is, Q. Wu, 2016, showed that the square graph of a tree *T* has a spanning 3-tree *F* in which every leaf of *T* has degree one or two and *F* has at most $\max\left\{0, \min\left\{\left\lfloor \frac{n-p(T)+3}{2} \right\rfloor, \left\lfloor \frac{n-5}{2} \right\rfloor\right\}\right\}$ vertices of degree three where *p(F)* is the length of the longest path of *F*. In the whole paper, we let *p(T)* be the length of a longest path of a tree *T*.

Theorem 1

Let *G* be a connected graph of order *n*. Then G^2 has a 3-tree *F* with at most

$$\min_{T \subseteq G} \max\left\{0, \min\left\{\left\lfloor \frac{n-p(T)+3}{2} \right\rfloor, \left\lfloor \frac{n-5}{2} \right\rfloor\right\}\right\}$$

vertices of degree three.

In this paper, we intend to improve the result above. Firstly, we give the following definitions. Let *T* be a tree of order *n* and *x* a vertex of *T*. We define $t_T(x)$ to be the number of edge-disjoint paths with length at least two that start at a vertex *x* and $W_3(T) = \{x \in V(T):$ there are at least three edge-disjoint paths of length at least two starting at $x\}$.

Obviously, $t_T(x) \le d_T(x)$ for any vertex *x* of *T*, where $d_T(x)$ denote the degree of *x* in *T*. For example, for a star $T = K_{1,k}$, it holds that $t_T(x) = 0$ and $d_T(x) = k$ for the center vertex *x* of $K_{1,k}$. From the definition of $W_3(T)$, one may obtain the following observation.

Observation 2

Let T be a tree of order *n*. Then

$$\sum_{x \in W_3(T)} t_T(x) - 2|W_3(T)| = \sum_{x \in W_3(T)} (t_T(x) - 2) \le \frac{n - p(T) - 1}{2}.$$

Proof

Let P_0 be a longest path of *T*. Then we may obtain *T* from P_0 by adding a path P_i of T such that P_i has a leaf of *T*, iteratively. In order to increase $\sum_{x \in W_3(T)} (t_T(x) - 2)$ at least one, these P_i (note that, in each step of the proceeding of adding path with a leaf of *T*, two leaves distance of at least two may be counted once in $\sum_{x \in W_3(T)} (t_T(x) - 2)$ should have length at least two. Therefore, Observation 2 follows.

By Observation 2, in this paper, we continue to give an upper bound for the number of vertices of degree three of spanning 3-tree *F* in square graph G^2 as

$$\min_{T \subseteq G} \max\left\{0, \sum\nolimits_{x \in W_3(T)} t_T(x) - 2|W_3(T)| - 2\right\}$$

where *T* is a spanning tree of *G*. Hence

Theorem 3

Let *G* be a connected graph of order *n*. Then G^2 has a spanning 3-tree *F* with at most

$$\min_{T \subseteq G} \max\left\{0, \sum_{x \in W_3(T)} t_T(x) - 2|W_3(T)| - 2\right\}$$

vertices of degree three, where T is a spanning tree of G.

Observation 2 shows that the bound in the theorem above improves the one gave in Theorem 1. In the next section, we shall give some auxiliary results, which will be used to proof of Theorem 3 in Section 4. In the last section, we shall show the sharpness of Theorem 3 and Observation 2 and also compare two upper bounds in Theorems 1 and 3, respectively.

Preliminaries and Auxiliary Results

For $S \subseteq V(G)$ or $E(G)$, we denote by $G[S]$ the subgraph of G induced by S. For a positive integer s, the graph $S(K_{1,s})$ is obtained from the complete bipartite graph $K_{1,s}$ by subdividing each edge once. The graph G is said to be $S(K_{1,3})$-free if it does not contain any induced copy of $S(K_{1,3})$. We use $N_G(u)$ and $d_G(u)$ to denote the neighbors and the degree of u in G. A *leaf* or *pendant vertex* is a vertex of degree one in a graph. A tree T is called a *caterpillar* if by deleting all pendant vertices of T we get a path. The following results will be used in our proofs.

Theorem 4

If G is a connected $S(K_{1,3})$-free graph of order at least three, then G^2 is hamiltonian.

Corollary 5

If G is a connected $S(K_{1,3})$-free graph, then G^2 has a hamiltonian path starting at any vertex of G.

Let $n_1(T), n_2(T)$ and $n_3(T)$ denote the number of vertices of degrees 1, 2 and 3 in a 3-tree T of order n, respectively. We have

$$n_1(T) + n_2(T) + n_3(T) = n \qquad (1)$$

and

$$n_1(T) + 2n_2(T) + 3n_3(T) = 2|E(T)| = 2(n-1), \qquad (2)$$

one may obtain that $n_2(T) + 2n_3(T) = n-2,$ and that

$$n_1(T) = n_3(T) + 2. \tag{3}$$

A well known and easily proved equality for 3-trees: they are the same as $n_2(T) = 0$ in the above equations, one can then show that $n_1(T) = n_3(T) + 2 = \frac{n - n_2(T) + 2}{2}$. So we only need to consider the upper bound of $n_3(T)$ for a 3-tree *T*.

Lemma 6

If $|W_3(T)| = 0,$ then T^2 has a hamiltonian path starting at any vertex.

Proof

Since $|W_3(T)| = 0, T$ is $S(K_{1,3})$-free. Then by Corollary 5, the lemma holds.

Lemma 7

For each caterpillar *T* (*i.e.*, $|W_3(T)| = 0$), T^2 has a spanning path starting at one end vertex *u* of longest path of *T* and ending at neighbor of *u*.

Proof

We prove by induction on $|V(T)|$. It is obvious when $|V(T)| \leq 4.$ Suppose that it holds when $|V(T)| \leq n-1 (n \geq 5).$ We now consider the case when $|V(T)| = n.$ We choose a longest path *P* of *T* and take an end vertex *u* of *P* and let $v \in N_p(u).$ Let $T_1 = T - u.$ Then $|V(T_1)| = n-1.$ By induction hypothesis and when $d_T(v) = 2, T_1^2$ has a spanning path Q starting at vertex *v* and ending at *h*, where $h \in N_T(v) \setminus \{u\}.$ Thus $Q \cup \{uh\}$ is a spanning path starting at vertex *u* and ending at vertex *v* in T^2. By induction hypothesis and when $d_T(v) \geq 3, T_1^2$ has a spanning path Q_1 starting at vertex *w* and ending at vertex *v*, where $w \in N_T(v) \setminus \{u\}$ with $d_T(w) = 1.$ Thus $Q_1 \cup \{uw\}$ is a spanning path starting at vertex *u* and ending at vertex *v* in T^2.

Lemma 8

Let T be a tree of order n with $W_3(T)=\{u\}$. Then T^2 has a spanning 3-tree F with $n_3(F)=t_T(u)-2$ such that $d_F(u)=1$ and each leaf in T has degree at most two in F.

Proof

Let the neighbors of u be labeled by $u_1,\ldots,u_t,u_{t+1},u_{t+2},\ldots,u_{d_T}(u)$ such that $d_T(u_i)\geq 2$ for $1\leq i\leq t$ and $d_T(u_i)=1$ for $t+1\leq i\leq d_T(u)$. Let T_i be the component of $T-u$ with at least two vertices and $u_i\in V(T_i)(1\leq i\leq t)$. Since $W_3(T)=\{u\}$, $W_3(T_i)=$ Ø. Let $T_u=T_1\cup\{uu_1\}$. Then by Lemma 7, T_u^2 has spanning path Q_u starting at vertex u and ending at vertex u_1. By Lemma 6, T_i^2 $(2\leq i\leq t)$ has a spanning path Q_i starting at u_i .

Then $F=T^2\left[E(\mathrm{Q_u})\cup(\cup_{i=2}^{t}E(Q_i))\cup E(u_1u_{t+1}u_{t+2}\ldots u_{d_T(u)}u_2u_3\ldots u_t)\right]$ is a spanning 3-tree in T^2. This implies that $n_3(F)=t_T(u)-2$ such that $d_F(u)=1$ and each leaf in T has degree at most two in F.

Lemma 9

The following statements hold:

(i) If $W_3(T)=\{u\}$, then T^2 has a spanning 3-tree F with $n_3(F)\leq\max\{0,t_T(u)-4\}$ such that each leaf in T has degree at most two in F.

(ii) If $|W_3(T)|=2$, then T^2 has a spanning 3-tree F with $n_3(F)=\sum_{x\in W_3(T)}t_T(x)-6$ such that each leaf in T has degree at most two in F.

Proof

Suppose that $W_3(T)=\{u\}$. Then by Lemma 6, it is easy to show that T^2 with $t_T(u)\leq 4$ is traceable. In the following, we assume that $t_T(u)\geq 5$. Let the neighbors of u be labeled by $u_1,\ldots,u_t,u_{t+1},u_{t+2},\ldots,u_{d_T(u)}$ such that $d_T(u_i)\geq 2$ for $1\leq i\leq t$ and $d_T(u_i)=1$ for $t+1\leq i\leq d_T(u)$. Let T_i be the component of $T-u$ with at least two vertices and $u_i\in V(T_i)$ $(1\leq i\leq t)$. Then $W_3(T_i)=\emptyset$. Let $T_{12}=T_1\cup T_2\cup\{uu_1,uu_2\}$ and $T_{34}=T_3\cup T_4\cup\{uu_3,uu_4\}$.

Obviously, T_{12} and T_{34} are both caterpillars. Then by Lemma 6, T_{12}^2 and T_{34}^2 have a spanning path P_l and P_s starting at vertex u, respectively. By Lemma 6, T_i^2 $(5\leq i\leq t)$ has a spanning path Q_i starting at vertex u_i. Then $F=T^2\left[E(P_l)\cup E(P_s)\cup\left(\cup_{i=5}^{t}E(Q_i)\right)\cup E(uu_{t+1}u_{t+2}\ldots u_{d_T(u)}u_5u_6\ldots u_t)\right]$ is a spanning 3-tree in T^2. This implies that $n_3(F)=t_T(u)-4$ such that each leaf in T has degree at most two in F.

Suppose that $W_3(T)=\{u,v\}$ and $uv\in E(T)$. Let T_1 and T_2 be two components of $T-\{uv\}$. Then by Lemmas 6 and 8, T_1^2 has a spanning 3-tree F_1 with $n_3(F_1)=t_T(u)-3$ and $d_{F_1}(u)=1$, T_2^2 has a spanning 3-tree F_2 with $n_3(F_2)=t_T(v)-3$ and $d_{F_2}(v)=1$. Then $F=F_1\cup F_2\cup\{uv\}$ is a spanning 3-tree of T with $n_3(F)=\sum_{x\in W_3(T)}t_T(x)-6$ and each leaf in T has degree at most two in F.

Suppose that $W_3(T)=\{u,v\}$ and $uv\notin E(T)$. Obviously, u and v are connected by path P. We assume that $uw,vw'\in E(P)$ (may $w=w'$). Let T_1 and T_2 be the component of $T-\{uw,vw'\}$ containing vertex u and v, respectively. Then by Lemmas 6 and 8, T_1^2 has a spanning 3-tree F_1 with $n_3(F_1)=t_T(u)-3$ and $d_{F_1}(u)=1$, T_2^2 has a spanning 3-tree F_2 with $n_3(F_2)=t_T(v)-3$ and $d_{F_2}(v)=1$. Let $T_0=(T-T_1-T_2)\cup\{uw,vw'\}$. Since T_0 is a caterpillar, T_0^2 has a spanning path Q with end vertices u and v. Then $F=F_1\cup F_2\cup Q$ is a 3-tree of T^2 with $n_3(F)=\sum_{x\in W_3(T)}t_T(x)-6$ and each leaf in T has degree at most two in F

Proof of Theorem 3

In this section, we present the proof of Theorem 3. In order to prove Theorem 3, we only need to show the following result.

Theorem 10

Let T be a tree. Then T^2 has a spanning 3-tree F with at most

$$\max\left\{0, \sum_{x\in W_3(T)} t_T(x) - 2|W_3(T)| - 2\right\}$$

vertices of degree three such that each leaf in a spanning tree T has degree at most two in F.

Now, we may present the proof of Theorem 10.

Proof of Theorem 10

We prove this theorem by induction on $|W_3(T)|$. If $|W_3(T)| \le 2$, then by Lemmas 7 and 9, the theorem holds. Suppose that the theorem holds when $|W_3(T)| < k$ $(k \ge 3)$.

In the following, we only need to show that the conclusion of Theorem 10 holds for the case when $|W_3(T)| = k$.

We may choose one pair of vertices $\{u, v\}$ where $u \in W_3(T)$ and $v \in N_T(u)$ such that $|W_3(T_1)| \le 1$ and $|W_3(T_2)| \le |W_3(T)| - 1$, where T_1 is a component of $T - \{uv\}$ and $T_2 = (T - T_1) \cup \{uv\}$.

By Lemmas 6 and 8, T_1^2 has a spanning 3-tree F_1 such that $n_3(F_1) = t_{T_1}(u) - 2 = t_T(u) - 3$ and $d_{F_1}(u) = 1$. Let F_2 be a spanning 3-tree of T_2^2.

By induction, $n_3(F_2) \le \max\left\{0, \sum_{x\in W_3(T_2)} t_{T_2}(x) - 2|W_3(T_2)| - 2\right\}$, and each leaf in T_1 and T_2 has degree at most two in F_1 and F_2, respectively. Then by $d_{T_2}(u) = 1$, $d_{F_2}(u) \le 2$. Let $F = F_1 \cup F_2$. Since $d_{F_1}(u) = 1$, F is a spanning 3-tree of T^2 with $n_3(F) \le n_3(F_1) + n_3(F_2) + 1$ such that each leaf in T has degree at most two in F.

We distinguish the following three cases to obtained our results.

Case 1

$t_T(v) = 2.$

Then $W_3(T) = W_3(T_2) \cup \{u\}$. Note that $\sum_{x \in W_3(T_2)} t_{T_2}(x) - 2|W_3(T_2)| - 2 \geq 0.$ Therefore,

$$
\begin{aligned}
n_3(F) &\leq n_3(F_1) + n_3(F_2) + 1 \\
&\leq t_T(u) - 3 + \max\left\{0, \sum_{x \in W_3(T_2)} t_{T_2}(x) - 2|W_3(T_2)| - 2\right\} + 1 \\
&= t_T(u) - 3 + \left(\sum_{x \in W_3(T)\setminus\{u\}} t_T(x) - 2\left(|W_3(T)| - 1\right) - 2\right) + 1 \\
&= \sum_{x \in W_3(T)} t_T(x) - 2|W_3(T)| - 2.
\end{aligned}
$$

Case 2

$t_T(v) = 3.$

Then $W_3(T) = W_3(T_2) \cup \{u, v\}$.

Note that possible $\sum_{x \in W_3(T_2)} t_{T_2}(x) - 2|W_3(T_2)| - 2 = -1,$ however, $\sum_{x \in W_3(T_2)} t_{T_2}(x) - 2|W_3(T_2)| - 2 + 1 \geq 0.$ Therefore,

$$
n_3(F) \leq n_3(F_1) + n_3(F_2) + 1
$$

$$
\begin{aligned}
n_3(F) &\leq t_T(u) - 3 + \max\left\{0, \sum_{x \in W_3(T_2)} t_{T_2}(x) - 2|W_3(T_2)| - 2\right\} + 1 \\
&\leq t_T(u) - 3 + \max\left\{0, \sum_{x \in W_3(T_2)} t_{T_2}(x) - 2|W_3(T_2)| - 2 + 1\right\} + 1 \\
&= t_T(u) + (t_T(v) - 3) - 3
\end{aligned}
$$

$$+\left(\sum_{x\in W_3(T)\setminus\{u,v\}} t_T(x)-2\left(\left|W_3(T)\right|-2\right)-1\right)+1$$

$$=\sum_{x\in W_3(T)} t_T(x)-2\left|W_3(T)\right|-2.$$

Case 3

$t_T(v)\geq 4.$

Then $W_3(T)=W_3(T_2)\cup\{u\}$. Note that $t_{T_2}(v)=t_T(v)-1$ and $\sum_{x\in W_3(T)\setminus\{u\}} t_T(x)-1-2(\left|W_3(T)\right|-1)-2\geq 0$. Therefore,

$$\begin{aligned}
n_3(F) &\leq n_3(F_1)+n_3(F_2)+1\\
&\leq t_T(u)-3+\max\left\{0, \sum_{x\in W_3(T_2)} t_{T_2}(x)-2\left|W_3(T_2)\right|-2\right\}+1\\
&= t_T(u)-3+\max\left\{0, \sum_{x\in W_3(T)\setminus\{u\}} t_T(x)-1\right.\\
&\qquad \left.-2(\left|W_3(T)\right|-1)-2\right\}+1\\
&= t_T(u)-3+\left(\sum_{x\in W_3(T)\setminus\{u\}} t_T(x)-2\left|W_3(T)\right|-1\right)+1\\
&= \sum_{x\in W_3(T)} t_T(x)-2\left|W_3(T)\right|-3\\
&< \sum_{x\in W_3(T)} t_T(x)-2\left|W_3(T)\right|-2.
\end{aligned}$$

In all cases, $n_3(F)\leq \max\left\{0, \sum_{x\in W_3(T)} t_T(x)-2\left|W_3(T)\right|-2\right\}$. This proves the theorem for the case when $\left|W_3(T)\right|=k$. Therefore, by induction, the theorem holds.

Concluding Remarks

Firstly, we have the following remark that shows the sharpness of the bound in Theorem 3.

Remark 11

The upper bound in Theorem 3 is sharp, because the square of the tree T with $W_3(T)=\{u\}$ and $t_T(u)=5$ has no hamiltonian path.

Observation 2 shows that the upper bound in Theorem 3 is better than Theorem 1. On the other hand, we may construct many examples to show that those two bounds in Theorems 1 and 3 may have many different. To see this, we let T_0 be a tree that is composed of k pathes of length exactly $l \geq 2$ (we may take $l=k$) with a common vertex and the length of a longest path of T_0 is $2l$ (i.e., the tree obtained by subdividing all edges in the star $K_{1,k}$ exactly $l-1$ times).

Then

$$|V(T_0)| = lk+1$$

$$\sum_{x\in W_3(T_0)} (t_{T_0}(x)-2)-2 = k-4$$

and

$$\frac{|V(T_0)|-p(T_0)+3}{2} = \frac{lk+1-2l+3}{2} = \frac{lk-2l+4}{2}.$$

From the equations above, one may know that the different $\frac{lk-2l+4}{2}-(k-4)=\frac{lk-2l-2k+12}{2}\left(=\frac{(k-2)^2+8}{2} \text{ if } l=k\geq 3, \text{ respectively}\right)$ between the two upper bounds in Theorems 1 and 3, respectively, may be any large $\left(\frac{(k-2)^2+8}{2} \text{ if } l=k\geq 5, \text{ respectively}\right)$.

Finally, we show that the inequality in Observation 2 is also sharp. To see this, we construct a tree as follows: we use T_0 to denote the resulting graph obtained a path P_0 by attaching at least one pendant edge on each vertex of P_0. Now we obtain the graph T_0' from T_0 by subdividing these pendant edges exactly once. Then $\sum_{x\in W_3(T_0')}(t_{T_0'}(x)-2)=\frac{|V(T_0')|-p(T_0')-1}{2}$ (here we suppose that $|V(T_0')|-p(T_0')-1$ is even). The sharpness shows that Observation 2 is itself interesting.

Acknowledgements

The work is supported by the Natural Science Funds of China (No: 11471037, 11671037).

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J. Myanmar Acad. Arts Sci. 2019 **Vol. XVII. No.3**

CLASSIFICATION OF SOME BUTTERFLIES BY USING RULES INDUCTION: CN2 ALGORITHM*

Su Myo Swe[1]

Abstract

Rule induction is an area of machine learning in which formal rules are extracted from a set of observations. Machine learning is a field of artificial intelligence that uses statistical techniques to give computer systems the ability to "learn" from data, without being explicitly programmed. Machine learning applications are classification, regression, clustering, density estimation and dimensionality reduction. The CN2 algorithm is a classification technique designed for the efficient induction of simple, comprehensible rules of form "if *cond* then predict *class*", even in domains where noise may be present. This research used *zoo* dataset and passed it to CN2 Rule Induction. In this research, classification of some butterflies species by using rules induction with CN2 algorithm system has developed. In this system, there are 29 species of butterflies are classified. In this system, MS Visual Studio 2010 as a programming tool and MS SQL Server as for database development are used.

Keywords: *Machine Learning, Rule Induction, CN2 Algorithm*

Introduction

In artificial intelligence, an expert system is a computer system that follows the decision-making ability of a human expert. Expert systems are designed to solve complex problems by reasoning through bodies of knowledge, represented mainly as if–then rules rather than through conventional procedural code.

The most common form of architecture used in expert and other types of knowledge-based systems is the production system, also called the rule-based system. In computer science, rule-based systems are used as a way to store and manipulate knowledge to interpret information in a useful way. They are often used in artificial intelligence applications and research.

This type of system use knowledge encoded in the form of production rules, that is, if…then rules. Rule have an antecedent or condition part, the left-hand side, and a conclusion of action pert, the right-hand side.

1. Lecturer, Department of Computer Studies, Dagon University

* Best Paper Award Winning Paper in Computer Studies, (2018)

IF: Condition-1 and Condition-2 and Condition-3 THEN: Take Action-4

The Representative Architectures of Expert System

An expert system is divided into two subsystems: the knowledge base and the interface engine. The knowledge base represents facts and rules. The inference engine applies the rules to the known facts to deduce new facts. Inference engines can also include explanation and debugging abilities.

The architectures of Expert Systems today reflect knowledge engineers understanding of how to represent knowledge and how to perform intelligent decision-making tasks with the support of knowledge base system. The Expert System architecture is independent of specific computer hardware. Determinants for computer hardware selection would include the size of the knowledge database, the desire speed of the system's responses and the level of sophistication for the user interface.

Figure 1 shows the architecture of a simple Expert System. The architecture of the simple Expert System could be extended. One common extension is to expand the knowledge base into a knowledge database and a domain database. These two databases could be managed by a database management system (DBMS).

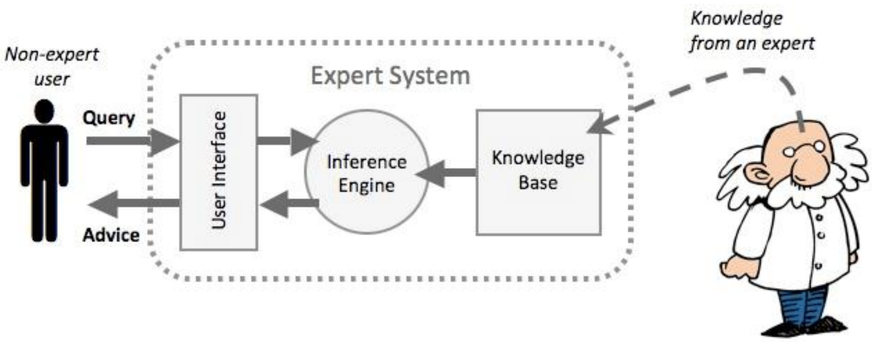


Figure 1: Architecture of a simple Expert System

Building A Knowledge Base

The procedure of building a knowledge base is called knowledge engineering. Investigation of a particular domain, determining what concepts are important in that the domain and creation of a formal representation of the objects and relations in the domain are the role of a knowledge engineer. Often, the knowledge engineer is trained in representation but is not an expert in the domain at hand, be it circuit design, space station mission scheduling or whatever. The knowledge will usually interview the real experts to become educated about the domain and to elicit the required knowledge, in a process called knowledge acquisition.

Architectures of a Rule-Based System

Each rule represents a small piece of knowledge relating to the given domain of expertise. A number of related rules collectively may correspond to a chain of inferences which lead from some initially known facts to some useful conclusions. The inference process is carried out in an interactive mode with the use providing input data needed to complete rule chaining process. Figure 2 shows the architecture of Rule-based system.

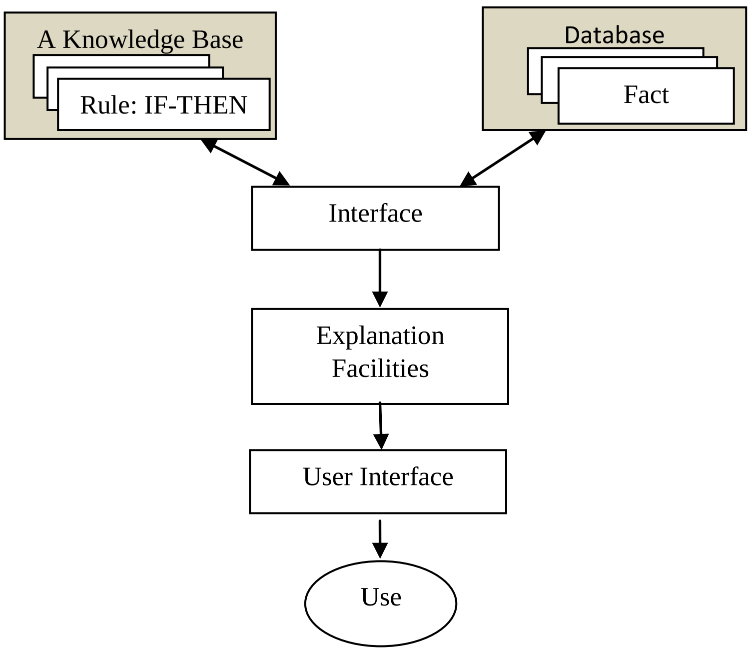


Figure 2: Architecture of Rule-Based System

A typical rule-based system consists of the following components are:

knowledge base : contains the rules embodying expert knowledge about the problem domain

database : contains the set of known facts about the problem currently being solved

inference engine : carries out the reasoning process by linking the rules with the known facts to find a solution

explanation facilities : provides information to user about the reasoning steps that are being followed

user interface : communication between the user and the system

In this research, rule induction with CN2 was used for classification of butterfly that is the simplest setting for classification.

Rule Induction

The rule induction system can create rule that fit the example cases. The rule can then be used to access other cases the outcome is not known. The heart of a rule induction system is an algorithm, which is used to induce the rules from the examples.

Induction methods use various algorithms to convert a knowledge matrix of attributes, values, and selections to rules. Some well-known rule learning algorithms are AQ, CN2, FOIL, RIPPER AND OPUS.

Rule Induction with CN2

The **CN2 induction algorithm** is a learning algorithm for rule induction. It is designed to work even when the training data is imperfect. It is based on ideas from the AQ algorithm to produce rules and combine decision tree learning (such as C 4.5, ID3) to handle noise. As a consequence it creates a rule set like that created by AQ but is able to handle noisy data like ID3.

Materials and Methods

In this research, keys of butterflies are used as materials or zoological data set and rule induction with CN2 algorithm is used as rule indention method.

Identifying Keys of the Butterfly

Butterflies are the most well-known of all insects. They are among the most beautiful creatures on Earth. They are popular among nature lovers as well as a subject for scientific study. Figure 3 shows the external features of the butterfly that are used in the scientific classification.

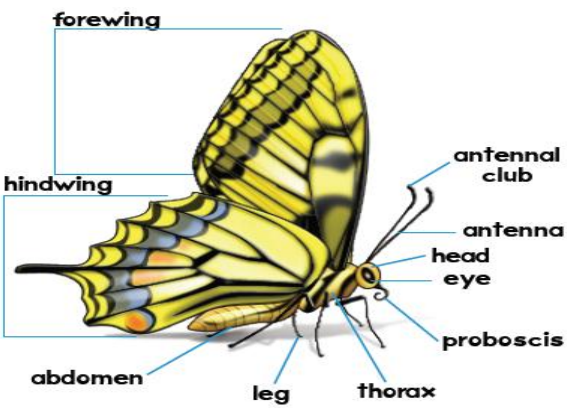


Figure 3: External Features of Butterfly

Butterflies and moths are insects that make up the order Lepidoptera, derived from the Greek words *lepidos* for scaly and *ptera* for wings. Four wings are present. The wings are membranous, with veins or nervures running longitudinal form base to the wing margins. The pattern formed by these veins (wing venation) is of primary importance in the classification of Lepidoptera. Wings of all the butterflies' families showed considerable variations in shapes and vein patterns reflecting their specific nature. Figure 4 shows the parts of wings and venation pattern of butterflies.

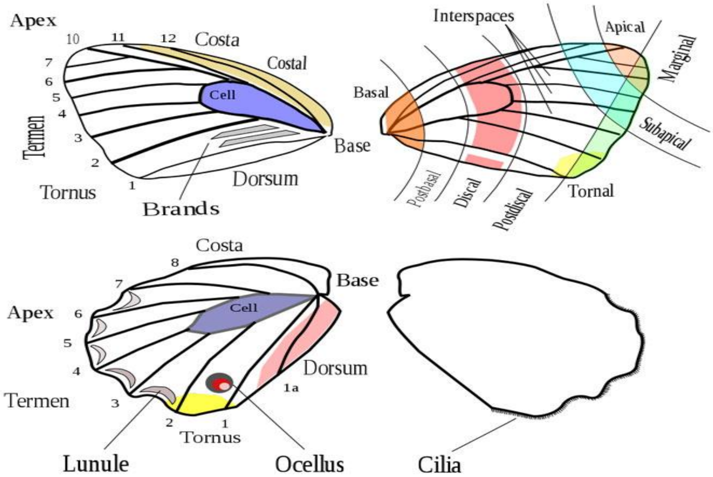


Figure 4 : Parts of Wings and Venation Pattern of Butterfly

Exploring the Rule Induction with CN2 Algorithm

Rule induction is a popular method that automates the knowledge acquisition process when knowledge is expressed in terms of rules in classification-type problems. Rule induction examines historical cases and generates the rules that were used to arrive at certain recommendations. Rule induction can be used by a system engineer, an expert, or any other system builder. Another advantage is that the builder does not have to be a knowledge engineer. He or she can be the expert or a system analyst. This is not only saves time and money, but it also solves the difficulties of dealing with the knowledge engineer who is an outsider unfamiliar with the domain.

CN2 is designed for the efficient induction of simple, comprehensible production rules in domains where problems of poor description language and/or noise may be present. CN2 based on the ID3 (Quinlan, 1983) and AQ (Michalski, 1969) algorithms. The ID3 algorithm provides itself to such modification by the nature of its general-to-specific search. The AQ algorithm's dependence on specific training examples during search makes it less easy to modify. CN2 was designed to modify the AQ algorithm itself in ways that removed this dependence on specific examples and increased the space of rules searched. CN2, a new induction algorithm combines the efficiency and ability to cope with noisy data of ID3 with the if-then rule form and flexible search strategy of the AQ family. Figure 5 shows the CN2 induction algorithm.

```
Let E be a set of classified examples.
Let SELECTORS be the set of all possible selectors.
        Procedure CN2(E)
        Let RULE-LIST be the empty list.
        Repeat until BEST.CPX is nil or E is empty:
        Let BEST.CPX be Find-Best.Complex(E).
                If BEST.CPX is not nil,
                Then let E' be the examples covered by
        BEST.CPX.
        Remove from E the examples E' covered by
BEST.CPX.
        Let C be the most common class of examples in E'.
        Add the rule 'If BEST.CPX then the class is C'
        to the end of RULE-LIST.
        Return RULE-LIST.
```

Figure 5: CN2 Induction Algorithm

Proposed System for Classification of Classification of Some Butterflies by Using Rules Induction: CN2 Algorithm

In this research, the proposed system contributed in system flow diagram, selected item for classification and implementation main form of proposed system.

System Flow Diagram

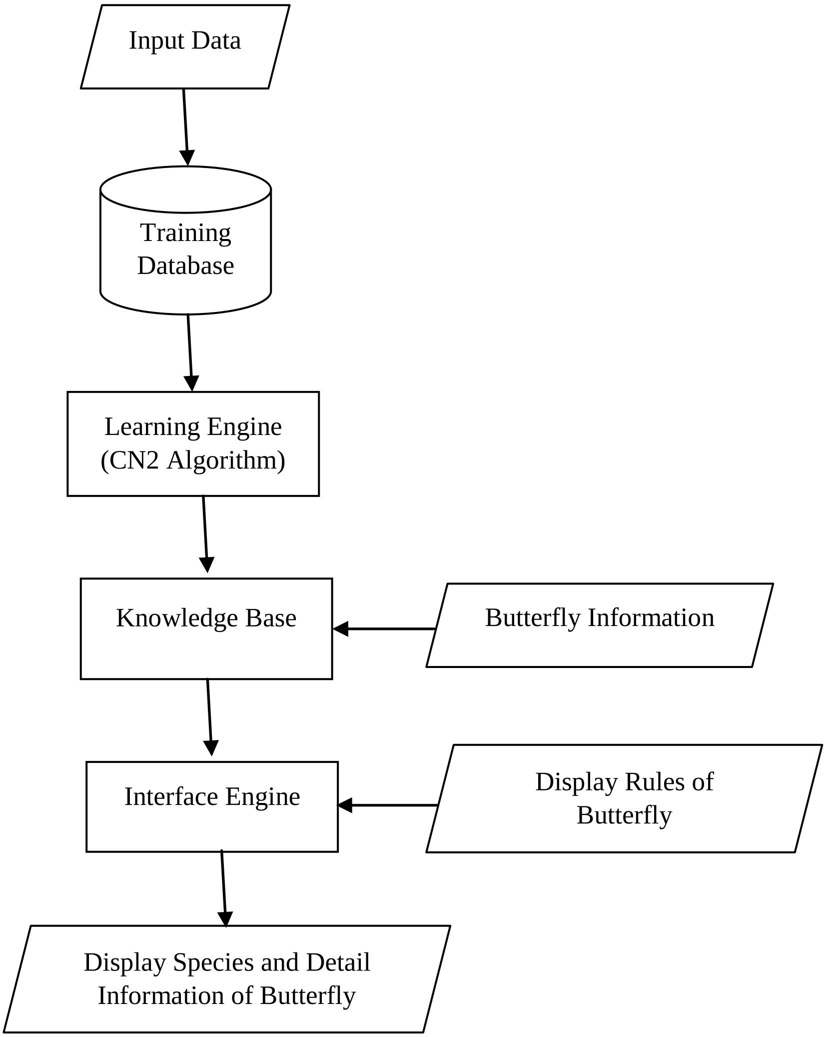


Figure 6: System Flow Diagram

Selected Item for Classification

A dichotomous key is a tool that can be used to classify objects. Dichotomous means "divided in two parts" or "binary classification". Therefore, this key uses a series of yes or no questions to place objects into groups. Butterflies are classified in the Kingdom: Animalia, Phylum: Arthropoda, and Order: Lepidoptera.

Kingdom	**Animalia** (animals)
Phylum	**Arthropoda** (arthropods, invertebrate animals with an exoskeleton, a segmented body, and jointed legs)
Class	**Insecta** (insects, arthropods with 6 legs, 2 antennae, and a 3-part body)
Order	**Lepidoptera** (butterflies and moths)

The butterflies are entirely depending upon their habitat. So, there are 29 butterflies were collected in Yangon Division for classify. The classification was made for the following procedure. First select an item as sample for classify and then classify the following keys. Figure 7 shows the selected item for classification.

Figure 7: Selected item for classification from Collected Butterfly

Key to the Families of Butterflies

1	A.	Antennae approximate at base, hind tibiae with only a terminal pair of spurs, one or more of the veins in the fore wing forked or coincident beyond the cell…………………………………......**2**
	B.	Antennae wide apart at base, hind tibiae generally with a medial as well as a terminal pair of spurs, all the veins in the fore wing from base or from cell, none forked or coincident beyond……………….…**Hesperiidae**
2	a.	Precostal nervure in hind wing present …………......….…..……**3**
	b.	Precostal nervure in hind wing absent……….........**Lycaenidae**
3	a1.	Front pair of legs imperfect in one or both sexes……………**..4**
	b1.	Front pair of legs perfect in both sexes…………………….......…**5**
4	a2.	Front pair of legs perfect in both sexes………………. …..**Nymphalidae**
	b2.	Front pair of legs imperfect in male, perfect in female................ .... **Nemeobidae**
5	a2.	Vein 1a in hind wing wanting, claws simple……… …..….**Papilionidae**
	b2.	Vein 1a in hind wing present, claws bifid……………………. …**Pieridae**

By using the rule induction with CN2 algorithm and identification key of family of butterfly, the following rule can be developed for rule 1. There will be four steps to produce rule 1.

Rule 1 (For Family)	**Family Name**
A (a) (a1) (a2)	Nymphalidae

Key to the Subfamilies of the Nymphalidae

1	A.	Discoidal cell in both fore and hind wing closed…….………..**2**
	B.	Discoidal cell open, or if closed, lower disco-cellular very slender, inconspicuous.
2	a.	Vein 1 in fore wing forked at base……………..……...***Danainae***
	b.	Vein 1 in fore wing not forked at base.

By using the rule induction with CN2 algorithm and identification key of subfamily of butterfly, the following rule can be developed for rule 2. There will be two steps to produce rule 2.

Rule 2 (For SbFamily)	**SubFamily Name**
A (a)	Danainae

Key to the Genera of the Danainae

1	A.	Claws furnished with paronychia and pulvilli.
	B.	Claws without paronychia or pulvilli………………………………..……***Danais***

By using the rule induction with CN2 algorithm and identification key of genus of butterfly, the following rule can be developed for rule 3. There will be only one step to produce rule 3.

Rule 3 (For Genus)	**Genus Name**
B	Danais

Key to the Species of Dannis

1	A.	Fore wing tawny, with black margins and white spots; larva with three pairs of fleshy tentacula…………………… ……………**2**
	B.	Fore wing fuliginous black, with subhyaline streaks and spots of bluish white; larva so far as known, with two pairs of fleshy tentacula.
2	a.	Middle discocellular in hind wing slightly curved inwards; veins in both wings conspicuously bordered with black.
	b.	Middle discocellular in hind wing bent inwards at almost a right angle in the middle; veins in wings not conspicuously bordered with black……………………………………...……..……………**3**
3	a1.	Apical third of fore wing black above, with a preapical obliquely-placed row of elongate white spots………………… …………….…**4**
	b1.	Apex of fore wing with narrow even margin only, of black spotted with white; preapical row of white spots quite obsolete.
4	a2.	Discoidal cell and disc of hind wing entirely tawny.................. ***chrysippus***
	b2.	Discoidal cell and disc of hind wing more or less marked with white……………………………………………………***alcipppus***

By using the rule induction with CN2 algorithm and identification key of species of butterfly, the following rule can be developed for rule 4. There will be four steps to produce rule 4.

Rule 4 (For Species)	**Species Name**
A (b) (a1) (a2)	chrysippus
A (b) (a1) (b2)	plexippus

Classification of the butterfly applying rule induction with CN2 also gives the result of scientific name. In the scientific name, the genus name and species name are called together. Therefore, the selected item is ***Danais chrysippus***.

Implementation Main Form of Proposed System

In the proposed system, there are seven parts. Among them, View, Searching and Detail Information are portion of the knowledge base and Classification is the portion of learning engine for CN2 algorithm and Rules is the output of the research. Figure 8 shows the main form of proposed system.

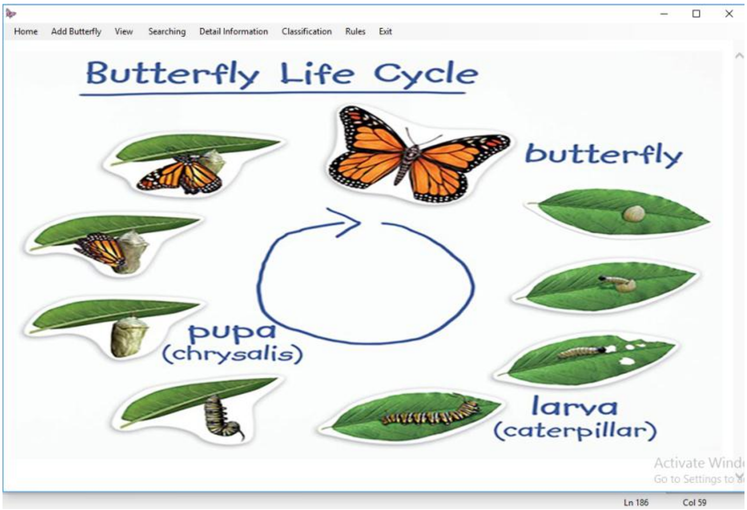


Figure 8 : Main Form of Proposed System

Result and Discussion

After classification by using rule induction with CN2 algorithm, the **family rule** for butterfly (**Rule 1**), the **subfamily rule** for butterfly (**Rule 2**), the **genus rule** for butterfly (**Rule 3**) and the **species rule** for butterfly (**Rule 4**) respectively come out as the result.

Rule for Family of Butterfly: Rule 1

Table 1 shows the results of the rules for butterfly family. There are sex families all together but five family are comes out for 29 butterflies.

Table 1: Rule for Family of Butterfly

Sr.No	Family Name	Rule for Family (Family Key)
1	Nymphalidae	A (a) (a1) (a2)
2	Nemeobidae	A (a) (a1) (b2)
3	Papilionidae	A (b) (a1) (a2)
4	Pieridae	A (b) (a1) (b2)
5	Lycaenidae	A (b)
6	Hesperidae	B

Rule for SubFamily of Butterfly: Rule 2

In the collected five families, the family Nymphalidae has three subfamilies: Danainae, Satyrinae and Nymphalinae; the family Papilionidae has one subfamily: Papilio and the family Lycaenidae has one subfamily: Lycaeninae. The family Nemobidae and Pieridae have not subfamily.

Rule for SubFamily of Nymphalidae

Table 2(a) shows the results of the rules for subfamily of family Nymphalidae. There are three subfamilies in Nymphalidae contain in the 29 butterflies.

Table 2(a): Rule for SubFamily of Nymphalidae

Sr.No	SubFamily Name	Rule for SubFamily (SubFamily Key)
1	Danainae	A (a)
2	Satyrinae	A (b) (a1)
3	Nymphalinae	B (b)

Rule for SubFamily of Papilionidae

Table (4.2(b)) shows the results of the rules for subfamily of family Papilionidae. There has one subfamily in Papilionidae contain in the 29 butterflies.

Table 2(b): Rule for SubFamily of Papilionidae

Sr.No	SubFamily Name	Rule for SubFamily (SubFamily Key)
1	Papilio	A

Rule for SubFamily of Lycaenidae

Table 2(c) shows the results of the rules for subfamily of family Lycaenidae. There has one subfamily in Lycaenidae contain in the 29 butterflies.

Table 2(c): Rule for SubFamily of Papilionidae

Sr.No	SubFamily Name	Rule for SubFamily (SubFamily Key)
1	Lycaeninae	B (b) (a1) (a2)

Rule for Genus of Butterfly: Rule 3

There are 13 genus contain in the five family of 29 butterflies which are collected in Yangon Division.

Rule for Genus of Danainae

Table 3(a) shows the results of the rules for genus of subfamily Danainae. There are only one genus in Danainae contain in the 29 butterflies.

Table 3(a) Rule for Genus of Danainae

Sr.No	Genus Name	Rule for Genus (Genus Key)
1	Dannis	B

Rule for Genus of Satyrinae

Table 3(b) shows the results of the rules for genus of subfamily Satyrinae. There are three genus in Satyrinae contain in the 29 butterflies.

Table 3(b): Rule for Genus of Satyrinae

Sr.No	Genus Name	Rule for Genus (Genus Key)
1	Mycalesis	A (a) (a1)
2	Orsotriaena	B (a)
3	Cyllogenes	B (b)

Rule for Genus of Nymphalinae

Table 3(c) shows the results of the rules for genus of subfamily Nymphalinae. There are only one genus in Nymphalinae contain in the 29 butterflies.

Table 3(c): Rule for Genus of Nymphalinae

Sr.No	Genus Name	Rule for Genus (Genus Key)
1	Euthalia	A (a)

Rule for Genus of Nemeobidae

Table 3(d) shows the results of the rules for genus of family Nemeobidae. There are only one genus in Nemeobidae contain in the 29 butterflies.

Table 3(d): Rule for Genus of Nemeobidae

Sr.No	Genus Name	Rule for Genus (Genus Key)
1	Abisara	A (b)

Rule for Genus of Papilio

Table 3(e) shows the results of the rules for genus of subfamily Papilio. There are two genus in Papilio contain in the 29 butterflies.

Table 3(e): Rule for Genus of Papilio

Sr.No	Genus Name	Rule for Genus (Genus Key)
1	memnon	A
2	polytes	B

Rule for Genus of Pieridae

Table 3(f) shows the results of the rules for genus of family Pieridae. There are four genus in Pieridae contain in the 29 butterflies.

Table 3(f) : Rule for Genus of Pieridae

Sr.No	Genus Name	Rule for Genus (Genus Key)
1	Delias	A (a) (b1)
2	Appias	B (a) (a1)
3	Hebomoia	B (b)
4	Catopsilia	A (a) (b1)

Rule for Genus of Lycaeninae

Table 3(g) shows the results of the rules for genus of subfamily Lycaeninae. There are only one genus in Lycaeninae contain in the 29 butterflies.

Table 3(g): Rule for Genus of Lycaeninae

Sr.No	Genus Name	Rule for Genus (Genus Key)
1	Abisara	B (b)

Rule for Species of Butterfly: Rule 4

There were 29 species for 29 butterflies were classified by using the rule induction with CN2 algorithm.

Rule for Species of Dannis

Table 4(a) shows the results of the rules for species of genus Dannis. There are two species in Dannis contain in the 29 butterflies.

Table 4(a): Rule for Species of Dannis

Sr.No	**Species Name**	**Rule for Species (Species Key)**
1	chrysippus	A (b) (a1) (a2)
2	alcippus	A (b) (a1) (b2)

Rule for Species of Mycalesis

Table 4(b) shows the results of the rules for species of genus Mycalesis. There are only one species in Mycalesis contain in the 29 butterflies.

Table 4(b): Rule for Species of Mycalesis

Sr.No	**Species Name**	**Rule for Species (Species Key)**
1	gotama	B (b)

Rule for Species of Orsotriaena

Table 4(c) shows the results of the rules for species of genus Orsotriaena. There are only one species in Orsotriaena contain in the 29 butterflies.

Table 4(c): Rule for Species of Orsotriaena

Sr.No	**Species Name**	**Rule for Species (Species Key)**
1	meda	Keys

Rule for Species of Cyllogenes

Table 4(d) shows the results of the rules for species of genus Cyllogenes. There are only one species in Cyllogenes contain in the 29 butterflies.

Table 4(d): Rule for Species of Melanitis

Sr.No	Species Name	Rule for Species (Species Key)
1	suradeva	A

Rule for Species of Euthalia

Table 4(e) shows the results of the rules for species of genus Euthalia. There are three species in Euthalia contain in the 29 butterflies.

Table 4(e): Rule for Species of Euthalia

Sr.No	Species Name	Rule for Species (Species Key)
1	lubentina	B (a)
2	phemius	B (b) (a1)
3	acontius	B (b) (b1) (c2)

Rule for Species of Abisara

Table 4(f) shows the results of the rules for species of genus Abisara. There are two species in Abisara contain in the 29 butterflies.

Table 4(f): Rule for Species of Abisara

Sr.No	Species Name	Rule for Species (Species Key)
1	fylla	A
2	echerius	B

Rule for Species of Memnon

Table 4(g) shows the results of the rules for species of genus Memnon. There are three species in Memnon contain in the 29 butterflies.

Table 4(g): Rule for Species of Memnon

Sr.No	Species Name	Rule for Species (Species Key)
1	agenor	A (a)
2	polymnestoroides	A (b)
3	mayo	B

Rule for Species of Polytes

Table 4(h) shows the results of the rules for species of genus Polytes. There are two species in Polytes contain in the 29 butterflies.

Table 4(h): Rule for Species of Polytes

Sr.No	Species Name	Rule for Species (Species Key)
1	polyts	A
2	pitmani	B

Rule for Species of Delias

Table 4(i) shows the results of the rules for species of genus Delias. There are two species in Delias contain in the 29 butterflies.

Table 4(i): Rule for Species of Delias

Sr.No	Species Name	Rule for Species (Species Key)
1	eucharis	A
2	hierta	B

Rule for Species of Appias

Table 4(j) shows the results of the rules for species of genus Appias. There are four species in Appias contain in the 29 butterflies.

Table 4(j): Rule for Species of Appias

Sr.No	Species Name	Rule for Species (Species Key)
1	nero	A
2	libythea	B (a)
3	leis	B (b) (a1)
4	wardi	B (b) (b1)

Rule for Species of Hebomoia

Table 4(k) shows the results of the rules for species of genus Hebomoia. There are two species in Hebomoia contain in the 29 butterflies.

Table 4(k): Rule for Species of Hebomoia

Sr.No	Species Name	Rule for Species (Species Key)
1	glaucippe	A
2	roepstorff	B

Rule for Species of Catopsilia

Table 4(l) shows the results of the rules for species of genus Catopsilia. There are four species in Catopsilia contain in the 29 butterflies.

Table 4(l): Rule for Species of Catopsilia

Sr.No	Species Name	Rule for Species (Species Key)
1	crocale	A (a)
2	scylla	A (b)
3	pyranthe	B (a)
4	florella	B (b)

Rule for Species of Castalius

Table 4(m) shows the results of the rules for species of genus Castalius. There are two species in Castalius contain in the 29 butterflies.

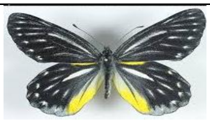
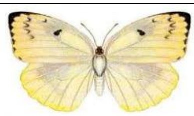
Table 4(m): Rule for Species of Castalius

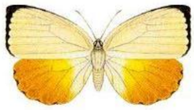
Sr.No	Species Name	Rule for Species (Species Key)
1	ananda	A
2	rosimon	B

Scientific Name of Butterflies

Table (4.5) shows the results of the scientific names of the collected butterflies by using rule induction with CN2 algorithm. For the zoologist and the users who have knowledge in classification in zoology are able to acquire more exposure knowledge. The results can also give the scientific name, that is the genus name and species name are called together.

Table 5 : Classified Butterfly with their Scientific Name

			
1. *Danaus chrysippus*	2. *Danaus alcippus*	3. *Mycalesis gotama*	4. *Orsotriaena meda*
			
5. *Cyllogenes suradeva*	6. *Euthalia lubentina*	7. *Euthalia phemius*	8. *Euthalia acontius*
			
9. *Abisara fylla*	10. *Abisara echerius*	11. *Papilio memnon agenor*	12. *Papilio memnon polymnestoroides*
			
13. *Papilio mayo*	14. *Papilio polytes polyts*	15. *Papilio polytes pitmani*	16. *Delias eucharis*
			
17. *Delias hierta*	18. *Appias nero*	19. *Appias libythea*	20. *Appias leis*
			
21. *Appias wardi*	22. *Hebomoia glaucippe*	23. *Hebomoia roepstorff*	24. *Catopsilia crocale*

			
25. *Catopsilia Scylla*	26. *Catopsilia pyranthe*	27. *Catopsilia florella*	28. *Castalius ananda*
			
29. *Castalius rosimon*			

Rule induction can be used by a system engineer, an expert, or any other system builder. The classification of butterfly by using rule induction with CN2 was simple, clear and useful for the researcher. But the steps of the procedure for classification were not the same. Some species of the butterfly were classified four steps and the others up to 10. The largest group is the family Papilionidae and the classification steps are more other family.

Conclusion

This research paper has introduced the idea of inducing rules from sets of examples in order to speed up the development of knowledge bases in expert systems. The rule induction with CN2 algorithm is the best technique for classification of biological dataset. The results give rules for classification and the implemented system is easy to use and understand for biologist; who is willing to classify the butterflies by computerization. A big advantage of the rule induction is that it enhances the thinking process of the expert. Butterflies are the best-known and best group of insects for examining pattern of terrestrial biotic diversity and distribution. The whole world takes an interest in the beautiful, exotic and colorful butterfly and many countries have established butterfly parks and organized special butterfly watching safaris for enthusiasts and eco-tourists in order to earn foreign exchange. In addition, children in schools are being encouraged to study the life and habit of butterflies and in this way come to appreciate the beauty of the natural environment and become involved in efforts for its conservation. So, this research will meet the aim of correct, easy, fast, simple and useful than traditional manual methods. As a future work, the evaluation of the classification method with rule induction will be carried out.

Acknowledgements

First, I would like to thank Dr. Yi Mon Win, Associate Professor (Head), Computer Studies Department, Dagon University, for her encouragement to prepare this research. Then I am grateful to Dr. Nwe Nwe Win, Professor Head, Department of Computer Studies (Retired), University of Yangon, for her valuable suggestions and review in details. Finally, I also want to express very special thanks to Dr. Soe Mya Mya Aye, Professor Head, Department of Computer Studies, University of Yangon, for her advices and comments.

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FINDING OPTIMAL TRAVELLING SALESMAN TOURS THROUGH MYANMAR CAPITAL CITIES

Kyaw Moe Min*

Abstract

The travelling salesman problem (TSP) is a combinational optimization problem in which the goal is to find the shortest path between different cities that the salesman takes. The travelling salesman problem involves finding the trip of minimum cost and the processing time that a salesman can make to visit the cities in a sales territory once and only once (represented by a complete graph with weights on the edges), starting and ending the trip in the same city. This paper adopts the nearest neighbour and two sided nearest neighbour algorithm to solve the well-known travelling salesman problem. The algorithms were implemented using (C++) and MS VC++programming language. The approach can be tested on two graphs that making a TSP tour instance of 5-city, 14-city and more. The computation results validate the performance of the proposed algorithm.

Keywords- travelling salesman problem (TSP), nearest neighbour, two sided nearest neighbour

Introduction

The travelling salesman problem (TSP) consists of a salesman and a set of Myanmar capital cities. The salesman has to visit each one of the capital cities starting from a certain one and returning to the same city. The challenge of the problem is that the travelling salesman wants to minimize the total length of the trip. A salesman must make a tour of a number of Myanmar cities using the shortest path available and visit each city exactly once and only once and return to the original starting point.

The travelling salesman problem can be described as follows:

TSP = {(G, f, t): G = (V, E) a complete graph, f is a function $V \times V \dashrightarrow Z$, $t \in Z$,

G is a graph that contains a travelling salesman tour with cost that does not exceed t}.

*. Dr, Associate Professor and Head, Department of Computer Studies, National Management Degree College

Example:

Consider the following set of cities:

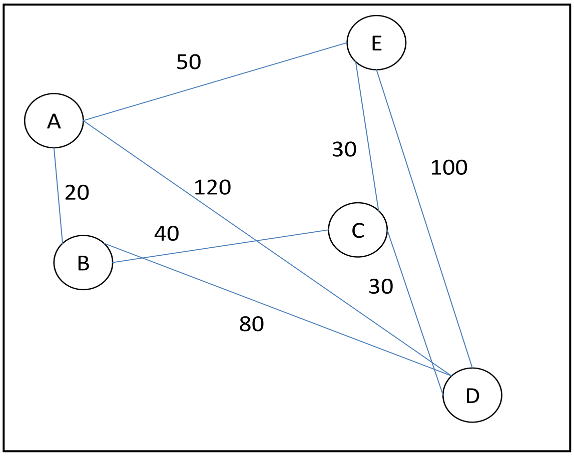

Figure 1: A graph with weights on its edges.

The problem lies in finding a minimal path passing from all vertices once. In the figure 1, the path ***Path1*** {A, B, C, D, E, A} and the path ***Path2*** {A, B, C, E, D, A} pass all the vertices but Path1 has a total length of 240 and Path2 has a total length of 310.

Statement of the Problem

The travelling salesman problem involves a salesman who must make a tour of a number of cities using the shortest path available and visit each city exactly once and only once and return to the original starting point. For each number of cities n, the number of paths which must be explored is n!, causing this problem to grow exponentially rather than as a polynomial. There are bunch of algorithms offering comparably fast running time and still yielding near optimal solutions.

Solution Methods

The following algorithms can be used to find the shortest path.

(i) Nearest Neighbour Algorithm

(ii) Two-sided Nearest Neighbour Algorithm

(iii)Nearest Insert Algorithm

(iv)Farthest Insert Algorithm

(v) Cheapest Insert Algorithm

(vi)Spanning Tree Algorithm

(vii)Chris to fides Algorithm

There will be coded Algorithms (i)Nearest Neighbour and (ii) Two-sided Nearest Neighbourin C^{++} language and apply them to find optimal or near optimal tours passing through sure specified cities of Myanmar.

Nearest Neighbour Algorithm

Input: V= {1, 2, 3,.... , n }, the set of labels of n cities

distancec_{ij}, between city i and city j.

Output: a TS tour T of optimal or near optimal length

Step 1. (Initialization)

Set visit [j]: = false for all j $\in$ V.

Choose any vertex i$\in$ V.

Set s: = i (s is the starting city)

T [1]: = s (T is the tour or sequence of cities)

visit [s] = true

tl:= 0 (tl is the length of the tour)

p: = s (p is the present city)

count: = 1.

Step 2. Choose a vertex k such that

$$c_{pk} = \min \{c_{pj} \mid \text{visit}[\ j] = \text{false}\ \}$$

Set count: = count + 1

T[count]: = k

visit [k]: = true

tl : = tl +c_{pk}

p: =k

Go to Step 2.

Step 3. Set T[n+1] = s.

Output T and tl. Stop.

The code will be described the above algorithm in C^{++} as follows. Firstly, the **distance matrix type** will be coded as follow.

```
int d[50][50]={ {0,0,0,0,0,0,0,0,0,0,0,0,0,0,0},
    {0,0,153,510,706,966,581,455,70,160,372,157,567,342,398},
    {0,153,0,422,664,997,589,507,197,311,479,310,581,417,376},
    {0,510,422,0,289,744,390,437,491,645,880,618,374,455,211},
    {0,706,664,289,0,491,265,4101,664,804,1070,768,259,499,310},
    {0,966,997,744,491,0,400,514,902,990,1261,947,418,635,636},
    {0,581,598,390,265,400,0,168,522,634,910,593,18,284,239},
    {0,455,507,437,410,514,168,0,389,482,757,439,163,124,232},
    {0,70,197,491,664,902,522,389,0,154,406,133,508,273,355},
    {0,160,311,645,804,990,634,482,154,0,276,43,623,358,498},
    {0,372,479,880,1070,1261,910,757,406,276,0,318,898,633,761},
    {0,157,310,618,768,947,593,439,133,43,318,0,582,315,464},
    {0,567,581,374,259,418,18,163,508,623,898,582,0,275,221},
    {0,342,417,455,499,635,284,124,273,358,633,315,275,0,247},
    {0,398,376,211,310,636,239,232,355,498,761,464,221,247,0} };
```

Secondly, the part of nearest neighbour algorithm used will be coded as follow.

```
while(count !=n)
{min =999999;
    for(i=1;i<=n;i++)
    {if(visit[i]==0){   if(d[pc][i]<min)
        {       min=d[pc][i];
```

```
                nc=i;
            }}}
        count=count+1;
        T[count]=nc;
        visit[nc]=1;
        tl=tl+min;
        pc=nc;
        cout<<min<<" + ";     }
        T[n+1]=fc;
        tl=tl+d[pc][fc];
        cout<<d[pc][fc]<<" = " <<tl;
        cout<<"\n\n A near optimal tour is "<<" ";
        cout<<"{ ";
        for(i=1;i<=n;i++)
        cout<<(T[i])<<", ";
        cout<<fc<<" }";
        cout<<"\n\nThe total length of the tour is"<<"
"<<tl<<".";}
```

Finding a Near Optimal Tour through 14 Cities in Myanmar by Using the Nearest Neighbour Algorithm

By using the above nearest neighbour algorithm we find out a near optimal tour passing through 14 cities in Myanmar: Yangon, Pathein, Sittwe, Hakha, Myitkyina, Mandalay, Taunggyi, Bago, MawlaMyine, Dawei, Hpa-an, Sagaing, Loikaw, Magwe. The distances, in kilometer, between these cities can be given by the following distance matrix in figure 2.

	Yangon	*Pathein*	*Sittwe*	*Hakha*	*MyintKyina*	*Mandalay*	*Taunggyi*	*Bago*	*MawlaMyine*	*Dawei*	*Hpa-an*	*Sagaing*	*Loikaw*	*Magwe*
Yangon	0	153	510	706	966	581	455	70	160	372	157	567	342	398
Pathein	153	0	422	664	997	589	507	197	311	479	310	581	417	376
Sittwe	510	422	0	289	744	390	437	491	645	880	618	374	455	211
Hakha	706	664	289	0	491	265	4101	664	804	1070	768	259	499	310
MyintKyina	966	997	744	491	0	400	514	902	990	1261	947	418	635	636
Mandalay	581	598	390	265	400	0	168	522	634	910	593	18	284	239
Taunggyi	455	507	437	410	514	168	0	389	482	757	439	163	124	232
Bago	70	197	491	664	902	522	389	0	154	406	133	508	273	355
MawlaMyine	160	311	645	804	990	634	482	154	0	276	43	623	358	498
Dawei	372	479	880	1070	1261	910	757	406	276	0	318	898	633	761
Hpa-an	157	310	618	768	947	593	439	133	43	318	0	582	315	464
Sagaing	567	581	374	259	418	18	163	508	623	898	582	0	275	221
Loikaw	342	417	455	499	635	284	124	273	358	633	315	275	0	247
Magwe	398	376	211	310	636	239	232	355	498	761	464	221	247	0

Figure2: The distance relationship among the capital cities

If the city 1is chosen as the first city, then the algorithm produces the following output:

The first city ---> 1

A near optimal tour is { 1, 8, 11, 9, 10, 2, 14, 3, 4, 12, 6, 7, 13, 5, 1 }

The sum of distances = 70 + 133 + 43 + 276 + 479 + 376 + 211+ 289 +259 +18 + 168 + 124 + 635 + 966 = 4047

The total length of the tour is 4047.

If the city 2 is chosen as the first city, then the algorithm produces the following output:

The first city ---> 2

A near optimal tour is { 2, 1, 8, 11, 9, 10, 13, 7, 12, 6, 14, 3, 4, 5, 2 }

The sum of distances = 153 + 70 +133 +43 + 276+ 633 + 124 +163 +18+ 239

+ 211 + 289 + 491 + 997 = 3840

The total length of the tour is 3840.

Table 1: Total distance and Time depending on the Starting City

City-Name	Starting City	Total Distance(km)	Time (ms)
Yangon	1	4047	3
Pathein	2	**3840**	**2**
Sittwe	3	4172	2
Hakha	4	3876	4
MyintKyina	5	4052	2
Mandalay	6	4207	1
Taunggyi	7	4059	2
Bago	8	3922	2
MawlaMyine	9	4728	2
Dawei	10	4016	2
Hpa-an	11	4706	3
Sagaing	12	4200	2
Loikaw	13	3922	5
Magwe	14	4092	2

By choosing the best of the above 14 tours in table 1, we obtain the following near optimal tour.

The tour = { 2, 1, 8, 11, 9, 10, 13, 7, 12, 6, 14, 3, 4, 5, 2 }

The total length of the tour = 3840 km

The total lengths on the starting city can be represented as a graph in the figure 3.

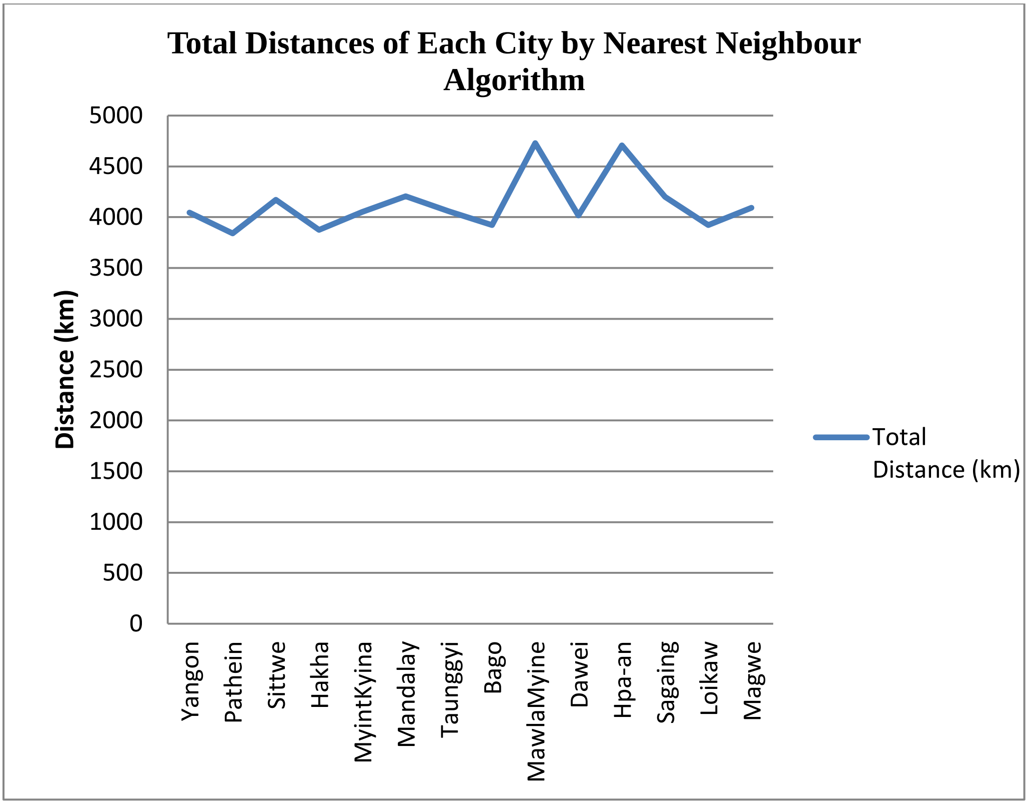

Figure 3: Total lengths of the tour on the starting cityby nearest neighbor algorithm

Two Sided Nearest Neighbour Algorithm

Input: V= {1, 2, ... , n }, the set of lables of n cities

distance sc_{ij}, between city i and city j

Output: a TS tour T

Step 1: (Initialization)

Set visit[j]:= false for all $j \in V$.

Choose any vertex $i \in V$.

Set s: = i (s is the starting city)

T[1] = s (T is the tour or the sequence of cities)

visit[s]: = true

tl: = 0 (tl is the length of the tour)

p_l: = s (p_l is the present left city)

p_r: = s (p_r is the present right city)

count:= 1

Step 2. If count = n, set T[n+1]: = T[1] (count is the number of already visited cities)

Output T and tl.

Step 3. Choose a vertex k such that

$c_{p_r k} = \min\{ c_{p_r j} \mid \text{visit}[j] = \text{false} \}$

Choose a vertex m such that

$c_{p_l m} = \min\{ c_{p_l j} \mid \text{visit}[j] = \text{false} \}$

If $c_{p_r k} \le c_{p_l m}$, then set

count: = count + 1

T[count] = k

visit[k] = true

tl = tl + $c_{p_r k}$

p_r : = k

Otherwise

for j = count to 1

Set T[j+1]: = T[j]

T[1]: = m

count: = count+1

visit[m]: = true

tl:= tl + $c_{p_l m}$

p_l: = m

Go to Step 2.

We can code the above algorithm in C^{++} as follows.

```
visit[sc]=1;
```

```
T[1]=sc;
inttl=0;
intplc = sc;
intprc = sc;
int count=1;
int min1,min2,nrc,nlc;
while(count !=n)
 {min1 =999999;
          for(i=1;i<=n;i++)       {
          if(visit[i]==0)  {         if(d[prc][i]<min1)
                    {         min1=d[prc][i];
                              nrc=i;}}}
          min2=999999;
          for(i=1;i<=n;i++)       {
                    if(visit[i]==0)  {         if(d[plc][i]<min2)
                              {min2=d[plc][i];
                              nlc=i;   }}}
          if(min1<=min2)          {         count=count+1;
                    T[count]=nrc;
                    visit[nrc]=1;
                    tl=tl+min1;
                    prc=nrc;}else{for(i=count;i>=1;i--)
                    T[i+1]=T[i];
                    T[1]=nlc;
                    count=count+1;
                    visit [nlc]=1;
                    tl=tl+min2;
                    plc=nlc;}
            }
```

```
T[n+1]=plc;
tl=tl+d[prc][plc];
cout<<"\n\n A near optimal tour is "<<" ";
cout<<"{ ";
for(i=1;i<=n;i++)
cout<<(T[i])<<", ";
cout<<plc<<" }";
cout<<"\n\nThe total length of the tour is"<<"
"<<tl<<".";}
```

Finding a Near Optimal Tour through 14 Cities in Myanmar by Using the Two-sided Nearest Neighbour Algorithm

By using the above nearest neighbour algorithm we find out a near optimal tour passing through 14 cities in Myanmar: Yangon, Pathein, Sittwe, Hakha, Myitkyina, Mandalay, Taunggyi, Bago, MawlaMyine, Dawei, Hpa-an, Sagaing, Loikaw, Magwe. The distances, in kilometer, between these cities can be given by the **Figure (2)** distance matrix.

If we choose the city 5 as the first city, the city 10 as the second city, and so on, then the algorithm produces the following outputs:

The starting city ---> 5

A near optimal tour is { 5, 6, 12, 7, 13, 14, 3, 4, 2, 1, 8, 11, 9, 10, 5 }

The total length of the tour is 4052.

The starting city ---> 10

A near optimal tour is { 10, 9, 11, 8, 1, 2, 14, 3, 4, 12, 6, 7, 13, 5, 10 }

The total length of the tour is 4016.

Table 2: Total distance and Time depending on the Starting City

City-Name	Starting City	Total Distance (km)	Time (ms)
Yangon	1	4016	2
Pathein	2	4016	3
Sittwe	3	3906	3
Hakha	4	**3876**	**3**
MyintKyina	5	4052	2
Mandalay	6	3921	2
Taunggyi	7	3921	2
Bago	8	4016	1
MawlaMyine	9	4016	2
Dawei	10	4016	4
Hpa-an	11	4016	2
Sagaing	12	3921	2
Loikaw	13	3921	3
Magwe	14	3908	2

By choosing the best of the above 14 tours in table 2, we obtain the following **near optimal tour.** The tour = { 4, 5, 12, 6, 7, 13, 14, 3, 2, 1, 8, 11, 9, 10, 4 }

The total length of the tour = 3876 km

The total lengths on the starting city can be represented as a graph in the figure 4.

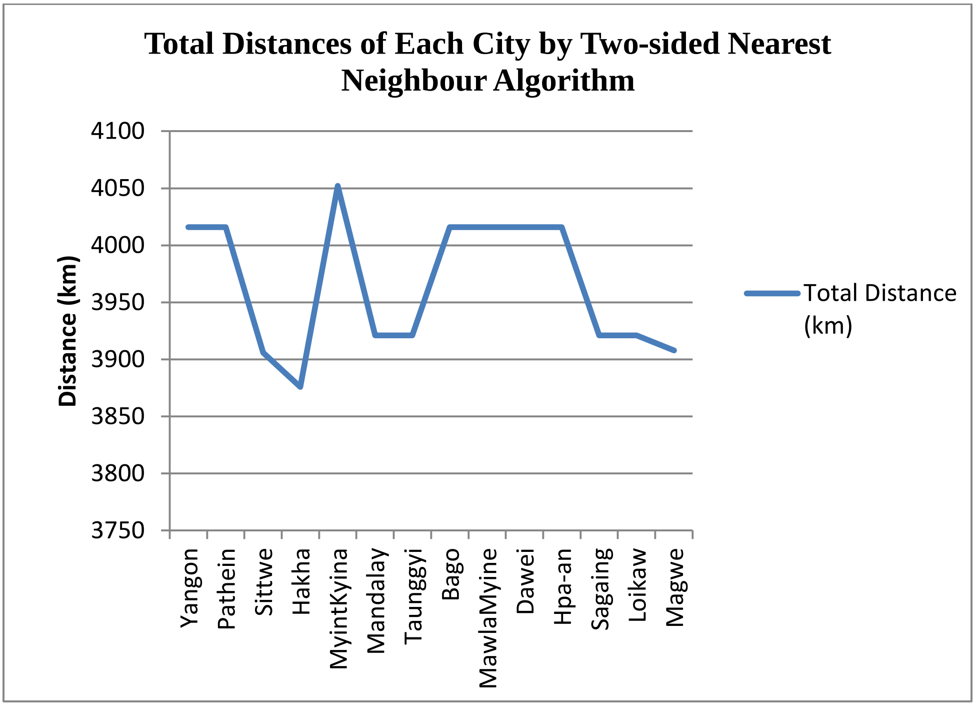

Figure 4: Total length of the tour on the starting city by two-sided nearest neighbor algorithm

Conclusion

We have coded the nearest neighbour algorithm and the two-sided nearest neighbour algorithm in C++ language used them to find near optimal tours through the 14 cities in Myanmar.

Table 3: Total distance and Processing Time depending on Starting City by the two Algorithms

City-Name	Starting City	nearest neighbour algorithm		two-sided nearest neighbour	
		Total Distance(km)	Time (ms)	Total Distance(km)	Time (ms)
Yangon	1	4047	3	4016	2
Pathein	2	**3840**	**2**	4016	3
Sittwe	3	4172	2	3906	3
Hakha	4	3876	4	3876	3
MyintKyina	5	4052	2	4052	2
Mandalay	6	4207	1	3921	2
Taunggyi	7	4059	2	3921	2
Bago	8	3922	2	4016	1
MawlaMyine	9	4728	2	4016	2
Dawei	10	4016	2	4016	4
Hpa-an	11	4706	3	4016	2
Sagaing	12	4200	2	3921	2
Loikaw	13	3922	5	3921	3
Magwe	14	4092	2	3908	2

We can choose the optimal tour path in order to table 3. By using the nearest neighbour algorithm, the total tour distance is less than that distance of the two-sided nearest neighbor used. The total lengths of the tour on the starting city by two algorithms can be represented as a graph in the figure 5.

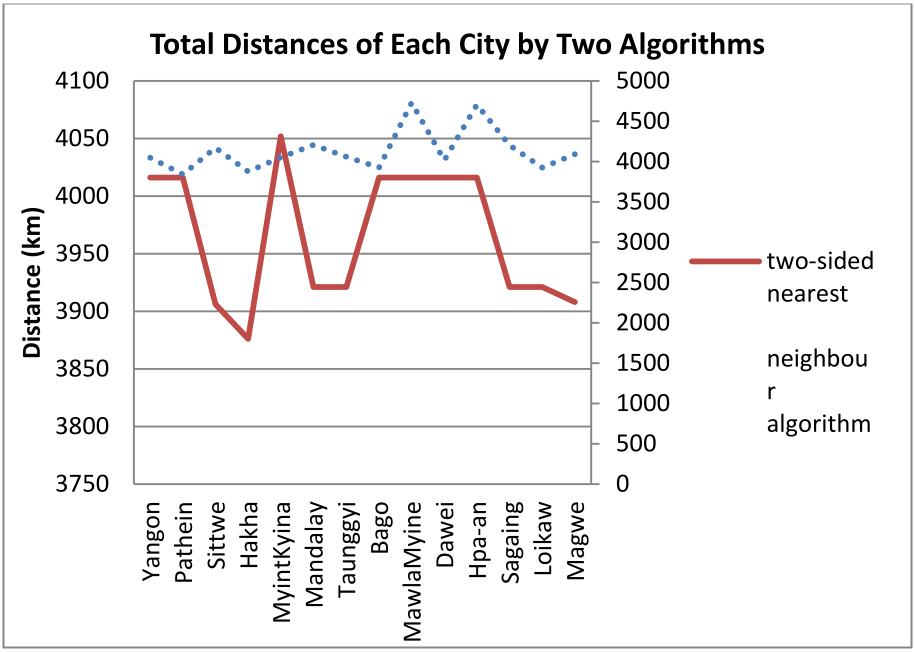

Figure 5: Total lengths of the tour on the starting city by two algorithms

We observe the processing time in both the nearest neighbour and the two sided nearest neighbour programs. The processing time is less. Moreover, these two algorithms can be applied to solve the travelling salesman problems containing more and more cities in Myanmar.

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J. Myanmar Acad. Arts Sci. 2019 **Vol. XVII. No.3**

A RECOMMENDER SYSTEM FOR INTERESTING PLACES IN MYANMAR BY USING COLLABORATIVE FILTERING METHOD

Yee Yee Aung*

Abstract

Tourists need information about places to visit, where to eat, activities, and so on. This information is typically found in guidebooks and on the Internet, requiring the tourist to actively search for relevant information. Instead, this information can be available on an electronic marketplace where it is personalized to suit each tourist. A recommender system is one of the types of personalization information filtering system that seeks to predict the "rating" or "preference" which a user would give to an item. Personalized recommender systems are becoming more interesting especially when not limited to just searching for information but they are also to recommend the items that would be more appropriate for the user's needs or preferences. The aim of this research, this recommender system is then used to predict interesting places in Myanmar (or ratings for places of items) that the users may have an interest in.

Keyword: Personalization, Recommender system, Collaborative Filtering System

Introduction

Tourism information on the World Wide Web is dynamic and constantly changing. It is not easy to get the relevant and updated information to each individual users' need. For the tourism domain, the internet has become a new media to deliver first-hand information about services to the customers from around the world. When surfing the Internet Web sites, users are demanding more powerful tools that are capable of integrating and interpreting the huge amount of mixed information available on the Web.

Personalized recommender systems are becoming more interesting, especially when not limited to just searching for information but that also to recommend the items that would be more appropriate for the user's needs or preferences. There are mainly two types of Recommender Systems(RSs): Content-Based Filtering (CBF) and Collaborative Filtering (CF). CF is one of the most commonly used methods in personalized recommendation systems

*. Dr, Associated Professor, Department of Computer Studied, Dagon University

and is used for dealing with this problem. The overwhelming amount of data necessitates mechanisms for efficient information filtering. Collaborative filtering aims at predicting the users' interest for a given item based on a collection of user profiles.

Memory-based approaches to collaborative filtering that can be divided into user-based and item-based that approaches alike. In fact, there are various reasons why service providers may want to develop this technology: they want to increase the number of items which are sold, sell more diverse items, increase the user satisfaction, and better understand what the user wants.

A travel plan may be composed of various attractions, destinations, and accommodation services that are located in delimited areas. From the point of view of the user these various alternatives can be considered and selected as a single travel destination. A travel RS is typically suggested by a travel agent or a destination management organization to increase its income, i.e., sell more hotel rooms, or to increase the number of tourists to the destination. Whereas, the user's primary motivation for accessing this system is to find a suitable hotel and interesting events/attractions when visiting a destination. Myanmar has a variety of travel attractions that are the huge cultural and geographical diversity, but has retained much of its own historic and unique character. This research is to provide information and services to people for News information about interesting places in Myanmar and to get the recommended place of items easily about user's satisfaction.

Data And Knowledge Sources From Recommender System

Recommender Systems (RSs) are information processing systems that actively gather various kinds of data in order to build their recommendations. Data is primarily about the items to suggest and the users will receive these recommendations. But, since the data and knowledge sources available for recommender systems can be very diverse, ultimately, whether they can be exploited or not depend on the recommendation technique. Recommendation techniques can either be of poor knowledge or knowledge dependent. While poor knowledge makes use of simple and basic data such as user ratings/evaluations for items, and knowledge dependent uses ontological descriptions of the users or the items, or constraints, or social relations and activities of the users. In this system, the use of knowledge is poor technique. Thus, as a general classification, three kinds of elements namely items, users and transactions construct the data used by recommender systems.

(i) Items T = {t_1, t_2,…,t_n}: Items are the objects or products in the recommender system for making suggestion to the user. Items may be characterized by their complexity and their value or utility. Travels are the most complex items that have been considered. All domain relevant items are stored in set **T**.

(ii) Users U = {u_1,u_2,…,u_n}: Elements of U comprises of all the users that have browsed items or contributed to the item ratings in the sites. In this system, site browsing patterns in a web-based recommender system or travel search patterns in a travel recommender system can be used to describe the users.

(iii) Transactions: Transaction, a recorded interaction between a user and the recommender system, are log- like data that stored important information generated during human-computer interaction used for the recommendation generation algorithm by the system. A reference to an item selected by the user and a description of the context (e.g., the user goal/query) for that particular recommendation is an instance of a transaction log and an explicit feedback, such as the rating for the selected item, and during a transaction is provided by the user. The rating is in fact the most popular form of transaction data collected by a recommender system which may be explicitly or implicitly. In the explicit rating, the user is asked to provide about an item on a rating scale.

(i) Explicit Profiling: The most apparent way to collect a customer's preferences is simply to ask the user to provide the information. This explicit profiling is often achieved by demanding the user to complete a preliminary set of questions in detail any appropriate preferences.

(ii) Implicit Profiling: Implicit profiling techniques build individual information by inferring users rating from so-called interest indicators depending on customer's interactions with the system.

User-item matrix: User-item matrix is a matrix of customers against products that have components as the explicit ratings of customers to products (user to item). Some of the user-matrix cells are not loaded, as there are products that are not rated by any user. For m items and k users, the user profiles are represented in a k x m user-item matrix X. Each element $x_{k,m}$ = r indicates that user k rated item m by r, where $r \in \{1, ..., |r|\}$. If the item has been rated $x_{k,m} = 0$; means that the rating is unknown. The user-item matrix can be decomposed into row vectors:

$$X=[u_1, \dots, u_k]^T, \quad u_k = [x_{k,1}, \dots, x_{k,m}]^T, \; k = 1, \dots, k \tag{1}$$

Where, T denotes transpose. Each row vector $\mathbf{u^T_k}$ corresponds to a user profile and represents a particular user's item ratings. As discussed below, this decomposition leads to user based CF. Alternatively, the matrix can also be represented by its column vectors:

$$X=[i_1, \dots, i_m], \quad i_m = [x_{1,m}, \dots, i_{k,m}]^T, \; m = 1, \dots, m \tag{2}$$

where, each column vector i_m corresponds to a specific item's ratings by all k users.

Recommendation Techniques

A recommender system is a subclass of personalization information filtering system. There are mainly two types of recommender systems: Content-Based Filtering (CBF) and Collaborative Filtering (CF). CF is one of the most commonly used methods in personalized recommendation systems and used for dealing with this problem. This system use a memory-based collaborative filtering approach build a model from a user's past behavior (selected and/or numerical ratings given to those visited in Myanmar) as well as similar decisions made by other users.

Collaborative Filtering Methods

Collaborative filtering (CF) can be classified into two main methods as user-based collaborative filtering (memory-based) and item-based collaborative filtering (model-based). The memory-based methods are most popular prediction techniques in collaborative filtering applications. This approach uses user rating data to compute the similarity between users or items. This is used for making recommendations. They utilize the entire user-item database to generate predictions. The user-based collaborative filtering algorithm is a memory-based algorithm. The model-based collaborative filtering methods use the user's preferences to learn a model, which is then used for predictions. Model-based methods are not suitable for environments in which user preference models must be updated rapidly or frequently.

Collaborative Filtering System Architecture

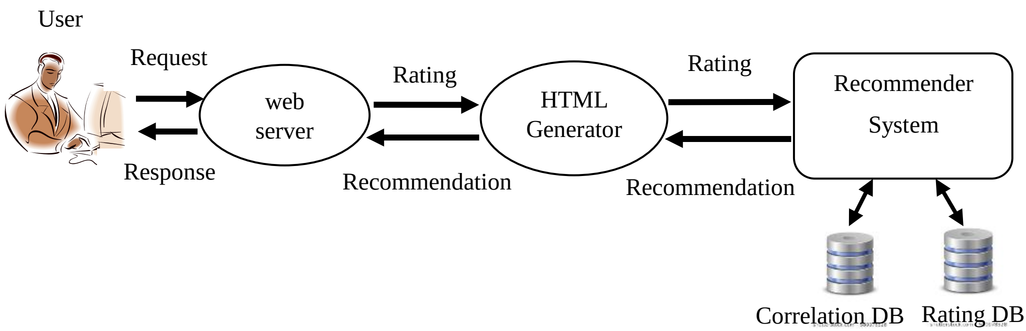

Figure 1 : Collaborative Filtering System Architecture

Collaborative filtering (CF) is a technique used by recommender systems, is shown in figure 1.

The implementation of this method recommends to the active user the items that other users with similar tastes liked in the past. The similarity in taste of two users is calculated based on the similarity in the rating history of the users. This system use a collaborative filtering method construct a model from a user's past behavior (selected and/or numerical ratings given to those visited in Myanmar) as well as similar decisions made by other users. In this system, implemented model is then used to ratings for items that the user may have an interest places in Myanmar.

Pearson Correlation Coefficient

It is used for converting similarity between two users or items by measuring obliquity of two series of preferences to act together in a comparative and linear manner. It considers preferences of conflicting users and items. It tries to find each users or items derivations from their average rates while recognizing linear adjustment between two items or users.

$$P,C_{(W,U)}=\frac{\sum_i (r_{w,i}-\overline{r}_w)(r_{u,i}-\overline{r}_u)}{\sqrt{\sum_i (r_{w,i}-\overline{r}_w)^2 \sum_i (r_{u,i}-\overline{r}_u)^2}} \tag{3}$$

w and **u** shows the two users or items for which the coefficient is calculated **i** is an item, $\boldsymbol{r_{w,i}}$ and $\boldsymbol{r_{u,i}}$ are individual rating from **w** and **u** for **i**, and average rating of $\overline{\boldsymbol{r_w}}$ and $\overline{\boldsymbol{r_u}}$ are, for user (or item) **w** and **u**.

Rule-based Personalization with Collaborative Filtering (RPCF) Algorithm

This RPCF algorithm aims to identify users that have relevant interest by calculating similarity between user profiles. In this system, Rule-based Personalization with Collaborative Filtering (RPCF) Algorithm is used and described as follows:

Step 1: Generate rules by using Rule-based Personalization from user query input.

This step generates the content rules based on the user query input. The content rules are in the form IF condition THEN action. Collaborative Filtering works by collecting user feedback in the form of ratings for items in a given domain and exploit similarities and differences among profiles of several users in determining how to recommend an item or how to give the prediction for the active user's interest. A subset of users is chosen based on their similarity to the active user, and a weighted combination of their ratings is used to produce predictions for the active user. Similarity is a powerful way to retrieve interesting information from large repository. The threshold of Correlation Coefficient ranges from -1.00 to +1.00. The value of -1.00 represents a perfect negative correlation while a value of +1.00 represents a perfect positive correlation. A value of 0.00 represents a lack of correlation.

Step 2: Compute the similarity measure between users by means of the Person Correlation Coefficient.

The following Pearson Correlation Coefficient function is used to compute the similarity measure between the user's preference functions.

$$S(a,b) = \frac{\sum_{i=1}^{N}(x_{a,i} - \bar{x}_a)(x_{b,i} - \bar{x}_b)}{\sqrt{\sum_{i=1}^{N}(x_{a,i} - \bar{x}_a)^2 * \sum_{i=1}^{N}(x_{b,i} - \bar{x}_b)^2}} \qquad (4)$$

Where, ***S (a, b)*** is the similarity of user ***a*** and ***b,*** **N** is the number of items. $x_{a,i}$ and $\boldsymbol{x_{b,i}}$ are the ratings given to the item ***i*** by user ***a*** and ***b***. $\bar{x}_a$ *and* $\bar{x}_b$ are the average ratings (mean) of user ***a*** and ***b.***

A higher collection value indicates more accurate recommendations. The Pearson's Correlation Coefficient only measures the overlapping items between users.

Step 3: Weight the similarity by the number of item ratings and select the neighboring user that have the highest similarity rating with the active user.

The Significance-Weighting method as shown in equation (5) is used to devalue the correlation based on few co-rated items,

$$W(a,b) = S(a,b) * \frac{n}{50} \qquad (5)$$

If two users have less than 50 commonly rated items, we apply a significance weight of **n/50**, where, **n** is the number of co-rated items.

If there are more than 50 co-rated items, then a significance weight of 1 is applied which means we leave the correlation unchanged.

Step 4: Compute a prediction from the ratings of neighbour.

Predictions are computed from the weighted combination of the neighbour, which is defined in as:

$$P_{a,i} = \bar{x}_a + \frac{\sum_{b=1}^{M}(x_{b,i} - \bar{x}_b)}{\sum_{b=1}^{M} W(a,b)} * W(a,b) \tag{6}$$

where, ***M*** is the number of users in the neighbour hood. $\boldsymbol{P}_{\mathbf{a,i}}$ is the prediction of the active user *a* on the target item ***i.*** [9]

Case Study of a Recommender System for Interesting Places in Myanmar by Using Collaborative Filtering Method

This system is implemented to recommend items from natural tourism/ecotorisum places and interesting places list to the user. In this system, user based filtering processes are as follow:

Step 1: Active user observe the information of the interested places in Myanmar, especially in natural places, historical places and other famous pagoda, beaches etc. In this system describe the interesting places, historical places and then user can do activities in Myanmar such as climbing, trekking, and dolphins and Ayeyarwady, …etc.)

Step 2: Active user give the rating (rating scale-1-5) for his/her preferences on the item places. Table 1 is shown for sample calculated rating based on seven interested places for number of twenty users.

Table 1: Sample Rating Scores for Interesting Places

User id	Chin	Kachin	Rhaing	Shan	Mandalay	Mgwae	Sagaing
1	1	2	3	4	5	5	4
2	3	2	2	1	5	4	3
3	2	2	3	3	4	5	1
4	2	4	5	2	3	1	5
5	5	5	4	3	2	2	3

User id	Chin	Kachin	Rhaing	Shan	Mandalay	Mgwae	Sagaing
6	3	1	4	2	3	5	4
7	1	4	5	2	3	5	4
8	4	5	2	3	4	2	1
9	1	5	3	2	1	4	2
10	2	4	5	3	2	1	3
11	1	4	5	3	1	5	1
12	3	3	1	4	5	3	2
13	4	5	1	2	3	4	5
14	2	1	3	4	5	3	1
15	4	5	3	1	2	4	1
16	3	5	2	1	4	3	5
17	2	3	4	5	2	2	3
18	5	2	3	1	4	2	4
19	3	4	5	2	1	4	3
20	1	2	3	4	5	2	3

Step 3: In this step, to estimate a prediction for an active user, the memory based algorithms first find the user's neighbours (the users who are similar to the active user). Then, the active user's rating is predicted by averaging the (weighted) known rating on the places item by his/her neighbors. It is based on the assumption that similar users have similar rating patterns.

Step 4: Find the measurement of the similarity between users by using Pearson's Correlation Coefficient. According to results of similarities measurement between user 20 (active user) and other users (user 2, user 3, …, user 20), the highest similarities is found between user 4 and user 20. According to results of similarities measurement between user 20 (active user) and other users (user 2, user 3, …, user 20), the highest similarities is found between user 4 and user 20.

Table 2: Show Similarity and User Pairs

User Pair	Similarity
user1, user 20	0.84
user2, user 20	0.70
user3, user 20	0.26
user4, user 20	**0.97**
user5, user 20	0.41
user6, user 20	0.61
user7, user 20	0.58
user8, user 20	0.60
user9, user 20	0.45
user10, user 20	0.68
user11, user 20	0.38
user12, user 20	0.82
user13, user 20	0.38
user14, user 20	0.89
user15, user 20	0.28
user16, user 20	0.58
user17, user 20	0.79
user18, user 20	0.56
user19, user 20	0.41

Similarity is greater than 0, this pair of users may be chosen and then user4 rating of each items and user20 rating of each items are the same, these items count are increased. These resulting counts are sorted and shown to the user in ascending order as shown in table 2. According to above the table result, Shan state is suitable place for visit in Myanmar for active user, is shown in figure 2.

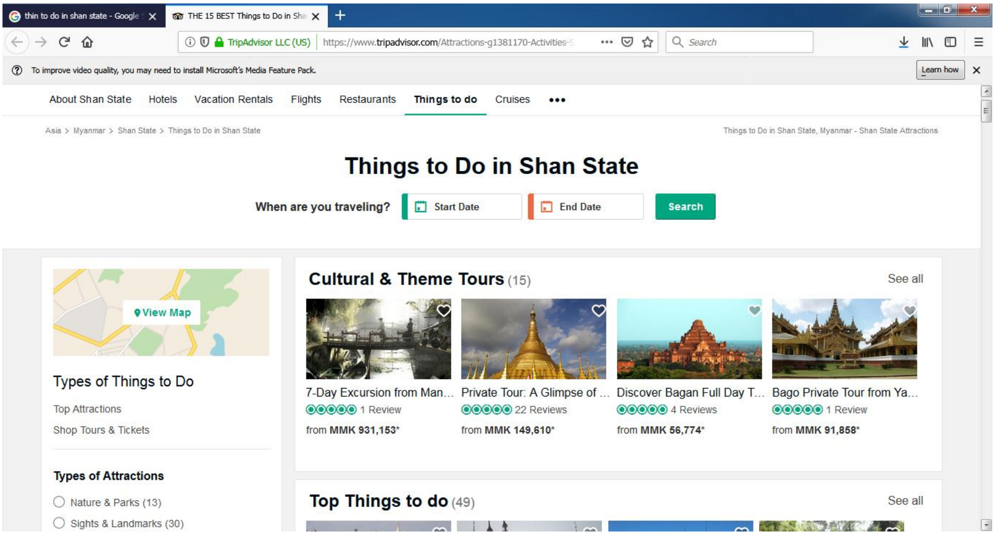

Figure 2: Recommended Place of Shan State Web Page

Discussion and Conclusion

A non-personalized recommender system is simpler to generate the popular places and top ten selections of places. This system is implemented by using memory-based technique, based prediction mechanisms and user based collaborative filtering for recommendations. This system will assist in achieving relevant recommendations combined with an active users' preferences and the same with past users' preferences and behaviors.

This recommender system helps to suggest and inform visitors about these natural tourism/ecotourism opportunities and in doing so, it hopes to promote the conservation of Myanmar's most beautiful areas and most extraordinary species. These suggestions relate to various decision-making processes, such as what to do and what places to visit. A tourist using this system can be helped to consider and select as a single travel destination. In future research, the community-based recommender system will implement for ecotourism and historical places in Myanmar. This type of RSs model acquires information about the social relations of the users and preferences of the user's friends.

Acknowledgements

First of all, I am grateful to Dr. Win Naing, Rector of Dagon University, and Dr.Yi Mon Win Associate Professor, Head of Department of Computer Studies, Dagon University, for their kind permission to carry out this work. I am greatly indebted to Professor Dr. Nwe Nwe Win, Head of Department of Computer Studies (Retired), University of Yangon, for her valuable guidance, overview and perpetual encouragement. Finally, I am very thankful to Dr. Soe Mya Mya Aye, Professor, Head of Department of Computer Studies, and University of Yangon, for her advices and comments.

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PHYLOGENY OF NATIVE CHICKENS IN CENTRAL AND WESTERN MYANMAR*

Theint Sandar Saing[1], Thida Lay Thwe[2], Khin Maung Saing[3], Tomofumi Shimada[4]

Abstract

The present work was conducted to assess the genetic status of some native Myanmar domestic chickens *(Gallus gallus domesticus)* collected from seven different sites in Ayeyarwady, Yangon and Magway Regions from December, 2016 to March, 2017. In this study, the phylogeographic relationships were investigated by analyzing the hyper variable segment (HVS- 1) in mitochondrial DNA of liver tissue sampled from a total of 20 domestic chickens including pygmy chickens. In a 319 bp fragment of HVS-1 DNA among 20 sequences, 33 variable sites that defined 16 haplotypes were identified. Phylogenetic analysis revealed four divergent clades (A, B, C and D) with distinct geographic pattern. The clades A, C, D consist of haplotypes only from Central Myanmar, while the clade B is made up of the haplotypes mainly from the Western Myanmar. Central Myanmar clades (A, C and D) contain the haplotypes mainly related to those of Southwest China and/ or surrounding regions (Japan and Indonesia), whereas the clade B clusters with haplotypes from India, and also from China and Japan. Pygmy chicken haplotype, closely related to one Magway haplotype, together cluster in the clade A. Evolutionary divergence (genetic distance) was lower within each clade of A, B, C and D but higher between the four clades. The distinct geographic pattern in the present phylogenetic tree and network suggests that Myanmar chickens have different geographic origins and have maintained their original geographic diversity in their local domestication history.

Keywords : Native chickens, phylogeny, Myanmar

1. Student, PhD Preliminary Course, Department of Zoology, University of Yangon
2. Professor and Head, Department of Zoology, University of Yangon
3. Senior Advisor (Retired), Ministry of Science and Technology
4. ILBC (Yangon)

*Best Paper Award Winning Paper in Zoology (2018)

Introduction

The domestic chicken (*Gallus gallus domesticus*) provides valuable animal protein for human consumption worldwide (Bhuiyan *et al*., 2013). There are many kinds of native fowls in Asia (Kawabe *et al*., 2014).

Myanmar possesses native chicken populations having well adapted phenotypes to the wet, dry, and hot environments. Farmers in Myanmar, as in many neighboring countries, rear small flocks of native domestic chickens for local consumption.

In contrast to the commercial breeds, the native chicken is mostly maintained in small populations with high genetic diversity and morphological variations (Yap *et al*., 2010).

The modern chicken breeds have lost many of the wild type genes possessed by native chickens. Thus it is very important to retain these wild type genes in the native chicken populations for future utilization in improving genotypes of commercial breeds (Kawabe *et al.,* 2014).

Numerous previous works have reported on the phylogeny and origin of the native chickens; some authors postulated single origin for native chickens from the red jungle fowl (*Gallus gallus*), whilst others suggested multiple origins from different species of jungle fowls (Liu *et al.,* 2006 in China, Kaginakudru *et al.,* 2008 in India, Yap *et al*., 2010 in Malaysia, Sawai *et al*., 2010 in Japan, Ngo Thi Kim Cuc *et al*., 2011 in Vietnam, Bhuiyan *et al*., 2013 in Bangladesh, Miao *et al*., 2013 in China, Hoque *et al.,* 2013 in Korea, Kawabe *et al*., 2014 in Laos.

However, phylogenetic works on native chickens have been still rare in Myanmar. In collaborative works, population structure and phylogeny among native chickens were analyzed for populations in Myanmar (Yangon, Pegu, Mandalay) and Indonesia (Aye Aye Maw *et al*., 2012), and for populations in Myanmar, Thailand and Laos (Aye Aye Maw *et al*., 2015).

Mitochondria DNA (mt DNA) has been extensively used as a genetic marker to clarify the phylogeny or maternal ancestral lineage or to compare populations in native domestic chickens as well as in junglefowls and commercial breeds (Fumihito *et al*., 1996, Kaginakudru *et al*., 2008).

Liu *et al*. (2006), in an extensive work, revealed the existence of nine distinct divergent clades related to the geographic distribution of a wide range of native chickens in Eurasian region.

Hence, the present work was conducted to assess the phylogeography and genetic status of some native chickens in Myanmar with the following objectives: to collect the native chickens from some parts of Western and Central Myanmar and extract genomic DNA, to find out genetic variation among the collected specimens based on HVS-1 mtDNA control region marker sequence, and to relate genetic variation to sample collection sites via molecular phylogenetic analysis.

Materials and Methods

Chicken sample collection and study period

A total of 20 native chickens *Gallus gallus domesticus* (Linnaeus, 1758), were collected live from seven different sites: Pathein, Ngwesaung, Tagongyi, Myaungmya in Ayeyarwady Region; Twantay and Hmawbi in Yangon Region ; and Magway in Magway Region of Myanmar. This study was conducted from December, 2016 to March, 2017. The laboratory work was conducted at the Molecular biology Laboratory at the Department of Zoology, University of Yangon.

Liver tissue sampling

Small pieces of liver tissue (1 gm each) were collected from each chicken (n = 20). The liver tissue samples were preserved in 70% ethanol in tissue sample tubes with caps.

Genomic DNA extraction

Total genomic DNA was extracted from 25 mg liver tissue (n =20) by Invitrogen Purelink Genomic DNA extraction Minikit (10), USA. The protocol followed was as directed by the kit manufacturer. Amount and purity of the extracted DNA were measured for each sample (n = 20) by NanoDrop One spectrometer

PCR amplification of HVS – 1 DNA

HVS – 1 DNA fragment (550 bp) was amplified by the set of primers L16750 (F) and H522 (R). (Fumihito *et al*., 1994; Fu *et al*., 2001). AmpliTag Gold 360 Master Mix was utilized.

The PCR running conditions were as follows :

Hold	95^{o}C	5 min
Denature	94^{o}C	1 min
Annealing	60^{o}C	1 min
Extension	72^{o}C	1 min
Final extension	72^{o}C	7 min

The PCR was run for 30 cycles in 20µL reaction

Sequencing PCR reaction

The amplified HVS-1 DNA fragment (550 bp) was utilized for sequencing reactions. Using the same primers: L16750 (F) and H522 (R) for sample template. The control primers – 21 M13 were used for pGEM-3zf(+) Control template. Big Dye Mix was utilized.

The running conditions for the sequencing PCR were as follows:

Hold	96^{o}C	1 min
Denature	96^{o}C	10 sec
Annealing	50^{o}C	5 sec
Extension	60^{o}C	4 min
Holding	4^{o}C	finite

The PCR was run for 25 cycles in 10.5 µL reaction

Sequencing in ABI 3500 Genetic Analyzer

A total of 20 HVS- 1 amplified DNA marker were sequenced and read for both forward and reverse primer extended template strands. The sequenced data were downloaded from ABI3500 Computer onto CD discs and Laptop computer using MEGA 7 software and the sequences were aligned by Proseq for 319 bp out of 550 bp amplified.

Agarose gel electrophoresis

Agarose gel electrophoresis were ran to check extracted genomic DNA and PCR amplified HVS-1 DNA of mitochondria. Agarose gel (1%) in TAE 1 x buffer (pH 8.0). Gel was run in TAE 1 x buffer at 135 VDC for 10 min – 15 min. The gel was stained with ethidium bromide (10 mg/L) for 30 min in distilled water, washed in tap water, and DNA bands were visualized and photographed under long wave UV light on a transilluminator.

Data analysis

A Maximum Likelihood (ML) phylogenetic tree and a Median-Joining network were constructed for 20 aligned haplotype sequences (319 bp) of HVS – 1 DNA marker from 20 collected chickens of different sites. Reference sequences for domestic chicken, (*Gallus gallus domesticus*) deposited in GenBank Database, were downloaded and compared to the sequences of the present study. Haplotype diversity, nucleotide diversity, variable sites and genetic distances were also calculated. MEGA 7 software, Proseq software, Arlequin ver 3.5 were utilized.

Results and Discussion

Morphological diversity such as variations in plumage colour and body size (e.g dwarfs) occur among Myanmar native domestic chickens. However, little is known about the origins and phylogeography of native chickens in different parts of Myanmar. Hence, the present study was conducted to assess the genetic status of some Myanmar native chickens.

In the present study, a mitochondrial DNA (mt DNA) D-loop Hyper Variable Segment (HVS-1) sequences (319 bp) were analyzed and compared among 20 sequences of Myanmar native chickens collected from seven different sites (populations) in Central and Western Myanmar (Figure.1, Table 1).

A. TS7, Myaungmya

B. TS11, Pathein

C. TS14, Tagongyi

D. TS16, Ngwesaung

E. TS17, Magway

F. TS21, Hmawbi

G. TS25, Twantay

Figure1. Some studied native chickens from different collection sites

Table 1 . Haplotypes and accession numbers of chicken mtDNA sequences used in this study

Haplotype		GenBank accession No.	Reference
A- TS15,TS 19, TS 21,TS24, TS 25, TS 26			This study
B- TS11,TS 12, TS 13, TS14, TS 16, TS 17 TS 22, TS 23			This study
Haplotype		GenBank accession No.	Reference
C- TS 7,TS 8, TS 9, TS18			This study
D- TS 10, TS 20			This study
G. gallus domesticus	Yunan, China	AF512057	GenBank
G. gallus domesticus	Sichuan, China	AF51206	
G. gallus domesticus	Yunan, China	AY392172	
G. gallus domesticus	India	AY644966	
G. gallus domesticus	Ryuku, Japan	AB007744	
G. gallus domesticus	Japan	AB268535	
G. gallus domesticus	Japan	AB268543	
G. gallus domesticus	H 10, China	AY588636	
G. gallus domesticus	Shizuoka, Japan	AB114076	
G. gallus domesticus	Shizuoka, Japan	AB114070	
G. gallus domesticus	Ibaraki, Japan	AB114069	
G. gallus domesticus	Cuanzi, China	AF512285	
G. gallus domesticus	Cuanzi, China	AF512288	
G. gallus	Vietnam	AB009434	
G. gallus	Japan	D82904	Out group
G. gallus	Indonesia	AB268545	Out group

Among studied chickens, a total of 33 variable sites (i.e nucleotide substitutions) that defined 16 mthaplotypes were found among the 20 sequences from seven populations of Myanmar. Overall haplotype and nucleotide diversity were 0.9737 ± 00250 and 0.027207 ± 0.016667 respectively for the total 20 native chickens studied (Tables 2 and 3).

These results indicated that studied Myanmar native chickens have higher haplotype and lower nucleotide diversities although they have moderate nucleotide polymorphism (variable sites) compared to Laotian native chickens which have 37 variable sites, 29 haplotypes, and haplotype diversity of 0.8798 and nucleotide diversity of 0.10158 among three populations (Kawabe *et al.,* 2014). In another work on four populations among Bangladeshi native chickens, 39 variable sites. 29 haplotypes, 0.901

haplotype and 0.016 nucleotide diversity respectively were reported by Bhuiyan *et al.* (2013).

Table 2. Variable sites between studied sequences

	Variable Sites																															
								1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	3
	1	3	3	4	6	8	9	2	3	3	4	4	5	5	6	6	7	7	7	8	8	0	1	2	3	3	3	3	5	6	7	8	1
	6	2	8	1	0	8	8	0	3	8	3	6	5	7	4	7	0	5	7	2	6	2	7	7	1	4	6	8	1	3	6	4	7
TS7Myaungmya	A	G	T	T	A	T	A	T	G	T	A	C	T	C	C	C	A	C	C	T	C	A	C	T	T	C	T	C	C	G	T	C	T
TS8Myaungmya	A	T	T	T	A	T	A	T	G	T	A	C	T	C	C	C	A	C	C	T	C	A	C	T	T	C	T	C	C	A	T	C	C
TS9Myaungmya	A	T	T	T	A	T	A	T	G	T	A	C	T	C	C	C	A	C	C	T	T	A	C	T	T	C	T	C	C	G	T	C	T
TS10Myaungmya	A	T	T	T	A	T	A	T	A	T	A	C	C	T	C	C	A	T	C	T	C	G	C	C	T	C	C	A	C	G	T	C	T
TS11Pathein	A	T	T	T	A	T	A	C	G	C	A	C	C	T	C	C	A	T	C	T	C	A	C	T	T	C	C	A	C	A	T	C	T
TS12Pathein	T	T	T	T	T	T	A	T	G	C	G	C	C	T	C	C	A	T	C	T	C	A	C	T	T	C	C	A	T	A	T	C	T
TS13Pathein	A	T	T	T	A	T	A	T	G	C	A	C	C	T	C	C	A	T	C	T	C	A	C	T	T	C	C	A	C	G	T	C	T
TS14Tagonegyi	A	T	T	T	A	T	A	C	G	C	A	C	C	T	C	C	A	T	C	T	C	A	C	T	T	C	C	A	C	A	T	C	T
TS15Tagonegyi	A	T	T	C	A	C	A	T	G	T	A	T	C	T	T	C	A	T	T	C	C	A	C	T	C	C	C	A	C	A	T	C	T
TS16Ngwesaung	A	T	T	T	A	T	A	T	G	C	G	C	C	T	C	C	A	T	C	T	C	A	C	T	T	C	C	A	T	A	T	C	T
TS17Magwae	A	T	T	T	A	T	A	C	G	T	A	C	C	T	C	C	A	T	C	T	C	A	C	T	T	C	C	A	C	A	T	C	T
TS18Magwae	A	T	G	T	A	T	A	T	G	T	A	C	T	C	C	C	A	C	C	T	C	G	C	T	T	T	T	C	C	A	T	C	T
TS19Magwae	A	T	T	T	A	T	A	T	A	T	A	C	C	T	T	T	A	T	T	C	C	A	T	T	C	C	T	A	C	A	T	C	T
TS20Magwae	A	T	T	T	A	T	T	T	G	T	A	C	C	T	C	C	A	T	C	T	T	G	C	C	T	C	C	A	C	G	T	T	T
TS21Hmawbi	A	T	T	T	A	C	A	C	G	T	A	T	C	T	T	C	A	T	T	C	C	A	C	T	T	C	C	A	C	A	T	C	T
TS22Hmawbi	A	T	T	T	A	T	A	T	G	C	G	C	C	T	C	C	G	T	C	T	C	A	C	T	T	C	C	A	C	A	C	C	T
TS23Hmawbi	A	T	T	T	A	T	A	T	G	C	G	C	C	T	C	C	G	T	C	T	C	A	C	T	T	C	C	A	C	A	C	C	T
TS24Hmawbi	A	T	T	T	A	C	A	C	G	T	A	T	C	T	T	C	A	T	T	C	C	A	C	T	C	C	C	A	C	A	T	C	T
TS25Twantay	A	T	T	T	A	T	A	T	A	T	A	C	C	T	T	T	A	T	T	C	C	A	T	T	C	C	T	A	C	A	T	C	T
TS26Twantay	A	T	T	T	A	T	A	T	A	T	A	C	C	T	T	T	A	T	T	C	C	A	T	T	C	C	T	A	C	A	T	C	T

Table 3. Polymorphic sites, haplotype and nucleotide diversity of four clades

Clade	n	No. of haplotypes	No. of polymorphic sites	Haplotype diversity (SD)	Nucleotide diversity (SD)
A	6	4	9	1.0000 (0.0962)	0.015047 (0.009873)
B	8	6	9	0.9286 (0.0844)	0.010636 (0.006925)
C	4	4	7	1.0000 (0.1768)	0.011494 (0.008734)
D	2	2	4	1.0000 (0.5000)	0.012539 (0.014019)
Total	20	16	33	0.9737 (0.0250)	0.027207 (0.014647)

Clade A: TS15, TS19, TS21, TS24, TS25, TS26
Clade B: TS11, TS12, TS13, TS14, TS16, TS17, TS22, TS23
Clade C: TS7, TS8, TS9, TS18
Clade D: TS10,TS20

Phylogenetic analysis, based on ML tree and Median- Joining network, (Figs. 2 and 3), revealed that four distinctly divergent clades (A, B, C and D) with distinct phylogeographic pattern, occurred among the seven different native chicken populations of Myanmar studied in the present work. Clades A, C and D consist of haplotypes from Central Myanmar related to mainly Chinese haplotypes. Clade B is composed of haplotypes mainly from Western Myanmar related to Indian haplotype. Interestingly, the Magway haplotypes are distributed in all four clades of the studied Myanmar native chickens, indicating gene flow or genetic admixture between the Magway population and the rest of the studied native chickens in Central and Western Myanmar (Fig. 3).

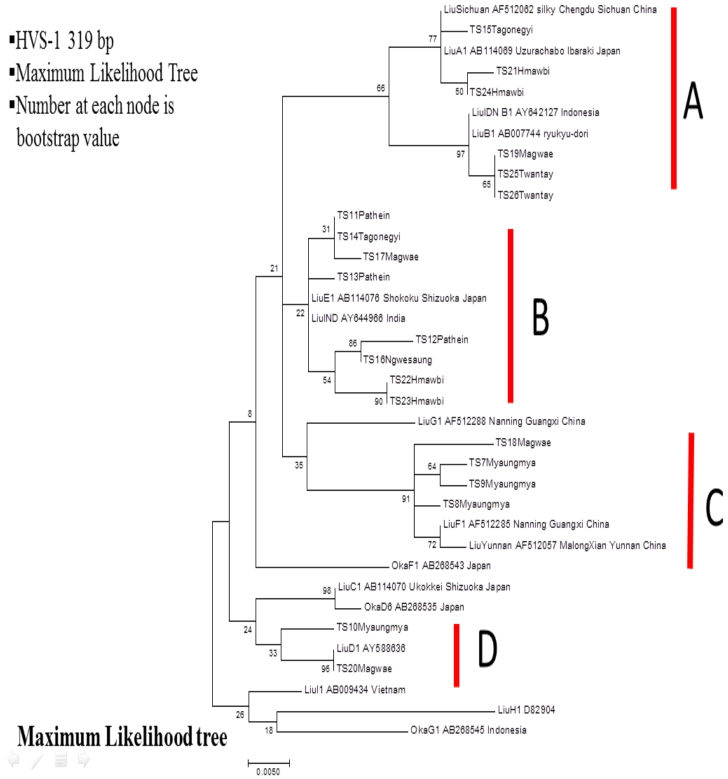

Figure 2. Maximum Likelyhood Phylogenetic tree constructed using HVS-1 data of the studied native chicken (n = 20) of Myanmar. Scale indicates number of nucleotide substitutions per site. Reference sequences are from GenBank.

Median-joining network analysis also showed that the native chicken haplotypes of Myanmar in clade B were also related to a Japanese haplotype indicating some genetic admixture from the northern areas of Myanmar (Fig. 3).

The pygmy or dwarf native chickens from Twantay are found to be closely related to one Magway haplotype, clustering together in clade A (Figs. 1, 2 and 3).

Laotian native fowls were distributed across five clades with mostly in two clades originated in China; the other three clades were reported to be probably originated in Southeast Asia (Kawabe *et al.,* 2014).

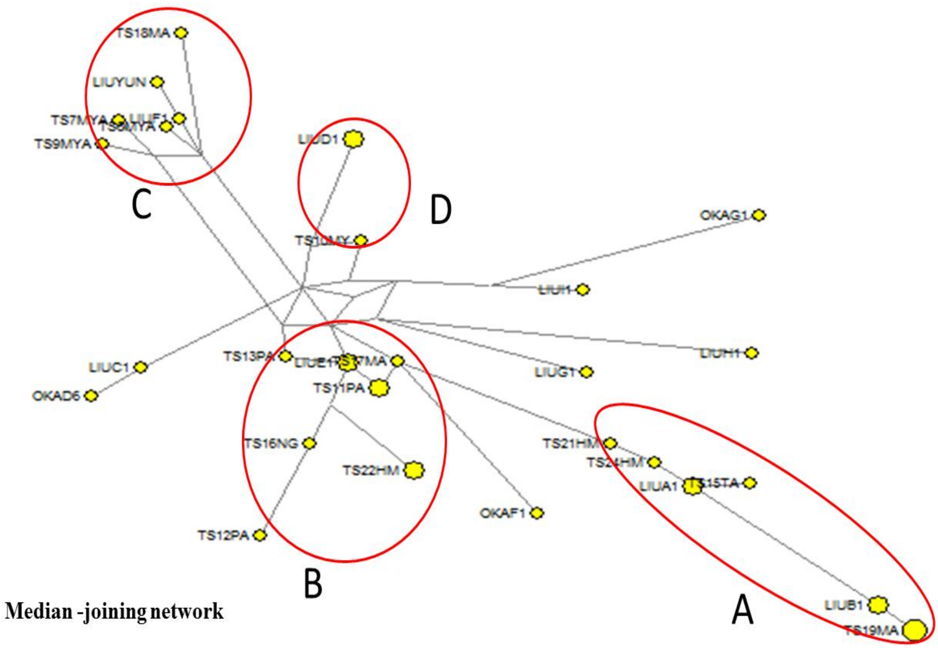

Figure 3. Median – joining network of HVS-1 sequencaes (n = 20) among the studied native chickens. Circle (yellow) size indicates haplotype frequency. Reference sequences are from GenBank.

Median – joining network of mtD-loop (648 bp fragment) revealed five clades with mostly in two clades among Bangladeshi native chickens;

they were thought to originate particularly from Myanmar and China (Bhuiyan *et al.*, 2013).

Regarding genetic distance (evolutionary divergence, expressed as number of substitutions of nucleotides per sequence site), between native chickens (Pathein, Hmawbi and Magway) and an Indian native chicken ranged 0.013 to 0.036, whereas, between Myanmar native chickens and a Chinese native chicken ranged 0.013 to 0.046. This indicated that native Western Myanmar chickens (clade B) are genetically closer to Indian native chicken than to Chinese native chickens (Table 4). But the gene flow seemed to be from Myanmar to neighbouring countries. This assumption is based on the results of work by Aye Aye Maw *et al.* (2015) who stated that genetic admixture (gene flow) was observed between Thai and Laotian native chickens, while foreign gene admixture was not found among Myanmar native chickens in Yangon, Pegu and Mandalay. The above researcher group also reported that the Neighbour-joining tree analysis showed Thai and Lao native chickens closely clustered in a single clade whereas Myanmar native chickens diverged into a different clade.

Table 4. Genetic distances between foreign and local native chickens

TS 13 Pathein	vs	India	0.013
TS 13 Pathein	vs	China	0.040
TS 24 Hmawbi	vs	India	0.036
TS 24 Hmawbi	vs	China	0.046
TS 18 Magway	vs	India	0.029
TS 18 Magway	vs	China	0.013

Aye Aye Maw *et al.* (2012) found that the genetic distance was 0.088 among native chicken populations of Myanmar and Indonesia.

Results of the present study show that genetic distance among studied Myanmar native chickens ranged 0.036 to 0.050 indicating moderate genetic diversity with less gene flow among the seven native Myanmar chicken populations studied which were diverged into four different clades with distinct geographic patterns (Figs. 2 and 3, Table 5).

Table 5. Genetics distances between local native chickens

TS 24 (Hmawbi)	vs	Indonesia	0.050
TS 24 (Hmawbi)	vs	TS 18 (Magway)	0.050
TS 15 (Tagongyi)	vs	TS 18 (Magway)	0.050
TS 9 (Myaungmya)	vs	TS 15 (Tagongyi)	0.046
TS 7 (Myaungmya)	vs	TS 24 (Hmawbi)	0.046
TS 18 (Magway)	vs	TS 25 (Twantay)	0.046
TS 18 (Magway)	vs	TS 12 (Pathein)	0.043
TS 26 (Twantay)	vs	TS 7 (Myaungmya)	0.043
TS 12 (Pathein)	vs	TS 9 (Myaungmya)	0.040
TS 26 (Twantay)	vs	TS 16 (Ngwesaung)	0.036

Evolutionary divergence estimates among the four clades of the studied Myanmar native chickens revealed that the divergence was low and similar, at 0.01 substitution of nucleotide per site of HVS-1 sequence (319 bp), within each clade of A, B, C and D. On the other hand, the divergence between the four clades ranged. 0.025 to 0.044, suggesting higher genetic diversity between the four clades compared to low diversity within each clade (Tables 6 and 7).

Table 6. Estimates of average evolutionary divergence over sequence pairs within clades

Clade	Average Evolutionary Divergence
Clade A	0.0154
Clade B	0.0108
Clade C	0.0117
Clade D	0.0128

Table 7. Estimates of evolutionary divergence over sequence pairs between clades

	A	B	C
Clade A			
Clade B	0.032		
Clade C	0.044	0.032	
Clade D	0.039	0.025	0.034

It is hoped that the data generated and the conclusion reached in the present work would contribute to future research on the phylogenetics of the native chickens of Myanmar.

Conclusion

Overall data of the present phylogenetic analysis revealed four divergent clades with distinct geographic patterns occurred among the studied native Myanmar chickens collected from seven different collecting sites or seven populations. Myanmar native chickens have different geographic origins within the country. Myanmar native chickens have thus maintained their original geographic genetic diversity in their local domestication history.

Acknowledgements

. Our special thanks go to Dr Moe Moe Aye, Assistant Lecturer, Zoology Department, Kyaing Ton University, Dr San Maung Maung Theint, Assistant Lecturer, Zoology Department, University of Yangon and Dr Khin Myat Myat Zaw for their kind help whenever and wherever needed during the course of this research.

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PROTOZOAN INFECTIONS IN *LABEO ROHITA* (HAMILTON, 1822), *PIARACTUS BRACHYPOMUS* (CUVIER, 1817) AND *PANGASIUS HYPOPTHALMUS* (SAUVAGE, 1878)

Yan Naung Tun[1], Hnin Hnin Htay[2], Hendrik Stolz[3], Fernando[4], Kay Lwin Tun[5]

Abstract

Labeo rohita, *Piaractus brachypomus* and *Pangasius hypopthalmus* are the most important fish species for Myanmar aquaculture and intensive investigation for protozoan parasites in these fishes are essential to develop methods for disease control. This study was conducted to examine the prevalence and infection intensity of protozoan parasites in cultured *Labeo rohita*, *Piaractus brachypomus* and *Pangasius hypopthalmus* collected from Lay Daung Kan fish farm. Thirty individuals from each species were collected monthly from January 2017 to December 2017. Gills, skin and internal organs were examined for protozoan infection. In total ten protozoan species were recorded including one species of *Thelohanellus*, two species of *Myxobolus*, one species of *Zschokkella* and one species of *Trichodina* in *Labeo rohita*. Two species of *Myxobolus* and one species of *Trichodina* were found in *Piaractus brachypomus*. In *Pangasius hypopthalmus*, one species of *Myxobolus* and one species of *Trichodina* were recorded. Among the recorded parasite species, the highest prevalence of infestation was found in *Trichodina* spp.

Keywords: Protozoan, *Labeo rohita*, *Piaractus brachypomus*, *Pangasius hypopthalmus*, Prevalence

[1.] PhD student, Department of Zoology, University of Yangon, kamayut Township, Yangon
[2.] Assistant Lecturer, Department of Zoology, University of Yangon, kamayut Township, Yangon
[3.] Scientist, Chair for Aquaculture and Sea-Ranching, University of Rostock, Germany, Development Advisor, Myanmar Sustainable Aquaculture Programme, Deutsche Gesellschaft fur Internationale Zusammenarbeit (GIZ)
[4.] Student, Department of Aquaculture, Bogor Agricultural University, MYCulture Project, Worldfish
[5.] Professor, Department of Zoology, University of Yangon, Kamayut Township, Yangon

Introduction

Aquaculture is the fastest-growing food production system globally, with a 9% increase in production of animal crops per year since 1985 (FAO 2007). In Myanmar, fish culture started in 1953 with imported *Tilapia mossambica*. Fish were realized to be a stable diet for its people and potential industry for the national economy. Freshwater fish culture is a major source of aquaculture production in Myanmar. The most cultivated freshwater fish in Myanmar are *Labeo rohita* (nga-myit-chin), *Pangasius hypopthalmus*(Nga-dan), *Piaractus brachypomus* (Pacu), *Puntius sophore* (Nga-kone-ma), *Catla catla* (Nga-gaung-pwa), *Hypophthalmichthys molitrix* (Ngwe-yaung-nga-gyin) and *Oreochromis niloticus* (Ti-la-pia). Among these rohu, *Labeo rohita* is the most produced species in fish hatcheries throughout Myanmar followed by *Pangasius hypopthalmus* and *Piaractus brachypomus* (FAO, 2006). They are widely cultured by extensive or semi intensive system in Southeast Asia. Freshwater pond fish culture is a major contributor to aquaculture production. They are widely cultured by extensive or semi intensive system in the Southeast Asia. Forty seven percent of the total fish produced in Myanmar were produced by freshwater fisheries (The State of World Fisheries and Aquaculture, 2016).

Fish naturally carry a variety of pathogenic viruses, bacteria, fungi and parasites. Parasitic infections are one of the most important factors for economic losses in aquaculture. Due to the intensification of culture systems, diseases related to e.g. nutritional deficiency, parasitic infection or thereby causes secondary infection increased and are responsible for significant economic losses (Snieszko, 1974, Martins *et al*, 2002).

The production from culture system is hampered by the infection of various fish parasite. Parasites and diseases are the most serious limiting factors in culture farm (FAO, 2004). Fish are usually cultured in high density in a restricted water bodies, 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). The number of fish parasitologists in fishery and aquaculture sectors of the country is small. 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 undertaken to investigate protozoan infection in three cultivated freshwater fish species. Prevalence and intensity of infections of protozoa in culture fish farm were also reported.

Materials and Methods

Sample collection and preparation of aquaria

The study period ran from January to September 2017 in Lay Daung Kan fish farm, Yangon Township (Fig-1). Fish were produced were induced breeding method and they were culture separately in 1436.67 m^2 pond very extensively (without feeding and water exchange). A total of 30 fish from each species (*Labeo rohita*, 5.1cm$\pm$1.4cm, *Piaractus brachypomus*, 5.6cm$\pm$0.7cm and *Pangasius hypopthalmus*, 5.4cm$\pm$1.2cm with initial body weight) was collected monthly from a pond to examine protozoan infections. Selected fish were taken to the laboratory in plastic bags filled with oxygen.

On arrival, they were kept in small glass aquaria (51cm ×45cm × 39cm) and aeration was given. One day prior to arrival of the fish, aquaria were thoroughly cleaned, filled with water and aerated. Some fish were immediately dissected to examine parasite load and incidence, and the remainder were kept in aquaria for about five days for later studies. Diagnostic symptoms were carefully recorded from individual fish.

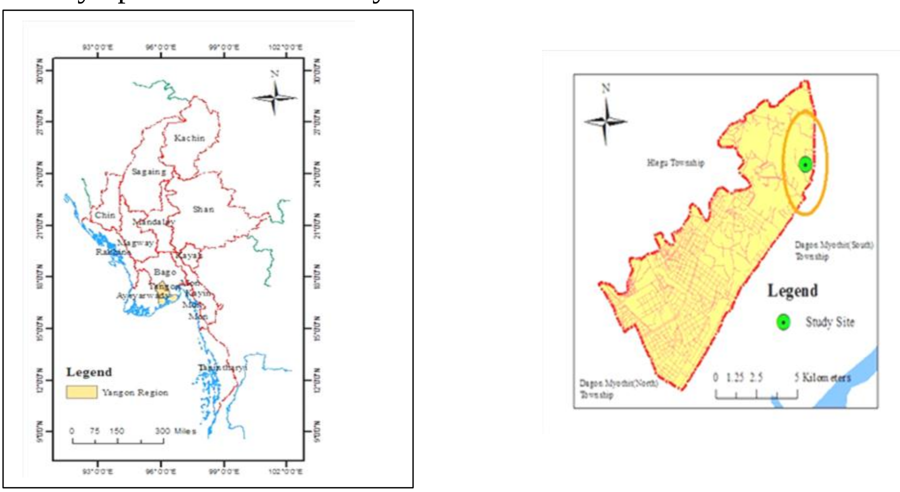


Figure 1. Lay Daung Kan fish farm located at Dagon Myothit(east)

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 analysis for parasite loads

Prevalence and mean intensity were calculated for each parasite species in accordance with the following method by Margolis *et al*. (1982).

$$\text{Prevalence (\%)} = \frac{\text{Number of infected host}}{\text{Total number of host examined}} \times 100$$

The intensity of infection were categorized into five stage according to Bachere *et al*., (1982)and Culloty *et al.,* (1999).

Stage 1: 1 – 20 parasites observed within 3-min of screening under 40× magnification.

Stage2: 21– 40 parasites observed within 3-min of screening under 40× magnification.

Stage3: 41– 60 parasites observed within 3-min of screening under 40× magnification.

Stage 4: 1 – 10 parasites observed in all fields of vision.

Results

1.1 Protozoans infection in three cultivated species of freshwater fish

During the study period, five species of parasite from two phyla were detected in the gills, skin and gallbladder of *Labeo rohita*, whereas four species of parasite belonging to two phyla were detected in the gills and skin of *Piaractus brachypomus* and in gills, skin and gallbladder of *Pangasius hypopthalmus*. Recorded species were *Thelohanellus* sp., *Myxobolus* sp. 1, *Myxobolus* sp. 2, *Zschokkella* sp., and *Trichodina* nigra in *Labeo rohita*. *Zschokkella* sp. was recorded only in the gallbladder of *Labeo rohita*.

In *Piaractus brachypomus*, *Myxobolus* sp. 3, *Myxobolus* sp. 4, and *Trichodina* sp. were observed, whilst *Myxobolus* sp. 5 and *Trichodina reticulata* were recorded from *Pangasius hypopthalmus* . Recorded parasites and their site of infection were described in Table (1).

1.2 Protozoan parasite recorded in *Labeo rohita*

Thelohanellus sp. (Plate 1a)

Host - *Labeo rohita*

Locality - Lay Daung Kan fish farm

Site of infection - Spores were mainly found in gills, brain and kidneys.

Characteristics of spores

The spores were pumpkin seed-like in front view. A single pyriform polar capsule reached the posterior half of the spore. Measurements of the spore were expressed as below;

Length of spore	= 12µm ± 1.5µm (n = 10)
Width of spore	= 11µm ± 0.5µm (n = 10)
Length of polar capsule	= 7.6µm ± 2.3µm (n = 10)
Width of polar capsule	= 1.5µm ± 0.4µm (n = 10)
Polar filaments	= 8

***Zschokkella* sp. (Plate 1b)**

Host - *Labeo rohita*

Locality - Lay Daung Kan fish farm

Site of infection - Spores were found in gallbladder

Characteristics of spores

The spores were ellipsoidal in sutural view and slightly bent or semicircular in valvular view, with rounded or bluntly pointed ends. Shell valves were either smooth or had ridges. The suture is straight, curved or sinuous. Polar capsules almost spherical, open slightly subterminally and both to one side; the sporoplasm is binucleate. The measurements of the spores were as follows;

Length of spore = 17.5µm ± 0.3µm (n=10)

Width of spore = 12.2µm ± 0.5µm (n=10)

Length of right polar capsule = 14.2µm ± 1.2µm (n=10)

Width of right polar capsule = 9.25µm ± 0.6µm (n=10)

***Myxobolus* sp. 1 (Plate 1c)**

Host - *Labeo rohita*

Locality - Lay Daung Kan fish farm

Site of infection - Spores were found in gills and cysts in fibrous layer of scales and fins.

Characteristics of spore

The spores were pear shape in front view, having two equal polar capsules at the anterior end. Polar capsules were pumpkin seed-like or pyriform shaped. The measurement of the spores was as follows;

Length of spore = 10µm (n = 10)
Width of spore = 7.5µm (n = 10)
Length of polar capsules = 7.5µm ± 0.3µm (n = 10)
Width of polar capsules = 5µm (n = 10)

***Myxobolus* sp. 2 (Plate 1d)**

Host - *Labeo rohita*

Locality - Lay Daung Kan fish farm

Site of infection - Spores were found in gills.

Characteristics of spore

The spores were pear shape in front view, having two unequal polar capsules at the anterior end. The larger polar capsule was tear-shaped and the smaller one was ovoid or spherical in shape. Measurements of the spores were as below;

Length of spore = 7.5µm ± 0.5µm (n = 10)

Width of spore = 3.4µm ± 0.2µm (n = 10)

Length of polar capsule = 5.2µm ± 1.5µm (n = 10)

Width of polar capsule = 2.5µm ± 0.1µm (n = 10)

***Trichodina nigra* (Plate 1e)**

Host - *Labeo rohita*

Locality - Lay Daung Kan fish farm

Site of infection - Parasites were found in gills

Characteristics of parasite

Trichodina nigra is medium-sized and disc-shaped. The denticles are reduced and angular with truncated distal margins. The anterior surface of the denticle is slightly curved. The posterior margin of the denticle forms a narrow semilunar curve with the deepest point. The measurements of the parasites were as follows;

Diameter of adhesive disc (da) = 50µm ± 4.2µm (n=10)

Diameter of denticulate ring (dd) = 20.5µm ± 2.5µm (n=10)

Diameter of clear area (dc) = 15.2µm ± 1.4µm (n=10)

Number of denticles = 21 ± 1.5 (n=10)

1.3 Protozoan parasite recorded in *Piaractus brachypomus*

***Myobolus* sp. 3 (Plate 2a)**

Host - *Piaractus brachypomus*

Locality - Lay Daung Kan fish farm

Site of infection - Spores were found in gills.

Characteristics of spore

The spores were pear shape in front view, characterized by two polar capsules at the anterior end. Polar capsules were pumpkin seed-like or pyriform spores with bilateral symmetry. 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. 4 (Plate 2b)**

Host - *Piaractus brachypomus*

Locality - Lay Daung Kan fish farm

Site of infection - Spores were found in gills.

Characteristics of spore

The spores were circle-shaped in front view, and had two unequal polar capsules at the anterior end. The large polar capsules were pumpkin seed-like in shape and the smaller one was tear-shaped. 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 = 24µm ± 5.2µm (n=10)

Width of left polar capsule = 14µm ± 5.2µm (n=10)

***Trichodina* sp. (Plate 2c)**

Host - *Piaractus brachypomus*

Locality - Lay Daung Kan fish farm

Site of infection - Parasites were found on skin and gills

Characteristics of parasite

Trichodina sp. is medium-sized and disc-shaped. The denticles are reduced and angular with truncated distal margins. The anterior surface of the denticle is slightly curved. The posterior margin of the denticle forms a narrow semilunar curve with the deepest point. 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)

1.4 Protozoan parasite recorded in *Pangasius hypopthalmus*

***Myobolus* sp. 5 (Plate 3a)**

Host - *Pangasius hypopthalmus*

Locality - Lay Daung Kan fish farm

Site of infection - Spores were found in gills, skin and gallbladder.

Characteristics of spore

The spores were spherical in front view, having two equal polar capsules at the anterior end. The polar capsules was tear-shaped. The measurements of the spores were as follows;

Length of spore = 17.5µm ± 0.3µm (n=10)

Width of spore = 12.2µm ± 0.5µm (n=10)

Length of right polar capsule = 14.2µm ± 1.2µm (n=10)

Width of right polar capsule = 9.25µm ± 0.6µm (n=10)

***Trichodina reticulata* (Plate 3b)**

Host - *Pangasius hypopthalmus*

Locality - Lay Daung Kan fish farm

Site of infection - Parasites were found in skin and gills

Characteristics of parasite

Trichodina reticulata is adhesive disc circular shaped. Denticles have needle-shaped well-developed thorns and blades. Rods are very conspicuous and parallel radial pins are inserted in the center of the disc. The measurements of the parasites were as follows;

Diameter of adhesive disc (da) = 50µm ± 4.2µm (n=10)

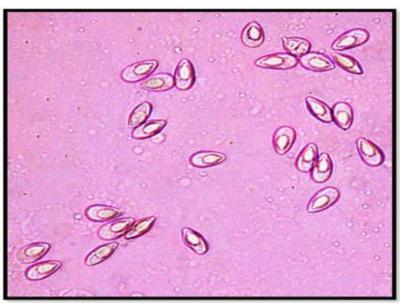
Diameter of denticulate ring (dd) = 20.5µm ± 2.5µm (n=10)

Diameter of clear area (dc) = 15.2µm ± 1.4µm (n=10)

Number of denticles = 21 ± 1.5 (n=10)

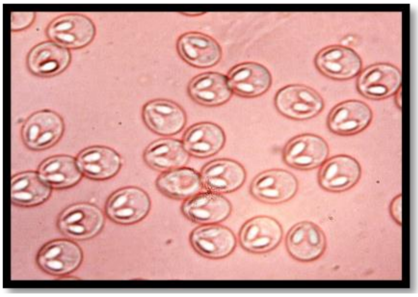
Table 1. List of parasites recovered and their site of infection

Parasite		Host	Site of infection
Cnidaria	*Thelohanellus* sp.	*Labeo rohita*	Gills
	Zschokkella sp.		Gills, skin (cyst) and fin
	Myxobolus sp. 1		Gills
	Myxobolus sp. 2		Gallbladder
Cilophora	*Trichodina nigra*		Gills, skin
Cnidaria	*Myxobolus* sp. 3	*Piaractus brachypomus*	Gills
	Myxobolus sp. 4		Gills
Cilophora	*Trichodina* sp.		Skin
Cnidaria	*Myxobolus* sp. 5	*Pangasius hypopthalmus*	Gills
Cilophora	*Trichodina reticulate*		Gills, skin



(a)

(b)



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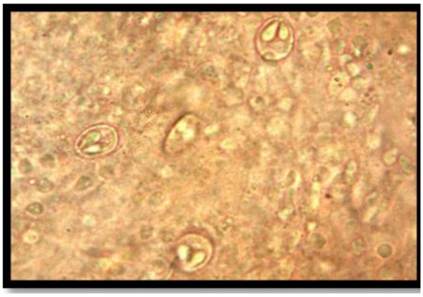
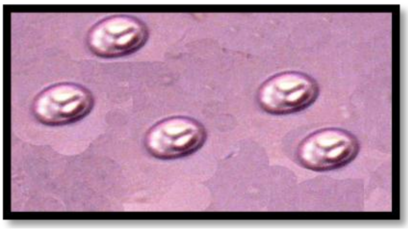


(d)

(e)

Plate. 1 Recorded protozoan parasite from *Labeo rohita* during study period.

(a) *Theohanellus* sp infected in *Labeo rohita* (b) *Zschokkella* sp. infected in *Labeo rohita* (c) *Myxobolus* sp. 1 infected in *Labeo rohita* (d) *Myxobolus* sp. 2 infected in *Labeo rohita* (e) *Trichodina nigra* infected in *Labeo rohita*



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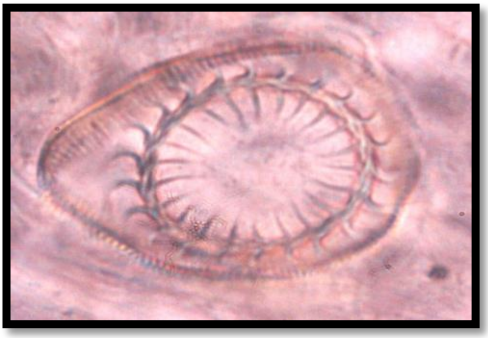
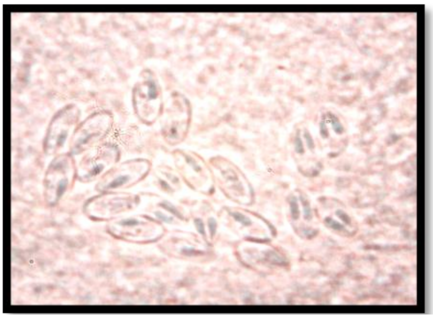


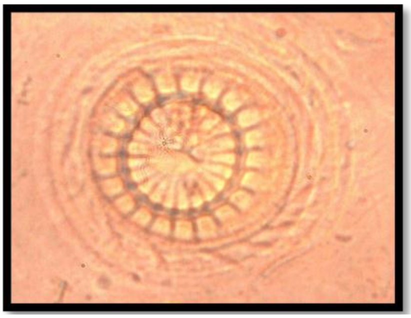
(c)

Plate. 2 Recorded protozoan parasite from *Piaractus brachypomus* during study period.

(a) *Myxobolus* sp. 3 infection in *Piaractus brachypomus* (b) *Myxobolus* sp. 4 infection in *Piaractus brachypomus* (c) *Trichodina* sp. infection in *Labeo rohita*



(a)



(b)

Plate. 3 Recorded protozoan parasite from *Pangasius hypopthalmus* during study period.

(a) *Myxobolus* sp. 5 infection in *Pangasius hypopthalmus* (b) *Trichodina reticulata* infection in *Pangasius hypopthalmus*

1.4 Prevalence and mean intensity of parasite in *Labeo rohita*

Figure. 2 shows prevalence and intensity of parasite in *Labeo rohita*. Prevalence of infestation of *Thelohanellus* sp. from gills of *Labeo rohita* during study period. The highest prevalence found in the month of April (40%) and lowest in July and September (10%). No infection was recorded in January, May and June. Monthly intensity of infestation was described in Figure. 3. High mean intensity of 2 was recorded in July and while it was decreased around about 1.7 to 1.5 in August and September.

Zschokkella sp. was found only in the gallbladder of *Labeo rohita*. Prevalence of infecting did not change very much during study period (one month to one month). High prevalence was recorded in April (Figure. 2). Monthly intensity of *Zschokkella* sp. infestation was described in (Figure. 3). Mean intensity ranged from 1-1.5. In August, mean intensity was slightly decreased to 1.2.

Myxobolus sp. 1 was found highest in April and lowest prevalence was found in July and August, 2017. No infection of *Myxobolus* sp. 2 was recorded in first month of sample period. Mean intensity of *Myxobolus* sp. 1 and *Myxobolus* sp. 2 infestation was described in Figure. 3. Mean intensity of *Myxobolus* spp. ranged from 1.1 to 2.1.

Among the recorded parasite species, the highest prevalence of infestation was recorded in *Trichodina nigra*. Prevalence of infestation fluctuated monthly ranging from 60% to 75%. The highest prevalence was recorded in September and the lowest prevalence was recorded in July (Figure. 2). Monthly intensity of *Trichodina nigra* infestation was described in (Figure. 3). Intensity was fluctuated from January to September, 2007. High mean intensity 1.5 and 2.1 was recorded in May and September, 2007.

1.5 Prevalence and mean intensity of parasite in *Piaractus brachypomus*

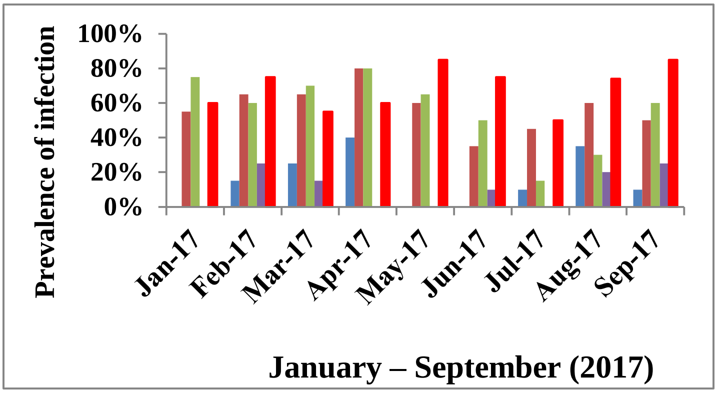
Prevalence and intensity of infection of parasites in Piaractus brachypomus were showed in Figure (4). *Myxobolus* sp. 3 was found highest in March and lowest prevalence was found in June and August. Prevalence of infection range from 15% to 50%. No infection of *Myxobolus* sp. 4 was recorded in the first four months. *Myxobolus* sp. 4 was recorded with the prevalence of 25% in May. And then, it was gradually decreased to 10% in June and 5% in July. But, it was disappeared in August. Mean intensity of *Myxobolus* sp. 3 and *Myxobolus* sp. 4 infestation was described in Figure 5. Mean intensity of *Myxobolus* sp. 3 ranged from 1.3 to 2. However, mean intensity of *Myxobolus* sp. 4 range from 1 to 1.3.

The highest prevalence of infestation was recorded in *Trichodina* sp. Prevalence of infestation fluctuated monthly ranging from 50% to 80%. The highest prevalence was recorded in April and the lowest prevalence was recorded in June (Figure 4). Monthly intensity of *Trichodina* sp. infestation was described in (Figure 5). Intensity was fluctuated from January to September, 2007. High mean intensity 2.5 was recorded in April 2007. Mostly, mean intensity of *Trichodina* sp. range was 1.2 during study period.

1.6 Prevalence and mean intensity of parasite in *Pangasius hypopthalmus*

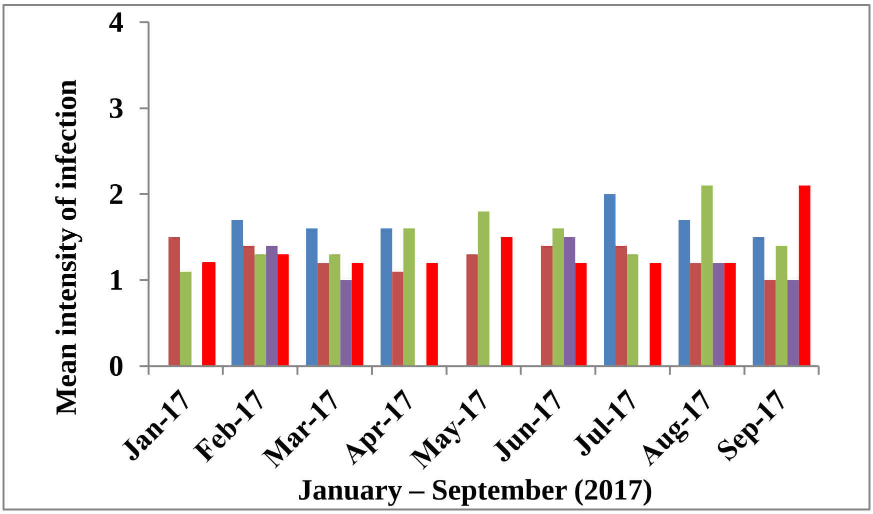
Figure 6 shows prevalence of infestation of *Myxobolus* sp. 5 from gills and gallbldder of *Piaractus brachypomus* during the study period. Prevalence of infection range from 15% to 60%. No infection of *Myxobolus* sp. 5 was recorded in June and July. Mean intensity of *Myxobolus* sp. 5 infestation was described in Fig. 7. Mean intensity of *Myxobolus* sp. 5 ranged from 1.2 to 2. High mean intensity of 2 was recorded in August.

Among the parasite species, the highest prevalence of infestation was recorded in *Trichodina reticulata*. Prevalence of infestation fluctuated monthly ranging from 45% to 65%. The lowest prevalence was recorded in July (Fig. 6). Monthly intensity of *Trichodina reticulate* infestation was described in (Fig. 7). Mean intensity of infection ranged from 1 to 1.5.



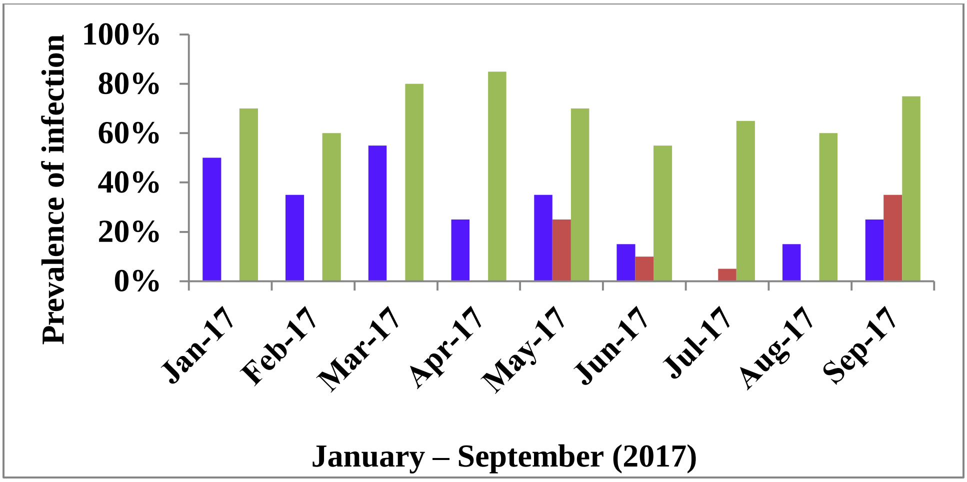
Thelohanellus sp. *Zschokkella sp.* *Myxobolus* sp. 1
Myxobolus sp. 2 *Trichodina nigra*

Figure. 2. Prevalence of protozoan infection in *Labeo rohita*



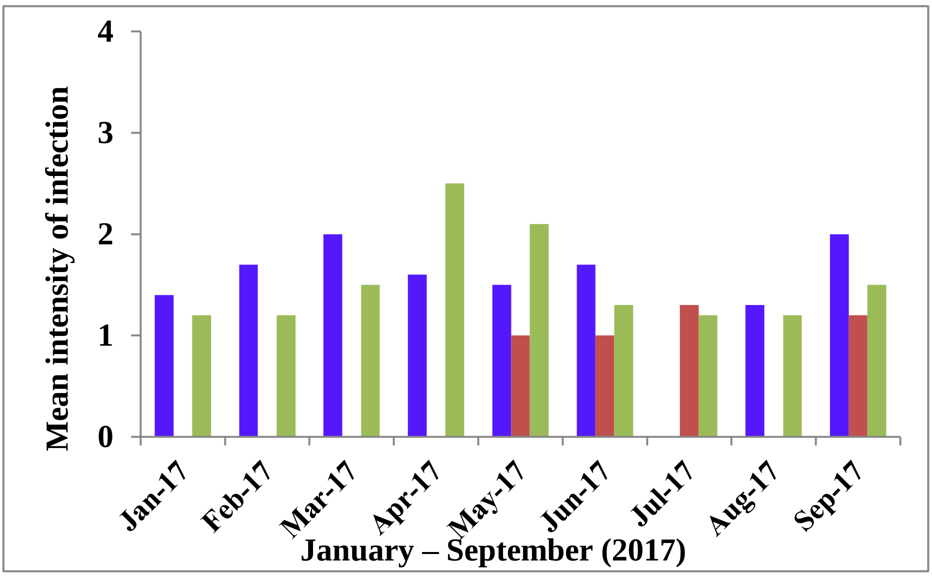
Thelohanellus sp. *Zschokkella sp.* *Myxobolus* sp. 1
Myxobolus sp. 2 *Trichodina nigra*

Figure. 3. Mean intensity of protozoan infection in *Labeo rohita*



Myxobolus sp. 3 *Myxobolus* sp. 4 *Trichodina* sp.

Figure. 4. Prevalence of protozoan infection in *Piaractus brachypomus*



Myxobolus sp. 3 *Myxobolus* sp. 4 *Trichodina* sp.

Figure. 5. Mean intensity of protozoan infection in *Piaractus brachypomus*

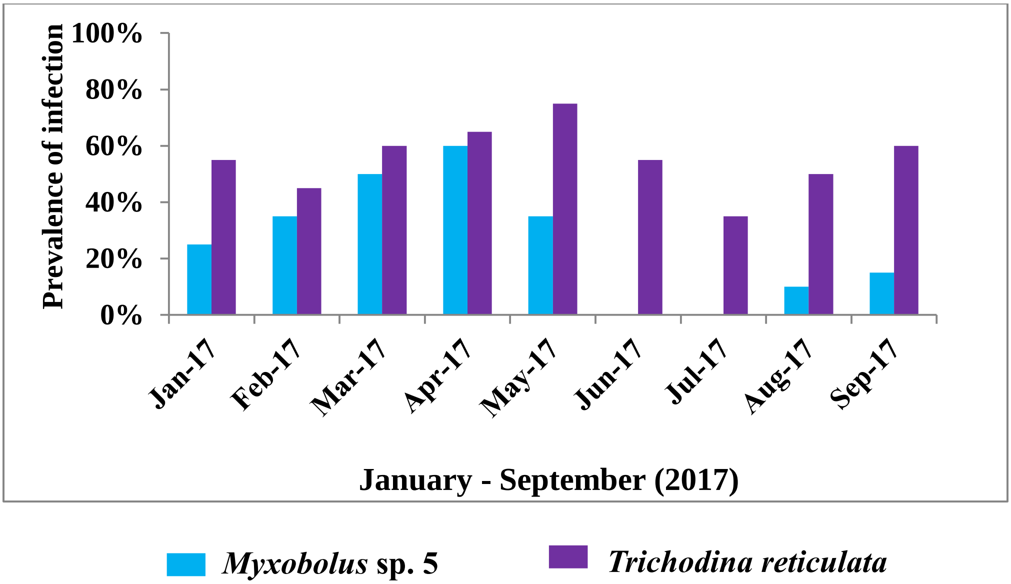


Figure. 6. Prevalence of protozoan infection in *Pangasius hypopthalmus*

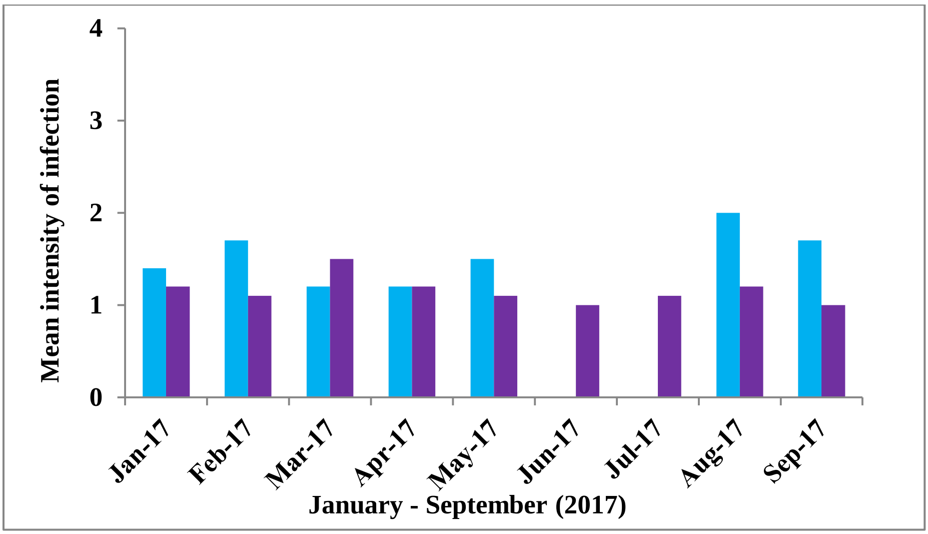


Figure. 7. Mean intensity of protozoan infection in *Pangasius hypopthalmus*

Discussion

In the present study, five parasites in *Labeo rohita*, three parasites in *Piaractus brachypomus* and two parasites in *Pangasius hypopthalmus* were recorded. One *Trichodina* species was found in each examined fish species in present study. In Myanmar, *Trichodina* spp were recorded in *Labeo rohita*, *Carassius auratus*, *Cyprinus carpio* and *Pangasius hypopthalmus* (Pa Pa Win, 2008; Thi Thi Thaw 2007; Khin May That, 2009 and Su Su Mon, 2014).

The genus *Trichodina* is the largest within the family Trichodinidae (Raabe, 1959). *Trichodina* spp. are the most famous and best known as ectoparasites of skin, fin and gill of the fish (Hoffman, 1998). They are typically found in aquaculture farms and sometime in natural water resources (Lom and Dykova, 1992). Although three *Trichodina* species were recorded in the present study, the intensity of infection was very low (range 1 -2). The impact of *Trichodina* species in the culture fish species will be low. However, since poor water quality enhance the production of *Trichodina* species, trichodinids become a problem in aquaculture if farmers do not maintain the water quality. Keeping the high water quality, feed residues and cleaning of the pool is very important in the control of *Trichodina* spp. (Ogut *et al*, 2005).

The shape and dimension of the *Myxobolus* spp. recorded in the present study was compared to those of other *Myxobolus* spp. Reported by Eiras *et al*., (2005). Moe Kyi Han (2007) recorded three species *Myxobolus* sp. in Rohu collected from That Yat Kone, Mandalay environ. Su Su Mon (2014) recorded two *Myxobolus* species in *Labeo rohtia* Yangon environ. In the present study, two *Myxobolus* sp was recorded in *Labeo rohita* and one species was found in *Piaractus brachypomus* and *Pangasius hypopthalmus.* Two *Myxobolus* species recorded in present are very similar shape and dimension of *Myxobolus* sp.1 and *Myxobolus* sp.2 reported by Moe Kyi Han (2007) and Su Su Mon (2014).

In the present study, one *Thelohanellus* sp. was recorded in *Labeo rohita.* The shape and dimension of *Thelohanellus* sp. found in this study were compared to those of some other species of genus *Thelohanellus* sp. reported by several authors (Lom and Dykova, 1992; Basu and Haldar, 2004; Moe Kyi Han, 2007; Su Su Mon, 2014). The shape and size of *Thelohanellus* sp. was similar to *Thelohanellus* sp. reported by Moe Kyi Han (2006).

Zschokkella sp. was found in *Labeo rohita* during study period. These myxozoans are primarily fish parasites usually infecting the gall-bladder of the hosts. *Zschokkella* sp. is very similar to *Zschokkella* sp. in *Labeo rohita* reported by Hnin Hnin Htay, 2014. Identification of myxosporeans so far described has been based on morphological variation in spore. Molnar *et al* (2002) stated that molecular biological methods such as DNA techniques including PCR might serve as an excellent tools for differentiation of morphologically similar species. DNA sequencing of recorded parasites should be conducted to identified the species as a future study.

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*., 2007, Yanagida *et al*., 2004). 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.

Five species of parasite were found in *Labeo rohita* but only three species in *Piaractus brachypomus* and two species in *Pangasius hypopthalmus* were recorded. Two reasons are considered; 1. Lay Daung Kan fish farm has been cultured for *Labeo rohita* more than 18 years and parasite and host has high relationship in that area than the other fish species and 2. *Piaractus brachypomus* and *Pangasius hypopthalmus* may have more disease resistance than *Labeo rohita*.

In conclusion, the data obtained from the present study are useful for fish culture. Protozoans cause disease outbreaks occur in cultured fish more than any other infection in intensive aquaculture systems can thus serious morbidity and mortality (Yokoyama, 2003). Studies on protozoan parasites in fish culture farms in Myanmar important to further develop aquaculture production.

Conclusion

Five parasites in *Labeo rohita*, three parasites in *Piaractus brachypomus* and two parasites in *Pangasius hypopthalmus* were recorded. Highest prevalence of infection was found in *Labeo rohita* while the lowest one is found in *Pangasius hypopthalmus.* 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.

Acknowledgements

I would like to thank Dr. Thida Lay Thwe, Professor and Head of Zoology Department for her useful advice. I would like to also knowledge Dr. Aye Mi San, Professor Department of Zoology, University of Yangon for her advice. The research is partially supported by USAID USAID's Myanmar Sustainable Seafood Project USAID-Burma-SOL-486-13-000012.

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EFFECT OF TEMPERATURE ON HATCHING AND SURVIVAL RATES OF NILE TILAPIA,*OREOCHROMIS NILOTICUS* (LINNAEUS, 1758)

Nay Lin Aung[1], Cho Cho Thin[2]

Abstract

Experiments were conducted to assess the effect of temperatures on hatching and survival rates of Nile tilapia, (*Oreochromis niloticus*). The present study was carried out at the Laboratory of Aquatic Bioscience Laboratory, Department of Zoology, University of Yangon from June 2017 to March 2018. The fertilized eggs were collected from the mouth of brood stock female tilapia and incubated at three different temperature treatments; 28ºC, 30ºC and 32ºC, each experiment was carried out with duplicate. For control, (ambient temperature, 26ºC) was used to compare with other temperature effects. The present results show that the longest incubation period was 39:02 hours at 26ºC and the shortest 21:51 hours at 30ºC. Incubation period decreased with increasing in incubation temperature. The optimum percentage of hatching rate was 75% at 30ºC, followed by 72% at 32ºC, 68.50% at 28ºC and 70.25% at 26ºC.Similarly, the survival rates were 98% at 26ºC, 97.2%at 28ºC and 97% at 30ºC and 32ºC respectively. The survival rates of fry decreased with the increasing of water temperature. The highest body weight fry (fish) was (4.6 ± 0.28) g at 32ºC and (3.03 ± 0.53) g at 26ºC (ambient) during the study period. Results of the present study showed that water temperature had significant ($P<0.000$) effect on hatching period. The temperature also has shown that it was significant ($P<0.03$) effect on growth. According to the present study, most suitable temperature for hatching the egg was 30ºC, while survivals rate was better at 26ºC. On the other hand, high body weight was recorded in fry maintained at 32ºC. The results of the present study found that temperature played an important role on the hatching and survival rates of Nile tilapia.

Keywords: Temperature, Hatching, Survival, Nile Tilapia

Introduction

Temperature is very essential for the egg formation, production and growth of fry in hatchery system. The effects of water temperature on growth

[1.] MRes Student, Department of Zoology, University of Yangon

[2.] Lecturer, Department of Zoology, University of Yangon

and development of fish have been well documented in Nile tilapia, shortnose sturgeon (*Acuoebser brevirastrum*), Atlantic sturgeon (*Acuoebser oxyrhynchus*) (Van Ham *et al*., 2003; Chatterjee *et al.*, 2004; Larsson and Berglund, 2005). Temperature is the main environmental factor governing the development of fish eggs (Nwosu and Holzlohnev, 2000). It determines certain morphological feature, hatching rate and the behaviour of larvae (Bagenal and Braun, 1978).

Physical and chemical parameters are known to affect the egg development. For example, temperature affects certain morphological features, hatching rate and larval behavior. In earlier studies, temperature influenced egg development and hatching in *Oreochromis niloticus* (Bhujel *et al*., 2000), *Tilapia zillii* (Omotosho, 1988), common carp, *Cyprinus carpio* (El-Gamal, 2009), and cod, *Gadus morhual* (Page and Frank, 1989; Geffen *et al*., 2006).

Optimal environmental conditions are essential to gain the best growth temperature. But due to excessive temperature on egg production and growth of fry hampered and the hatchery operator cannot produce in its optimal production. Thus, at higher or lower temperatures, feeding and growth rates are reduced, and at 20°C or less, feeding and growth are completely stopped (Caluton, 1982).

The climate models referenced by the Intergovernmental Panel on Climate Change (IPCC, 2001) predicted that global temperatures are likely to increase by 1.4 to 6.4°C in this century. So, the present study has been conducted to forecast the optimal temperature on hatching, growth and survival of Nile tilapia.

The world’s total tilapia production in 2010 was 3.49 million tons (FAO, 2012).Culture of this species has expanded rapidly under a wide range of farming environments from extensive to intensive scale in both fresh and brackish water in Asia and many other countries of the world. They can also feed on locally available foods since they are herbivorous and omnivorous, and are resistant to diseases (Beveridge and McAndrew, 2000).

The "Miracle Fish" *Oreochromis niloticus,* is one of 77 tilapia species described by Thys in 1968 and belongs to the Family Cichlidae of the Tribe *Tilapiine* (Thys, 1968 and George, 1955). It is indigenous to Sudan among other tilapia species namely, *Tilipia zillii* and *Sarotherodon galilaeus* (George, 1955 and Sandon, 1950). Tilapia is a worldwide fish of great commercial importance and it is recognized as one of the most important aquaculture species of the 21st century. Tilapia is currently ranked second only to carps in global production (Ridha, 2006).

The tilapia species are preferred species for aquaculture because they exhibit tolerance to harsh water conditions such as temperature changes, high salinity and low water quality (Maluwa and Brood, 1996).Tilapia was first introduced to Malawi in 1999 in order to develop the enormous geothermal water resources in the south of the country. Their tropical and subtropical origins are clearly reflected in their thermal preference these fish do not grow well at temperature below 16°C and cannot usually survive for more than a few days below 10°C (Chervinski, 1982), but they are remarkably tolerant to high temperature, up to 40°C (Azaza, 2004).

The Nile tilapia is a mouth-brooder. Mouth brooding is an advanced reproductive tactic, a form of intensive care by mother whereby the seed can be protected from the outside world until their development is more advanced. The male establishes a territory and builds a round nest in the pond bottom. (Usually the diameter of a nest is 30-60 cm. The size of the nest is correlated to the size of the male.) The female enters the nest and lays the eggs. The eggs are fertilized by male. The female then collects and incubates the eggs in her mouth. The eggs are yellow in color. Eggs hatch after about five to seven days. After hatching the fry remain in the mouth of the female for another 4-7days. Female spawns every four to six weeks interval, but may spawn sooner if the eggs are removed. The number of eggs per spawning is related to the size of the female. A female of about 100 grams may produce approximately 100 eggs per spawning while a female weighting 100-600 g can produce 1,000-1,500 or more per spawning (Hepher and Prugunin, 1981).

Brummett, (1995) found that Nile tilapia did not lay eggs when water temperatures went down below 19°C. The most productive period coincided with the rise in water temperature to 2-27°C where spawning rate averaged 40 and 73% of total females under dark and natural photoperiod conditions, respectively. Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758), is one of the most commonly farmed species in freshwater aquaculture. The species is cultured extensively by small holder farmers and commercial intensive operators in Myanmar.

Tilapia species are favored by consumers for white meat good flavor, shiny appearance and bigger size. The species that has been overfished from the major water quantities is necessary to meet both market demand for consumption and restocking purposes (Kaunda *et al*., 2005).So, the present study was chosen the tilapia fish species to test the temperature effect on hatching, survival and growth rate.

Due to above mentioned reasons; the present study has been carried out with the following objectives:

- to determine hatching period and hatching rates at different temperatures
- to find out the survival rates at different temperatures
- to carry out the optimum temperature for hatching and survival rates

Materials and Methods

Study site

Fertilized eggs of female tilapia were collected at the Hlaw-ga fish farm, Htauk Kyant Township, Yangon (Plate 1. A). After collecting the eggs from the mouth of fish from Hlaw-ga Fish Farm. The following experiments were conducted in the Aquatic Bioscience Laboratory, Department of Zoology, University of Yangon.

Study period

Study period lasted from June 2017 to March 2018. Laboratory experimental period was conducted for duration of six months from August 2017 to January 2018.

Materials

The following materials were used in the present study.

1. Aquarium jar tanks (120cm×60cm×45cm)
2. Handmade incubation jar (1.5Litre)
3. Thermostat for controlling temperatures (SOBO AC-220V 50/60Hz 200W)
4. DO meter
5. pH meter
6. SOBO aquarium top filter (WP-1800F, AC220-240V 50/60Hz, 25W)

Methods

This experiment was conducted using the following methods.

Identification and classification of the studied fish species

The studied species was identified and classified according to Fish Base, (2013).

Preparation of the tanks, incubation jars and installation the thermostat

Glass aquaria were cleaned with the sodium chloride and filled with water (12inches depth). A total of four glass aquaria (120cm×60cm×45cm) were prepared to observe the effect of different temperatures on hatching duration of fish (Plate 3.A). To imitate the McDonald jar, the incubation jars were prepared with Coca Cola plastic bottle (1.5L), piece of net, PVC pipe and SOBO aquarium top filter (WP-1800F, AC220-240V 50/60Hz, 25W) (Plate 3. B).Water entered each incubation jar through a vertical tube that caused an up-welling effect to maintain the eggs suspended. This effect imitates similar to the natural process in the buccal cavity of a female during the incubation process. Tilapia eggs are negatively buoyant. And then, thermostat (SOBO AC-220V 50/60Hz 200W) was installed into each aquarium to control the water temperature.

Sample collection

Fertilized eggs were directly collected from the mouth of brood stock female tilapia and the eggs were counted with plastic siphon pipe in small size of beakers (Plate 1.B and C).

Incubation of eggs at different temperatures

The eggs were transferred into the incubation jars (round plastic container) (1.5L). The jar stocked with 200 eggs were set up in the aquarium tanks. Four set of aquaria tanks were prepared, replicated twice (Plate 2) and incubated the eggs (Plate 3. F). Four different water temperatures; 26°C (ambient), 28°C, 30°C and 32°C were set up in the aquarium to assess the effect of temperature on hatching and survival rates using thermostat(Plate 3. C). Thermometer was installed in the aquarium and checked the temperatures twice a day to maintain the constant temperature. Water parameter such pH and DO were taken and recorded (Plate 3. D and E). Stability of four different water temperature of respective aquarium was checked twice a day 8:00am and 11:00pm in separate tank.

Data analysis

The hatching, survival and growth rates of the studied species were determined using the following formulae according to (El-gamal, 2009 and Naeem *et al*., 2011).

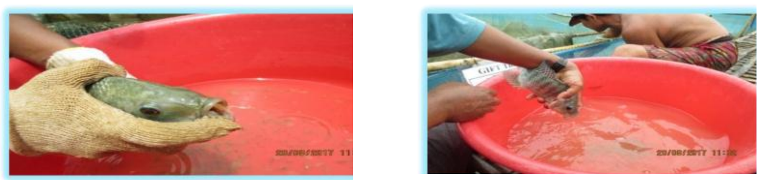
(i) $$\text{Hatching rate (\%)} = \frac{\text{No. of hatched larvae}}{\text{Fertilized eggs}} \times 100$$

(ii) $$\text{Survival rate (\%)} = \frac{\text{Survived hatchling after 3 days}}{\text{Total no. of hatched eggs}} \times 100$$

Significant difference of incubation period, hatching rate, survival rate and body weight among the various water temperatures were tested using one-way analysis of various (ANOVA), SPSS version 2.5. Multiple comparison test (HSD) was also performed.

A. Hlaw ga Fish Farm (Department of Fisheries)

B. Maintaining brood stocks in controlled tanks



C. Collecting the fertilized eggs from mouth of brood stock female

Plate 1. Hlaw-ga Fish Farm and taking the fertilized eggs from brood stocks female

A.Temperature at 26°C (ambient) B. Expt. Temperature at 28°C

C. Expt. Temperature at 30°C

D. Expt. Temperature at 32°C

Plate 2. Experimental aquarium with different water temperatures

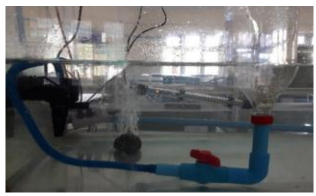
A. Preparation of the tanks

B. Handmade incubation jar

C. Thermostat

D. DO meter

E. pH meter



F. Incubating of the eggs

Plate 3.Setting up the Equipment

Results

Systematic position

The systematic position of the species was identified according to FishBase, (2013). (Plate 4)

Phylum	- Chordata
Class	- Vertebrata
Sub-phylum	- Actinopterigii
Order	- Cichliformes
Family	- Cichlidae
Genus	- *Oreochromis*
Species	- *niloticus*

Morphological characters

Body shape generally laterally compressed to oval and deep, through variable depending on the environment. D XVI-XVIII, 12-14, A III, 9-11. Lateral line interrupted with 30-34 cycloid scales. Mouth terminal. Gill rakers on lower limb of first gill arch 20-26. Vertebrate 30-32. Caudal fin has 7-12 distinct vertical stripes. Breeding males with red flush to head, lower body, dorsal and caudal fins. Caudal fin truncate. The most diagnostic features are the regular and definitive stripes on the caudal fin, the red flush of the breeding male and the dark margin of the dorsal fin. Body coloration varies depending on environmental, physiological and dietary factors (Plate 4).

There were clear differences between the sexes and tilapia species particularly in a round of the urogenital opening. The tilapia of the male tends to be elongated with one opening. The papilla of the female tends to be wider and has to opening, one of which is a transverse slit (Plate 5)

Incubation period and hatching rate of Nile tilapia at different temperatures

The influence of temperature on incubation period and hatching rates of *Oreochromis niloticus* was shown in Fig. 1 and 2 respectively. The mean values of incubation period were also described in Table 5. The longest incubation period was 39:02:30 (hours) at 26°C (ambient) (Table 1).The shortest incubation period was 21:51 (hours) at 30°C (Table 3). The results of this study also revealed that incubation period was38:48:30 (hours) at 28°C

(Table 2) while it was 22:05 (hours) at 32°C (Table4).In the present study, the incubation period was significant at 26°C(ambient) and28°Ccompared with 30°C and 32°C(p<0.000). It is also significant at 30°C and 32°C compared with 26°C and 28°C (p<0.000). There was significant different between incubation period and temperatures (p<0.000) (Appendix II).

The highest hatching rate was highest75% at 30°C (Table 3) and lowest68.5% at 28°C (Table 2). The hatching rates were 70.25% at 26°C (ambient) and 72% at 32°C (Table 1 and 4).

Variation in Survival rate of Nile tilapia at different temperature

In the present study, the mean values of survival rates were recorded in Table 5. The effect of different temperatures on survival rates was shown in Fig.3. The temperature treatment of 26°C (ambient) showed the highest survival rate of 98% (Table 1).The survival rates were also recorded 97.20 % at 28°C(Table 2) and 97% at 30°C and 32°C (Table 3and 4).

Variation in Body weight of Nile tilapia at different temperatures

After one month of experimental period, wet body weights of Tilapia were measured from each experimental tank. Among different temperatures treatments, the mean values of body weight were varied (Table 5). The effect of different temperatures on body weights was shown in Fig. 4. The highest fish body weight was observed; (4.6 ± 0.28) g at 32°C (Table. 4) and the lowest; (3.03 ± 0.53) g at 26°C (Table 1). The fish body weight was also recorded (3.38 ± 0.46) g at 28°Cand (4.1 ± 0.28) g at 30°C (Table 2 and 3).In the present study, the body weight was significant effect at 26°C compared with 32°C (p<0.037).

Plate 4. External features of Nile tilapia (*Oreochromis niloticus*)

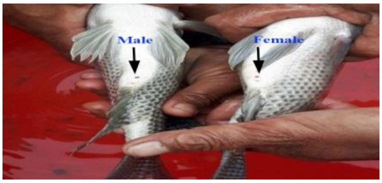


Plate 5. Male and female of Nile tilapia (*Oreochromis niloticus*)

Table 1. Biological parameter of Nile tilapia at 26°C (control)

Sr. No	Biological parameter	Replication		Mean±SD
		1st	2nd	
1	Fertilized eggs	200	200	
2	Incubation Period(hours)	39:20	38:30	38:55±0.025
3	Hatching rate%	53.50	87	70.25±0.237
4	Fry Survival rate%	98	97.7	98±0.002
5	Body weight	3.4	2.65	3.03±0.53

Table 2. Biological parameter of Nile tilapia at 28°C

Sr. No	Biological parameter	Replication		Mean±SD
		1st	2nd	
1	Fertilized eggs	200	200	
2	Incubation Period(hours)	39:07	38:30	38:48±0.018
3	Hatching rate (%)	62.5	74.5	68.5±0.085
4	Fry Survival rate (%)	98.4	96	97.2±0.017
5	Body weight	3.7	3.05	3.38±0.46

Table 3. Biological parameter of Nile tilapia at 30°C

Sr.No	**Biological parameter**	**Replication**		**Mean±SD**
		1st	2nd	
1	Fertilized eggs	200	200	
2	Incubation Period(hours)	21:30	21:12	21:51±0.04
3	Hatching rate (%)	62	88	75±0.184
4	Fry Survival rate (%)	96.8	96.6	96.7±0.001
5	Body weight	4.3	3.9	4.1±0.28

Table 4. Biological parameter of Nile tilapia at 32°C

Sr.No	**Biological parameter**	**Replication**		**Mean±SD**
		1st	2nd	
1	Fertilized eggs	200	200	
2	Incubation Period(hours)	21:18	22:53	22:05±0.05
3	Hatching rate (%)	52	92	72±0.283
4	Fry Survival rate (%)	96.2	97.3	96.75±0.008
5	Body weight	4.8	4.4	4.6 ±0.28

Table 5. Mean values of biological parameter in Nile tilapia at different temperatures

	Temperature 26°C	**Temperature 28°C**	**Temperature 30°C**	**Temperature 32°C**
Incubation Period(hours)	38:55±0.025	38:48±0.018	21:51±0.04	22:05±0.05
Hatching rate (%)	70.25±0.237	68.5±0.085	75±0.184	72±0.283
Fry survival rate (%)	98±0.002	97.2±0.017	96.7±0.001	96.75±0.008
Body weight	3.03±0.53	3.38±0.46	4.1±0.28	4.6 ±0.28

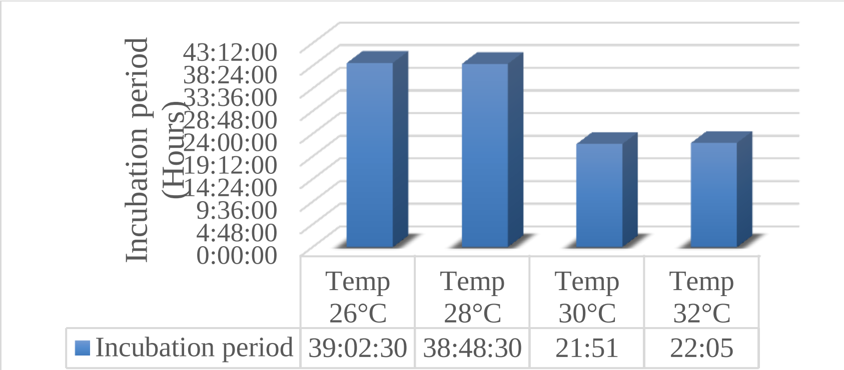


Figure 1. Variation of the incubation period of *Oreochromis niloticus* at different temperatures

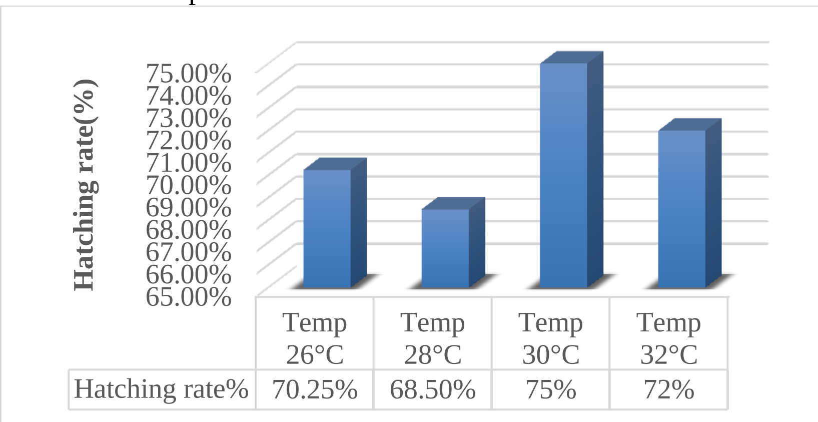


Figure 2. Variation of the hatching rate of *Oreochromis niloticus* at different temperatures

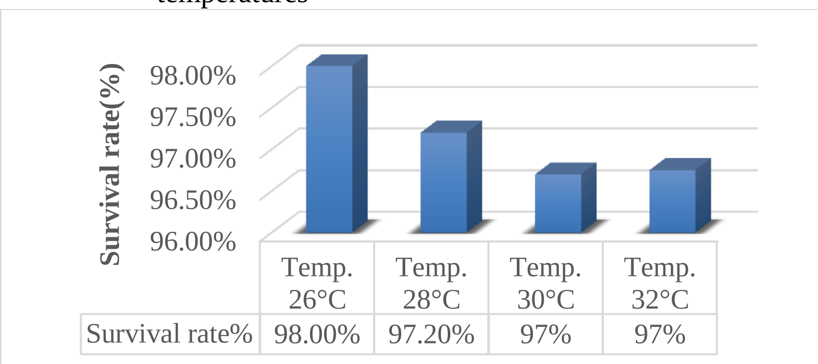


Figure 3. Variation of the survival rate of *Oreochromis niloticus* at different temperatures

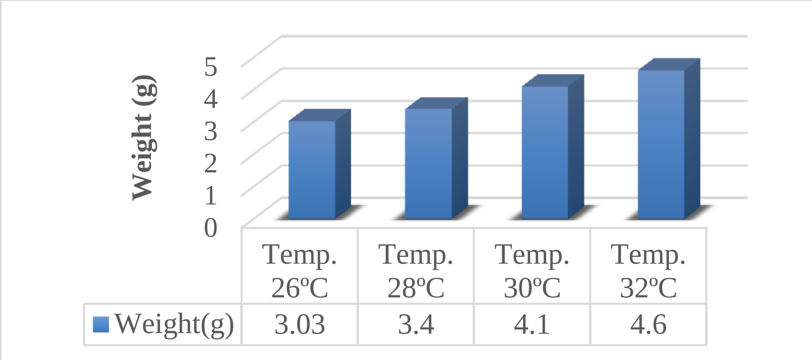


Figure 4. Final body weight of *Oreochromis niloticus* at different temperatures

Discussion

Temperature exerts a major influence on the biological activity and growth for aquatic organisms. It has also effect on the incubation and hatching rates of eggs. Incubation temperature rather than pond water temperature. It is important in hatching success. Development and hatching of fish is delayed at low temperatures and accelerated at high temperatures (Peters, 1983). Tilapia eggs tend to sink in water and when in permanent contact with a substrate, the embryo suffers and will eventually die. So, air diffuser was placed directly below the incubation jars to create an up-welling effect. During incubation, continuous water flow is essential for preventing accumulation of waste products and allowing gas exchange between the egg and the surrounding water. Continuous motion also appears to be necessary for successful hatching for some species of fish. Proper water flow also reduces mechanical abrasion (Green, 2006).

Oreochromis niloticus is known to tolerate high water temperatures. It can tolerate for a long period low water temperature between 10°C and 15°C (Ballarin and Hatton, 1979), and does not survive below 10°C (Caulton, 1982). Thus, at higher or lower temperatures, feeding and growth rates are reduced, and at 20°C or less, feeding and growth are stopped (Caulton, 1982).

The fertilized eggs of tilapia were incubated in an experimental hatchery system at different temperature in the present study. The embryonic development of eggs, hatchability, survival and wet body weight of studied species were investigated in this study. The present results show that the incubation period was (38:55) hours at 26°C (ambient temperature), (38:48) hours at 28°C,(21:51) hours at 30°C, and (22:05) hours at 32°C respectively. The incubation period increase with decreasing in incubation temperatures in the present study. There was a significant difference between incubation period and temperatures ($p<0.000$). Similar results were observed in Common carp, *Cyprinus carpio* (El-Gamal, 2009) and *Oreochromis niloticus* (Bhujel *et al.*, 2000) where hatching period decrease with increase in incubation temperature. Peng and Tang (1988) also indicated that a lower temperature could longer the time of hatching of *O. barbatulum.* Korwin-Kossakowski (2008) proposed that lower water temperature delay embryo growth, resulting in a decreased hatching rate, and fry postponed foraging. The present finding was in agreement with the results obtained from *Cyprinus carpio* (El-Gamal, 2009).

In the present work, the maximum percentage of hatching rates was (75 ± 0.18) % at 30°C. The minimum percentage of hatching was (68.5 ± 0.09) % at 28°C. The results in this study found that the optimum temperature for hatching rate of fertilized eggs was at 30°C. This finding was in agreement with the results obtained by in other fish species, Nwosu and Holzlohnev, 2000. Similar results were also observed in *O. niloticus.* It has an optimum temperature of growth at 30°C and *Tilapia zilli* has an optimum feeding temperature at 31.4°C (Stickney, 1986).

In case of a proper increase in the water temperature usually enhances the metabolism, respiration, and growth rate, shortens sex maturation period. It also reduces the high mortality of fry in the planktonic stage, and increases the fry survival rate (Pankhurst and Munday, 2011). Pandit and Nakamura (2010) reported that high water temperature above 32°C significantly reduced the survival, growth and feeding efficiency of Nile tilapia. *O. niloticus* is known to tolerate high temperatures, up to 40 - 42°C (Philippart and Ruwet, 1982). However, the optimum temperature for feeding, growth and reproduction is between 26°C and 30°C (Hauser, 1977). The survival rate was 98% at 26°C, 97.2% at 28°C, 97% at 30°C and 97% at 32°C respectively in the present study. The present study showed that the survival rate decreases with increases in water temperature. The cannibalism was found among unequal sizes of fishes in experimental tanks. The mortality in fish is considered due to cannibalism. Similar result Dynamics of pond aquaculture found that at the age of 10-30 days was cannibalism (Hillary and Claude, 1997).

In fish biology, temperature influence is the single most important factor that determines growth rates in fish (Brett *et al.,* 1975). Studies on several fish species have revealed that in the temperature ranges tolerated by fish, growth rates increase with increasing temperature, showing a parabolic pattern (Xiao-jun and Ruyung, 1992; Larsson and Berglund, 2005). When experimental temperature reached the upper extreme limit of the tolerance range, performance of growth decreased. This depression of growth is due to the higher energy cost for maintenance metabolism and seems to be related mainly to a loss of appetite (Azaza *et al.,* 2008). Results of our study showed that performance of body weight is better at 32°C (4.6 ± 0.28) g than at 26°C (ambient) (3.03 ± 0.53) g, which confirms the thermophilic character of *O. niloticus.* Water temperature also found to have a significant ($P<0.03$) effect on body weight of fry. El-gamal (2009) reported that growth of larvae increased at the optimum temperature rate of 27°C-30°C and no eggs hatched at 20°C and 38°C for *Cyprinus carpio*.

The optimum temperature for incubation period and hatching rate was found at 30°C, while the survival rate at 26°C and body weight at 32°C. So, this study provided an insight into the thermal effect on developmental stages, hatching rates, survival rates and body weight of Tilapia fish species.

Conclusion

The experiments were conducted to find out the hatching, survival and growth of Nile tilapia at different temperatures of 28°C, 30°C, 32°C and ambient water temperature with 26°C. It was found that the water temperature increase, the incubation period will increase. Significant difference was detected among the temperatures and incubation periods (P<0.000) and also between temperatures and growth (body weight), (P<0.03). Incubation period was significantly different at the temperatures of 26°C versus 30°C and 32°C. It was also significant between the temperature of 28°C versus 30°C and 32°C. In the case of growth (body weight), it was significant only between the temperature of 26°C and 30°C. Hatching rate and survival rate were not significantly difference at the temperature tested at ambient temperature of 26°C.

Acknowledgements

Firstly, I would like to express my gratitude to Dr. Thida Lay Thwe, Professor and Head of Department of Zoology, University of Yangon, for her permission to work in this interesting topic and suggestions. I am indebted to Dr. Aye Mi San, Professor, Department of Zoology, University of Yangon, for her interest and encouragement. Special thanks go to Dr. Sann Aung, Advisor/Fishery Specialist, Ministry of Livestock, Fishery and Rural development, Nay Pyi Taw for his invaluable help in this research work. I also wish to express my gratitude to Dr. Kyu Kyu Win, Professor, Department of Zoology, University of Lashio. My thanks are due to Associate Professor Dr. Kay Lwin Tun, Department of Zoology, University of Yangon, for her valuable suggestion during the study. My profound gratitude goes to Dr. Cho Cho Thin, Lecturer, Department of Zoology, University of Yangon, for her supervision, suggestions and guidance throughout the study period. I sincerely thank to all staffs from Hlawga Hatchery, Department of Fisheries. Lastly, I would like to give my special thanks to my grandparents for their moral and financial support throughout this work.

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LARVAL DEVELOPMENT OF BLACK SEA URCHIN, *DIADEMA SETOSUM* (LESKE, 1778)

Naing Naing Oo[1], Hua Thai Nhan[2]

Abstract

Diadema setosum is one of the common marine forms of echinoids widely distributed in the Indo-West Pacific Ocean, where it occurs from the Red Sea, Persian Gulf and the east coast of Africa to Japan, Australia and Malaysia. The sea urchin aquaculture is mainly based on the production of marketable gonads, which are valuable seafood product in Asian and European markets. The investigation on the developmental basis of morphological changes in larvae of *D. setosum* was conducted in a controlled Wet laboratory at the College of Aquaculture and Fishery (CAF) of Can Tho University, Vietnam, during July to August, 2017. The fertilized eggs of sea urchin were obtained by the induced spawning. Induced spawning was done using 1.5 to 2 ml of 0.5 M Potassium Chloride (KCl,) on their oral (mouth) surface. The daily larval developmental stages were studied in the wet laboratory from 10 days after fertilization to 49 days after fertilization. During investigation, the larvae reached metamorphic competence within 43 days after fertilization. This study represented the successful investigation on larval and early juvenile development of *D. setosum*. The findings would be helpful towards the development of breeding and seed production techniques for aquaculture of sea urchins.

Keywords; sea urchin, *Diadema setosum*, induce spawning, larval development

Introduction

Sea urchins are members of the phylum Echinodermata, They are typically spiny, globular animals, echinoderms in the class Echinoidea. About 950 species inhabit all oceans and zones from the intertidal to 5,000 meters (16,000 ft) deep (Animal Diversity Web, 2012). Sea urchins can be found in all climates, from warm seas to polar oceans (Andreas, 2010*).*

[1]. Dr., Associate Professor, Zoology Department, Kyaing Tong University
[2]. Dr., Lecturer, College of Aquaculture and Fisheries, Can Tho University, Vietnam.

Diadema setosum (Leske, 1778) is one of the common echinoids widely distributed in the Indo-West Pacific Ocean, where it occurs from the Red Sea (Gulf of Suez, Gulf of Aqaba, Northern and Southern Red Sea), and the east coast of Africa to Japan and Australia (Lessios *et al*.,2001). In Myanmar *D. setosum* are called Than Pa Chup and they are found along the coastal region especially Bouldern Island at Myeik (Mergui) Archipalago, Adman Sea and Rakhine coastal region.

This species is nocturnal and commonly observed around reefs and shallow rocky habitats (1–6 m depth), where it hides in crevices and under overhangs by day, and forages at night, at a distance of a few meters away from its daytime hideout. There is no external morphological difference between male and female sea urchins. The sex can be determined only after gamete shedding has begun or following biopsy of the gonads (Hinegardner 1967, 1975).

Several months are needed for the larva to complete its development; the change into the adult form beginning with the formation of test plates in a juvenile rudiment which develops on the left side of the larva, its axis being perpendicular to that of the larva. Soon, the larva sinks to the bottom and metamorphoses into a juvenile urchin in as little as one hour (*Ruppert, et al.*, 2004). In some species, adults reach their maximum size in about five years (*Barnes, 1982*) The purple urchin becomes sexually mature in two years and may live for twenty years (Alisa , 2001*)*.

Sea urchins are important objects of research in different fields of biology, ecology, biodiversity and aquaculture. At the same time, they are used as raw material to produce foodstuff, in particular, the product of processing gonads known as "Sea urchin Roe or Uni" (Kaneniwa and Takagi, 1986; Oshima *et al*., 1986; Ichihiro, 1993). The roe is considered a prized delicacy in Asia, Mediterranean, and Western Hemisphere countries such as Barbados and Chile (Lawrence *et al*., 1997).

People of the Asian Pacific Region have also used sea urchin gonads for many years as a remedy for improving general body condition, treatment for a number of diseases and strengthening of sexual potency of men (Yur'eva *et al.*, 2003). Gonads of sea urchins have long been a luxury food in Japan (Shimabukuro, 1991).

Sea urchin is one kind of seafood that has high nutritional value and beneficial effects on health. Their gonads are also rich in valuable bioactive compounds, such as polyunsaturated fatty acids (PUFAs) and β-carotene (Dincer and Cakli, 2007). PUFAs, especially eicosapentaenoic acid [EPA, C20:5) (n-3)] and docosahexaenoic acid [DHA C22:6 (n-3)], they have significant preventative effects on arrhythmia, cardiovascular diseases and cancer (Pulz and Gross, 2004). On the other hand, the high levels of arachidonic acid (AA) and EPA recently detected in *D. setosum* supported the development of aquaculture of this urchin (Chen *et al*., 2010), since PUFAs are important for human nutrition (Lawrence, 2007).

According to the importance of *D. setosumin* in the field of nutritional food and medicine, the information of the early life history is also important for enhancing large scale seed production, culture and management. Therefore, the present study was conducted to study the detailed larval and early juvenile development of *D. setosum* in a captive lab-rearing condition.

Materials and Methods

Study site

The present study was conducted in a controlled wet laboratory at the Collage of Aquaculture and Fishery (CAF) of Can Tho University, Vietnam.

Study Period

The recent study was lasted from July to December, 2017.

Specimen collection

The matured urchins *Diadema setosum* was collected from Kien Giang Province, Viet Nam during their natural breeding season in July. The collected specimens were transported to the Laboratory of CAF and then maintained in the recirculation system with well aerated sea water at salinity of 30 ppt.

Induced spawning

Induced spawning was done by injection of 2.0 ml of 0.5 M KCl (Potassium Chloride) into the coelomic cavity from the oral disc. About 10-15 min after injection, fertilized eggs were siphoned via scoop net (125 µm) then waste carefully 2-3 times news clean seawater. Then fertilized eggs were placed into the aerated larval rearing tank.

Larval rearing

Larval densities up to the four-armed pluteus stage were maintained at 2–3 individuals/ml, following the methods described by Rahman *et al*., 2000, 2005, 2012b. When the larvae attained feeding stage (four-armed pluteus), they were cultured in the same system with a larval density of 1 individual/ml. Larvae were supplemented with a laboratory cultured phytoplankton, *C. calcitrans* at concentrations of 6,000-8,000 cells per ml (Rahman *et al*., 2000). All the developmental stages of larva were daily observed from the pluteus larval stage until they reached metamorphic competence.

The morphometric measurements of ten individual larvae were made on freshly prepared specimens. After that, it was observed and finally measured and photographed under the compound microscope.

(A) Preparation for induced spawning

(B) Algae culture for larval feed

(C) Larval rearing tanks

(D) Larval and juvenile rearing tanks

Plate 1. Wet laboratory, induced spawning and larval rearing tanks

Results

Systematic position of *Diadema setosum*

Kingdom: Animalia
Phylum: Echinodermata
Subphylum: Echinozoa
Class: Echinoidea
Subclass: Euechinoidea
Super order: Diadematacea
Order: Diadematoida
Genus: *Diadema*
Species: *setosum*

Diadema setosum (Adult)

Larval development

The present study was observed from late four arms pluteus larva at 10 day after fertilization (daf). Two long post oral arms and two short anterolateral arms were prominent at this stage. Mouth, oesophagus and stomach were prominent (Plate 3A). The anus was formed in the lower half of the aboral side. Post oral arms (POA) elongated larval stage was recorded from 12 daf to 14 daf (Plat 3B). In this stage red pigmentation occurs throughout the body especially more concentrated around the oral hood and at the tip of the arms. The rest of the body was transparent. Basal portion of the larval was nearly pointed. Arms muscle visible at the near of stomach, arms skeleton (fenestrated rod) prominent and the larval was moveable using with them. Body transverse rod was prominent at the aboral surface. At 14 daf the beginning of larval arms degeneration was occurred in recent study.

During the three weeks after fertilization (15–21daf), the larval development was continuous observed. Body transverse rod was more prominent and become rounded and more pigmentation. Basal portion of the larval was gradually blunt and enlarged to form the body rod. All of the arms were gradually degenerate. Adult rudiment was visible at the left side of the larval body. Ventrolateral process and body transverse rod were more prominent. The appearance of the larva was not changed, except for the arms degeneration until the 18 daf. At 19 daf arms become shorter and the tip becomes the club shape. The pigments were concentrated in dark at the tip of the arms. Larval skeleton was visible on both aboral and oral sides at third week after fertilization (Plate 3C).

During four weeks after fertilization (22-28 daf), the premature larval stage was recorded. The pigment spot more accumulated on the body during this stage. Stomach was become folded and contracted during the study period. Long arms tip were continuously degenerate together with the arm epidermis and pigmented sport was more concentrated in this area. Short arms were completely reduced as appear only the oral hood of mouth. Body rod was more prominent and the basal portion with the pigmented arches. Larval skeletons were appeared on the mouth and body. Larval spicules were visible inside the larval body. Body rod was prominent instead of basal disc. Larval skeleton was more prominent on the lower half of the aboral portion (Plate 3D).

The arms absorption was continuously occurred during five weeks after fertilization (29- 35 daf). Short arms become reduced together with larval mouth tissue. Larval tissue began to regress from the mouth and other parts of the body, forming a globoid structure. The pigments become concentrated around the oral hood and at the end of the arms. The rest of the larva body was transparent. Body transverse rod and body rod were more prominent and rounded. Fusion of these two portions was resembled nearly global shape (Plate 3E).

Competent larva was recorded within the sixth week after fertilization (36 -42 daf). Star shape muscles were prominent on the oral hood in this stage. Adult body rudiment growing and gradually accumulation of larval tissue on the rudiment was appeared. Arms become more and more short and body of larval size was small because of larval tissue resorption was formed in this stage. The arms degenerated muscles were accumulated at the base of the arms and muscle of arms were more prominent. Furthermore a pair of muscle fiber was occurred that attached to the posterior end of the stomach. Arms rods and epidermis were together receded toward the body. The larval arms and tissue degeneration was nearly completed during this week (Plate 4A). In this stage, pedicelleriaes were emerged and larval body was started to circle from the mouth as center point to form the adult globoid body and tissue more compact on the adult rudiment.

The arms were completely degenerated and the larva started metamorphosis to build up the juvenile form during seventh week after fertilization (43-49 daf). The accumulation of resorptive arms muscle was found as dark color portion on the each side of the larval body. Mouth opening with five radial discs and anus were more prominent (Plate 4B). In this stage the larval movement was more rapidly rotated around the body side to form the adult globoid and compact body. The larval size was too small about 5µm in diameter. Larval was appeared pentagonal shape and purple pedicellariae were emerged around the body in 44 days after fertilization. At the same time, larval movements were slow and start to settle at the bottom to do the metamorphosis into juvenile form. At the 45 days after fertilization, larval body shape was more rounded. The body surface of larval had a lot of prominent purple pedicelleriaes and translucent tube feet were occurred at the end of this stage (Plate 4C). Oral disc more prominent and body was formed the globoid shape like the adult form except the long spines. Larval was absolutely settle at the bottom and gradually do the metamorphosis into Juvenile stage at the end of this week (Plate 4D)

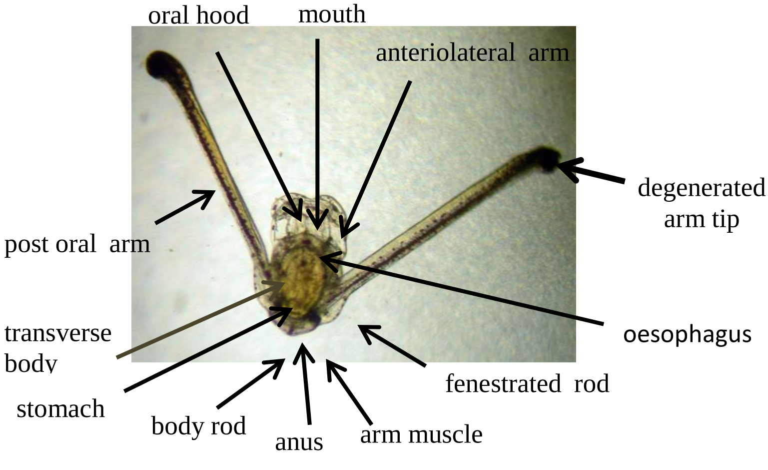
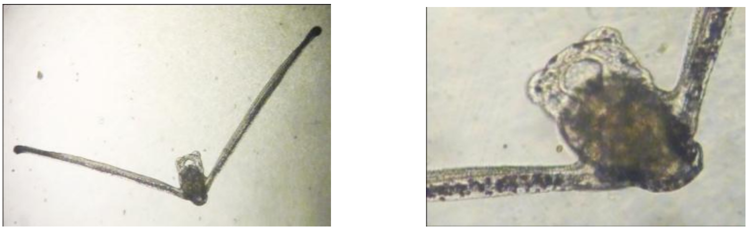
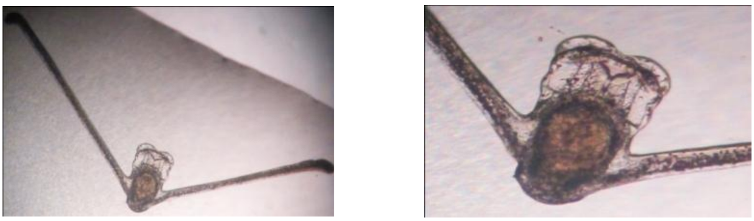


Plate 2. Distinctive characters of the larva (aboral view) (32daf)

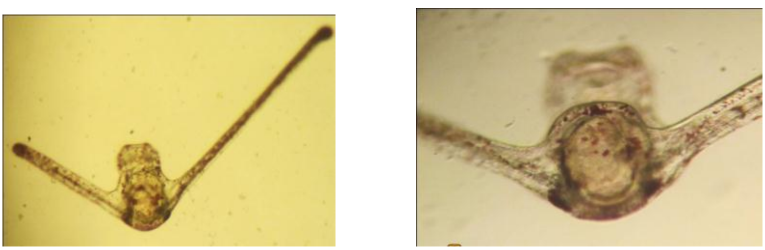
(A) Late four arms pluteus larva (40X) (B) Two weeks old larva (100X)



(40X) (C) Three weeks old larva (100X)

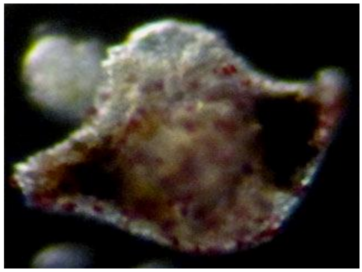


(40X) (D) Four weeks old larva (100X)

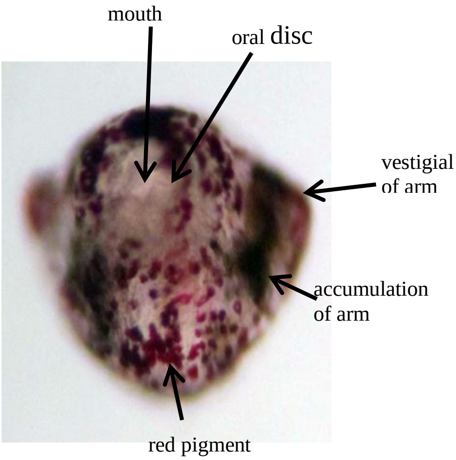


(40X) (E) Five weeks old larva (100X)

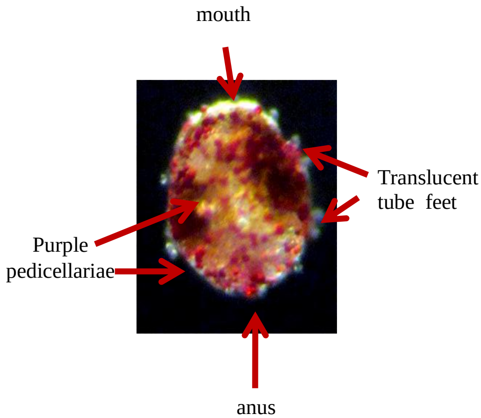
Plate 3. Weekly developmental stages of sea urchin (*Diadema setosum*) larval



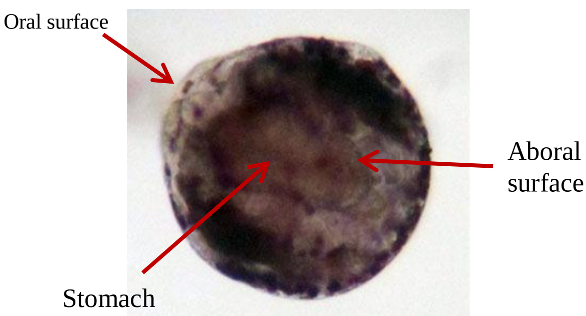
(100X) (A) Six weeks old (100X)



(B) 43days old metamorphosing larva (100x)



(C) 45days old metamorphosing larva (100x)



(D) 49 days old juvenile (100x)

Plate 4. Developmental stages of sea urchin larval to start of juvenile

Measurements of sea urchin larvae

In present study, the total lengths of larvae were highest in number (952.85 ±138.77µm) during three weeks after fertilization, follow by two weeks (904.28± 147.40 µm), four weeks(627.14 ± 169.38 µm) and five weeks (522.85 ± 147.84 µm) old larval respectively. After five weeks old larval stage, total lengths of larvae were quickly slowdown until the end of larval stage. Therefore the lowest numbers of larval length were recorded in six weeks (58.5 7 ± 12.14 µm) after fertilization.

The body lengths of larvae were also recorded in highest numbers during two weeks (101.42 ± 8.99 µm) after fertilization, followed by three weeks (98.57 ± 12.14 µm) , four weeks (94.28 ± 9.75 µm) and five weeks (91.42 ± 10.69 µm) old larvae respectively. The lowest numbers of larval body length were observed during seven weeks (5.85 ± 1.21 µm) after fertilization.

During the study period, the body widths of larvae were a little changed during the two (51.42 ± 6.90 µm) to three weeks (50 ± 5.77 µm) old larval stages. The larval widths were not changed until the end of larval stage (50 ± 0 µm).

Table 1. Measurements of sea urchin larvae during study period

Period (daf)	Larval stage	Total length (µm)	Body length (µm)	Body width (µm)
14 daf	POA elongated stage-1	904.28± 147.40	101.42 ± 8.99	51.42 ± 6.90
21 daf	POA elongated stage-2	952.85 ±138.77	98.57 ± 12.14	50 ± 5.77
28 daf	Pre-competent	627.14 ± 169.38	94.28 ± 9.75	50 ± 0
35 daf	competent	522.85 ± 147.84	91.42 ± 10.69	50 ± 0
39 daf	metamorphosis	258.57 ± 121.16	77.14 ± 4.87	50 ± 0
44 daf	Early juvenile	58.5 7 ± 12.14	58.57 ± 12.14	50 ± 0

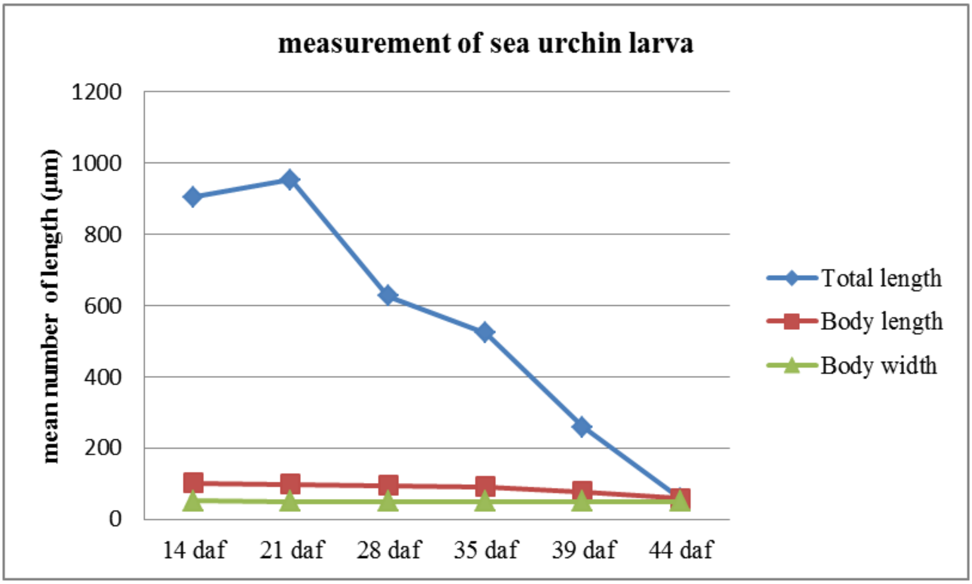


Figure 1. Measurements of sea urchin (*Diadema setosum*) larva during study period

Discussions

In recent study, *Diadema setosum* larval developments were achieved until they reach to the juvenile stage. Many morphological changes of larval were occurred during studying period. Within first two week old larva, postoral arms (POA) of larval were conspicuously elongated from the four arms pluteus to postoral arms (POA) elongated stage 1 (904.28 ± 147.40 µm) .

At three weeks old larva was (POA) elongated stage 2, the total lengths of larvae were highest in number (952.85 ±138.77 µm) in present study. But during first week old larva could not measure in the recent study because of this larval stage was too small and difficult to measure.

This record was more or less similar with the report of Rahman *et. al*, 2015, who also stated that the measurement of POA elongated stage 1 larva (1011.76 ± 15.44 µm) and POA elongated stage 2 larva (1186.67 ± 18.39 µm) was the highest number in their observation.

According to the Rahman *et. al*, 2015, they stated that the premature (precompetent) larval stage started to form 28 days after fertilization. During this stage, the basal portion of the larva was enlarged and the pigmented arches appeared to form, and the pedicellaria was encircled with a ciliated ring. The measurement of this stage was 894.28 ± 14.82 µm.

This report supported to the recent study except the emerging of pedicelleriaes, during four weeks (28 daf) old larva stage, the premature larval stage like that the pigment spots accumulation in basal portion, stomach activation, arms epidermis degeneration, larval skeletons and larval spicules formation were recorded in their body. The mean number of total larval length was 627.14 ± 169.38 µm.

Mean length of larval were continuously reduced in both result of Rahman *et. al*, 2015 and present study because the larval arms were gradually degenerated until absolutely disappear at the end of larval stage. Therefore total lengths of larvae were slowdown to end of larval stage (258.57 ± 121.16 µm). The lowest mean numbers of larval length were recorded in early juvenile stage (58.5 7 ± 12.14 µm.)

Rahman *et al*., 2015, pointed out that the mature (competent) larvae of *D. setosum* had pedicellariae during the late larval period and after metamorphosis as those documented in other regular urchins, *P. lividus* (Gosselin and Jangoux, 1998), *Strongylocentrotus fanciscanus* (Miller and Emlet, 1999) and *S. sphaeroides* (Rahman *et al*., 2012b), whereas pedilellariae of *S. purpuratus* appear sometime after metamorphosis.

The present observation was more or less similar to those reported in competent larva within the sixth week (42 daf) old larva. Larval tissue resorption and accumulation on the rudiment, arms rods and epidermis completely degenerated, pedicelleriaes were emerged.

This finding was also agreed with the report of Rahman *et. al*,(2015), they stated that the larval arms in newly metamorphosed juvenile of *D. setosum* were completely absorbed together with the skeleton and epidermis.

But the duration of armless metamorphosed juvenile formation was different with their report (approximately 35 daf) and the present study (42 daf). The time variations of developmental stages were suggested that it may be different situation of fertilization and larval rearing methods. Their experiment was done by small skill using with the glass beaker and petridish in the laboratory, but the recent study was done by large skill using with the tank at room temperature in the wet laboratory.

Furthermore larval body circulation was occurred in the recent observation. It behavior was assumed that the global shape of their juvenile body formation and to resorption and compact their larval tissue on the adult rudiment together with their vestigial of arms were formed. No any reported concern with their circling behavior during metamorphosis stage.

Later, the larva was started to metamorphosis and settle at the bottom to build up the juvenile form during seventh week old larva. Like the juvenile forms, mouth with five radial discs pentagonal shape, purple pedicellariae and translucent tube feet were emerged around the globoid shape body.

The body lengths of larvae were also recorded in highest numbers during two weeks after fertilization (101.42 ± 8.99 μm). In this stage, larval body was look like the biggest size among the larval developmental period. During four weeks after fertilization, larvae body lengths were gradually reduced (91.42 ± 10.69 μm) because of the larval body tissue absorption was began together with arms epidermis. The lowest numbers of larval body length were observed in seven weeks after fertilization (58.57 ± 12.14 μm) because of the larval tissue absorption was completed.

During the study period, the mean body widths of larvae were a little changed during the two (51.42 ± 6.90 μm) to three weeks (50 ± 5.77 μm) old larval stages. After that larval width was no more changed until the end of larval stage and early juvenile stage (50 ± 0 μm). Their stability of larval width suggested that the adult rudiment was developed as the frame of their adult body shape like the globoid body.

The findings emerged from the present study would be useful towards the understanding of ontogeny and life-history strategies of sea urchin, which will ultimately help to seed production and aquaculture techniques of commercially important sea urchins in captive rearing conditions.

Summary

The larval development of *D. setosum* was observed from four arms pluteus to early juvenile stage during the recent study. The present study was achieved the understanding of ontogeny and part of life-history events of sea urchin. The competent larval circling behavior to form the compact and globoid juvenile body was recorded during metamorphosis stage. This recorded behavior was not reported in all of sea urchin research field.

Acknowledgements

I would like to express my deepest gratitude to professors Dr. Thet Thet Lwin (Head), and Dr Moe Kyi Han Department of Zoology, Kyaing Tong University for their kind permission to conduct the paper presentation. I also thank a lot to the USAID association for financial support and Dr Kay Lwin Tun, Professor, Zoology Department, University of Yangon for their supporting to do the recent work. I am greatly indebted to the Ministry of Education and Training, Viet Nam (B2016-TCT-12DT) for financial support to conduct the present research paper. I also thankful to Dr Aye Aye Thint, Associate Professor, Kyaing Tong University, for her help to do the present work.

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J. Myanmar Acad. Arts Sci. 2019 **Vol. XVII. No.3**

SEASONAL VARIATION OF ESSENTIAL AND TOXIC METAL CONTENTS IN WATER, SEDIMENT AND SOME FISHES AT AYEYAWADY RIVER SEGMENT OF SALAY ENVIRONS

Cho Cho Thin[1], Myinzu Minn[2], Min Thu Aung[3]

Abstract

Seven metals concentrations in the muscular tissue of 15 fish species and water and sediments collected from Ayeyawady River segment of Salay environs were seasonally examined by Flame Atomic Absorption Spectrometer (FAAS) (Perkin Elmer AAanalyst 800 and Winlab-32 software) in Universities' Research Centre (URC) during the period from February 2015 to January 2016. The mean concentrations of toxic metals in all studied fishes with different feeding habits were lower than the WHO permissible limits except the arsenic in cold season. However essential metal concentrations were found within the permissible FAO range. The cadmium concentrations of water in rainy and cold seasons and arsenic in cold season were higher than WHO guideline values. Essential metal concentrations of water were within the WHO permissible limits. Toxic metals of sediments were within probable effect concentrations (PEC).

Keywords: muscle, water, sediment, element concentration, heavy metal, seasons

Introduction

Heavy metals are environmentally ubiquitous, readily dissolved and transported by water and readily taken up by aquatic organisms (Alam *et al*., 2002). Fishes are often at the top of aquatic food chain in aquatic ecosystems and fish living in the polluted waters may accumulate toxic trace metals. It is well known that fish, as a regular constituent of the human diet, can represent a dangerous source of certain heavy metals (Mansour and Sidky, 2002).

[1]. Dr., Lecturer, Department of Zoology, University of Yangon
[2]. Dr., Pro-rector, Mandalay University
[3]. Dr., Professor, Department of Zoology, Magway University

The discharge of wastewater and industrial effluents whether treated or not can be regarded as constant pollution source which is negatively have an impact on water quality. Knowledge of water quality parameters can produce a better understanding of the environmental situation and assist policy makers to design priorities for sustainable water management. Water quality includes all physical, chemical and biological variables influencing the suitability of water for any intended use (Hung *et al*., 2010). Relationships between two parameters may influence in the concentration of others.

In this study, concentrations of seven metals (calcium, magnesium, sodium, potassium, cadmium, lead and arsenic) were determined in 15 fish species as well as in water and sediment samples from the Salay segment of Ayeyawady River.

Humans primarily consume fish muscle tissue. It is important to verify whether the concentrations of contaminants like heavy metals in the muscle are within the recommended limits which are suitable for human consumption. Therefore, the metal contents in fish muscle tissue were analyzed in the present study and especially, the mean concentrations of the metals listed above were evaluated in terms of international guidelines.

Materials and Methods

Study Area

Salay Township, Magway Region of Ayeyawady 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, 2015 to February, 2016.

Collection and Preparation of Fish Specimens

From the three study sites, a total of 111 specimens of 15 fish species were collected from local fishermen in hot, rainy and cold seasons. Feeding habits of recorded fish species were designated in accordance with Talwar and Jhingran (1991). From among the collected species, five species in each of herbivores, carnivores, and omnivores were selected for determination of metal concentrations. The specimens were washed by tap water until the body surface was clean. Total length (cm) and body weight (g) of the specimens were measured. Subsequently 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 Preparations

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 of 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 seven elements (calcium, magnesium, sodium, potassium, 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).


(A). Ashing samples in furnace


(B). Filteration of samples


(C). Samples ready for AAS


(D). Analysis by AAS

Plate 1. Apparatus used in sample analysis

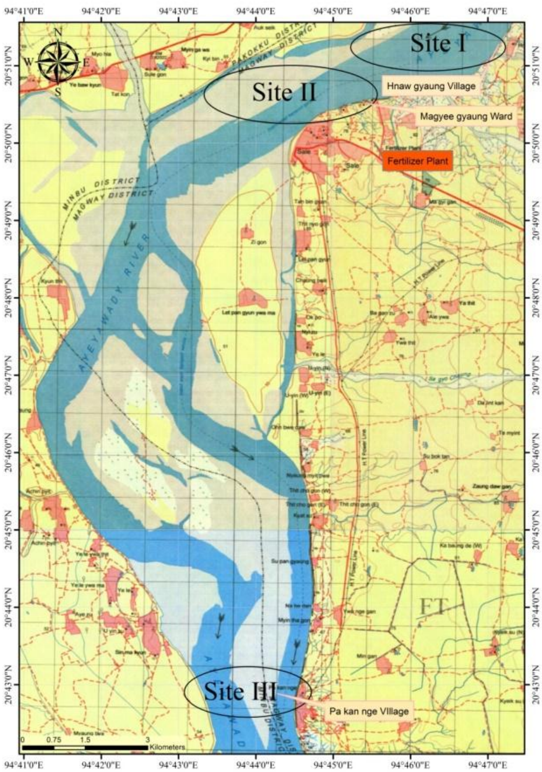


Figure.1 Map of the study area and study sites

Results

A total of 15 fish species which included five species of herbivores (*Cirrhinus mrigala*, *Labeo boga*, *Labeo calbasu*, *Labeo rohita*, *Oreochromis mossambicus)*, five species of carnivores *(Notopterus notopterus*, *Separata aor*, *Mystus cavasius*, *Eutropiichthys vacha*, *Channa punctatus)*, and five species of omnivores *(Tenualosa ilisha*, *Salmostoma sardinella*, *Rhinomugil corsula*, *Macrognathus zebrinus*, *Mastacembelus dayi*) were collected from the Ayeyawady River segment in Salay Environs.

Concentration of the essential metals (Ca, Mg, Na and K) in all studied fish species with different feeding habits were found within FAO standard ranges for all seasons (Table 1, 2, 3 and 4). The mean concentrations of the essential metals in the fishes were presented in Table 5 and Fig. 2, A, B, C, and D.

Cadmium concentrations of all studied fish species with different feeding habits for all seasons, were found to be lower than those of maximum permissible limits (MPL) recognized by WHO/ FAO, except in the carnivorous *Separata aor* in rainy season which have the cadmium concentrations of 0.48 mg/L was higher than the MPL of 0.2 mg/L (Table 6).

Lead concentrations of all studied fish species with different feeding habits in all seasons, were found to be lower than those of MPL recognized by WHO/ FAO except in *Labeo rohita,* a herbivorous fish in rainy season. The mean value of lead concentrations in latter species at 1.13 mg/L was found to be higher than the recommended highest standard of 1mg/L (Table 7).

Arsenic concentrations of *Labeo boga* (0.76 mg/L), *Oreochromis mossambicus* (0.57 mg/L) among the herbivorous fishes; *Salmostoma sardinella* (0.95 mg/L) *Macrognathus zebrinu* (0.98 mg/L) and *Mastacembelus dayi* (1.98 mg/L) among the omnivorous fishes in hot season; *Labeo boga* (1.08 mg/L) and *Labeo calbas* (1.2 mg/L) among the herbivorous fishes; and *Salmostoma sardinella* (2.59 mg/L) among omnivorous fishes were found to be lower than those of maximum permissible limits (0.26 mg/L) recognized by WHO/ FAO. In cold season, arsenic concentrations of all studied fish species in different feeding habits were found to be lower than those of maximum permissible limits except in carnivorous *Notopterus notopterus* (0.00 mg/L) (Table 8). The mean concentrations of the toxic metals were shown in Table 9 and Fig. 3, A, B and C.

Calcium, magnesium, sodium and potassium concentrations of water samples in all seasons were found to be lower than those of the maximum permissible limits recognized by WHO/ FAO. Cadmium concentrations (0.08 mg/L in rainy and 0.07 mg/L in cold seasons) and arsenic (1.40 mg/L) in cold season were higher than the MPL (Table 10 and 11. Fig. 4 and Fig. 5). Cadmium, lead, and arsenic concentrations of sediment in all seasons were observed to be lower than the TEC, MEC, and PEC (Table 12 and 13, Fig. 6 and 7).

Table 1. Seasonal variation of calcium concentration (mg/L) in fishes with different feeding habits

Types of Feeding habit	Species	Calcium			FAO Standard range
		Hot	Rainy	Cold	
Herbivores	*Cirrhinus mrigala*	2.13	13.47	12.63	19 - 881
	Labeo boga	4.49	40.71	27.12	
	Labeo calbasu	1.50	47.99	20.75	
	Labeo rohita	1.11	13.89	28.97	
	Oreochromis mossambicus	1.51	14.18	36.35	
Carnivores	*Notopterus notopterus*	1.85	11.73	17.55	
	Separata aor	1.63	27.69	11.11	
	Mystus cavasius	57.45	39.91	19.29	
	Eutropiichthys vacha	1.50	19.44	20.77	
	Channa punctatus	22.06	17.17	17.87	
Omnivores	*Tenualosa ilisha*	4.87	51.47	17.55	
	Salmastoma sardinella	39.12	90.64	41.48	
	Rhinomugil corsula	5.97	48.55	18.66	
	Macrognathus zebrinus	6.43	33.38	30.09	
	Mastacembelus dayi	18.83	40.58	39.21	

Table 2. Seasonal variation of magnesium concentration (mg/L) in fishes with different feeding habits

Types of Feeding habit	Species	Magnesium			FAO Standard range
		Hot	Rainy	Cold	
Herbivores	*Cirrhinus mrigala*	3.20	9.51	8.29	4.5-452
	Labeo boga	5.46	9.29	9.24	
	Labeo calbasu	3.59	9.05	9.09	
	Labeo rohita	4.53	9.29	8.99	
	Oreochromis mossambicus	2.49	9.26	9.19	
Carnivores	*Notopterus notopterus*	1.58	9.43	9.03	
	Separata aor	0.80	9.29	7.82	
	Mystus cavasius	9.80	9.57	8.81	
	Eutropiichthys vacha	1.41	9.40	8.94	
	Channa punctatus	9.38	9.31	9.14	
Omnivores	*Tenualosa ilisha*	3.05	9.33	9.18	
	Salmastoma sardinella	6.37	9.42	9.40	
	Rhinomugil corsula	7.35	9.42	9.21	
	Macrognathus zebrinus	9.41	9.25	9.11	
	Mastacembelus dayi	9.29	9.24	9.32	

Types of Feeding habit	**Species**	**Magnesium FAO Standard range**			**FAO Standard range**
		Hot	**Rainy**	**Cold**	
Omnivores	*Tenualosa ilisha*	3.05	9.33	9.18	
	Salmastoma sardinella	6.37	9.42	9.40	4.5-452
	Rhinomugil corsula	7.35	9.42	9.21	
	Macrognathus zebrinus	9.41	9.25	9.11	
	Mastacembelus dayi	9.29	9.24	9.32	

Table 3. Seasonal variation of sodium concentration (mg/L) in fishes with different feeding habits

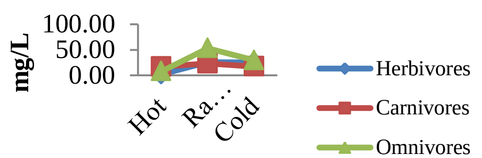
Types of Feeding habit	**Species**	**Sodium**			**FAO Standard range**
		Hot	**Rainy**	**Cold**	
Herbivores	*Cirrhinus mrigala*	13.51	27.8	10.41	
	Labeo boga	9.76	29.81	22.17	
	Labeo calbasu	9.09	25.34	14.60	
	Labeo rohita	6.96	35.78	16.26	
	Oreochromis mossambicus	10.99	29.11	21.06	
Carnivores	*Notopterus notopterus*	4.46	29.36	22.60	
	Separata aor	1.98	28.33	9.89	
	Mystus cavasius	24.13	36.13	13.87	30-134
	Eutropiichthys vacha	6.77	31.89	17.25	
	Channa punctatus	28.52	25.15	16.46	
Omnivores	*Tenualosa ilisha*	7.68	25.60	22.87	
	Salmastoma sardinella	14.10	33.65	25.56	
	Rhinomugil corsula	16.64	36.13	18.11	
	Macrognathus zebrinus	31.37	27.96	20.46	
	Mastacembelus dayi	29.36	32.92	28.10	

Table 4. Seasonal variation of potassium concentration (mg/L) in fishes with different feeding habits

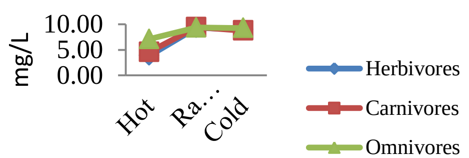
Types of Feeding habit	Species	Potassium			FAO Range
		Hot	Rainy	Cold	
Herbivores	*Cirrhinus mrigala*	142.20	12.63	12.54	
	Labeo boga	142.30	12.58	12.50	
	Labeo calbasu	143.20	12.51	12.50	
	Labeo rohita	141.10	12.58	12.52	
	Oreochromis mossambicus	141.50	12.58	12.52	
Carnivores	*Notopterus notopterus*	50.10	12.56	12.50	
	Separata aor	8.41	12.56	12.55	
	Mystus cavasius	143.00	12.66	12.52	19-502
	Eutropiichthys vacha	141.50	12.60	12.51	
	Channa punctatus	12.59	12.54	12.51	
Omnivores	*Tenualosa ilisha*	141.10	12.53	12.50	
	Salmastoma sardinella	143.70	12.64	12.52	
	Rhinomugil corsula	141.90	12.61	12.50	
	Macrognathus zebrinus	144.20	12.59	12.51	
	Mastacembelus dayi	145.50	12.58	12.52	

Table 5. Mean values of essential metals in fishes with different feeding habits

Feeding habit	Calcium			Magnesium			Sodium			Potassium		
	Hot	Rainy	Cold	Hot	Rainy	Cold	Hot	Rainy	Cold	Hot	Rainy	Cold
Herbivores	2.15	26.05	25.16	3.85	9.28	8.96	10.06	29.57	16.90	142.06	12.58	12.52
Carnivores	16.90	23.19	17.32	4.59	9.40	8.75	13.17	30.17	16.01	71.12	12.58	12.52
Omnivores	7.84	52.92	29.40	7.09	9.33	9.24	19.83	31.25	23.02	143.28	12.59	12.51



(A) Calcium



(B) Magnesium

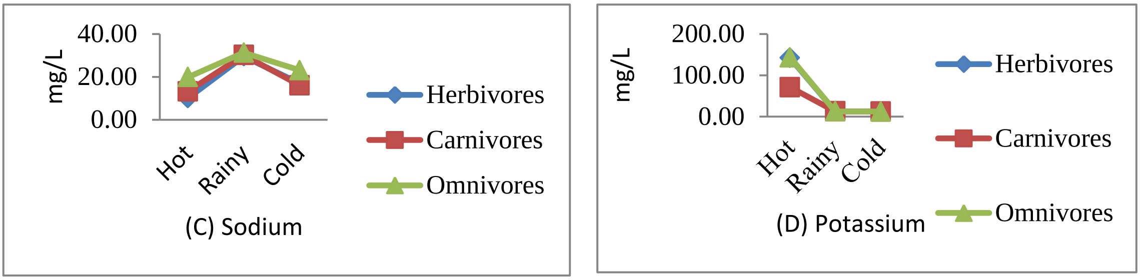


Figure 2. Seasonal variation of essential metal content in fishes with different feeding habits

Table 6. Seasonal variation of cadmium concentration (mg/L) in studied species with different feeding habits

Types of Feeding habit	**Species**	**Cadmium**			**WHO/FAO MPL**
		Hot	Rainy	Cold	
Herbivores	*Cirrhinus mrigala*	-0.05	0.08	0.07	0.2
	Labeo boga	-0.06	0.01	0.07	0.2
	Labeo calbasu	-0.04	0.15	0.08	0.2
	Labeo rohita	-0.06	0.08	0.08	0.2
	Oreochromis mossambicus	-0.06	0.10	0.08	0.2
Carnivores	*Notopterus notopterus*	-0.04	0.07	0.07	0.2
	Separata aor	-0.06	0.48	0.07	0.2
	Mystus cavasius	-0.07	0.10	0.07	0.2
	Eutropiichthys vacha	-0.04	0.10	0.07	0.2
	Channa punctatus	0.10	0.07	0.08	0.2
Omnivores	*Tenualosa ilisha*	-0.04	0.14	0.09	0.2
	Salmastoma sardinella	-0.04	0.11	0.09	0.2
	Rhinomugil corsula	-0.04	0.33	0.08	0.2
	Macrognathus zebrinus	-0.04	0.09	0.08	0.2
	Mastacembelus dayi	-0.03	0.09	0.09	0.2

Table 7. Seasonal variation of lead concentration (mg/L) in fishes with different feeding habits

Types of Feeding habit	**Species**	**Lead**			**WHO/FAO MPL**
		Hot	Rainy	Cold	
Herbivores	*Cirrhinus mrigala*	-1.31	0.07	-0.03	1
	Labeo boga	-3.38	0.10	0.00	1
	Labeo calbasu	-3.64	0.06	0.08	1
	Labeo rohita	-1.86	1.13	0.03	1
	Oreochromis mossambicus	-2.80	0.04	0.29	1
Carnivores	*Notopterus notopterus*	-0.87	0.14	0.25	1
	Separata aor	-2.03	0.41	-0.05	1
	Mystus cavasius	-2.34	0.15	-0.04	1
	Eutropiichthys vacha	-2.60	0.09	-0.03	1
	Channa punctatus	-0.11	0.06	-0.03	1
Omnivores	*Tenualosa ilisha*	-0.63	0.13	0.32	1
	Salmastoma sardinella	-3.58	0.21	0.05	1
	Rhinomugil corsula	-2.97	0.38	0.36	1
	Macrognathus zebrinus	-3.57	0.04	-0.04	1
	Mastacembelus dayi	-4.09	0.09	0.07	1

Table 8. Seasonal variation of arsenic concentration (mg/L) in fishes with different feeding habits

Types of Feeding habits	Species	Arsenic			WHO/FAO MPL
		Hot	Rainy	Cold	
Herbivores	*Cirrhinus mrigala*	-2.53	-0.91	0.83	0.26
	Labeo boga	0.75	1.08	2.16	0.26
	Labeo calbasu	1.37	1.20	1.61	0.26
	Labeo rohita	-2.20	-0.99	1.50	0.26
	Oreochromis mossambicus	0.57	-0.86	2.46	0.26
Carnivores	*Notopterus notopterus*	-3.07	-0.47	0.00	0.26
	Separata aor	-1.93	-0.80	0.80	0.26
	Mystus cavasius	-1.78	0.13	1.29	0.26
	Eutropiichthys vacha	-1.00	-0.52	2.11	0.26
	Channa punctatus	4.89	-1.10	1.69	0.26
Omnivores	*Tenualosa ilisha*	-2.75	-1.50	0.52	0.26
	Salmastoma sardinella	0.95	2.59	2.46	0.26
	Rhinomugil corsula	-0.06	-0.99	1.98	0.26
	Macrognathus zebrinus	0.98	-1.00	1.52	0.26
	Mastacembelus dayi	1.98	-0.66	2.84	0.26

Table 9. Mean values of toxic metals in fishes with different feeding habits

Feeding habits	Cadmium			Lead			Arsenic		
	Hot	Rainy	Cold	Hot	Rainy	Cold	Hot	Rainy	Cold
Herbivores	-0.05	0.08	0.08	-2.60	0.28	0.07	-0.41	-0.10	1.71
Carnivores	-0.02	0.16	0.07	-1.59	0.17	0.02	-0.58	-0.55	1.18
Omnivores	-0.04	0.15	0.09	-2.97	0.17	0.15	0.22	-0.31	1.86

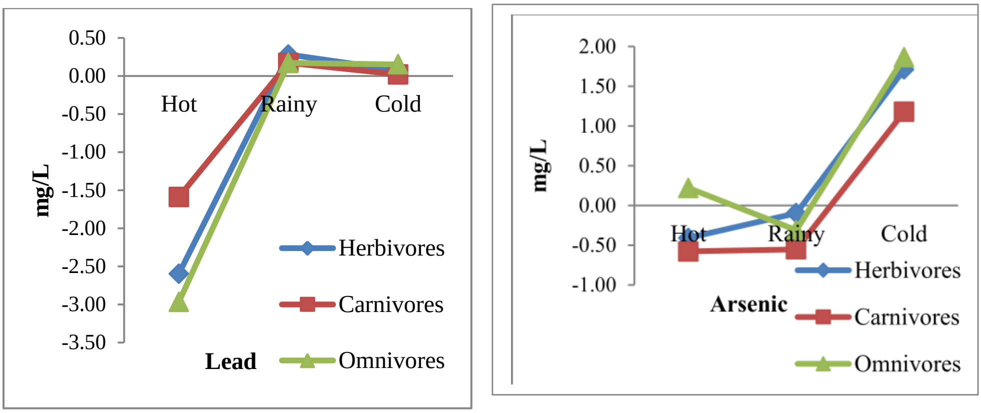


Figure 3. Seasonal variation of toxic metal content in fishes with different feeding habits

Table10. Seasonal variation of essential metal content in water samples of Ayeyawady River segment of Salay environs

Elements	Concentration (mg/L)			WHO/FAO MPL
	Hot	Rainy	Cold	
Calcium	-0.01	7.22	9.61	100
Magnesium	0.33	8.45	8.60	150
Sodium	0.33	12.33	17.01	200
Potassium	-0.01	0.69	3.99	12

MPL = maximum permissible limit

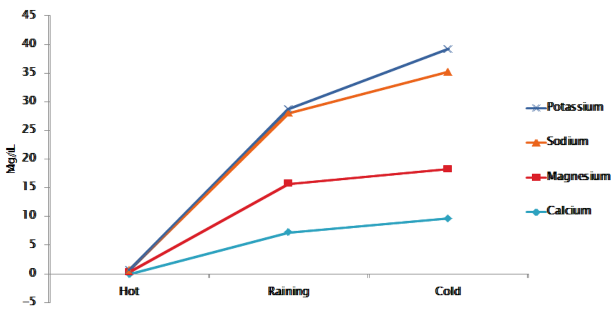


Figure 4. Seasonal variation of essential metal content in water samples of Ayeyawady River segment in Salay environs

Table 11. Seasonal variation of toxic metal content in water samples of Ayeyawady River segment of Salay environs

Elements	Concentration (mg/L)			WHO/FAO MPL
	Hot	Rainy	Cold	
Cadmium	-0.02	0.08	0.07	0.01
Lead	-0.86	−0.15	−0.17	0.05
Arsenic	-4.71	-1.26	1.40	0.01

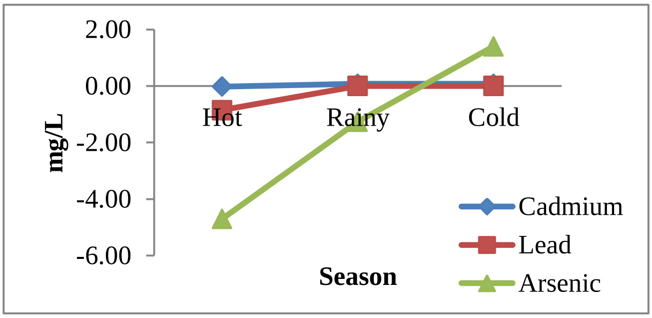


Figure 5. Seasonal variation of toxic metal content in water samples of Ayeyawady River segment, Salay environs

Table 12. Seasonal variation of essential metals content in sediment samples of Ayeyawady River segment of Salay environs

Elements	Concentration (mg/L)		
	Hot	Rainy	Cold
Calcium	4.93	5.72	1.32
Magnesium	9.09	9.04	8.93
Sodium	18.42	18.00	5.90
Potassium	8.47	7.79	5.73

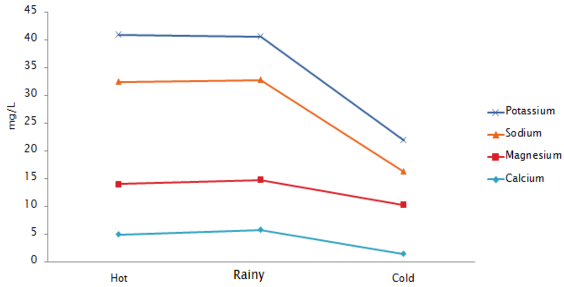


Figure 6. Seasonal variation of essential metal content in sediment samples of Ayeyawady River, Salay environs

Table 13. Seasonal variation of toxic metal content in sediment samples of Ayeyawady River segment of Salay environs

Element	Concentration (mg/L)			MPL		
	Hot	Rainy	Cold	TEC	MEC	PEC
Cadmium	0.12	0.12	0.08	0.99	3	5
Lead	0.13	0.11	−0.18	36	83	130
Arsenic	4.62	2.37	2.46	9.8	21.4	33

TEC = Threshold effect concentration, MEC = Midpoint effect concentration
PEC = Probable effect concentration, MPL = maximum permissible limit

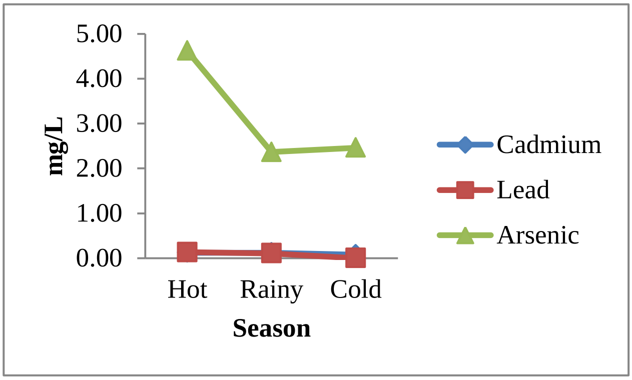


Figure 7. Seasonal variation of toxic metal content in sediments of Ayeyawady River, Salay environs

Discussion

Knowledge of element concentrations in fish is important for both human health and environmental management. Forstner and Wittmann (1981) reported that aquatic organisms fish are capable of accumulating metal concentrations much higher than those present in water, sediments and micro flora in their environment. Toxic metalsin the environment and food are very harmful because of their potential to bioaccumulate in different body parts of plants, animals and humans.

In this study, the effects of feeding habits and the seasons on element accumulation in muscle of fishes and their environs (water and sediments) were determined. The values observed for metal concentrations of all studied fish species of different feeding habits and their environs were lower than the maximum permissible limit in all seasons except for As in cold season.

Ca is needed for muscle development as well as heart and digestive system function. It is also essential for the normal development and maintenance of bones (Norman and Joseph, 1996). Mg plays important role in enzyme activities and maintaining electrical potential in nerves and membranes; it improves insulin sensitivity, protect against diabetes and also reduces blood pressure (Kiran *et al*., 2011). Na is one of the chief extracellular ions in the body: it involves in the production of energy and transport of amino acids and glucose into the body cells. Its deficiency results in hypothermia (Donatelle, 2005). K is the principle intracellular cation. It helps to regulate osmotic pressure and maintain pH. Its deficiency causes muscle weakness, respiratory paralysis and decreases reflex responses (Norman and Joseph, 1996).

Khin Myint Mar (2011) stated that Cd and Pb concentrations of all studied fish species were markedly lower than the permissible limit. WHO (2007) stated that Cd exposures are associated with kidney and bone damage. Cd has also been identified as a potential human carcinogen, causing lung cancer. Pb is toxic metal and non-essential element for the human body and it causes a rise in blood pressure, kidney damage and miscarriage (Kiran *et al*., 2011). Toxic effects appear when As is ingested in excess for long periods, resulting in cancer, cutaneous malignancies, etc.

The essential element concentrations recorded in the present study in all studied fishes with different feeding habits and their environs were within the maximum permissible limits of WHO/FAO. Toxic metal concentrations of all studied fishes were found to be lower than the maximum permissible limit except for As in the cold season. Toxic metal concentrations of water and sediment were found within the maximum permissible limit. Based on the present results, the fish species tested were found to be suitable for human consumption.

Conclusion

In the present study, essential and toxic metal concentrations of all studied fish species and in their environs (water and sediment), were found to be lower than the maximum permissible limits except the arsenic in cold season. Based on the results, it could be concluded that both the fishes and their environment were suitable for human health.

Acknowledgements

I am greatly indebted to Dr. Thida Lay Thwe, Professor/Head, Department of Zoology, Yangon University for her kind encouragement. I would like to specially thank to Dr. Aye Mi San, Professor, Department of Zoology, Yangon University for her invaluable help in this work. My profound gratitude goes to Dr. Nyan Tun, Associate Professor, Department of Chemistry, Bago University, for his invaluable help in this research work.

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J. Myanmar Acad. Arts Sci. 2019 **Vol. XVII. No.3**

REPRODUCTIVE BIOLOGY OF FEMALE INDO- PACIFIC KING MACKEREL, *SCOMBEROMORUS GUTTATUS* (BLOCH& SCHNEIDER, 1801) FROM KYAUKPHYU ENVIRONS, RAKHINE STATE

Khin July Han[1], Kalayar Win Maung[2], Sandar Win[3]

Abstract

In this study, a total of 240 samples were collected and dissected, from Kyaukphyu Market during the study period from February 2016 to January 2017. Identification was followed after Fisher and Whitehead (1974) and FAO (1984).The gonads were extracted from the specimens. Total body length, standard length, body weight and gonad weight were recorded and preserved in neutral buffer for further histological examination and to analyze the Gonosomatic indices (GSI).The GSI value of female is the highest (4.23± 3.12) in August and the lowest is (1.16 ± 0.06) in June. According to GSI value and based on its histological characteristics, four stages of cyclical changes i.e. maturity in March and July, spawning in April and August, , post spawning in May, September and October, resting in November, December, January, February and June were found in studied species. The breeding season of Spanish mackerel were two peaks in a year such as pre-monsoon and post-monsoon.

Keywords: Gonosomatic indices (GSI), histological examination, breeding

Introduction

Indo – Pacific king mackerel, it is also known as seer fish. *Scromberomorus guttatus* was one of the number of different species of pelagic fish. It belongs to the family Scrombridae. These fishes are found in both temperate and tropical sea, mostly inhabiting along the coast or offshore in the oceanic environment. Its sides are silvery white with several longitudinal rows of round dark brownish spots scattered in about 3 irregular

[1] Assistant Lecturer, Department of Zoology, University of Yangon
[2] Dr. Lecturer, Department of Zoology, University of Yangon
[3] Dr. Associate Professor, Department of Zoology, University of Yangon

rows along lateral line. There are over 30 different species of mackerel, of these, 21 species were Spanish mackerel in the world. (FAO, 2003). Seer fishes are considered as one of the high value resources due to high economic return and export markets (Muthiah,2002). Among the five species of seer fishes the king seer fish, *Scomberomorus commerson* and Spotted seer fish, *Scromberomorus guttatus* are commonly caught in the Myanmar coasts.

Myanmar has three coastal regions with a long coastline of nearly 3,000 km. It possesses a considerable diversity of coastal habitats (FAO, 2003). In addition, fisheries in Myanmar plays an important role in the development of a nation of considerable economic value, especially in the coastal fisheries (FAO, 2003). The mackerel species are found in the marine water. Those species are economically important in Rakhine State studied. Mackerel inhabit marine, mostly living along the coast or offshore in the oceanic environment. Knowledge on reproductive biology is essential not only for the benefit in life history and stock management but also for evaluating the potential in successful culture (Rahman *et al*, 2006). Spawning periods differ with different species of fish and environmental conditions (Thant Zin, 1988).

The reproductive cycle must ensure a sufficient quantity of mature egg cells, which is possible only within the regular process of the oogenesis. The oogenesis is a very dynamic process in the ovaries, in which the oocyte passes through various phases of development that are very similar in different fish species. The fish oocyte development can be divided into oocyte growth and oocyte maturation. Vitellogenesis plays an important role in the oocyte growth. (Nagahama *et al*., 1983, Yueh and Chang 2000).

This study was thus undertaken with the following objectives:

(1) To determine the annual reproductive cycle of Gonosomatic Index (GSI) and maturity stages of *S. guttatus*

(2) To find out the morphological and histological development of ovaries of *S. guttatus*

Materials and Methods

Study area and study periods

The study area was coastal area of Kyaukphyu environs, Rakhine State between 18° 30'- 20° 35' N and 93° 20' - 94° 22' E was selected as study site and the study periods lasted from February 2016 to Feb 2017(Fig., 1)

Collection of specimen and data

The specimens were collected monthly from the catches from Kyaukphyu Market. These were measured Total length (TL), standard length (SL), body weight (BW) and gonad weight (GW) and liver weight (LW) to the nearest millimeter. The photograph of mackerels were taken. The specimens were dissected and the gonads are extracted. And then gonads were blotted dry, weighed and preserved in neutral buffered formalin, for further histological examination (Fig 2)

Identification and classification

These species were identified based on Fischer and Whitehead (1974), and FAO (1984).

Analysis of Gonosomatic Index (GSI) Agarwal (1996)

Conditions of ovaries were checked monthly and recorded. This process was conducted in order to estimate the peak periods and decline of the breeding conditions.

The Gonosomatic Index is an indicator of the breeding pattern of fish and Gonosomatic Index (GSI) was calculated as follow:

$$\mathrm{GSI} = \frac{\text{Gonad weight}}{\text{Whole body weight}} \times 100\%$$

Histological study of gonads

Monthly histological examinations of gonads (ovaries) were taken into consideration to determine the spawning season. The gonads (ovaries) were preserved in neutral buffered formalin for further histological examination. Gonads samples (ovaries) were taken from anterior, middle and posterior region for further sectioning and staining. Double staining method was used according to Harris's Haematoxylin and eosin methods (1977). The stages of oocyte were identified according to Agarwal (1996), Htun Han (1978) and Kitsawat, Chinabut and Limsuwan (1991).

The sectioned ovaries were observed under the image analyzer microscope. Six stages of oogenesis were defined as oogonia (stage I), primary oocyte (stage II), secondary oocyte (stage III), primary vitellogenesis (stage IV), secondary vitellogenesis (stage V), tertiary vitellogenesis (stage VI) were recorded by utilizing image analyzer microscope to the nearest micro millimeter (μmm). Reproductive cyclical changes of species were recorded based on the cell morphology and histological examination.

Figure 1. Map of the study site

Results

Systematic position

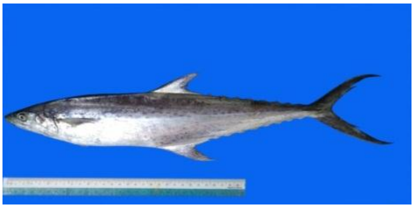
Kingdom - Animalia
Phylum - Chordata
Subphylum - Vertebrata
Class - Actinopterygii
Order - Perciformes
Suborder - Scombroidei
Family - Scombridae
Genus - *Scomberomorus*
Species -*S. guttatus*
Common name- Indo-Pacific king mackeral or Indo-Pacific Spanish mackerel
Local name - Nga – Kun- Shut

External features of *Scromberomorus guttatus*

Mackerels **a**re swift-moving, streamlined body and sport fishes mostly found in temperate and tropical seas around the world, allied to tunas in the family Scombridae (order Perciformes). Mackerels are rounded and torpedo-shaped, with a slender, keeled tail base, a forked tail, and a row of small finlets behind the dorsal and anal fins. They are carnivorous fishes and feed on crustaceans, mollusks, fish eggs, and small fish. They congregate in schools and swim actively in the upper 25–30 fathoms of the water in the warmer months and then descend to as deep as 100 fathoms during the winter. They spawn during the spring and early summer along coastlines.

Morphological characters of ovaries of *Scromberomorus guttatus*

The ovaries of *S. guttatus* were paired and not too elongated. The ovaries are generally equal in length but sometimes one may be longer than the other. The weight, length, width and color of the ovaries vary greatly with the stages of their maturity. In early stage, they were opaque giving pale pink to yellowish coloration. The colour of the ovaries changed brightly yellowish when they reached the maturing stage. During breeding season, the ovaries were much larger and almost occupying the major portion of the body cavity and changed the pale yellowish color.



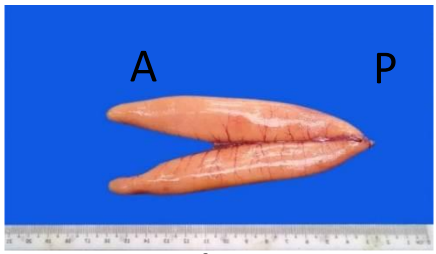
External feature of *S. guttatus*

Weighing of ovaries

Variations sizes of *S. guttatus of the samples*

Plate 1. External feature, weight of ovary and different sizes of *Scromberomorus guttatus*

A. Size of Immature ovaries



B. Size of Mature ovaries

C. Size of Ripe ovaries

D. Size of Spent ovaries

Plate 2. Macroscopic developmental stages of female reproductive organ of *Scomberomorus guttatus*

A = Anterior part, P = Posterior part

Histological study

Oogonesis of *Scromberomorus guttatus*

There were six stages of oogenesis in female *Scromberomorus guttatus*

Oogonia (Stage 1)

Perinucleolar oocytes dominant. Yolk nucleus in cytoplasm, nucleolus was found in the periphery of nuclear membrane. The oogonia of *Scromberomorus guttatus* were rectangular shape.

Primary oocyte (Stage II)

Cortical alveoli stage oocyte appeared containing cortical alveoli and yolk granules in cytoplasm. The number of nucleoli increased and was visible on the surface of the nucleus. The size of nucleus and cytoplasm were bigger than stage I.

Secondary oocyte (Stage III)

Large vitellogenic oocyte dominant containing well developed zona radiata and yolk globule. The size of nucleus and cytoplasm were increased.

Primary vitellogenesis (Stage IV)

The production and accumulation of the yolk began. This process was known as vitellogenesis. Mature follicles showing germinal vesicle migration and yolk granules in cytoplasm were found. At this stage, the nucleus enlarges and the nuclear membrane become irregular. During this stage the nucleus and cytoplasm continued to grow in these species.

Secondary vitellogenesis (Stage V)

The size and number of vacuoles were gradually increased in the peripheral and central zone of the oocyte. The whole cytoplasm was densely filled with vacuoles. In this stage, the nuclear membrane disintegrated.

Tertiary vitellogenesis (Stage VI)

Final stage of vitellogenesis, yolk vesicles were initially accumulated in the periphery of the oocyte. During this stage, the chorion started to form around the oocyte (vitelline membrane and follicular cells). Both the oocytes and follicular cells had microvilli in their apical surfaces. The chronics materials were deposited around these microvilli and became perforated by pore channels. The size of the cells was largest.

Seasonal cyclicity

Monthly variation of Gonadosomatic index of female *S. guttatus* was recorded as shown in Table 1 (below). Based on the data recorded from GSI, four stages of cyclical changes (maturing in March and July) spawning in (April and August), postspawning in (May, September and October), resting in (November to February and June) were found in *Scromberomorus guttatus* (Table 2).

Table 1. Monthly variation of Gonadosomatic index of female *S. guttatus*

Months	GSI%
February	1.17± 0.03
March	1.23± 0.12
April	3.58 ± 2.10
May	2.21 ± 0.08
June	1.16 ± 0.06
July	1.56± 0.91
August	4.23± 3.12
September	2.47± 0.64
October	1.31± 0.33
November	1.21± 0.11
December	1.22± 0.06
January	1.23± 0.04

Gonadosomatic index(GSI) female

The GSI value of female was the highest (4.23± 3.12) in August and second highest (3.58± 2.10) in April and the lowest GSI value was recorded (1.16 ± 0.06) in June.

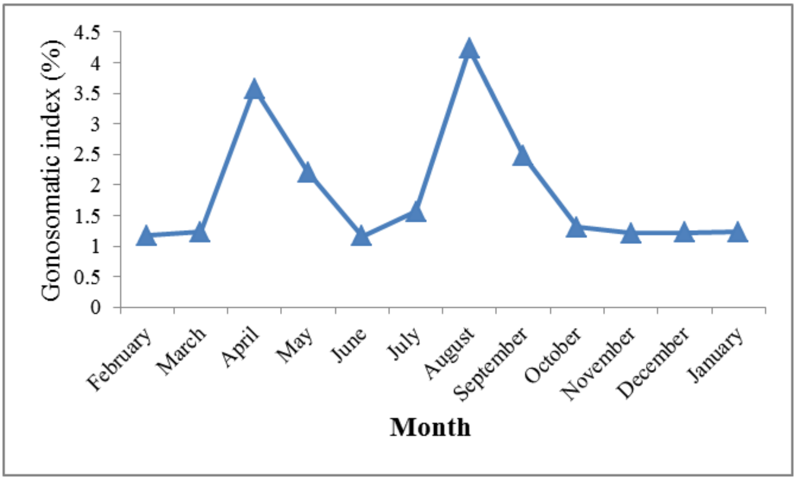
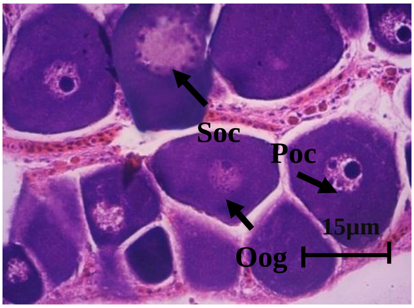


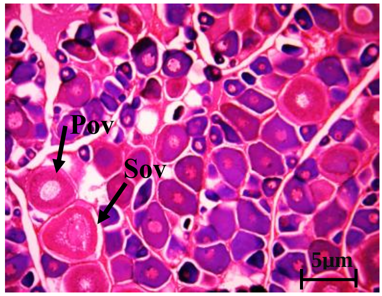
Figure 2. Monthly GSI of female *Scromberomorus guttatus*

Table 2. Annual reproduction cycle of *S.guttatus*

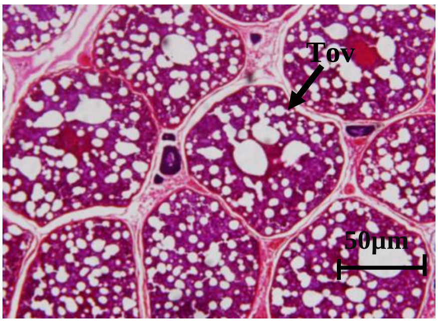
Months	Stages
February	Resting
March	Maturing
April	**Spawning**
May	Post spawning
June	Resting
July	Maturing
August	**Spawning**
September	Post spawning
October	Post spawning
November	Resting
December	Resting
Jan	Resting



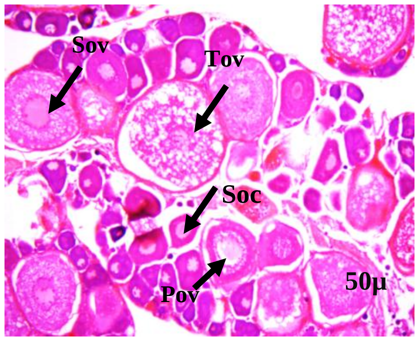
A. Immature stage



B. Mature stage

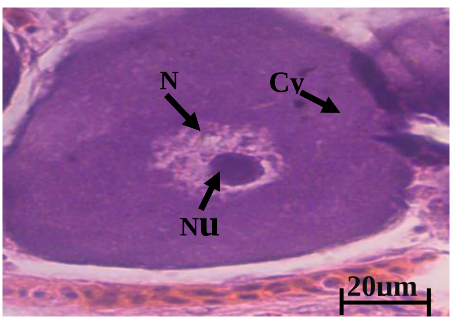


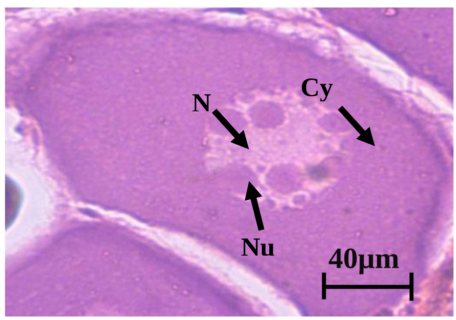
C. Ripe stage



D. Spent stage

Plate 3. Microscopic gonadal maturation stages in female *S.guttatus*; Oog(oogonia), Soc (secondary oocyte), Poc (primary oocyte), Pov (primary vitellogenesis), Sov (secondary vitellogenesis) and Tov (tertiary vitellogenesis)





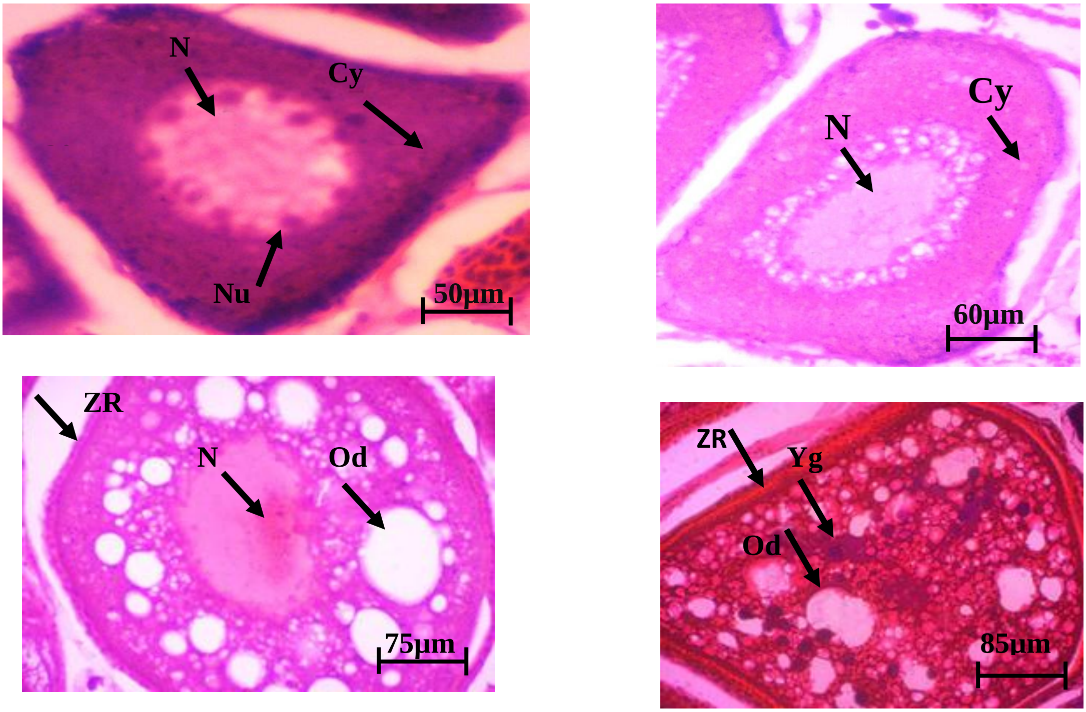


Plate 4. Microscopic gonadal maturation section, stages in female *S.guttatus*; transverse Cy (Cytoplasm), N (Nucleus), Nu (Nucleolus), Zr (Zona radia), Od (Oil droplets) and Yg (Yolk granule)

Discussion

In the present study, Reproductive biology of female Indo-Pacific king mackerel, *Scomberomorus guttatus* were undertaken in Kyaukphyu during the study period from Feb2016 to Jan 2017.The spawning season of *Scomberomorus guttatus* began April to May and June Krishnamoorthi. B. (1958).During nonbreeding season the gonads were small in size and hence a great difference in the ratio of gonad weight to body weight resulted in very low GSI value.

Ovarian development and spawning period of *S. brasiliensis* were investigated using both macroscopic and histological techniques. Mean monthly value of GSI and ovarian maturation indicated that the main spawning period occurs during the rainy season stated by Chellappa *et al.*, Reproductive peak of *Scomberomorus commerson* was occurred in October and November in QueeDBland east coast waters stated that G.R. McPHERSON (1993). Histological study of ovaries of *Alosa fallax fallax* was delineated a total of eight developmental stages recorded by Thresa pina,et.al. (2003). Oogenesis stages of *Rastrelliger brachysoma* was total of seven stages. In the present study, there were six stages of oogenesis was found and recorded.

Spanish mackerels were multiple spawners with asynchronous oocyte development and indeterminate fecundity. They spawn in the Chesapeake Bay area from June through August, June being the peak spawning month. (Cynthia L. Cooksey, 1996).She recorded that females spanish mackeral do not spawn after August, because all females were in either spent or resting stages in September, October and December. In this study, the GSI value showed highest in April and August Similar conditions were also reported by Kalayar Win Maung, 2007.She has recorded the peak of the spawning season for *Tenualosa ilisha* was May and Ocober, *Tenualosa toil* was recorded in April and October. It could be assumed that they breed twice a year in study areas. According to GSI values, breeding season of mackerel species showed two peaks in a year. During the study period, the months of November, December, January and February were found and recorded as the resting stages.

Acknowledgements

My great thankful goes to Dr. Thida Lay Thwe, Professor and Head of Zoology Department, University of Yangon, for her encouragement and permission to conduct this research.

Special thanks is expressed to, Professor Dr. Aye Mi San, Department of Zoology, University of Yangon, for her suggestion and valuable advice.

My sincerely gratitude is referred to Dr. Kalayar Win Maung, Lecturer, University of Yangon, who stood by my side and gave me full support and encouragement throughout my study periods.

Moreover, Co-supervisor Dr. Sandar Win, Associate professor, Zoology Department, University of Yangon, is the person who gets my heartfelt thank for her supervision, encouragement throughout the research work.

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MOTHER - INFANT RELATIONSHIPS AND INFANT BEHAVIOUR OF RHESUS MACAQUE (*MACACA MULATTA*) IN SHINMA TAUNG AREA, YESAGYO TOWNSHIP, MAGWAY REGION

Nwe Nwe Win[1], Aye Pyae Sone[2]

Abstract

Mother - infant relationships and infant behaviour of rhesus macaque (*Macaca mulatta*) from Shinma Taung area, Yesagyo Township, Magway Region was investigated during June, 2013 to February, 2014. The maternal care showed various motherly activities such as suckling, grooming, ventral and dorsal carrying. The behavioral data included the time spent on various infant activities. The activities patterns were observed on the focal sampling using five minutes scan at ten minutes intervals from 08:30hr to 16:30hr. A total of 288 scan samples in 1440mins of scan time were used. Based on nine months data, rhesus infant spent 20.21% of total time on grooming, followed by 16.88% on suckling, 15.14% on feeding, 14.03% on playing , 10.56% on moving (off mother), 10% on resting, 6.74% carrying infant ventrally and 6.46% carrying infant dorsally. Rhesus infant spent more time on grooming compared to the other activities. The strongest social bonds were linked between mother and infant.

Keywords: *Macaca mulatta,* mother-infant relationships, infant behaviour, Shinma Taung

Introducion

A characteristic feature of primate's life history is the prolonged period of postnatal development in which newborns depend on adults especially their mothers, for nutrition, transport and protection. Many researchers have focused on the development of infant nonhuman primates, attempting to identify factors that influence the ontogeny of social behavior, with strong emphasis on the mother – infant relationship (Nicolson, 1987).

[1.] Dr., Associate Professor, Department of Zoology, Banmaw University
[2.] MSc Student, Department of Zoology, Pakokku University

Studies on infant development in Old World monkeys have concentrated on macaques, especially *Macaca mulatta* (Forster and Cords, 2002). Animals allocate resources, both time and energy, to offspring care at a cost to their own maintenance, survival or future reproduction (Altmann and Samuels, 1992).

Most studies on primate maternal styles have been conducted with Cercopithecine species in which variation in infant - directed behaviors usually fall along two dimensions: protection and rejection (Lathouwers and Elsacker, 2004).

Rhesus mother and infant relationship are the most intimate and long lasting of all the relationships (Rowell *et al.,* 1964). The infants of rhesus monkeys begin life completely development on their mother for survival, receiving all nourishment, physical warmth and other basic biological support. Rhesus mothers are usually very restrictive as they restrict the movements and social contacts of their infants from approaching or being approached by other monkeys (Sambyal *et al.,* 2009). The physical contact between mother and infant is influenced by the mother's feeding behavior (Jhnson, 1986).

Rhesus monkey, infants develop distinctive social relationships with their mothers and with same-age peers during their initial weeks and months of life respectively. However, these relationships differ substantially, from one another in several fundamental ways. An infant attachment relationship with its mother is firmly established long before it begins any interactions with peers. The resulting attachment is specific to its mother whereas the infant typically develops multiple relationships with several different peers. The infant's frequency and duration of interactions with its mother are highest during its first month of life and decline steadily thereafter, especially following the birth of younger siblings (Suomi, 2005).

Data on behavior of macaques from different places already existed Naw Phaw Phaw Say, 2005; Aye Mi San, 2007 and Nwe Nwe Win, 2013. Therefore there is sometime needed to carry out the same trend work in Shinma Taung area. The monkeys become so much accustomed to the human presence, making at easier to conduct study. Thus Shinma Taung was chosen as the area of study.

The objectives of the present study are:

- to study the relationship between rhesus mother and different ages of infant
- to compare the activity patterns of infant in rhesus macaque.

Materials and Methods

Study area

The study site is Shinma Taung area located at Yesagyo Township, Magway Region. The location of the area is situated between North latitudes (21° 35' N and 21° 38' N) and East longitudes (95° 03' E and 95° 10' E). According to the data from Dry Zone Greening Department, Pakokku District, the highest peak of Shinma Taung is 525.52 meters (1723 ft) and total area is 687.17 hectares (18995 acre) wide. It is covered with deciduous forest (Fig 1, Plate 1).

Study period

The study was conducted from June, 2013 to February, 2014.

Study group

From the study site, nine pairs of mother with offspring were selected as focal sampling monkeys. All individuals were recognized by features of their faces, tails and others body parts.

Activity pattern

The field’s trip was conducted once a month (one day) during the study period. Data was collected using five minutes scan sampling with ten minutes intervals from 08:30 hr to 16:30hr.A total of 288 scan samples in 1440 mins of scan time were used. Behavioral data included time spent on various infant activities and activity between mother-infant relations was observed according to Foster and Cords (2002).

Behavioral categories

Suckling behavior	- Infant sucks nipple in mouth (whether clinging or not)
Grooming behavior	- Infant groomed by mother
Resting behavior	- Sitting off mother, sleeping, not engaged in social interaction, nor feeding.
Ventral carrying	- carrying position ventrally by mother
Dorsal carrying	- carrying position dorsally by mother
Moving (off the mother)	- Infant locomoting off mother
Playing	- social play with peers
Feeding	-searching for, manipulating and swallowing food items

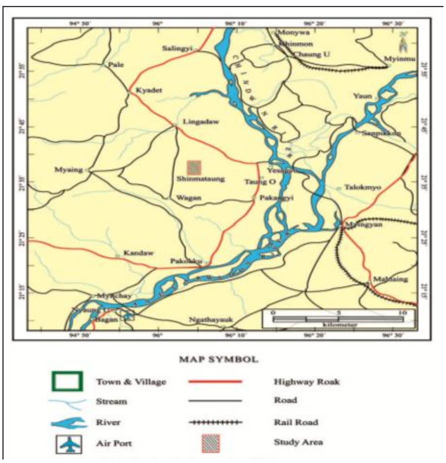

Figure 1. Map of Shinma Taung area (Source: Geology Department, Pakokku University)

Plate 1. Study area of Shinma Taung

Results

Mother-infant relationship and infant behaviour

The newborn offspring was usually taken care of by females. Newborn infant spent a great deal of time nursing or sleeping on chest of its mother (Plate 1, and 2). Mother may restrict and control the activities of its infant by physically restraining it and by making contact with it frequently.

Time on mother (ventrally)

While the infant was on the mother, it regularly changed positions. During one month old infant, the infant was almost always positioned ventrally to the carrying mother (Plate 3). But infant was not found ventrally to the carrying mother during the six months old (Table 1). The infant spent on the mother (ventrally) was 21.88% of time at the 1st month, 15.63% of time at the 2nd month, 13.75% of time at the 3rd month, 6.55% of time at the 4th month and 3.13% of time at the 5th month, respectively (Fig. 1, Table 2).

Time on mother (dorsally)

The infant began riding dorsally during the two months old infant (Plate 4). As the infant achieved locomotion independence, the percentage decreased to 9.38% during the 6th month and 6.25% by the 7th month. By the 8th month, the mother seldom carried the infant when traveling (3.13%) but never observed by the 9th month (Fig. 1 and Table 2).

Suckling

Mother helps with hands only pressing the infant to ventral side while carrying or suckling. Mother was generally very attentive to her infant but sometimes had difficult supporting infant in gaining nipple access, especially while moving or foraging. The infant spent on the nipple 39.38% of time at the 1st month, followed by the 2nd month with 37.50%, the 3nd month with 30%, the 4th month with 23.13%, the 5th month with 15% and the 6th month with 6.88% (Fig.2, Table 2). Although the mother rejected the infant for the first time in the five months old, the infant was being nursed still at the six months old (Plate 5).

Grooming

Infant received a great deal of grooming from its mother beginning on its first day of life (Plate 6). The highest time spent on grooming of infant rhesus was found 28.13% of time by the 1st month, the 2nd month with 25%, the 3rd month with 23.13%, the 4th and 5th months with 18.75%, the 6th and 7th months with 17.50%, the 8th month with 16.88% and the 9th month with 16.25% (Fig. 2, Table 2).

Time off mother (moving)

The time of an infant spent off the mother increased with age (Plate 7). The infant spent moving (off the mother) 3.13% of time at the 3rd month, 9.38% of time at

the 4th month, 10.63% of time at the 5th month, 15.63% of time at 6th month, 17.50% of time at 8th and 20% of time at the 9th month. (Fig. 2, Table 2).

Resting

The infant spent time sitting off mother, sleeping, not engaged in social interaction and nor feeding. The infant spent resting 10.63% of time at the 1st month, 9.38% of time at the 2nd month, 8.13% of time at the 3rd month, 9.38% of time at the 4th month, 10% of time at the 5th month, 11.25% of time at the 6th month, 10.63% of time at the 7th month, 10% of time at the 8th month and 10.63% of time at the 9th month (Fig.2, Table 2).

Playing

First social play occurred during the two months old of an infant. Early social play occurred with infant peers or small juveniles (Plate 8). When no playmates were available, solitary object play was observed and usually consisted of manipulation twigs and small sticks with hands and mouth. The infant spent 3.13% of time at the 2nd month, followed by the 3rd month with 9.38%, the 4th month with 12.50%, the 5th month with 15.63%, the 6th and 7th

months with 20.63%, the 8th month with 21.88% and 9th month with 22.50% (Fig.2, Table 2).

Feeding

Infant began to consume solid foods during the two months old, while riding dorsally the mother or moving about independently (Plate 9). It consumed solid foods 3.13% of time during the 2nd and 3rd months, 10% of time during the 4th month, 13.75% of time during the 5th month, 18.75% of time during the 6thmonth, 27.50 of time during the 7th month, 29.38% time during the 8th month and 30.63% time during the 9th month (Fig.2, Table 2).

Based on the data for nine months, it was found that rhesus infants spent 20.21% (32.33 ± 13.29min) of their total time on grooming, followed by 16.88% (27 ± 7.45 min) on suckling, 15.14% (24.22 ± 25.93 min) on feeding, 14.03% (22.44± 6.69 min) on playing, 10.56% (16.89 ± 12.77 min) on moving, 10% (16 ±1.50 min) on resting, 6.74% (10.78 ± 13.42 min) carrying infant ventrally and 6.46% (10.33 ± 19.22 min) carrying infant dorsally (Fig. 3).

Table 1 Monthly duration on activity pattern of rhesus infant in Shinma Taung (observation time = 1440 mins).

Activity pattern	Time spent on activity pattern (mins)										
	June, 13 1st	July, 13 2nd	Aug, 13 3rd	Sep, 13 4th	Oct., 13 5th	Nov, 13 6th	Dec, 13 7th	Jan, 14 8th	Feb, 14 9th	Total	Mean ± SD
Ventral carrying	35	25	22	10	5	0	0	0	0	97	10.78 ± 13.29
Dorsal carying	0	10	15	17	21	15	10	5	0	93	10.33 ± 7.45
Suckling	63	60	48	37	24	11	0	0	0	243	27 ± 25.93
Grooming	45	40	37	30	30	28	28	27	26	291	32.33 ± 6.69
Moving (off the mother)	0	0	5	15	17	25	28	30	32	152	16.89 ± 12.77
Resting	17	15	13	15	16	18	17	16	17	144	16 ± 1.50
Playing	0	5	15	20	25	33	33	35	36	202	22.44 ± 13.42
Feeding	0	5	5	16	22	30	44	47	49	218	24.22 ± 19.22

Table 2 Monthly activity pattern of infant spent (observation time = 1440 mins).

Month	Activity pattern of infant (%)							
	Ventral carrying	Dorsal carrying	Suckling	Grooming	Moving	Resting	Playing	Feeding
June, 13 (1st)	21.88	0	39.38	28.13	0	10.63	0	0
July, 13 (2nd)	15.63	6.25	37.50	25.00	0	9.38	3.13	3.13
Aug, 13 (3rd)	13.75	9.38	30.00	23.13	3.13	8.13	9.38	3.13
Sep, 13 (4th)	6.25	10.63	23.13	18.75	9.38	9.38	12.50	10.00
Oct., 13 (5th)	3.13	13.13	15.00	18.75	10.63	10.00	15.63	13.75
Nov, 13 (6th)	0	9.38	6.88	17.50	15.63	11.25	20.63	18.75
Dec, 13 (7th)	0	6.25	0	17.50	17.50	10.63	20.63	27.50
Jan, 14 (8th)	0	3.13	0	16.88	18.75	10.00	21.88	29.38
Feb, 14 (9th)	0	0	0	16.25	20.00	10.63	22.50	30.63
Percentage	6.74	6.46	16.88	20.21	10.56	10.00	14.03	15.14

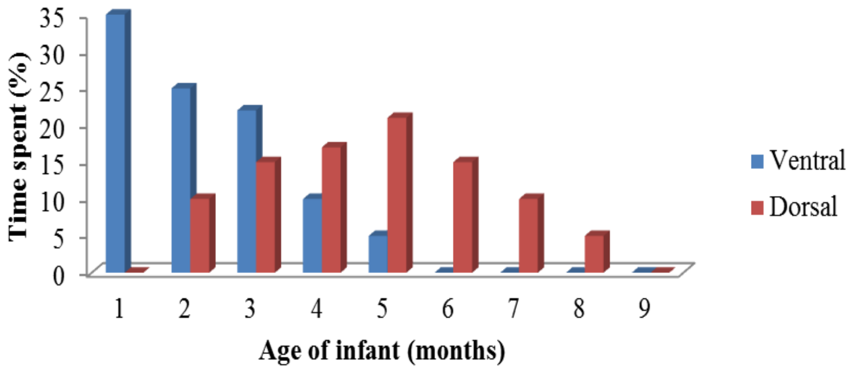

Figure1. Monthly time spent according to the position adopted by infant staying on the mother

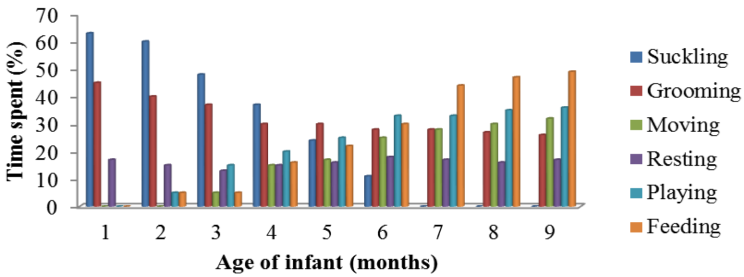

Figure2. Monthly time spent on infant according to mother-infant relationship

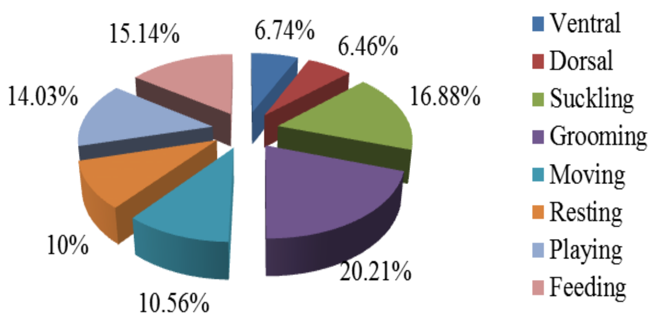

Figure3. Percentage of the activity patterns of infant *Macaca mulatta*

Plate1. Focal study groups of mother and infant

Plate 2. Embraced her infant with one arm

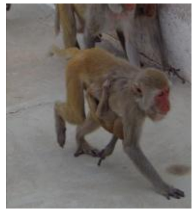

Plate3. Mother carrying her infant (Ventral side)

Plate 4. Mother carrying her infant (Dorsal side)

Plate 5. Suckling behaviour

Plate 6. Grooming behaviour

Plate7. Moving behaviour

Plate 8. Playing behaviour

Plate 9. Feeding behaviour

Discussion

Mother-infant relationship and infant behavior of rhesus macaque (*Macaca mulatla*) were conducted during the study period of June, 2013 to February 2014. In this study, transference of infants between their parents was rarely observed during the study months Dixson and Fleming (1981) reported that after the infant *Aotus lemurinus* had suckled, transfer from mother to father occurred. This is probably due to different habit of species.

In the present study, ventral carrying position occurred most frequently during the one month old infant rhesus but thereafter the infant was increasingly positioned on the dorsal surface of the mother. Moreover the infants spent 6.74% of their total time on mother (ventrally) and 6.46% on mother (dorsally). This finding is in agreement with the statement of Dixson and Fleming (1981) that the mother carries the single offspring during the 1^{st} week in *Aotus Lemurinus*. It may be assumed that this shows a strong and affectionate social bond between mother and her infant.

In this study the infants spent 16.88% of their total time on suckling. The infant did not remain on the nipple of the mother often at about 6 months old. This finding is in agreement with the statement of Fooden *et al.* (2000), who found that at about four or five months of age, mothers begin to resist the attempts of their offspring to suck or nurse.

Immature rhesus monkeys resemble many other primate species in enduring grooming relationship with their mothers. In this study during the one month old, rhesus infants spent the most time on grooming (20.21%) compared to other months. This is similar to a study of Aye Mi San (2007) that young infants were groomed more often by their mothers than older infants. It is assumed that the frequency of mother to infant grooming depends on the age of infant. Moreover most immaturity groomed with their mothers more than with any other partner. This is similar to a study of Nwe Nwe Win (2013) that mothers continue to groom their offsprings while they are moving out from the group. It is assumed that the social bonds are the strongest ones between mothers and infants.

During the three or four month old infant, they began gradually to break contact with their mothers. Moreover mothers also gradually rejected the attempt of their infants to make contact and gain access to nipple. The infants spent time of 10.56% moving (off the mother). This finding is supported by Hinde and Spencer-Booth (1967), who reported that during the first few weeks of infant life, mothers are almost entirely responsible for maintaining contact and proximity of their infants. During the two or three months old infant life, mothers frequently break contact with and walk away from their infants, while the infants follow their mothers and make contact with them. It may be assumed that mothers encourage their infants staying independently.

In this study, during the two months old infants spent time playing with twigs and leaves. As infants became more independent, they spent increasing amount of time in rough and tumble play with their peers. The infants spent time of 14.03% on playing. This is similar to a study of Nwe Nwe Win (2013), who reported that the juveniles and infants spent more of playing behavior of than the adults. This finding is similar to the report of Rotundo, *et al.* (2005), who found that during the second month most play consisted of the infant moving and jumping around other members of the group on playing with twigs and leaves. When the infants were three months old, they started chasing juveniles in the group. When the infants were older, play chases continued. It is assumed that it is associated with their physical and behavioral development and socialization.

In this study infant began to consume solid food at the two months old and it was weaned by seventh month. The infant spent 15.14% of this total time on feeding. This finding is supported by Rotundo, *et al.* (2005), who reported that in Argentina, infants began to manipulate twigs and food items during the two months old and that by four months old they eat fruits. It is assumed that infants explore the environment and start foraging independently.

It was concluded that rhesus infants spent the most time on grooming compared to other activities. The grooming between mother and her younger infant was frequently observed especially in the birth season. So the strongest social bonds are linked between mother and infant. Thus it is important for the group living primates. The finding of the present study is contributing to future researchers who study with reproductive biology and animal behaviors of macaques in Myanmar.

Acknowledgements

First author greatly indebted to Dr Zaw Jat, Rector and Dr Aye Aye Han, Pro-Rector, Banmaw University for his encouragement. Thank are also due to Dr Sann Sann, Head of Professor and Dr Win Mar, Professor, Department of Zoology, Banmaw University for their suggestion in preparing this paper.

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J. Myanmar Acad. Arts Sci. 2019 **Vol. XVII. No.3**

ASSESSMENTS ON CURRENT STATUS OF ELEPHANT PRODUCTS TRADE IN SOME AREAS OF MYANMAR

Su Su Naing[1], Kyi Thar Khaing[2], Sapai Min[3] and Khin War War[4]

Abstract

Current status of Elephant product trade in some areas of Myanmar was studied from January 2017 to March 2018. The four main study areas were Yangon, Mandalay, Kyaiktiyo (Golden Rock) and Bhamo. The research was based on field studies and involved interviews with local dealers and on direct observation. The pieces of skin and teeth were recorded in the pagoda markets from Kyaiktiyo. The ivory products and product shops were recorded as highest in Bogyoke market from Yangon areas. Items were observed at the study sites included skins, teeth, and bones mainly for use in traditional medicine and various ivory products were on sale to foreigners, especially from China border country.

Keywords- ivory products

Introduction

Myanmar is the largest country in mainland Southeast Asia. Bordering five nations, Bangladesh, India, Thailand, Lao PDR and the People's Republic of China where it is strategically located as a land bridge between South and East Asia. The country is endowed with rich natural resources–arable land, forestry, minerals including gas and oil, and freshwater and marine resources (ADB, 2006). Although rich in wildlife, habitat loss, illegal and unregulated hunting for domestic and international trade, threaten the existence of many species in Myanmar (Rao *et al*., 2005; Shepherd and Nijman 2007 a,b, 2008).

Myanmar is thought to have the largest remaining population of wild Asia elephants in Southeast Asia, and is second only to India in all of Asia (Kemf and Santiapillai, 2000). In the past elephants were smuggled into Thailand for use in the logging industry. Since Thailand's logging ban in

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[1.] Dr., Lecturer, Department of Zoology, University of Yangon
[2.] Dr., Lecturer, Department of Zoology, University of Yangon
[3.] Dr., Lecturer, Department of Zoology, University of Yangon
[4.] Dr., Associate professor, Department of Zoology, Bhamo University

1990, the illegal market targets and sells to the lucrative tourism industry (Shephard and Nijman, 2008).

The Asian elephant is listed as Endangered under the IUCN Red List classification system. The progressive decline of the original population across Asia is largely due to unrelenting human–elephant conflict, habitat loss and fragmentation, as well as ongoing illegal capture, killing and trade. Asian elephants are protected in Myanmar as a “Totally Protected Species” under the protection of Wildlife and Wild Plants and Conservation of Natural Areas Law. Violation of this law is an offence punishable with imprisonment for a term which may extend to seven years, or with a fine which may reach 50,000 MMK ($1,490) (Aung, 1997).

Myanmar is the most significant country in South-east Asia for elephant conservation, with an estimated 2,000 elephants remaining in the wild, with a further 5,600 elephants registered in captivity. There are numerous threats to elephants in Myanmar, including loss of

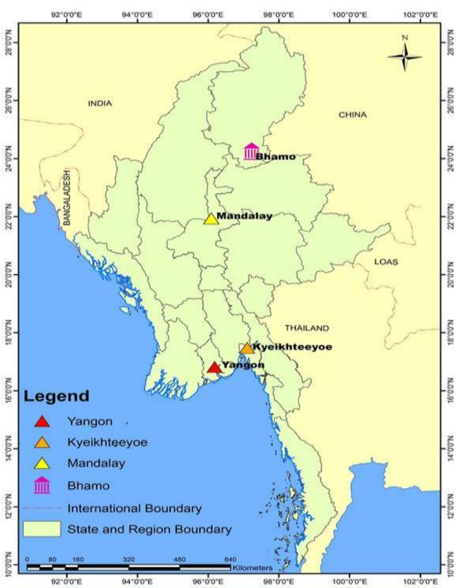

Figure1. Map of the study areas

Source by Geography Department, University of Yangon

habitat, and poaching for the wildlife trade. The Forest Department has recorded 98 elephants killing in the last six years; this is likely to be just a small proportion of the true number.

The present research is carried out with the following objectives: to collect data on: the number of trades identified, the quantity of stock, or the reported availability of additional stock.

Materials and Methods

The study was undertaken from January 2017 to March, 2018. The four main study areas; Yangon, Mandalay, Kyaihtiyo (Golden Rock) and Bhamo which is located in the Kachin State were conducted. The research was based mainly on field studies and involved interviews with dealers to assess the source of the material and on direct observation. Visits to the four study sites; once for Mandalay and Golden Rock each sites and three times for Yangon and Bhamo were undertaken during the survey period. Information relating to identification of the number of traders, the quantities of stocks were collected via interviews with traders and observations and counting of elephant parts and derivatives at the markets. All specimens and parts observed were recorded by taking photos. All elephant parts, such as ivory, skin, teeth, and any other parts were assessed at public and private markets.

Conservation status

The conservation status of recorded species was also identified in accordance with global and national status of threatened categories; The World Conservation Union (formerly the International Union for the Conservation of Nature and Natural Resources) (IUCN) (2017), Conservation on International Trade in Endangered species of Wild Flora and Fauna (CITES) (2017) and Myanmar Wildlife Protection Law (MWPL) (1994).

Results

In 2017, a total of 30 shops were recorded. In Yangon, 13 shops were recorded. In these, three retail outlets along the south entrance of Shwedagon pagoda and ten shops from Bogyoke market were observed In Mandalay, 8 shops were recorded and seven retail outlets along the south entrance of Mahamuni pagoda and only one shop at downtown were observed. In Kyaiktiyo (Golden Rock pagoda), eight retail outlets shops were recorded along the entrance of pagoda market. In Bhamo, only one traditional medicine shop was observed. In 2018, the total of 28 elephant parts shops and 1017 items were found from all study areas.

A total of 16 ivory shops were observed from Shwedagon pagoda and Bogyoke market. In these, three retail outlets along the south entrance of Shwedagon pagoda and 13 shops from Bogyoke market were observed with 844 pieces of ivory and elephant products. In Bogyoke market, there are openly displayed the ivory products such as bead necklaces, pendants, bangles, pipe smokings, name seals, earrings and others. Moreover, mostly numbers of elephant bones products were found as bangles, bead necklaces. Along the southern entrance of Shwedagon pagoda, three shops were observed in ivory products such as the statue of gong with ivory carved, small forks and spoons, small hairpin the highest items of ivory products were found as 311pendants and 109 bangles from Yangon area. In Yangon, 457 items of elephant products were observed in the last year and more markedly increase in this year. According the interview surveys with the shop owners in market, mostly the ivory products are sold to Chinese visitors.

Mandalay, the ancient city of Myanmar, a total of seven shops were recorded in the entrance of Mahamuni pagoda. Among these, three ivory product shops occurred and another three shops were non ivory parts as the religious beads necklaces, pendants of elephant's bone. A total of 127 elephant products items were observed that pendants were most numbers of items. Also an ivory shop was found in the city but we didn't get the photo record of ivory products item. Although there are decreased the ivory products shops than the last year, one owner who showed many of creative ivory carved photos in his phone for sale but we didn't get photo record.

Kyaiktiyo (Golden rock) is very famous pagoda where many shops are found as traditional medicine. A total of four shops have found the ivory and elephant parts such as skins, molar teeth and tip of ivory. There were observed three molar teeth, 22 pieces of molar teeth, nine skin pieces and one tip of ivory during the survey period. The numbers of elephant parts were found for sale in traditional medicine shop. According to the interview with dealers, mostly of elephant's parts are used for traditional medicine. In this year, the items of elephant parts for sale were observed about half of the fewer than the last year.

Bhamo is a city of Kachin State in the northernmost part of Myanmar, lies within 65 km (40 ml) of the border with Yunnan Province, China. Only one traditional medicine shop was observed with six skin pieces and five pieces of molar teeth of elephant parts. During the surveys period, three kg of elephant's bone powder were found for sale. According to the interview with dealers, most of elephant parts were used for traditional medicine. The ivory and elephant part items were fewer than the last year.

According to compare of two years survey, although the elephant parts shops decreased markedly in Kyaiktiyo (Golden rock) pagoda market, increased in Yangon area.The elephant parts such as hoof, tail, raw pieces of tusk didn't found in all study sites in this year.

Table 1. Numbers of ivory products shop and non-ivory products shop from four study areas in 2017

No.	Study areas	Study sites	Numbers of shop	Total
1	Yangon	Shwedagon pagoda	3	13
		Bogyoke market	10	
2	Mandalay	Maharmuni pagoda	7	**8**
		Ivory shop	1	
3	Kyaiktiyo (Golden pagoda)	Pagoda market	8	**8**
4	Bhamo	TM shop	1	1
		Total	**30**	**30**

Table 2. The numbers of elephant products' shop in 2018

Sr.	**Study areas**	**Study sites**	**Numbers of shop**		**Total**	**Remarks**
			Ivory products shop	Non-ivory products shop		
1	Yangon	Shwedagon pagoda	3	-	3	
		Bogyoke market	6	7	13	
2	Mandalay	Maharmuni pagoda	3	3	6	
		Ivory shop	1	-	1	did not get photo
3	Kyaiktiyo (Golden pagoda)	Pagoda market	1	3	4	
4	Bhamo	TM shop	-	1	1	
		Total	**14**	**14**	**28**	

Table 3. Compare of the Ivory product's shop in four study areas (2017 and 2018)

Sr.	Study areas	2017		**Total**	2018		**Total**	Remarks
		IPS	NIPS		IPS	NIPS		
1	Yangon	13	-	**13**	9	7	**16**	
2	Mandalay	8	-	**8**	4	3	**7**	
3	Kyaiktiyo (Golden Rock)	-	8	**8**	1	3	**4**	
4	Bhamo	-	1	**1**	-	1	**1**	
	Total	21	9	**30**	14	14	**28**	

IPS -Ivory products shop

NIPS - Non ivory products shop

Table 4. Elephant parts showing the different items and products offered for sale in four study sites in 2017

Items used	Products	Study sites				Total	Remark
		Yangon	Mandala y	Kyaiktiyo	Bham o		
Ivory parts	Bead necklace	22	4	26	-	26	
	Earrings	3	5	8	-	8	
	Hairpin	6	13	19	-	19	
	Religious icons carved	23	115	138	-	138	
	Bangle	32	8	40	-	40	
	Pendants	216	173	389	-	389	
	Ring	-	10	10	-	10	
	Ivory carving	12	12	24	-	24	
	Name seals	5	2	7	-	7	
	Brooch	6	2	8	-	8	
Raw tusks	Piece of tusk	46	18	93		93	
	Tip of tusk	-	-	6	-	6	
Non-ivory parts	Pendants	2	-	2	-	2	
	Bead necklace	58	8	66	-	66	
	Hair pin	24	63	87	-	87	
	Hair rings	2	18	20	-	20	

Items used	**Products**	**Study sites**				**Total**	**Remark**
		Yangon	Mandalay	Kyaiktiyo	Bhamo		
	Rings (bone)	-	-	-	-	-	
Ivory parts	Skin piece	-	-	38	5	43	
	Molar teeth	-	-	5	-	5	
	Piece of molar teeth	-	-	42	8	50	
	Tail	-	-	4	-	4	
	Piece of tail	-	-	6	-	6	
	Hoof	-	-	2	-	2	
	Piece of hoof	-	-	10	-	10	
	Total	**457**	**451**	**142**	**13**	**1063**	

Table 5. Elephant parts showing the different items and products offered for sale in four study sites in 2018

Items used	**Products**	**Study sites**				**Total**	**Remark**
		Yangon	Mandalay	Kyaiktiyo	Bhamo		
Ivory parts	Bead necklace	35	-	-	-	35	
	Earrings	8	10	-	-	18	
	Hairpin	35	-	-	-	35	
	Religious icons carved	8	22	-	-	30	
	Bangle	109	7	-	-	116	
	Pendants	311	28	-	-	339	
	Ring	24	2	-	-	26	
	Ivory carving		1	-	-	1	Not get photo
	Pipe smoking	11	2	-	-	13	
	Name seals	18	3	-	-	21	
	Animal's statue (elephant)	6	3	-	-	9	

Items used	**Products**	**Study sites**				**Total**	**Remark**
		Yangon	Mandalay	Kyaiktiyo	Bhamo		
	Stick of tusk	4	-	-	-	4	
	Gong and tusk statue	3	-	-	-	3	
	Small fork	15	-	-	-	15	
	Small spoon	8	-	-	-	8	
Raw tusks	Piece of tusk	-	-	-	-	-	
	Tip of tusk	-	-	1		1	
Non-ivory parts	Pendants (bone)	5	-	-	-	5	
	Bangle (bone)	132	-	-	-	132	
	Bead necklace (bone)	58	21	-	-	79	
	Hairpin (bone)	32	18	-	-	50	
	Tail hair rings	10	-	-	-	10	
	Rings (bone)	12	10	-	-	22	
	Skin piece	-	-	9	6	15	
	Molar teeth	-	-	3	-	3	
	Piece of molar teeth	-	-	22	5	27	
	Bone powder (kg)	-	-	-	-	-	3 kg
	Total	**844**	**127**	**35**	**11**	**1017**	

Table 6. Compare of the ivory products and non ivory products in four study areas (2017 and 2018)

Items used	Products	2017	2018	Remarks
Ivory parts	Bead necklace	26	35	
	Earring	8	18	
	Hairpin	19	35	
	Religious icons carved	138	30	
	Bangle	40	116	
	Pendants	389	339	
	Ring	10	26	
	Ivory carving	24	1	
	Name seals	7	21	
	Brooch	8	-	
	Pipe smoking	-	13	
	Animal's statue (elephant)	-	9	
	Stick of tusk	-	4	
	Gong with ivory carved	-	3	
	Small falk	-	15	
	Small spoon	-	8	
Raw tusks	Piece of tusk	93	-	
	Tip of tusk	6	1	
Non-ivory parts	Pendants	2	5	
	Bangle	-	132	
	Bead necklace	66	79	
	Hairpin	87	50	
	Hair rings	20	10	
	Rings (bone)	-	22	
	Skin piece	43	15	
	Molar teeth	5	3	
	Piece of molar teeth	50	27	
	Tail	4	-	
	Piece of tail	6	-	
	Hoof	2	-	
	Piece of hoof	10	-	
	Bone powder (kg)	-		3 kg
	Total	**1063**	**1017**	

Table 7. Compare of elephant products in four study sites (2017 and 2018)

No.	Study sites	Products	
		2017	2018
1.	Yangon	457	844
2.	Mandalay	451	127
3.	Kyaiktiyo	142	35
4.	Bhamo	13	11
	Total	**1063**	**1017**

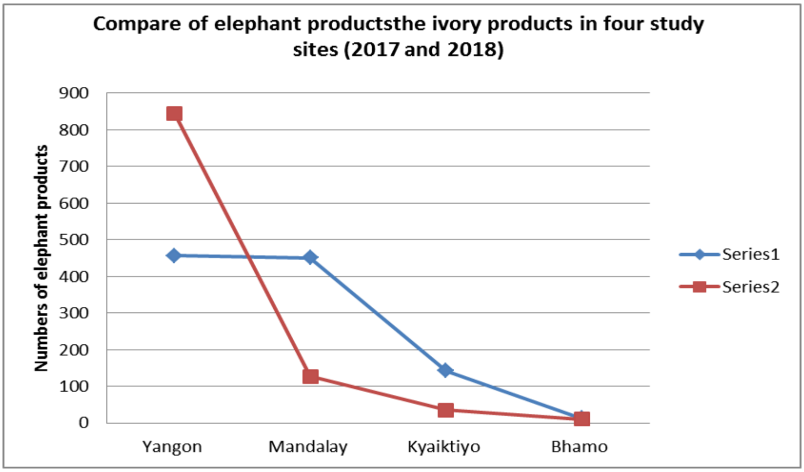

Figure 2. Compare of elephant productsthe ivory products in four study sites (2017 and 2018)

Discussion and Conclusion

Elephant ivories and parts were traded across the country such as Yangon, Mandalay, Kyaiktiyo and Bhamo areas. Many of the products are traded with neighbor countries mainly with China and marketed to foreign visitors rather for domestic use. Products were observed to be commonly used for traditional medicine, accessories, souvenirs and decoration such as gong with ivory, small forks and spoons, beads necklaces, earrings, bangles, many pendants.

According the results from market surveys of four study sites, ivory products were observed to be found in Yangon and Mandalay, and elephant parts were observed to be found in Kyeiktiyo and Bhamo. Ivory products were most commonly found in Bogoke Market and Shwedagon Pagoda in Yangon. There has been an increase in numbers of products as well. According to interviews, many of the buyers are from China. There are a few buyers from Japan as well. It was observed that the main market for ivory products trade is Bogyoke Market in Yangon. Owners of the shops stated that tips of the ivory can be purchased from elephant veterinarians, and raw materials come from Hle Ku Township. It can be assumed that products are purchased from elephant hunters from around Bago mountain ranges.

Numbers of products for sale at Shwedagon Pagoda has decreased compare to last year. Sculptors are usually from older generations and the new generation did not inherit the career. As a result, new generation is unable to sculpt ivory but sell products that are already in stock. They also said that it is uneasy to obtain elephant ivory. The decrease in numbers of products shows that the market at the Pagoda has weakened in comparison with last year.

Also, in Kyeikhtiyo, the number of shops and elephant products sales has decreased by half in contrast with last year. According to interviews with shop owners, ivory products sales are not in accordance with the laws, and there are severe penalties for selling them. However, it was observed that there are secret sales of ivory products. Effectiveness in law enforcement has increased compare to last year.

There has been a decline in number or elephant parts in Bhamo as well. Overall, it is observed that sales in all study sites except Bogyoke market have declined.

All dealers stated that law enforcement is taken more seriously during this year. However, traders in Mandalay still have a black market for ivory and elephant products. Although retail shops are now less visible in general trade market except Bogyoke Market, connections and hidden markets for ivory and elephant products still exist. It is observed that Mandalay and

Yangon are commercial towns of ivory and elephant products. Dealers intend to sell it to foreign buyers, both tourists and foreign middlemen, who would then in turn take it out of the country (Shephard, 2002).

According to the results, the highest numbers of ivory product were observed in Bogyoke market from Yangon area. In Mandalay, although the displayed of ivory product items for sale were decreased than the last year, the secret markets flow to China. Similarly, dealers stated that in Kyeiktiyo (Golden Rock) and Bhamo, elephant parts were carried to China. There is evidence to trade across the border with China (Li and Wang, 1999; Yi Ming *et al.*, 2000).

Rule of law and enforcement is necessary for commercial cities, border areas and gates. It is vital to increase border gate patrols for illegal ivory and elephant products trade, and restrict rules and regulations to control illegal trades in border areas. To prevent

Acknowledgements

I extend my profound gratitude to Professor Dr. Thida Lay Thwe, Head of Zoology Department, University of Yangon for her permission to conduct this research topic. I wish to express my sincere thank to Professor Dr.Aye Mi San, Department of Zoology, University of Yangon ,for her suggestion and encouragement. My deepest obligation is next extended to Former Professor Dr. Khin Maung Swe, Department of Zoology, Dagon University, for his advice, valuable suggestion, supervision and guidance of this research work.

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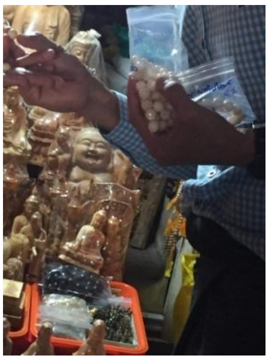

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Displayed for sale of Ivory products and non ivory products in south entrance of Shwedagon Pagoda

Plate1.Displayed for sale of ivory and non ivory products in Pagoda market from Mandalay



(a) Molar teeth (b) Molar teeth (c) Molar teeth

(d) Pieces of molar teeth (e) Pieces of molar teeth (f)Tip of ivory

(g) Elephant skins (h) Elephant skins (i) Elephant skin

Plate 2. Showing for sale of elephant parts in traditional medicine shops from Kyeikytiyo


17-12-2017 16:12


17-12-2017 16:07


17-12-2017 16:17

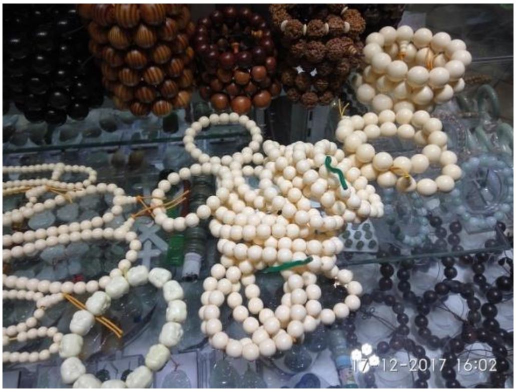

17-12-2017 16:18


17-12-2017 16:17


17-12-2017 16:28

Plate 3. Displayed for sale the ivory products in jewelry shops from Bogyoke market

(a) Ivory products (b) Ivory products (c) Necklace (bone)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(d) Beads bangles (bone) (e)Bangles(bone)

(f) Beads bangles (bone) (g) Bangles (bone)

Plate 4. Displayed for sale of ivory products in jewelry shops (a), (b) and non ivory products in accessories shops (c), (d), (e), (f) and (g) from Bogyoke market

(a) Elephant skin (b) Ring (ring)

(c) Tip of Ivory (d) Piece of molar teeth

(e) Bone powder

Plate 5. Displayed for sale of ivory products in Bhamo (a) Elephant skin, (b) Ring (c) Tip of ivory, (d) Piece of molar teeth, (e) Bone powder

MOTHER - INFANT RELATIONSHIPS AND INFANT BEHAVIOUR OF RHESUS MACAQUE (*MACACA MULATTA*) IN SHINMA TAUNG AREA, YESAGYO TOWNSHIP, MAGWAY REGION

Nwe Nwe Win[1], Aye Pyae Sone[2]

Abstract

Mother - infant relationships and infant behaviour of rhesus macaque (*Macaca mulatta*) from Shinma Taung area, Yesagyo Township, Magway Region was investigated during June, 2013 to February, 2014. The maternal care showed various motherly activities such as suckling, grooming, ventral and dorsal carrying. The behavioral data included the time spent on various infant activities. The activities patterns were observed on the focal sampling using five minutes scan at ten minutes intervals from 08:30hr to 16:30hr. A total of 288 scan samples in 1440mins of scan time were used. Based on nine months data, rhesus infant spent 20.21% of total time on grooming, followed by 16.88% on suckling, 15.14% on feeding, 14.03% on playing , 10.56% on moving (off mother), 10% on resting, 6.74% carrying infant ventrally and 6.46% carrying infant dorsally. Rhesus infant spent more time on grooming compared to the other activities. The strongest social bonds were linked between mother and infant.

Keywords: *Macaca mulatta*, mother-infant relationships, infant behaviour, Shinma Taung

Introducion

A characteristic feature of primate's life history is the prolonged period of postnatal development in which newborns depend on adults especially their mothers, for nutrition, transport and protection. Many researchers have focused on the development of infant nonhuman primates, attempting to identify factors that influence the ontogeny of social behavior, with strong emphasis on the mother – infant relationship (Nicolson, 1987).

Studies on infant development in Old World monkeys have concentrated on macaques, especially *Macaca mulatta* (Forster and Cords, 2002). Animals allocate resources, both time and energy, to offspring care at a cost to their own maintenance, survival or future reproduction (Altmann and Samuels, 1992).

Most studies on primate maternal styles have been conducted with Cercopithecine species in which variation in infant - directed behaviors usually fall along two dimensions: protection and rejection (Lathouwers and Elsacker, 2004).

Rhesus mother and infant relationship are the most intimate and long lasting of all the relationships (Rowell *et al.*, 1964). The infants of rhesus monkeys begin life completely development on their mother for survival, receiving all nourishment, physical warmth and other basic biological support. Rhesus mothers are usually very restrictive as they restrict the movements and social contacts of their infants from approaching or being approached by other monkeys (Sambyal *et al.,* 2009). The physical contact between mother and infant is influenced by the mother's feeding behavior (Jhnson, 1986).

Rhesus monkey, infants develop distinctive social relationships with their mothers and with same-age peers during their initial weeks and months of life respectively. However, these relationships differ substantially, from one another in several fundamental ways. An infant attachment relationship with its mother is firmly established long before it begins any interactions with peers. The resulting attachment is specific to its mother whereas the infant typically develops multiple relationships with several different peers. The infant's frequency and duration of interactions with its mother are highest during its first month of life and decline steadily thereafter, especially following the birth of younger siblings (Suomi, 2005).

Data on behavior of macaques from different places already existed Naw Phaw Phaw Say, 2005; Aye Mi San, 2007 and Nwe Nwe Win, 2013. Therefore there is sometime needed to carry out the same trend work in Shinma Taung area. The monkeys become so much accustomed to the human presence, making at easier to conduct study. Thus Shinma Taung was chosen as the area of study.

The objectives of the present study are:

- to study the relationship between rhesus mother and different ages of infant
- to compare the activity patterns of infant in rhesus macaque.

Materials and Methods

Study area

The study site is Shinma Taung area located at Yesagyo Township, Magway Region. The location of the area is situated between North latitudes (21° 35' N and 21° 38' N) and East longitudes (95° 03' E and 95° 10' E). According to the data from Dry Zone Greening Department, Pakokku District, the highest peak of Shinma Taung is 525.52 meters (1723 ft) and total area is 687.17 hectares (18995 acre) wide. It is covered with deciduous forest (Fig 1, Plate 1).

Study period

The study was conducted from June, 2013 to February, 2014.

Study group

From the study site, nine pairs of mother with offspring were selected as focal sampling monkeys. All individuals were recognized by features of their faces, tails and others body parts.

Activity pattern

The field's trip was conducted once a month (one day) during the study period. Data was collected using five minutes scan sampling with ten minutes intervals from 08:30 hr to 16:30hr.A total of 288 scan samples in 1440 mins of scan time were used. Behavioral data included time spent on various infant activities and activity between mother-infant relations was observed according to Foster and Cords (2002).

Behavioral categories

Suckling behavior	- Infant sucks nipple in mouth (whether clinging or not)
Grooming behavior	- Infant groomed by mother
Resting behavior	- Sitting off mother, sleeping, not engaged in social interaction, nor feeding.
Ventral carrying	- carrying position ventrally by mother
Dorsal carrying	- carrying position dorsally by mother
Moving (off the mother)	- Infant locomoting off mother
Playing	- social play with peers
Feeding	-searching for, manipulating and swallowing food items

Figure 1. Map of Shinma Taung area (Source: Geology Department, Pakokku University)

Results

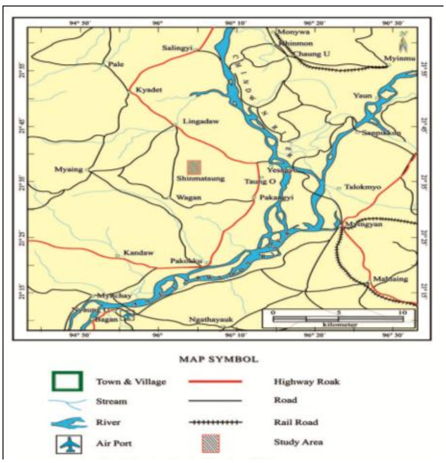
Mother-infant relationship and infant behaviour

Plate 1. Study area of Shinma Taung

The newborn offspring was usually taken care of by females. Newborn infant spent a great deal of time nursing or sleeping on chest of its mother (Plate 1, and 2). Mother may restrict and control the activities of its infant by physically restraining it and by making contact with it frequently.

Time on mother (ventrally)

While the infant was on the mother, it regularly changed positions.



During one month old infant, the infant was almost always positioned ventrally to the carrying mother (Plate 3). But infant was not found ventrally to the carrying mother during the six months old (Table 1). The infant spent on the mother (ventrally) was 21.88% of time at the 1st month, 15.63% of time at the 2nd month, 13.75% of time at the 3rd month, 6.55% of time at the 4th month and 3.13% of time at the 5th month, respectively (Fig. 1, Table 2).

Time on mother (dorsally)

The infant began riding dorsally during the two months old infant (Plate 4). As the infant achieved locomotion independence, the percentage decreased to 9.38% during the 6th month and 6.25% by the 7th month. By the 8th month, the mother seldom carried the infant when traveling (3.13%) but never observed by the 9th month (Fig. 1 and Table 2).

Suckling

Mother helps with hands only pressing the infant to ventral side while carrying or suckling. Mother was generally very attentive to her infant but sometimes had difficult supporting infant in gaining nipple access, especially while moving or foraging. The infant spent on the nipple 39.38% of time at the 1st month, followed by the 2nd month with 37.50%, the 3rd month with 30%, the 4th month with 23.13%, the 5th month with 15% and the 6th month with 6.88% (Fig.2, Table 2). Although the mother rejected the infant for the first time in the five months old, the infant was being nursed still at the six months old (Plate 5).

Grooming

Infant received a great deal of grooming from its mother beginning on its first day of life (Plate 6). The highest time spent on grooming of infant rhesus was found 28.13% of time by the 1st month, the 2nd month with 25%, the 3rd month with 23.13%, the 4th and 5th months with 18.75%, the 6th and 7th months with 17.50%, the 8th month with 16.88% and the 9th month with 16.25% (Fig. 2, Table 2).

Time off mother (moving)

The time of an infant spent off the mother increased with age (Plate 7). The infant spent moving (off the mother) 3.13% of time at the 3rd month, 9.38% of time at the 4th month, 10.63% of time at the 5th month, 15.63% of time at 6th month, 17.50% of time at 8th and 20% of time at the 9th month. (Fig. 2, Table 2).

Resting

The infant spent time sitting off mother, sleeping, not engaged in social interaction and nor feeding. The infant spent resting 10.63% of time at the 1st month, 9.38% of time at the 2nd month, 8.13% of time at the 3rd month, 9.38% of time at the 4th month, 10% of time at the 5th month, 11.25% of time at the 6th month, 10.63% of time at the 7th month, 10% of time at the 8th month and 10.63% of time at the 9th month (Fig.2, Table 2).

Playing

First social play occurred during the two months old of an infant. Early social play occurred with infant peers or small juveniles (Plate 8). When no playmates were available, solitary object play was observed and usually consisted of manipulation twigs and small sticks with hands and mouth. The infant spent 3.13% of time at the 2nd month, followed by the 3rd month with 9.38%, the 4th month with 12.50%, the 5th month with 15.63%, the 6th and 7th

months with 20.63%, the 8th month with 21.88% and 9th month with 22.50% (Fig.2, Table 2).

Feeding

Infant began to consume solid foods during the two months old, while riding dorsally the mother or moving about independently (Plate 9). It consumed solid foods 3.13% of time during the 2nd and 3rd months, 10% of time during the 4th month, 13.75% of time during the 5th month, 18.75% of time during the 6thmonth, 27.50 of time during the 7th month, 29.38% time during the 8th month and 30.63% time during the 9th month (Fig.2, Table 2).

Based on the data for nine months, it was found that rhesus infants spent 20.21% (32.33 ± 13.29min) of their total time on grooming, followed by 16.88% (27 ± 7.45 min) on suckling, 15.14% (24.22 ± 25.93 min) on

Table 1.	Time spent	Mean ± SD	10.78 ±	10.33 ±	27 ± 25.93	32.33 ±	16.89 ±	16 ± 1.50	22.44 ±	24.22 ±	Table 2	Activity	Feeding	0	3.13	3.13	10.00	13.75	18.75	27.50	29.38	30.63	15.14

feeding, 14.03% (22.44± 6.69 min) on playing, 10.56% (16.89 ± 12.77 min) on moving, 10% (16 ±1.50 min) on resting, 6.74% (10.78 ± 13.42 min) carrying infant ventrally and 6.46% (10.33 ± 19.22 min) carrying infant dorsally (Figure. 3).

Activity pattern	June,	July, 13 2nd	Aug, 13 3rd	Sep, 13 4th	Oct, 13 5th	Nov, 13 6th	Dec, 13 7th	Jan, 14 8th	Feb, 14 9th	Total
Ventral carrying	35	25	22	10	5	0	0	0	0	97
Dorsal carrying	0	10	15	17	21	15	10	5	0	93
Suckling	63	60	48	37	24	11	0	0	0	243
Grooming	45	40	37	30	30	28	28	27	26	291
Moving (off the	0	0	5	15	17	25	28	30	32	152
Resting	17	15	13	15	16	18	17	16	17	144
Playing	0	5	15	20	25	33	33	35	36	202
Feeding	0	5	5	16	22	30	44	47	49	218

Month	Ventral	Dorsal	Suckli	Groom	Moving	Resting	Playing
June, 13 (1st)	21.88	0	39.38	28.13	0	10.63	0
July, 13 (2nd)	15.63	6.25	37.50	25.00	0	9.38	3.13
Aug, 13 (3rd)	13.75	9.38	30.00	23.13	3.13	8.13	9.38
Sep, 13 (4th)	6.25	10.63	23.13	18.75	9.38	9.38	12.50
Oct, 13 (5th)	3.13	13.13	15.00	18.75	10.63	10.00	15.63
Nov, 13 (6th)	0	9.38	6.88	17.50	15.63	11.25	20.63
Dec, 13 (7th)	0	6.25	0	17.50	17.50	10.63	20.63
Jan, 14 (8th)	0	3.13	0	16.88	18.75	10.00	21.88
Feb, 14 (9th)	0	0	0	16.25	20.00	10.63	22.50
Percentage	6.74	6.46	16.88	20.21	10.56	10.00	14.03

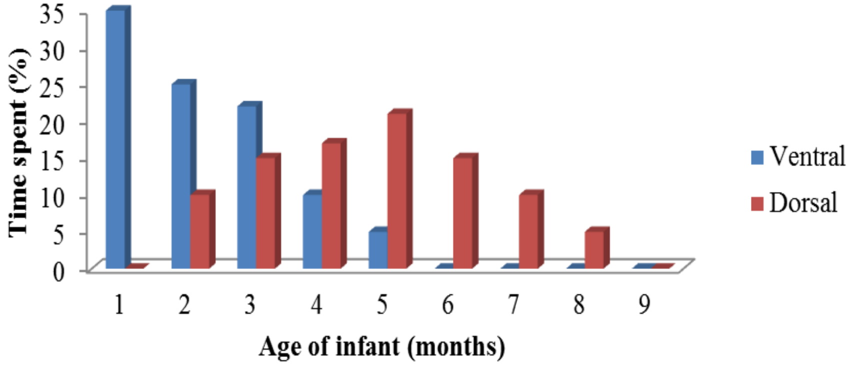


Figure1. Monthly time spent according to the position adopted by infant staying on the mother

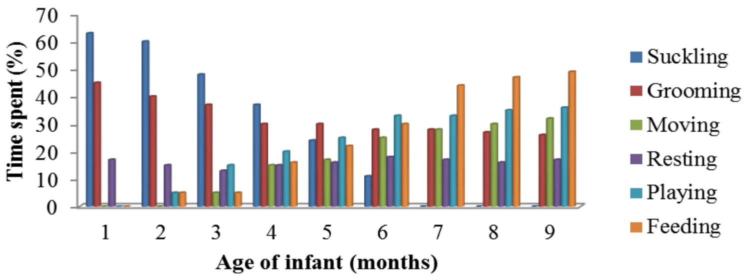


Figure2. Monthly time spent on infant according to mother-infant relationship

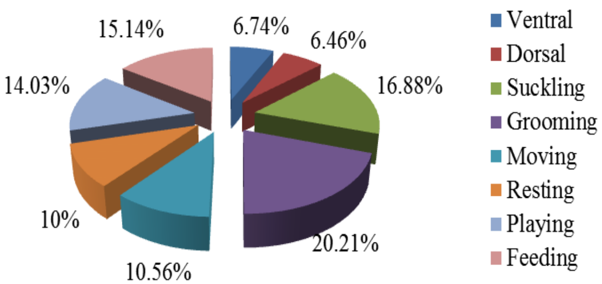


Figure3. Percentage of the activity patterns of infant *Macaca mulatta*

Plate1. Focal study groups of mother and infant

Plate 2. Embraced her infant with one arm

Plate3. Mother carrying her infant (Ventral side)

Plate 4. Mother carrying her infant (Dorsal side)

Plate 5. Suckling behaviour

Plate 6. Grooming behaviour

Plate7. Moving behaviour

Plate 8. Playing behaviour

Plate 9. Feeding behaviour

Discussion

Mother-infant relationship and infant behavior of rhesus macaque (*Macaca mulatla*) were conducted during the study period of June, 2013 to February 2014. In this study, transference of infants between their parents was rarely observed during the study months Dixson and Fleming (1981) reported that after the infant *Aotus lemurinus* had suckled, transfer from mother to father occurred. This is probably due to different habit of species.

In the present study, ventral carrying position occurred most frequently during the one month old infant rhesus but thereafter the infant was increasingly positioned on the dorsal surface of the mother. Moreover the infants spent 6.74% of their total time on mother (ventrally) and 6.46% on mother (dorsally). This finding is in agreement with the statement of Dixson and Fleming (1981) that the mother carries the single offspring during the 1st week in *Aotus Lemurinus*. It may be assumed that this shows a strong and affectionate social bond between mother and her infant.

In this study the infants spent 16.88% of their total time on suckling. The infant did not remain on the nipple of the mother often at about 6 months old. This finding is in agreement with the statement of Fooden *et al.* (2000), who found that at about four or five months of age, mothers begin to resist the attempts of their offspring to suck or nurse.

Immature rhesus monkeys resemble many other primate species in enduring grooming relationship with their mothers. In this study during the one month old, rhesus infants spent the most time on grooming (20.21%) compared to other months. This is similar to a study of Aye Mi San (2007) that young infants were groomed more often by their mothers than older infants. It is assumed that the frequency of mother to infant grooming depends on the age of infant. Moreover most immaturity groomed with their mothers more than with any other partner. This is similar to a study of Nwe Nwe Win (2013) that mothers continue to groom their offsprings while they are moving out from the group. It is assumed that the social bonds are the strongest ones between mothers and infants.

During the three or four month old infant, they began gradually to break contact with their mothers. Moreover mothers also gradually rejected the attempt of their infants to make contact and gain access to nipple. The infants spent time of 10.56% moving (off the mother). This finding is supported by Hinde and Spencer-Booth (1967), who reported that during the first few weeks of infant life, mothers are almost entirely responsible for maintaining contact and proximity of their infants. During the two or three months old infant life, mothers frequently break contact with and walk away from their infants, while the infants follow their mothers and make contact with them. It may be assumed that mothers encourage their infants staying independently.

In this study, during the two months old infants spent time playing with twigs and leaves. As infants became more independent, they spent increasing amount of time in rough and tumble play with their peers. The infants spent time of 14.03% on playing. This is similar to a study of Nwe Nwe Win (2013), who reported that the juveniles and infants spent more of playing behavior of than the adults. This finding is similar to the report of Rotundo, *et al.* (2005), who found that during the second month most play consisted of the infant moving and jumping around other members of the group on playing with twigs and leaves. When the infants were three months old, they started chasing juveniles in the group. When the infants were older, play chases continued. It is assumed that it is associated with their physical and behavioral development and socialization.

In this study infant began to consume solid food at the two months old and it was weaned by seventh month. The infant spent 15.14% of this total time on feeding. This finding is supported by Rotundo, *et al.* (2005), who reported that in Argentina, infants began to manipulate twigs and food items during the two months old and that by four months old they eat fruits. It is assumed that infants explore the environment and start foraging independently.

It was concluded that rhesus infants spent the most time on grooming compared to other activities. The grooming between mother and her younger infant was frequently observed especially in the birth season. So the strongest social bonds are linked between mother and infant. Thus it is important for the group living primates. The finding of the present study is contributing to future researchers who study with reproductive biology and animal behaviors of macaques in Myanmar.

Acknowledgements

First author greatly indebted to Dr Zaw Jat, Rector and Dr Aye Aye Han,
Pro-Rector, Banmaw University for his encouragement. Thank are also due to Dr Sann Sann, Head of Professor and Dr Win Mar, Professor, Department of Zoology, Banmaw University for their suggestion in preparing this paper.

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J. Myanmar Acad. Arts Sci. 2019 **Vol. XVII. No.3**

INSECT PESTS AND THEIR PREDATORS ON POMELO PLANT IN HMAWBI TOWNSHIP

Thinzar Oo[1], Pwint Thu Aye[1], Khin Myat Hmwe[2], Su Su[3]

Abstract

Insect pests are the major enemies for the agriculture sector. This research was thus conducted at the pomelo private farm of Let Pan Tan Su village located in Hmawbi Township, Yangon Region from November, 2016 to October, 2017. Biweekly sampling was focused on 30 trees in 10 acres. A total of 18 species of insect pests belonging to 17 genera under 13 families of 7 orders and 12 species of the predators belonging to 11 genera under 4 families of 3 orders were recorded on the pomelo plants. The highest pest species composition was found in *Pseudoccus citriculus* (58738 individuals) while the lowest as *Chiridopsis saclaris* (22 individuals). The study insect pest was most infested in the month of November. *Oecophyllas maradina,* was observed as dominant predator species (125196 individuals) while *Coecinellas septempunctata*as least predator species with only one individual during the study periods. The research could be investigated not only the incident and population of insect pest but also the pest seasonal and peak of outbreak time.

Keywords: Insect pests, predators, population, pomelo plant

Introduction

Insect diversity is the highest in the living world. Most are infected in all plant species, the storage grains and seeds as pest Smithsonian Information, (2016). Major pests are called serious pests of a crop (or crops) in a restricted locality, or are economic pests over a large part of the distributional range of the crop plant. Insect pests can destroy any parts of the plants and fruit loss. Due to their wide spread, frequent occurrence, biological interest, wide range of host plants and other aspects of academic interest, insect species can be recognized as major pests.

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[1] Assistant Lecturers, Department of Zoology, University of Yangon.
[2.] Lecturer, Department of Zoology, University of Yangon
[3.] Professor (Retired), Department of Zoology, University of Yangon.

The farm owners in Southeast Asia lost at least 300 million USD per year due to pests.

According to FAO, (1991) mentioned that this is one of the most important factors of economic aspects owing to fruit loss by insect pests. Predators are carnivores feed only on living prey and some feed on insects. Wise, (1993) also stated that predator are important role in the control of pest ecological balance.

Pomelo is very important plant for economic aspects because of its nutrition value and high demand in the world. In Southeast Asia, pomelo is grown in home gardens, in mixed citrus orchards and in pure pomelo orchards. Aung Soe, (1999) mentioned that 18,211 hectares of pomelo plantation in Myanmar. Hmawbi Township is one of the main agricultural Township in Yangon Region, Myanmar. Due to the high demand of pomelo fruit, most of the orchards farm owners in Hmawbi Township are desired to plant them. Flowering season of pomelo plants is from January to February. Fruiting season takes for five months (March to July), harvest time is October to December and the yield rate is 80-150 fruits per plant.

Casey Ng, (2015) recorded more than 74 insect pest species on pomelo trees. Hence, Insect pests are important for the yield of pomelo fruits. Because of insect pests, pomelo farm owners in Hmawbi Township faced reducing in pomelo yield in every year. Fruit loss is about 100-200 fruits per day. According to interview, about 30,000 fruits were lost in one crop season. Main cause of fruit loss is due to insect pests. Thus, the supply of demand is inadequate. No one had been conducted on the study of insect pests of pomelo plants in Myanmar and also in Hmawbi Township as yet. Thus the present study has been addressed to this issue and conducted at the pomelo private farm in Let Pan Tan Su Village, Hmawbi Township with the following objectives:

- To record the occurrence of insect pest species and their predators
- To evaluate the populations of insect pest and their predators

Materials and Methods

Study area

In Let Pan Tan Su Village, Hmawbi Township between latitude 17° 12′ 58″ and 17° 13′ 4″ and longitude 96° 2′ 58″ and 96° 3′ 7″ was chosen as the study area.

Study site

Pomelo private farm is Let Pan Tan Su Village was chosen as study site about ten acres. A total of 1200 trees is planted in 6 plots and 20 ft apart the plants in each plot have 17 rows and 15 trees in each row (Figure. 1).

Study design

Research design was targeted to the corner and center of pomelo plots in the farm. Five plants were chosen in each plot (one in each corner and one plant in the center of each plot). A total of 30 plants were studied as sample in study area (Fig. 2).

Study period and Sample collection

The study period lasted from November, 2016 to October, 2017. Biweekly data collection on the pest population was done from 7:00 am to 5:00 pm. The population of infested species on five selected plants was counted and recorded.

Identification

Identification was followed after Bland and Jaques (1978), Davidson and Lyon (1979), Hill (1983) and McGavin, (2000).

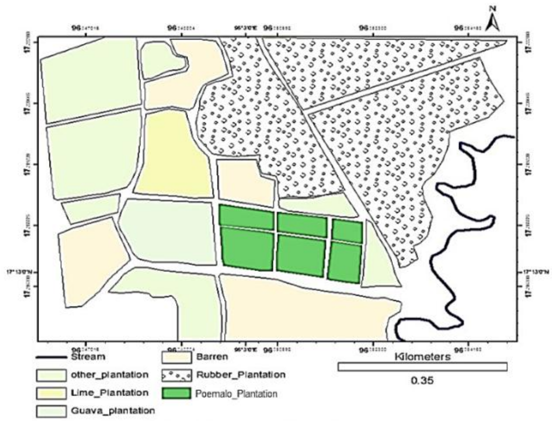

Figure 1. Layout plan of study sites in pomelo orchard farm

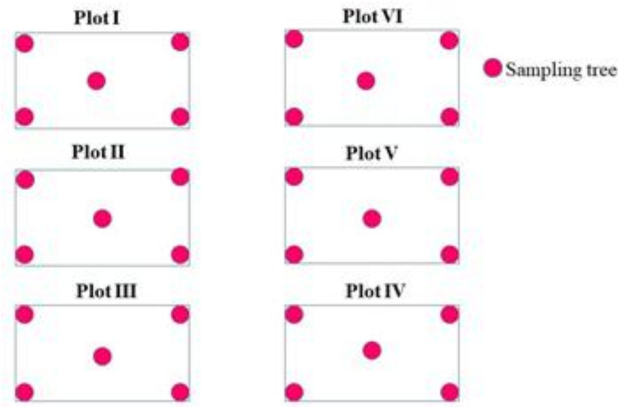

Figure 2. Flow chart of research design

Results

A total of 18 species of insect pests belonging to 17 genera under 13 families of 7 orders was recorded on studied plants. Order wise contribution of the recorded insect pest species were Order Homoptera (5 species, 28%); Lepidoptera and Coleoptera (3 species each, 17%); Hemiptera, Heteroptera and Hymenoptera (2 species each, 11%) and, Diptera with only one species (5%). Of the recorded families, Pseudococcidae was highest species composition (17%, 3 species) (Table 1, Fig. 3, 4 and Plate 1).

With respect to monthly fluctuation in occurrence, insect pest species were more recorded in October (15 species) while less in March (6 species). Among the species, *Aleurocanthus woglumi, Coccus hesoerdium* and *Phyllocnistis citerlla* were observed throughout the studied months except November and *Pseudoccus citriculus* was not in September while *Holotrichia serrata* was found only in May and June (Table 2, Fig. 6).

Concerning with infestation rate, the peak infestation rate was observed in November with the population number (31332 individuals) while bottom in April (988 individuals). Regardless of the studied months and plots, the upper most abundance species was *Pseudoccus citriculus* (58738 individuals) while the lowermost as *Chiridosis saclaris* (22 individuals). Moreover, *Holotrichia serrata* had the noticeable number of population (4738 individuals) appeared only in May and June during the study periods.

Comparison among the study plots, Plot I had the highest population (33430 individuals) while the lowest in Plot IV (22001 individuals). *Pseudoccus citriculus* was dominant in Plot I, III, IV and VI with (18981, 6788, 10469 and 7163 individuals), respectively. *Pseudoccus longispinus* was dominant in Plot II and V with (9885 and 8615 individuals), respectively (Table 2, Fig. 5).

Regarding to the predators, 12 species of the predators belonging to 11 genera under 24 families of 3 orders were recorded during the study period (Table 3, Plate 2). Of the recorded orders, Order Hymenoptera represented with 2 predator species, Coleoptera with 4 species and Aranea with 6 species. From the point of families wise, the highest species composition was found in the family Araneidae (42%, 5 predator species) and followed by Coccinellidae (33%, 4 species), Formicidae (17%, 2species) and then Oxyopidae (8%, only one species). The occurrence of highest predator species was found in February (12 species) while lowest in April and May (3 species each). Among the recorded predator species, *Oecophylla maradina* and *Camponotus* species were found throughout the study periods but *Coecinella septempunctata* was found only in February (Table 3, Plate 2 and Fig. 4).

The predator infestation was peak in May (23395 individuals) while bottom in April (3893 individuals). *Oecophyllas maradina, Camponotus* species and *Eriovixia laglaisei* were regarded as dominant species with (125196, 15352 and 1217 individuals) respectively in all study plots. Only one individual *Coecinella septempunctata* was recorded during the study periods (Table 3, 4, Plate 2).

Discussion

Total record of (18 insect pest species) and 12 predator species in the present study was less than those reported by Casey Ng, (2015) who reported 74 species in SEA (except Myanmar). Thormann *et al,* (2016) mentioned that insect are extremely richness and unexplored species in every country because of difficult in sorting and identifying and; existing of methodological challenge Achieving less recorded pest species may be due to experience, time duration or different environmental condition. Having more experience and longer study time may achieve more species recorded.

In addition, only five species (*P. longispinus, P.citriculus, C.hesperidum, D. citri,* and *B. dorsalis*) among the recorded species were corresponded to the previous research by Mi Zin Mar Khine, (2015). Therefore, a total of thirteen insect pest species was reported as new record for pomelo plant which grown in Myanmar. The first step of Integrated Pest Management (IPM) in controlling a problem, whether it is an insect, disease or weed, is to identify it correctly Barkley, (2011).

According to the guide to crop protection (2016), control pests were achieved by implementing agronomic practices. It will help to manage to reduce certain insect species, as well as the levels of some disease organisms. The regular water supply is important before flowering and until after harvesting of pomelo fruits. Loquias, (2006) also mentioned that regular supply of water is need to sustain new shoot growth and development of flowers and fruits. The present study was noted that lesser insect's population level or extent of crop damage in plot III and IV may be related to delay irrigation and culture practices.

Hill, (1983) and Ahmed *et. al.*, (2014) reported that the list of major pests (including *A. woglumi*, *P. citriculus*, *P. demoleus* and *B. dorsali*) at citrus farms including pomelo in South East Asia and in Bangladesh with their infestation rate and active months which were accorded with the present study.

Thus the present study could be revealed the major pest species of the post-harvest time of pomelo plant in the present study area owing to noticeable population number and highest damage by these mention insect pest species especially in the months of November.

Ghosh, (1940) stated that predator can properly manage the infestation of insect. Spiders are predators of invertebrates, especially insects. They immobilized the prey them with venom inserted through their jaws ('fangs'). Natural predators may also be used in the control the pest. Barkley, (2011) also reviewed that biological controls are natural enemies of pests such as predatory and parasitic insects as well as birds.

The present study was supported above the statements as the predator species of *C.septempunctata* and *O.smaradina* were observed feeding on insect pest species *P. longispinus*, *P. citriculus* and *C. hesoerdium* during the study periods.

Moreover, as for suggestion the weaver ant species, *O. smaradina* can be used as control agent since they were recorded in highest number of population of in study farm throughout the study months.

Table 1. Recorded insect pest species on pomelo plants in study farm

No	Order	Family	Species	Common name	Plot I	Plot II	Plot III	Plot IV	Plot V	Plot VI	Total
1	Hemiptera	Aleyrodidae	*Aleurocanthus woglumi*	Citrus black fly	4208	3422	1617	646	3392	4653	17938
2			*Dialeuro descitri*	Citrus white fly	207	186	39	123	33	100	688
3	Homoptera	Pseudococcidae	*Ferrisi avirgata*	Striped mealybug	906	465	691	187	544	923	3716
4			*Pseudococcus longispinus*	Longtail mealybug	4593	9885	3202	1595	8615	4317	32207
5			*P. citriculus*	Longtail citrus mealybug	18981	7761	6788	10469	7576	7163	58738
6		Diaspididae	*Aonidiella aurantii*	California red scale	476	801	2565	1307	1385	1249	7783
7		Coccidae	*Coccus hesperidum*	Brown soft scale	726	884	2563	1322	323	1802	7620
8	Heteroptera	Coreidae	*Leptocoris aacuta*	Rice seed bug	231	178	123	185	147	161	1025
9		Pentatomidae	*Halyomorpha halys*	Brown marmorated stink bug	215	357	258	207	247	216	1500
10	Lepidoptera	Phyllocnistidae	*Phyllocnistis citrella*	Leaf minor	1854	2316	2993	3772	2168	1856	14959
11		Arctiidae	*Cyana coccinea*	Moth	33	25	15	12	18	24	127
12		Papilionidae	*Papillo demoleus*	Citrus butterfly	63	64	79	65	44	43	358
13	Diptera	Tephritidae	*Bactrocera dorsalis*	Fruit fly	295	552	133	165	629	484	2258
14	Coleoptera	Coccinellidae	*Holotrichia serrata*	White grub	518	650	960	1584	520	506	4738
15		Chrysomelidae	*Alticaly thri*	Beetle green	25	45	29	22	9	14	144
16			*Chiridopsis saclaris*	Golden beetle	0	12	4	1	2	3	22
17	Hymenoptera	Curculionidae	*Hypomeces squamous*	Gold- dustweevil	87	152	71	56	95	49	510
18			*Nematocerus spp.*	Shiny cereal weevil	12	325	346	283	145	47	1158
					33430	28080	22476	22001	25892	23610	155489

Table 2. Monthly insect pest populations on pomelo plant

No.	Species	Nov	Dec	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Total
1	*A. woglumi*	0	263	436	9869	422	241	3491	1294	199	768	351	604	17938
2	*D. citrifolii*	0	0	0	0	0	0	265	24	105	85	78	131	688
3	*F. virgata*	0	0	0	0	0	0	810	806	878	234	131	857	3716
4	*P. longispinus*	11616	3326	4590	5162	114	21	4953	966	185	0	0	1274	32207
5	*P. citriculus*	**17747**	**24881**	**4294**	**7543**	**174**	**22**	**2556**	**546**	**252**	**37**	**0**	**686**	**58738**
6	*A. aurantii*	0	0	0	0	0	0	12	0	0	2988	2226	2557	7783
7	*C. hesperdium*	0	112	402	1716	153	125	2463	1948	236	147	37	281	7620
8	*L. acuta*	206	117	207	1	0	0	255	123	9	1	0	106	1025
9	*H. halys*	283	314	251	0	0	1	159	73	64	49	106	200	1500
10	*P. citerlla*	0	29	164	335	173	563	8335	2645	137	691	1327	560	14959
11	*C. coccinea*	27	12	45	43	0	0	0	0	0	0	0	0	127
12	*P. demoleus*	59	30	37	38	0	2	0	1	24	62	82	23	358
13	*B. dorsalis*	1349	30	0	0	0	0	0	0	0	237	352	290	2258
14	*H. serrata*	0	0	0	0	0	0	3035	1703	0	0	0	0	4738
15	*Alticalythri*	0	0	0	0	0	4	96	34	4	0	0	6	144
16	*C. saclaris*	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**8**	**0**	**0**	**12**	**22**
17	*H. squamous*	45	76	34	26	0	9	135	50	22	22	16	75	510
18	*Nematocerus* spp.	0	0	0	0	0	0	631	326	80	49	72	0	1158
	TOTAL	31332	29190	10460	24734	1037	988	27196	10539	2203	5370	4778	7662	155489

(A) *Aleurocanthus woglumi*

(B) *Dialeurodescitri*

(C) *Ferrisiavirgata*

(D) *Pseudococcus longispinus*

(E) *Pseudococcuscitriculus*

(F) *Aonidiellaaurantii*

(G) *Coccus hesperidum*

(H) *Leptocorisaacuta*

(I) *Halyomorphahalys*

(J) *Phyllocnistis citrella*

Plate 1. Recorded insect pest species on Pomelo plants

(K) *Cyana coccinea*

(L) *Papillodemoleus*

(M) *Bactrocera dorsali*

(N)*Holotrichia serrata*

(O) *Alticaly thri*

(P) *Chiridopsis saclaris*

(Q) *Hypomeces squamous*

(R) *Nematocerus spp.*

Plate 1. Continued: Recorded insect pest species on Pomelo plants

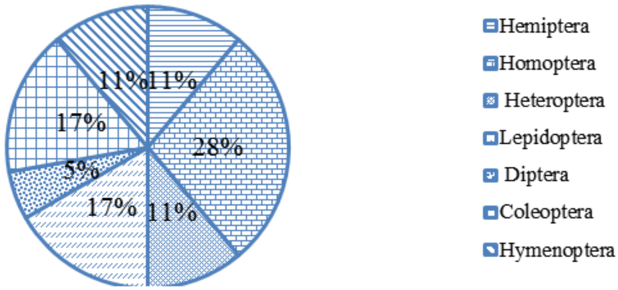

Figure 3. Insect species composition in recorded Order

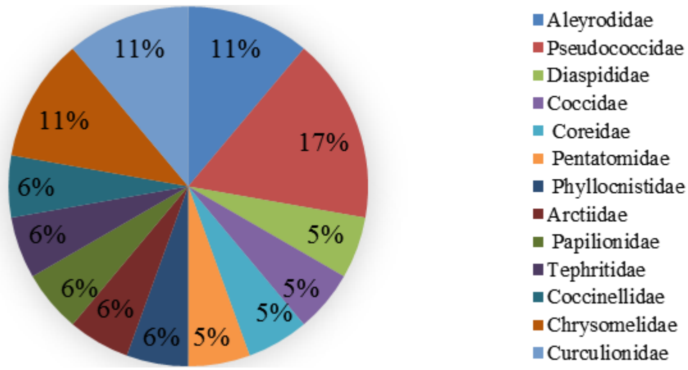

Figure 4. Insect species composition in recorded Families

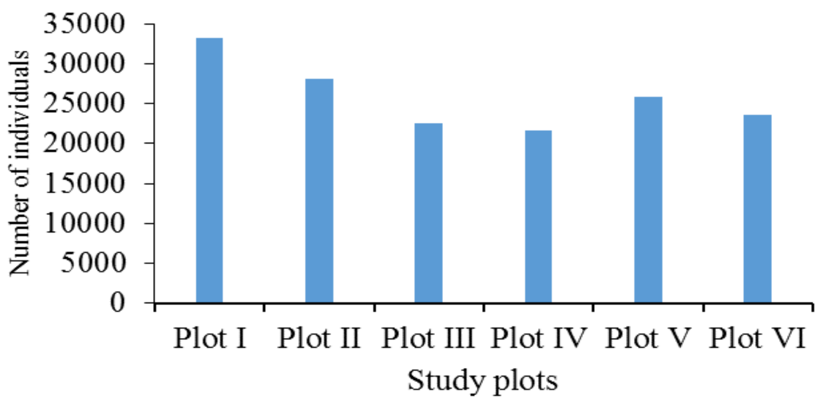

Figure 5. Populations of insect pest species among study plots during the study period

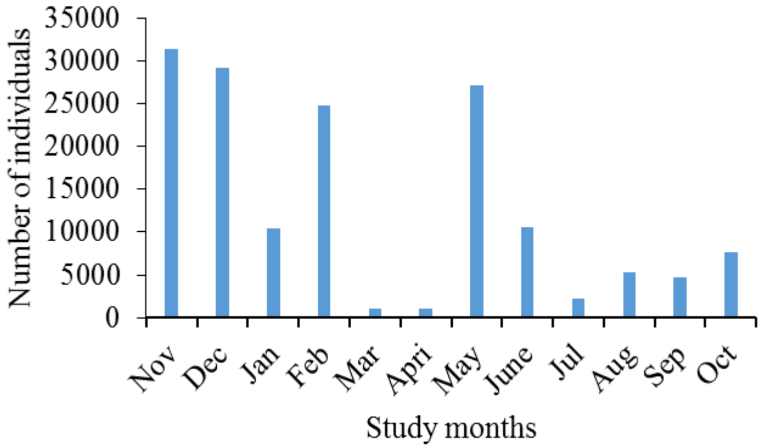

Figure6. Comparison of monthly populations of insect pest in study farm

Table 3. Recorded predator species on pomelo plants in study farm

No.	Order	Family	Species	Common name	Plot I	Plot II	Plot III	Plot IV	Plot V	Plot VI
1	Hymenoptera	Formicidae	*Oecophyllas maradina*	Weaver ant	34078	16725	12673	17564	21531	22626
2			*Camponotus* sp.	Carpenter ant	1579	4398	4290	1784	1934	1331
3	Coleoptera	Coccinellidae	*Coccinella transversalis*	Transverse ladybird beetle	27	6	4	5	10	8
4			*Chilomenessexmaculata*	Six-spoted ladybird	3	9	8	3	6	2
5			*Chilocorous stigma*	Twice-stabbed Lady Beetle	6	9	3	2	1	14
6			*Coccinella septempunctata*	Beetle seven spots	0	0	0	0	0	1
7	Araneae	Araneidae	*Argiope versicolour*	Multi-coloured St. Andrew's cross spider	11	14	4	71	77	12
8			*Gasteracantha hasseltii*	Hasselt's spiny spider	5	10	5	1	14	3
9			*G. khuli*	Black and white spiny spider	7	7	11	6	4	7
10			*Eriovixia laglaisei*	Laglaise's garden spider	1101	209	357	203	144	335
11			*Telamoni adimidiata*	five spot head spider	36	16	407	20	39	34
12		Oxyopidae	*Oxyopes shweta*	Black and white spider	9	7	10	75	6	15

Table 4. Monthly predator species on pomelo plants in the study farm

No.	Species	Nov	Dec	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Total
1	*O. smaradina*	12190	14479	14191	14056	4484	4542	21878	17737	6930	6678	3649	4382	125196
2	*Camponotus* species	1340	354	2458	3587	1249	717	1508	2940	468	429	169	133	15352
3	*C. transversalis*	18	16	5	26	0	0	0	0	0	0	0	0	65
4	*C. sexmaculata*	11	12	5	3	0	0	0	0	0	0	0	0	31
5	*C. stigma*	14	7	15	3	1	0	0	0	0	0	0	0	40
6	*C. septempunctata*	0	0	0	1	0	0	0	0	0	0	0	0	1
7	*A. versicolor*	0	0	0	5	0	0	0	1	0	125	1	1	133
8	*G. hasseltii*	0	0	0	3	0	0	0	0	0	0	0	11	14
9	*G. Khuli*	0	0	0	5	0	0	0	2	0	2	0	5	14
10	*E. Laglaise's*	0	0	0	1119	1	0	0	1	5	22	41	28	1217
11	*T. dimidiata*	0	0	0	18	1	2	9	0	9	25	14	39	117
12	*Oxyopesshweta*	0	0	0	1	0	0	0	0	2	91	19	9	122
	TOTAL	13573	14868	16674	18827	5736	5261	23395	20681	7414	7372	3893	4608	142302


(A) *Oecophyllas maradina*


(B) *Camponotus* sp.


(C)*Chilomenes transversalis*


(D) *Chilomenes sexmaculata*


(E) *Chilocorous stigma*


(F) *Coccinella septempunctata*


(G)*Argiope versicolour*


(H) *Gasteracantha hasseltii*


(I) *Gasteracantha khuli*


(J) *Eriovixia laglaisei*


(G) *Telamonia dimidiata*


(H) *Oxyopes shweta*

Plate 2. Recorded predator species on Pomelo plants

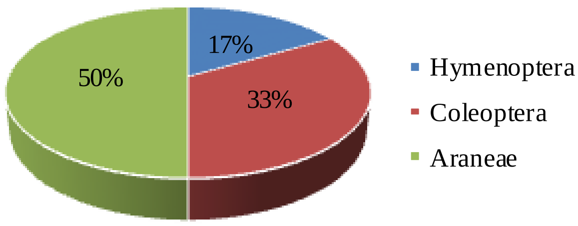

Figure 7. Predator species composition in recorded Orders

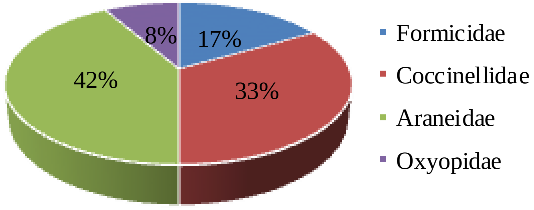

Figure 8. Predator species composition in recorded families

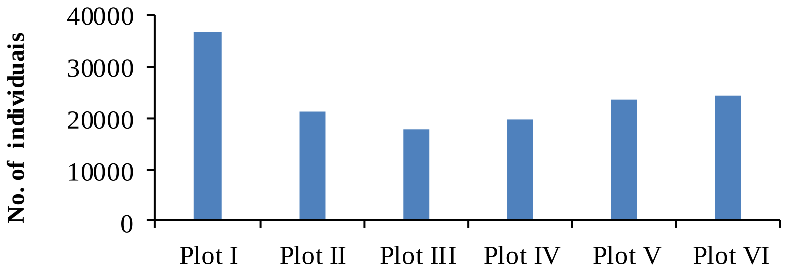

Figure 9. Occurrence of predator species among study plots during the study area

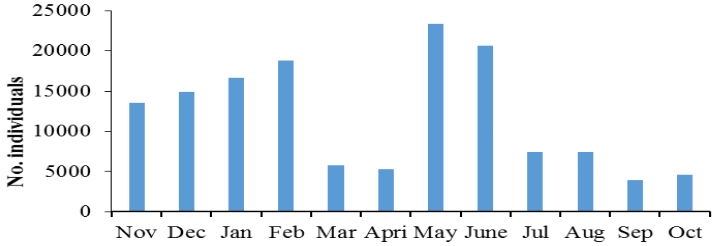

Figure 10. Comparison of monthly occurrence of predator species in study area

Conclusions

The present research was conducted at the pomelo private farm of Let Pan Tan Su Village, Hmawbi Township, Yangon Region to record the occurrence of insect pest species and their predators on pomelo plant. Biweekly data of insect pests and predators were identified and counted the population number to roughly predict when and which insects infected on pomelo plants. A total of 18 insect pest species and 12 predators were recorded. Predation was observed. Pest populations could identify in studied farm at risk from various pests. To carry out management of pest problems on pomelo plants, regular checking is needed on the targeted plants in the orchard for the presence of pests throughout the entire growing season.

Acknowledgements

We would like to greatly indebted to Dr Thida Lay Thwe, Professor/Head, Department of Zoology, University of Yangon for her permission to conduct this study. Our special thanks go to Dr Aye Mi San, Professor, Department of Zoology, University of Yangon for moral support. Heartfelt thanks go to Dr Aye Mya Phyu, lecturer, Department of Zoology, Sittway University for providing the literature. We are also grateful to U Myo Naing, Orchard's owner of pomelo farm, Let Pan Tan Su Village for allowing as do this research in his farm.

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J. Myanmar Acad. Arts Sci. 2019 **Vol. XVII. No.3**

PLANT-PARASITIC NEMATODES ASSOCIATED WITH MONSOON RICE IN BANMAW ENVIRONS

Khin War War[1] and Cho Nandar[2]

Abstract

A total of 17 species of plant-parasitic nematodes belongs to 15 genera, 11 families and two orders were recorded in soil and root samples at different monsoon rice fields from Banmaw environs. In two orders, the total percent of genera (73.33%) in order Tylenchida was higher than in the order Aphelenchida (26.67%). In soil samples, the highest individual population of plant-parasitic nematodes (2280/100cc soil) was recorded in after harvest sample from rice field III (RF III) and the lowest (7/100cc soil) was in second sample (S II) from rice field I (RF I). All the root samples, the highest (2008/10g root) and lowest (33/10g root) population of plant-parasitic nematodes was recorded in sixth sample (S VI) and fourth sample (S IV) respectively from RF III. Among the recorded plant-parasitic nematodes, three species (*Hirschmanniella oryzae*, *Meloidogyne graminicola*, *Aphelenchoides besseyi*) were the major rice nematodes. In all collected samples, the highest (15246 individuals) and lowest (413 individuals) individual population were observed in *H. oryzae* and *A. besseyi* respectively. The highest individual number of major rice nematodes, lowest seed weight and yield were recorded in RF III. The lowest number of nematodes, highest seed weight and yield were observed in RF I.

Keywords: monsoon rice, plant-parasitic nematodes

Introduction

Rice (*Oryza sativa* L.) is a monocotyledonous plant belonging to the grass family (Gramineae) and the genus *Oryza*. Rice is the major staple food for the 57% of the world's population and it provides approximately 23% of daily caloric intake, especially in Southeast Asia (Rehm and Espig, 1976). In many Asian countries where rice is the principal food, the importance of this crop in relation to food security and socio-economic development is evident.

1. Associate Professor, Department of Zoology, Banmaw University
2. Demonstrator, Department of Zoology, University of Yangon

In Myanmar, rice is the national food crop and is needed for local consumption as well as for export (Dobermann and Fairhurst, 2000). There are a lot of rice diseases commonly classified into four groups which are fungus, bacteria, virus, and nematodes. Nematodes can be found in almost any type of environment and include both free-living and parasite species. Plant-parasitic nematodes typically live in soil and feed on cells in plant roots. They threaten agricultural crops throughout the world, particularly in tropical and sub-tropical regions (Oteifa, 1997). They are one of the main pathogen of rice diseases, cause annual yield loss ranging from 10 to 20% (Sharma *et al*., 2000). Nematode parasites of rice include rice white tip nematode (*Aphelenchoides besseyi*), rice stem nematode (*Ditylenlenchus angustus*), root rot 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.) (Butler, 1913; 1919). A number of genera of plant-parasitic nematodes are associated with rice in Myanmar, but only four genera of proven or potential economic importance are found. They are rice stem nematode, white tip nematode, root-knot nematode and root rot nematode (Mya Mya, 1983). In the past, emphasis had been placed on nematode disease of rice such as Ufra, white tip, root-knot in Myanmar. In the present, root-rot disease occurred in many rice growing areas of the country according to a few local reports. The present study focus on Banmaw environs because there is no information and recorded data of rice nematodes in this environs. This research seems useful to gather additional information on the incidence of nematode species on rice in Myanmar.

The objectives of the present study were to identify and record the plant-parasitic nematodes from soil, root and seed samples of rice fields, to assess the plant-parasitic nematode species composition in collected soil, root and seed samples and to compare the population of major rice plant-parasitic nematodes, seed weight and yield.

Materials and Methods

Study areas and period

Survey was carried out at different sites in Sihe (RF I), Siin (RF II) and Mophein (RF III) villages in Banmaw Township, Kachin State. This study was conducted from June 2017 to January 2018 (Plate 1 A, B, C).

Collection of soil and plant samples

Before planting, soil samples were taken with garden trowel from four corners and one center of each site and at a depth between 10 cm to 20 cm. Two weeks after planting, soil and root samples were collected fortnight interval from the same location and depth of each site. The samples were placed in an individual plastic bag with a complete label.

Extraction of nematodes from soil, root and seed

Soil samples were thoroughly mixed and 100 cc of the composite soil were used for nematode extraction by Whitehead tray method (Whitehead and Hemming, 1965). The composite soil sample was evenly spread on a cloth which was in a plastic basket that attached in a plastic tray. About 250 ml of water was carefully added down the inside edge of the tray until the soil looked wet. The tray was kept at room temperature for 24 hr. Then the basket was slowly and carefully removed and nematodes suspension from the tray was concentrated by pouring into a 250 ml beaker and the nematodes suspension was left to settle for 2 to 3 hr for nematodes to sink in the bottom. The upper layer of water was carefully decanted to remain 20 ml nematode suspension. It was thoroughly mixed by pipetting for 15 sec then 1 ml of the suspension was transferred to a counting dish. They were counted and identified under compound microscope according to Chidwood (1950), Siddiqi (1985), Hunt (2002) and Bridge (2005). Root samples were washed and cut into small pieces then mixed together. A 10 g of mixed root pieces was spread on a two layers of tissue paper in a sieve attached with a glass Petri-dish. Water from wash bottle was poured between the sieve and the Petri-dish just to cover the roots. The rest steps were the same above method.

A total of 100 seeds were bisected with a nail clipper. The split seeds and husks were placed on a two layers of tissue paper in a sieve which attached with glass Petri-dish. The rest steps were the same as mentioned above (Plate 1 D, E, F).

Results

Occurrence and Composition of Plant-Parasitic Nematodes

A total of 17 species of plant-parasitic nematodes belongs to 15 genera, 11 families, six superfamilies and two orders were recorded in soil and root samples at different monsoon rice fields from Banmaw environs (Table 1). There were *Tylenchus* sp., *Polenchus* sp., *Sakia* sp., *Boleodorus* sp., *Tylenchorhynchus* sp., *Psilenchus* sp., *Helicotylenchus dihystera*, *H. multicinctus*, *Pratylenchus* sp., *Hirschmanniella oryzae*, *Meloidogyne graminicola*, *Macroposthonia xenoplax*, *Aphelenchus* sp., *Aphelenchoides besseyi*, *A. bicaudatus*, *Ektaphelenchoides* sp., and *Bursaaphelenchus* sp. (Plate 2, 3, 4). Among them, seven species was identified to species level and the rest nematodes were classified to genera level. In two orders, the total percent of genera (73.33%) in order Tylenchida was higher than the Aphelenchida (26.67%). The highest percent of genera (26.7%) was recorded in family Tylenchidae, followed by Hoplolaimidae (13%). The lowest and similar percent of genera (6.7%) were found in each of the rest nine families (Figure. 1, 2).

Population of Plant-parasitic Nematodes in Soil and root Samples from Study Sites

The plant-parasitic nematodes were found in initial soil samples (before planting) of all different rice fields. Among them, the highest individual nematodes population (153/100cc soil) was found in rice field III (RF III) from Mophein village and the lowest (53/100cc soil) in rice field II (RF II) from Siin village. The individual population of plant-parasitic nematodes in rice field I (RF I) from Sihe was moderate (131/100cc soil). After planting, in RF I, the highest individual population of plant-parasitic nematodes in soil sample (520/100cc soil) was observed in fifth sample, S V and the lowest population (7/100cc soil) was recorded in second sample, S II. In RF II, the highest individual population of plant-parasitic nematodes (1827/10g root) was observed in after harvest. The lowest population (53/100cc soil) was recorded in initial and second sample, S II. In RF III, the highest individual population of plant-parasitic nematodes (2208/100cc soil) was observed in after harvest sample. The lowest individual population (153/100cc soil) was recorded in initial and fourth sample, S IV. Among the all soil samples, the highest and lowest individual population of plant-parasitic nematodes was recorded in after harvest sample of RF III and second sample, S II in RF I respectively (Fig. 3). According to root samples, in RF I, the highest individual population of plant-parasitic nematodes (973/10g root) was observed in fourth sample, S IV and the lowest population (47/10g root) was recorded in second sample, S II. In RF II, the highest individual population of plant-parasitic nematodes (1173/10g root) was observed in fifth sample, S V and the lowest population (316/10g root) was recorded in first sample, S I. In RF III, the highest individual population of plant-parasitic nematodes (2008/10g root) was observed in sixth sample, S VI and the lowest individual population (33/10g root) was recorded in fourth sample, S IV. Among the all root samples, the highest and lowest individual population of plant-parasitic nematodes was recorded in sixth sample, S VI and fourth sample, S IV respectively in RF III (Fig. 4).

Population of Major Rice Nematodes in Soil and Root Samples and seed weight

Three major rice species, *Hirschmanniella oryzae* (root rot nematode), *Meloidogyne graminicola* (root knot nematode) and *Aphelenchoides besseyi* (white tip nematode) were found during study period. The highest individuals population of *H. oryzae* (7876 individuals) was observed from initial to harvested soil and root samples from RF II followed by the same population of RF I and RF III (3685 individuals/each). The highest individual population of *M. graminicola* (6825 individuals) was observed in RF III followed by RF II (1401 individuals) and RF I (554 individuals). The highest individual population of *A. besseyi* (357 individuals) was observed in RF III, followed by RF I (42 individuals) and RF II (14 individuals) (Fig 5). In seed extraction for *A. besseyi* nematode, it was not found in all seeds samples before planting. After harvested, the seed samples from RF I was found the only one *A. besseyi* female nematode. The rest seed samples from RF II and RF III were not observed. During study period, the highest total population of major rice plant-parasitic nematode (10867 individuals) was found in RF III, followed by RF II (9291 individuals) and RF I (4281 individuals) from initial to harvested soil and root samples. In seed weight, the highest seed weight was recorded in RF I (25g/1000 seeds) followed by RF II (24g/1000 seeds) and RF III (22g/1000 seeds). The highest yield from interview survey of rice field owners was recorded in RF I (4122kg/ha) followed by RF II (3866kg/ha) and RF III (3092kg/ha). The highest individual number of major rice plant-parasitic nematodes and the lowest seed weight and yield were recorded in RF III. The lowest number of nematodes and highest seed weight and yield were observed in RF I (Figure. 6). According to the results, major rice plant-parasitic nematodes, seed weight and yield were related each other.

A. Rice field I (RE I) in Sihe village

B. Rice field II (RF II) in Siin village

C. Rice field III (RF III) in Mophein village

D. Extraction of nematode from soil

E. Extraction of nematode from root

F. Extraction of nematode from seeds

Plate1. Studied of rice fields and extraction of nematodes

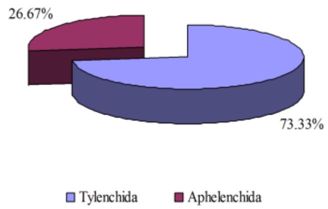


Figure 1. Percent composition of plant parasitic nematode genera in different orders

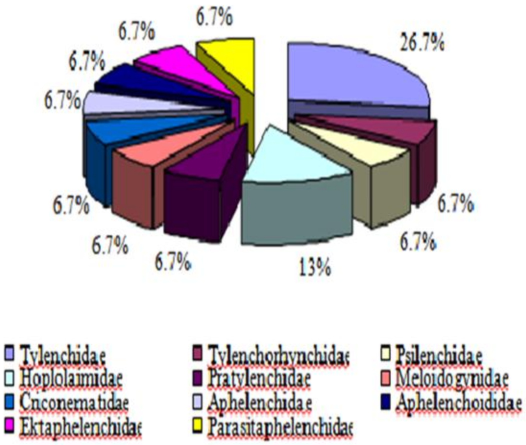


Figure 2. Percent composition of plant parasitic nematode genera in different families

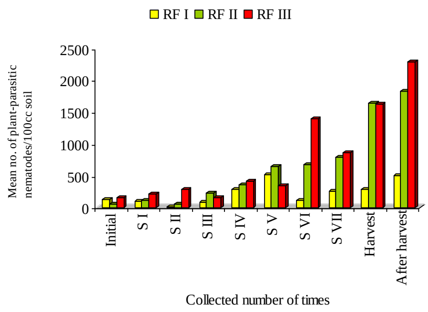


Figure 3. Plant-parasitic nematodes in all soil samples

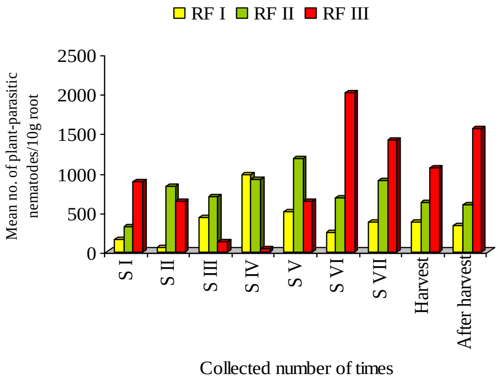


Figure 4. Plant-parasitic nematodes in all root samples

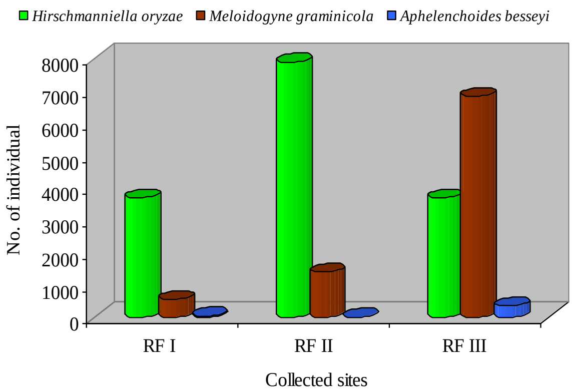


Figure 5. Individuals number of major rice nematodes population in different sites

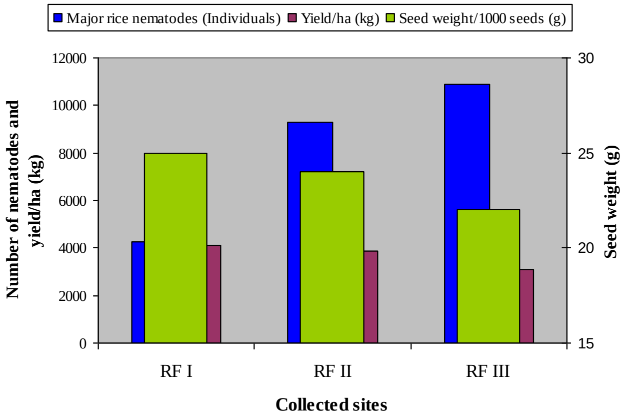


Figure 6. Major rice plant-parasitic nematodes and seed weight in different sites

A. *Tylenchus* sp. 400X

B. *Polenchus* sp. 400X

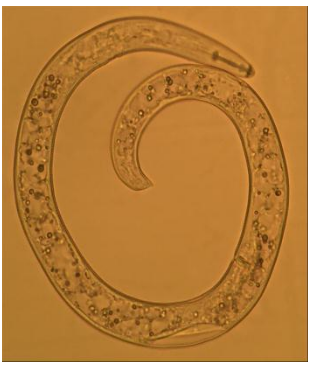
C. *Sakia* sp. 400X

D. *Boleodorous* sp. 400X

E. *Tylenchorhynchus* sp. 400X

F. *Psilenchus* sp. 400X

Plate 2. Plant-parasitic nematodes under family Tylenchidae, Tylenchorhynchidae, Psilenchidae



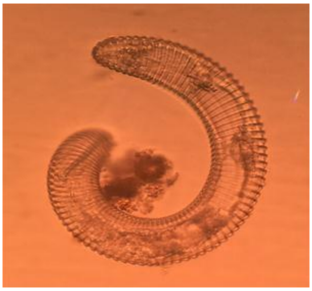
A. *Helicotylenchus dihystera* 400X

B. *H. multicintus* 400X

C. *Pratylenchus* sp. 400X

D. *Hirschmanniella oryzae* 400X

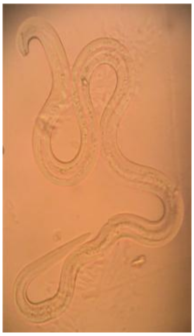
E. *Tylenchorhynchus* sp. 400X



F. *Psilenchus* sp. 400X

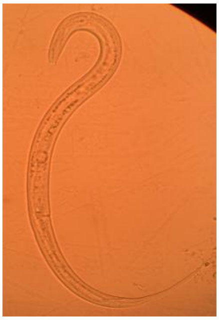
Plate 3. Plant-parasitic nematodes under family Hoplolaimidae, Pratylenchidae, Meloidogynidae, Creconematidae

A. *Aphelenchus* sp. 400X

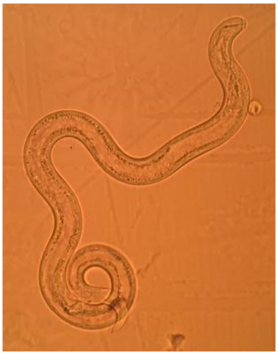


B. *Aphelenchoides besseyi* 400X

C. *Aphelenchoides bicaudatus* 400X



D. *Ektaphelenchoides* sp. 400X



E. *Bursaphelenchus* sp. 400X

Plate 4. Plant-parasitic nematodes under family Aphelenchidae, Aphelenchoididae, Ektaphelenchidae, Parasitaphelenchidae

Table 1. Plant-parasitic nematodes genera recorded in all samples of different rice fields

Order	Superfamily	Family	Genus	Species
Tylenchida	Tylenchoidea	Tylenchidae	*Tylenchus*	*Tylenchus* sp.
			Polenchus	*Polenchus* sp.
			Sakia	*Sakia* sp.
			Boleodorus	*Boleodorus* sp.
	Dolichodoroidea	Tylenchorhynchidae	*Tylenchorhynchus*	*Tylenchorhynchus* sp.
		Psilenchidae	*Psilenchus*	*Psilenchus* sp.
	Hoplolaimoidea	Hoplolaimidae	*Helicotylenchus*	*H. dishystera*
				H. multicintus
		Pratylenchidae	*Pratylenchus*	*Pratylenchus* sp.
			Hirschmanniella	*H. oryzae*
		Meloidogynidae	*Meloidogyne*	*M. graminicola*
	Criconematoidea	Criconematidae	*Macroposthonia*	*M. xenoplax*
Aphelenchida	Aphelenchoidea	Aphelenchidae	*Aphelenchus*	*Aphelenchus* sp.
	Aphelenchoidoidea	Aphelenchoididae	*Aphelenchoides*	*A. besseyi*
				A. bicaudatus
		Ektaphelenchidae	*Ektaphelenchoides*	*Ektaphelenchoides* sp.
		Parasitaphelenchidae	*Bursaphelenchus*	*Bursaphelenchus* sp.

Discussion

In Myanmar, rice is the major agricultural crop and is grown throughout the country. The Chin thone rice variety is grown in monsoon season at Banmaw environs. The biotic and abiotic factor can reduce rice yield and seed quality. Among the biotic constraints, it contains the plant-parasitic nematodes. In the present study, a total of 17 species of plant-parasitic nematodes belonging to 15 genera, 11 families, and two orders were recorded in soil and root samples. Previous survey carried out in 1983 in Myanmar, 28 species belonging to 22 genera of plant-parasitic nematodes from 57 host plants were reported (Mya Mya, 1983). In 2003, Po Po Than reported that 17 genera of plant-parasitic nematodes were found from 17 different crops. According to Bridge *et al*. (1978), 17 genera were found from the survey of plant-parasitic nematodes in Gambia. In U.S.A, Wehunt *et al*. (1989) reported a total of 110 species of plant-parasitic nematodes from various crops in Arkansas. The total number of nematode genera in previous records was not similar to this record. It may be due to different crops, soil types, collected time and weather condition.

During the study period, the highest (73.33%) and lowest (26.67%) percent of plant-parasitic genera was found in order Tylenchida and Aphelenchida respectively. In 2009, Aye Aye Thint, Naing Naing Oo and Aye Aye Thant reported that the high percent of genera (87%, 73.33% and 69% respectively) was found in order Tylenchida. Tylenchida was occurred in all possible habitats in soil, water and plants. Their greatest diversity of forms occurred amongst parasites of roots (Siddiqi, 1985). The finding of present work is agreed with the statements of above authors. The plant-parasitic nematodes (337 individuals/100cc soil) were found in initial sample in all study sites. A single acre of soil from arable land may contain as many as 3,000,000,000 nematodes (Hunt, 2002). The total number of nematodes is different from the statement of Hunt. It may be due to the different location, soil type and crop. Moreover the initial nematode population report was rare in previous record in Myanmar. The population of plant-parasitic nematodes in all root samples was not similar in different study sites. According to the recent results, the highest population was found in fourth sample in RF I, fifth in RF II and sixth in RF III. The differences of population may be due to the flood condition in rice fields, the growth of plant and other managements. The lowest population in all root samples was recorded in S IV at RF III. This rice field was flooded between the collected time of third and fourth samples. It is assumed that the flooding influences the nematode population. A number of plant parasitic nematodes are associated with rice in Myanmar. There were *Ditylenchus angutus*, *Hirschmanniella oryzae*, *Meloidogyne* graminicola and *Aphelenchoides besseyi* (Aung Swe, 1997; Ohnmar Thein, 1998). During study period, *H. oryzae*, *M.* graminicola and *A. besseyi* were observed and *D. angutus* was not completely in all samples. This finding is different from the above finding. It may be due to the different study sites, variety, weather parameters, irrigation and other environmental factors.

Hirschmanniella oryzae has also been reported on rice in other Asian countries such as India, Pakistan, Bangladesh, China Korea and Japan (Bridge *et al*., 2005). Zin Thu Zar Maung (2011) conducted the collection of soil and root samples from 539 fields from 11 monsoon rice varieties in 12 regions of Myanmar. Diseased rice plants that caused by *H. oryzae* were found from rice fields of Hlaingtharyar and Htantapin Township, Yangon Region (Hla Hla Maw, 2009). In the present study, *H. oryzae* was observed in all rice soil and root samples from different rice fields. This finding is agreed with the statement of above authors. In fact, *H. oryzae* may be common in all cultivated rice fields. In the Ayeyarwady River Delta, *M. graminicola* and *H. oryzae* were found in both summer and monsoon rice growing season in delta region. Moreover no root galls were observed in monsoon rice (Pa Pa Win *et al*., 2013). During study period, both species were observed in monsoon rice in Banmaw environs and was not found the root galls. This result coincided with the above finding. According to both results, these two species may be found rice growing areas in Myanmar. The white tip nematode, *A. besseyi* was recorded in collected seeds from different rice varieties in Taikkyi, Thanutpin, Yinmarpin, Kyauktan, Thonegwa, Hlegu and Yaezin Townships. The highest individual (21/100seeds) was reported from Manawthukha rice variety in Taikkyi Township (Khin War War, 2009). In the present survey, *A. besseyi* nematode (1/100 seeds) was found in seed sample although it was found in initial soil sample from RF I and II. It may be due the different rice variety and transporting of seed from one place to another.

Among rice disease, nematode infestation can result in yield losses of up to 30 percent (Dobermann and Fairhurst, 2000). Yield loss due to the root rot disease caused by *H. oryzae* may be as much as 50-60% (Miah and Mondal, 1988). In the screenhouse experiments, infection with *M. graminicola* caused on average a yield reduction with 31.1% in lowland rice varieties vs 44.9% in the upland rice varieties (Pa Pa Win, 2015). The numbers of *A. besseyi* nematode were found 17-34/100 seeds to reach the economic threshold level and 20-36% yield loss (Khin War War, 2009). The result of present study showed that the most important rice growing areas in Banmaw environs were all heavily infested by *H. oryzae* and *M. graminicola*. The interview survey from rice field owners, the highest yield (4122kg/ha) in Sihe was followed by Siin (3866kg/ha) and Mophein (3092kg/ha). According to this research, the highest number of major rice nematodes and lowest seed weight and yield was recorded. Moreover the lowest number of nematodes and the highest seed weight and yield were also observed.

Conclusion

A total of 17 species of plant-parasitic were recorded from different rice fields. Among them, three major rice nematodes (*H. oryzae*, *M. graminicola*, *A. besseyi*) were found in all study sites. The percent of recorded genera in order Tylenchida were higher than the Aphelenchida. Monsoon rice in Banmaw environs were heavily infested in plant-parasitic nematode. According to the nematological point of view, combination of control methods should be used to reduce nematode population. And then, finally my suggestion, seed and soil treatment should be used before planting. The ratoon and weed were burned after planting. The local farmers should be used resistant varieties, crop rotation and biological control methods

Acknowledgements

We would like to express my gratitude to Dr. Zaw Jat, Prorector, Banmaw University for his kind permission to read the research paper. We always thank to Dr Sann Sann (Professor and Head of Zoology Department) for her advice and laboratory supporting. We also thank to Dr Win Mar, Professor, Zoology Department for her advice. Finally, special thanks to rice field owners for their permission of rice samples through out the study period.

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SPECIES COMPOSITION, ABUNDANCE AND DIVERSITY OF AVIAN FAUNA IN PAKOKKU ENVIRONS, MAGWAY REGION

Yadanar Myo[1], Thant Zin[2], Sann Sann Htay[3]

Abstract

Avifauna was investigated in four study sites of the Pakokku environs during July 2015 to June 2016. A total of 126 bird species belonging to 81 genera, 41 families and 16 orders were recorded. Of these, 35 species were water birds and 91 species were terrestrial birds. The bird species were represented with 25 migrants and 101 residents. During the study period, two endemic species, namely *Turdoides gularis* (White-throated Babbler) and *Mirafra microptera* (Burmese Bushlark) were recorded. The highest species composition was found in order Passeriformes (51.59%, 65 species), followed by Pelecaniiformes (8.73%, 11 species). Relative abundance indicated six species as very common, 10 species as common and 110 species as uncommon in Pakokku environs. In the study area, the highest species richness and diversity values were evaluated in Site I ($d = 11.643$, $D = 0.077$, $H' = 3.199$) and Site III ($d = 10.934$, $D = 0.039$, $H' = 3.646$). This situation may be related to the habitat conditions and availability of food sources. Pakokku environs may be considered rich in bird diversity due to occurrence of high number of bird species and individuals.

Keywords: Composition, Abundance, Diversity, Avian Fauna, Pakokku environs

Introduction

Birds are certainly not the only group of species affected by global change, nor even the most affected. But they are certainly among the most visible and evocative to us as a society and are thus an important window into the broader changes in ecosystems (Altwegg *et al*., 2009).

[1] Dr., Assistant Lecturer, Zoology Department, Yenanchaung Degree College
[2] Dr., Professor and Head, Zoology Department, University of Mandalay
[3] Dr., Associate Professor, Zoology Department, University of Kalay

Bird abundance and species composition vary in response to different degrees of change in vegetation structure. Considerable variation in species composition may occur along successional gradients, and many species are restricted to either initial or advanced stages (Casas *et al*., 2016).

The relative abundance of a species is often associated with the vegetation community, food resources and habitat structural complexity. Determining the avian relative abundance is highly important because this variable indicates the proportion of an existing population in a particular habitat. Microclimate and habitat structure are major factors that influence avian survival rate, reproduction success, time of breeding, species dispersal and habitat selection (Rajpar and Zakaria, 2015).

The effect of habitat heterogeneity on species diversity is a fundamental concept in community ecology often invoked to account for the absence of a species area effect. The relationship between habitat heterogeneity and bird species diversity is a well documented pattern in community ecology. Bird species richness and diversity in terrestrial landscapes is closely related to habitat structure and floristically, that bird species can occupy, resulting in greater bird diversity (Azlan *et al.,* 2015).

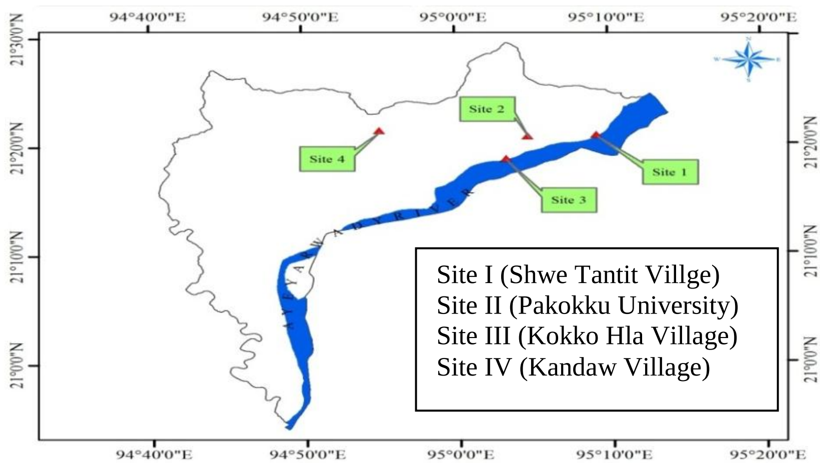
Conducting the bird diversity in Pakokku is an important need to understand the avian assemblages and distribution in different habitats for effective conservation and better management in future. Thus, it is required to assess how habitat variables affect avian distribution patterns and richness. The objectives of this study were conducted to determine the composition and abundance of bird species in different study sites and to assess the species richness, diversity and evenness of birds in Pakokku environs.

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 is located in the dry zone of central Myanmar. Pakokku lies between latitude 20°

20' to 21° 30' North and longitude 94° 40' to 95° 20' East. 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) and these sites were situated at the east, north, south and west of Pakokku Township respectively (Fig. 1). The 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 woody trees, medium and tall trees, artificial pond, bushy area and buildings were found in Site II. The 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 woody trees, medium and tall trees, bushes, 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 birds, the transect method is used. Transect surveys are used to record a variety of birds. It is a simple method that provides a uniform way of counting birds over time or across locations. An appropriate transect lines were drawn in each study site. Birds were viewed by a pair of binocular while traversing the transect routes. Birds watching were taken from 6:30 AM to 10:30 AM. In each occasion of bird watching, bird species and their individuals were recorded. Field marks such as streaks, bars, wings, eyes, tail and birds' behavior were noted down. The photos of birds 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 Smythies (2001), Robson (2015) and Robson (2016). Classification of birds was followed after Birdlife International (2015).

Analysis of data

The number of individuals for each species obtained during two data collections of each month was pooled and mean value was taken to represent the monthly data of each species.

The data were analyzed for the following parameters:

Relative abundance

$$\text{Relative abundance} = \frac{\text{No. of individuals of a species}}{\text{Total no. of individuals of all the species}}$$ (Bisht *et al*., 2004)

The average relative abundance was categorized as by method of Bisht *et al*. (2004).

uC = (uncommon) having relative abundance less than 0.0100

C = (common) having relative abundance of 0.0100 and above but less than 0.0500

vC = (very common) having relative abundance of 0.0500 and above.

Status

Status of the birds such as migrant or resident was worked out based on the presence or absence of bird species in each month in the study area according to King and Dickinson (1995).

M = migrant (bird migrate from cold northern to warm southern temperature regions at a definite time of each year to avoid hazard winter)

R = resident (birds that spend throughout the year in one place but some species show local movement).

Diversity

The diversity of birds was evaluated by using five methods: Margalef (1958), Simpson (1949), Shannon-Wiener (1948) and Hill (1973) (Cited by Ludwing and Reynolds, 1988) and Sorensen's Similarity Index (Odum, 1971) (Cited by Gonzalez, 1995).

Species richness

Species richness of bird species was determined by using the formula of Margalef's index (1958).

$$d = \frac{S-1}{\ln (N)}$$

Where, d = Margalef's species richness index, N = total number of individuals

S = number of species, ln = Natural Logarithm

Species diversity

Species diversity was determined by using two formulae of Simpson's index of diversity "D" (1949) and Shannon-Wiener's index "H'" (1948).

Simpson's index of diversity

$$D = \sum_{j=1}^{s} \frac{n_i (n_i-1)}{n (n-1)}$$

Where, D = Simpson's index of diversity, n_i = number of individuals in the "i" species

n = total number of individuals

Shannon-Wiener's index of diversity

$$H' = \sum_{j=1}^{S} \left[\left(\frac{n_i}{n} \right) \ln \left(\frac{n_i}{n} \right) \right]$$

Where,

H' = Shannon-Wiener's index of diversity, n = total number of individuals

n_i= number of individuals in the "i" species, S = number of species

ln = Natural Logarithm

Hill diversity number

$$N_1 = e^{H'}$$

′Where,

H' = Shannon's index, N_1 = number of abundant species in the sample

$$N_2 = \frac{1}{D}$$

D = Simpson's index, N_2 = number of very abundant species in the sample

Species evenness

The measurement of bird species evenness or equitability (or relative species abundances) was assessed by using Hill's evenness index (1973).

$$E = \frac{\left(\frac{1}{D} \right)}{e^{H'}} = \frac{N_2}{N_1}$$

Where, E = Hill's evenness index, H' = Shannon's index of species diversity

D = Simpson's index of diversity, N_1 = number of abundant species in the sample

N_2 = number of very abundant species in the sample

Results

Throughout the study period from July 2015 to June 2016, a total of 126 bird species and 12791 individuals under 81 genera belonging to 41 families and 16 orders were observed from four study sites in Pakokku (Table 1). Among the bird species recorded, 91 species of terrestrial birds belonging to 55 genera, 30 families and nine orders and 35 species of water birds comprising under 26 genera, 11 families and seven orders were represented. Moreover, the bird species recorded were categorized into 25 migrants and 101 residents. Based on the relative abundance data of species from Pakokku environs, six species were recorded as very common, ten species as common and 110 species as uncommon (Table 2).

Composition of species in bird orders and study sites

Among the 16 orders concerned in this work, the order Passeriformes was represented with the largest number of 65 species (51.59% in composition), followed by the order Pelecaniformes (11 species, 8.73%), Charadriiformes (nine species, 7.14%), Gruiformes (eight species, 6.35%), Cuculiformes (six species, 4.76%), Columbiformes and Coraciiformes (five species, 3.97% each), Anseriformes and Accipitriformes (three species, 2.38% each), Suliformes, Falconiformes, Strigiformes and Piciformes (two species, 1.59% each) and Podicipediformes, Ciconiiformes and Bucerotiformes (one species, 0.79% each) (Fig. 2).

The highest number of 19 families was recorded under order Passeriformes, followed by order Charadriiformes containing four families and order Coraciiformes containing three families.

Moreover, with regard to the different sites, the highest number of 102 species was found in Site I and then followed by Site III (86 species), Site II (64 species) and Site IV (58 species) (Table 1).

Abundance and relative abundance of birds in different study sites

Site I

Total number of 102 bird species and 5851 individuals were recorded in Site I. The highest number of species was observed in February (92 species), followed by January (84 species) and December (77 species). The highest total number of individuals was found in February (1680 individuals) and followed by March (719 individuals), January (619 individuals) and December (575 individuals) (Table 1).

In this study site, six species of bird were recorded as very common, seven species as common and 89 species as uncommon. Among the species recorded, 81 species were residents and 21 species were migrants. Moreover, 67 species of terrestrial birds and 35 species of water birds were recorded (Table 2).

Site II

In Site II, total number of 64 species and 2849 individuals were observed. The highest number of species was recorded in February (55 species), followed by December (49 species) and November (48 species). With regard to the individuals, the highest was recorded in February (334 individuals), followed by November (316 individuals) and December (299 individuals) (Table 1).

In the case of relative abundance, three species were considered as very common, 11 species as common and 50 species as uncommon. Among the recorded bird species, 62 species were residents and two species were migrants. The species recorded were represented with 62 species of terrestrial birds and two species of water birds (Table 2).

Site III

A total of 86 species representing 2377 individuals were found in Site III. The highest number of 74 bird species in February was observed. This was followed by December (68 species) and January (63 species). Among the total number of individuals, the highest number was recorded in February (434 individuals), followed by January (272 individuals) and March (268 individuals) (Table 1).

In the case of relative abundance, three species were taken as very common, 20 species as common and 63 species as uncommon. With regard to the species, 71 species were residents and 15 species were migrants. Sixty-two species of terrestrial birds and 24 species of water birds were represented in the avian fauna in this site (Table 2).

Site IV

Bird fauna was represented with 58 bird species and 1714 individuals in Site IV. The highest number of species was found in January (47 species), followed by February (43 species) and December (40 species). Among the bird individuals, the highest total number was recorded in February (191 individuals), followed by January and October (186 individuals each) and December (183 individuals) (Table 1).

In this study site, seven species were regarded as very common, 12 species as common and 39 species as uncommon. Among the recorded bird species, 56 species were residents and two species were migrants. In this study site, the bird fauna was represented with 55 species of terrestrials and three species of water birds (Table 2).

Comparison of diversity values among study sites

According to diversity indices evaluated among four study sites, the value of d was the highest (11.643) in Site I and the lowest (7.655) in Site IV. The highest values of D (0.039), H' (3.646), N_1 (38.329), N_2 (25.861) and E (0.675) were observed in Site III, whereas the lowest values of D (0.111), H' (2.931), N_1 (18.748), N_2 (8.999) and E (0.480) were recorded in Site II (Table 3).

Table 1. Total number of bird species and individuals in different months of each site during July 2015 to June 2016

Site	Species / Individuals	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Ma y	Jun	Total
I	Species	40	42	52	53	52	77	84	92	61	36	32	37	102
	Individuals	314	306	254	198	465	575	619	1680	719	256	235	230	5851
II	Species	33	30	36	44	48	49	43	55	37	28	26	28	64
	Individuals	220	195	227	290	316	299	260	334	222	188	135	163	2849
III	Species	27	27	42	46	53	68	63	74	47	37	20	25	86
	Individuals	111	111	151	215	228	243	272	434	268	130	101	113	2377
IV	Species	28	30	31	32	32	40	47	43	30	24	19	22	58
	Individuals	110	114	125	186	158	183	186	191	124	115	106	116	1714
Total number of species		60	58	73	79	89	105	108	119	83	58	45	54	126
Total number of individuals		755	726	757	889	1167	130 0	133 7	2639	133 3	689	577	622	12791

Table 2. Comparison on the status of bird species among study sites during July 2015 to June 2016

Status of species	Site I	Site II	Site III	Site IV	Combined sites (Pakokku environs)
Very common	6	3	3	7	6
Common	7	11	20	12	10
Uncommon	89	50	63	39	110
Migrant	21	2	15	2	25
Resident	81	62	71	56	101
Terrestrial bird	67	62	62	55	91
Water bird	35	2	24	3	35

Table 3. Comparison on diversity indices among four study sites of Pakokku environs durig July 2015 to June 2016

Indice \ Site	I	II	III	IV
S	102	64	86	58
N	5852	2849	2377	1714
d	11.643	7.920	10.934	7.655
D	0.077	0.111	0.039	0.077
H′	3.199	2.931	3.646	3.102
N_1	24.525	18.748	38.329	22.242
N_2	13.048	8.999	25.861	12.985
E	0.532	0.480	0.675	0.584

S=Total number of species, N=Total number of individuals

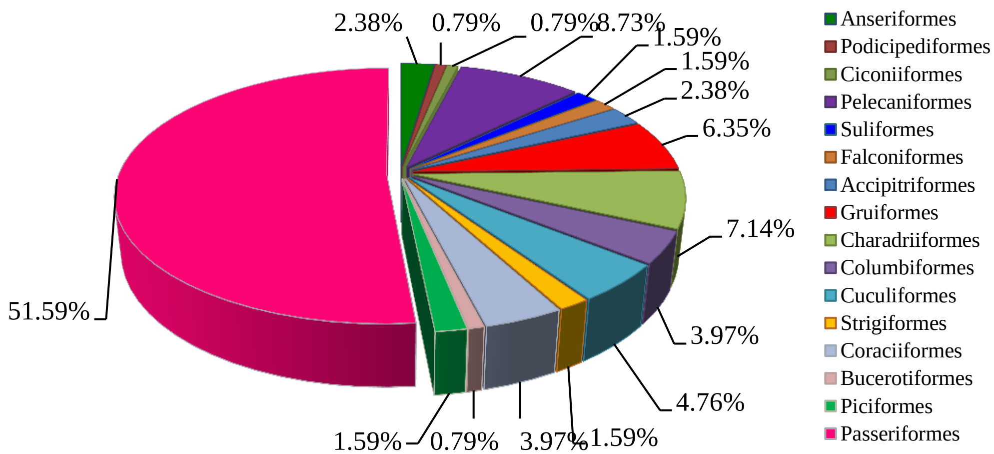


Figure 2. Percentage composition of bird species in different orders in Pakokku environs during the study period

Discussion

Throughout the study period from July 2015 to June 2016, a total of 126 bird species confined to 81 genera belonging to 41 families of 16 orders were identified and recorded from four study sites in the environs of Pakokku. Among them, 91 species were terrestrial birds and 35 species were water birds. Moreover, 101 species of residents and 25 species of migrants were included.

Concerned with studies on the occurrence of bird species in different areas in Upper Myanmar undertaken by local researchers, Khin Hnin Thet (2013) observed 105 species of terrestrial birds and 35 species of water birds in Monywa environs; Chaw Su Shwe (2014) recorded 64 species of terrestrial birds and 14 species of water birds in Minbu environs. In this work 91 species of terrestrial birds and 35 species of water birds were recorded in Pakokku environs. Some species are same and some are different between present work and previous works. The differences may be due to environmental conditions, habitat conditions, availability of food sources and habitat sensitivity of some species.

Regarding the different orders of birds in South-East Asia, the largest order was found as Passeriformes according to Smythies (2001) and Robson (2015, 2016). In the study area, not only the highest number of bird species but also the highest species composition was found in order Passeriformes. Most of bird species recorded in the study area are terrestrial birds and included under Passeriformes.

With regard to study sites, the highest number of species was found in Site I (102 species) and the second highest number of species in Site III (86 species). The area of paddy fields was significantly more in Site I than Site III. Therefore, more number of water bird species was found in Site I than Site III. Although the number of terrestrial bird species showed less variation among the study sites, the number of water bird species were significantly more in Site I (35 species) and Site III (24 species) than Site IV (three species) and II (two species). The habitat of Site I and III possess the vast area of water body for water bird species. The flood plain area, dam and river create the wetland for water birds to swim, forage, breed and roost. Many hundreds of birds from foraging ground and paddy field come to roost together on the trees of Site I and III. The highest total number of individuals was recorded in Site I (5851 individuals), followed by Site II (2849 individuals) and Site III (2377 individuals). The lowest number of species (58) and individuals (1714) were recorded in Site IV due to less diversified habitat and closeness to human residents.

During the study period, more number of bird species and individuals were found during December, January and February represented with winter migrants especially in Site I and III.

This study showed the high relative abundance of both water birds and terrestrial birds as well. Based on the relative abundance values, the habitats were not only preferred by land birds but are also utilized by water bird species for food, shelter and reproduction.

In the study area, the highest species richness (d) was found in Site I and second highest in Site III. However, Shannon-Wiener's diversity index (H′) and Simpson's index (D) were the highest at Site III and second highest in Site I due to aggregation of large individual number of water birds depended on the availability of the vast area of wetland. These habitats were highly productive and good foraging, nesting and roosting sites for many water birds and terrestrial birds.

But in the case of evenness index, the highest value of evenness was found in Site III and Site IV showing more even distribution of birds than the other sites. The number of individual birds recorded for each species in Site III and Site IV showed less variable whereas Site II showed the lowest value of evenness because birds were found from one individual to large flocks for each species.

Kang, *et al*. (2015) reported that local improvements to habitat structure through increased patch area, reduced human disturbance, or increased vegetation complexity could positively contribute to local species diversity.

In the present study, high diversity of birds in Pakokku environs was found to be related to the suitable and diversified habitats. Two Myanmar endemic species of *Mirafra microptera* (Burmese Bushlark) and *Turdoides gularis* (White-throated Babbler) were included in the bird fauna of Pakokku environs. Based on IUCN (2015), all species are in the status of least concern.

The habitats of Pakokku environs are well inhabited with terrestrial and water birds as well as residents and migrants. The area is considered good habitat for bird species of both terrestrial and water birds because this area has good food resources, shady trees, bushes, agricultural land and vast area of wetland.

Acknowledgements

Firstly, I am greatly indebted to Dr. Soe Win, Principal, Yenanchaung Degree College, for his encouragement. Thanks are also due to Dr. Thet Wah Kyaw Myint, Professor and Head, Department of Zoology, Yenanchaung Degree College, for her helps in many ways in preparing this paper.

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POPULATION, BREEDING STATUS, AND HABITATS UTILIZATION OF SARUS CRANE *GRUS ANTIGONE* IN AYEYARWADY REGION

Myo Sandar Winn[1], Tran Triet[2], Ah Mar Yi[3], Thet Naing[4], Aung Aung [5]

Theingi Soe Myint[6], Kaythi Khine[7], May Kadipar Cho[8]

Abstract

The present research deals with the estimate population, breeding status, and habitats of the globally threatened vulnerable species of Sarus Crane *Grus antigone* in Ayeyarwady Region. Data recorded by two methods of point transect and direct observation method in breeding season (June–October) and non-breeding season (November-May). Estimate population status survey was carried out toward end of the breeding season. This is the time when new chicks have fledged and recruitment rate can be estimated. At each Crane site, habitats were collected by rapid assessment by level 4 of Asian Wetland Inventory Handbook (Finlagson et al, 2002). According to the data, 299 individuals of Sarus Crane and 78 nests were recorded from June 2017 to May 2018.

Keywords: Population, habitats, breeding, Sarus Crane, wetland, Ayeyarwady

Introduction

Ayeyarwady Delta is the Region where Sarus Crane have been seen most frequently and highest numbers. Surveys conducted by the International Crane Foundation and Myanmar Forest Department in Ayeyarwady Delta recorded 122 and 61 Sarus Cranes in 1996 and 1998, respectively (Barzen *et al.*,1996). 88 Sarus Crane in April 2004 and 128 in May 2005 were recorded

1. Lecturer, Department of Zoology, University of Yangon
2. Director (South East Asia program), International Crane Foundation, USA
3. Lecturer, Department of Zoology, University of Yangon
4. Lecturer, Department of Zoology, University of Yangon
5. Lecturer, Department of Zoology, University of Yangon
6. Lecturer, Department of Zoology, Taunggok College
7. Assistant Lecturer, Department of Zoology, Dawei University
8. Assistant Lecturer, Department of Zoology, University of Yangon

at Tawtay Township, Ayeyarwady Delta (Thet Zaw Naing, 2005). Sarus Crane is currently listed as Vulnerable on the IUCN Red list and the only resident crane. There is a need to assess and monitor birds' populations since their numbers, distribution and activities reflect the ecosystem's quality and status (Ismail *et al.*, 2012). The current population size, seasonal movements and habitat use of Sarus Crane in Myanmar are still poorly known. No attempt has been made in Myanmar to conduct a country wide survey of Sarus Crane to estimate the total population and identify and describe habitats used. The research objectives are the estimate population, breeding status, and habitat utilization of the Sarus Crane *Grus antigone* in Ayeyarwady Region.

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 four districts (Maubin, Myaungmya, Pyapon, and Labyutta).

Study period

The survey was conducted from June, 2017 to May, 2018.

Field data collection

The present survey was conducted crane population count at all known and potential sites in Ayeyarwady Region by field survey and interviewed survey. Field surveys were carried out in the breeding season (June- October) and non-breeding season (November- May). Status of current breeding behavior and nesting sites were also recorded. Population surveys were conducted towards the end of the breeding season (June- October). This is time when new chicks have fledged and recruitment rate can be estimated. Interviewed surveys conducted with a wide variety of people such farmers, villagers, boatmen, fishermen and other local residents who well known about the Crane. Data were collected using two methods, point transect and direct observation method. Birds were counted as bird seen and heard. Field data were carried out three days per trip and couple times every month during the survey period.

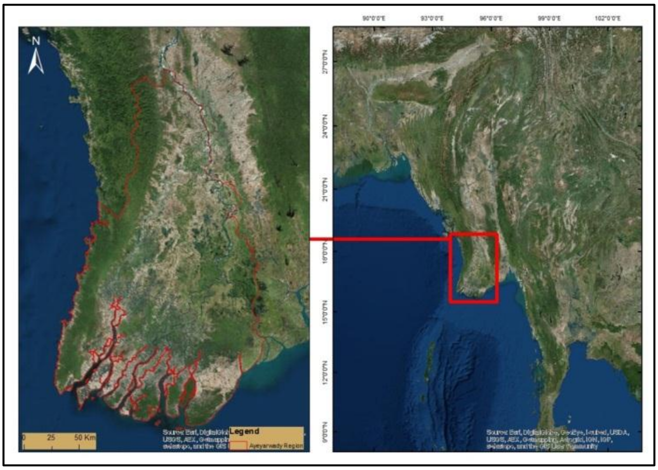


Figure 1. Map of the Study site

Results and Discussion

Population and nest recorded in the study sites

The present research conducted at 97 villages in nine townships of four Districts. According to the survey result, 299 individuals of Sarus Cranes and 78 nests were recorded from June 2017 to May 2018 (Table 1). Nests were recorded in 28 villages under the study sites. The highest numbers of nest were recorded in the month of August. Among the total individuals, 90 individuals of Sarus Crane found in Maubin District, 188 individuals of cranes in Myaungmya District, 13 individuals of cranes in Pyapon District and eight individuals of cranes in Labytta District (Table 2). Some area of nesting sites were not easy to survey because there were no boat way and footpath due to thick, tall grass and marshy.

Maubin District

Population number and nest recorded in Maubin Township

A total of 28 villages were surveyed at Maubin Township. There were 61 individuals of Sarus Crane recorded in non-breeding season and 12 nests were recorded in breeding season in seven villages (Table1).

Population number and nest recorded in Nyaungdon Township

In non-breeding season, nine individuals of Sarus Crane were occurred in three villages. Nest was not observed in this township. The local people were interviewed by survey team and they said that flocks of Sarus Crane could be found in non-breeding period of December to February every year. Seasonal wetland and flooded paddy field were used as a foraging and roosting habitat.

Population number and nest recorded in Pantanaw Township

Researches were conducted in ten villages in Pantanaw Township. During the survey period, 18 individuals of Sarus Crane were recorded in non-breeding season. The only one nest was recorded in Kattiyar village. This nesting site was observed in flooded paddy field and the nest site was chosen at the same place more than three years.

Population number and nest recorded in Danuphyu Township

Researches were conducted in four villages. Two individuals of Crane were carried out in Set Tu Chaunggalay village in flying position. Local people said that Sarus Cranes could be seen in flooded paddy field habitat in every year. Nest data was not recorded during the survey period.

Myaung Mya District

Population number and nnest recorded in Myaung Mya Township

Three villages were conducted during the research period. Four individuals of Sarus were recorded in near Tha Min Chan village in non-breeding season.

Population number and nest recorded in Einme Township

Field and interviewed survey were carried out at 13 villages. Out of total village, 29 individuals of Sarus Crane were conducted in five villages during the research period (Table 1). In breeding season, four nests were observed in three villages. Local people of other village said that many Cranes (over 100 individuals) of Sarus Crane had been seen in July to August along the side of the road of Enime to Myaungmya Township.

Population number and nest recorded in Wakhema Township

Researches were conducted in 31 villages. A total of 155 individuals of Sarus Crane were recorded in non-breeding season. In the breeding season, 56 nests were recorded in 11 villages. A total of 56 nests, the highest numbers of nests (24 nests) were recorded in Shwe Laung village. According to the survey result, Wakhema Township in Myaungmya District is the crucial important habitats for Crane species. Many Sarus Cranes were utilized as foraging, roosting and nesting in natural wetlands and flooded paddy field habitats in this area.

Pyapon District

Population number and nest recorded in Kyaiklat Township

Survey conducted in three villages. During the survey period, 13 individuals of Sarus Crane and two nests were observed in each Hlaing Tar village and Kyweku Khayar Yoe village in both season of breeding and non-breeding.

Labutta District

Population number and nest recorded in Mawlamyingkyun Township

Eight individuals of Sarus Crane were recorded two villages in non- breeding season. Two nests and four individuals were observed in Kyaw Zan village during the study period.

Courtship and Breeding behavior of Sarus Crane

Courtship and breeding in Sarus Crane is an annual spectacular event, initiated by loud trumpeting calls. Breeding pairs may resort to spectacular displays of calling in unison and different posturing, which include ‘dancing’ movements that are performed both during and outside the breeding season, and involved a short series of jumping and bowing movements made as one of the pair circles around the other. The parental birds are also known to display during the incubation period and when young birds are being reared to threatened intruders or to display territorial behavior. Sometimes, display is done to reassure each other after a predator is chased away.

Nesting behavior of Sarus Crane

The Sarus Cranes breed mainly during the monsoon (June to October). They build large nest platforms made of reeds and vegetation in wet marshes or paddy fields. The nest can be upon to a meter in diameter and more than a meter height. The nest constructed in shallow water by pilling up rushes, straw, grasses with their roots and mud so that the plat form rises above the level of the water to form a small island. The elevated nest is conspicuous and is visible from a long distance. The clutch size is one or two eggs (rarely three), which are incubated by both the parents for about four to five weeks. The chalky white eggs are about four inches long and two and half inch wide. After hatching, the eggs shells are sometime swallowed by the parents or removed from the nesting site.

Behavior of Sarus Crane' chick

The chicks depend on the parents to be fed for the first few days after which they are able to feed independently, mostly following the parents. It is, therefore, common to see Sarus chicks being continuously guarded by both the parent. Young bird still with their parents for more than three months.

Habitat utilization of Sarus Crane

During the breeding season, breeding pairs and non- breeding pairs used different wetland sites. Foraging, nesting, and roosting in three kinds of habitats such as seasonal wetlands, natural wetlands and flooded paddy fields were used. Nesting sites were most found in natural wetlands and flooded paddy field. The flooded paddy field is mingled with natural wetlands. Sarus Cranes forage in shallow water of wetlands and agricultural fields probing to depths that their long bills can reach. Natural wetlands appear to be the most preferred habitat of the Sarus followed by crop fields interspersed with water bodies. The natural wetlands are crucial to ensure maximum breeding success of Sarus Crane, even though a mosaic of surrounding paddy fields serves the territorial requirements of the breeding pairs. Reedy marshes and marginal wetland are supporting a fairly good breeding population of Sarus Crane in this area. It may be assumed that habitat quality often plays a major role until the fledging stage of the juvenile Sarus Crane.

Threats on Sarus Crane

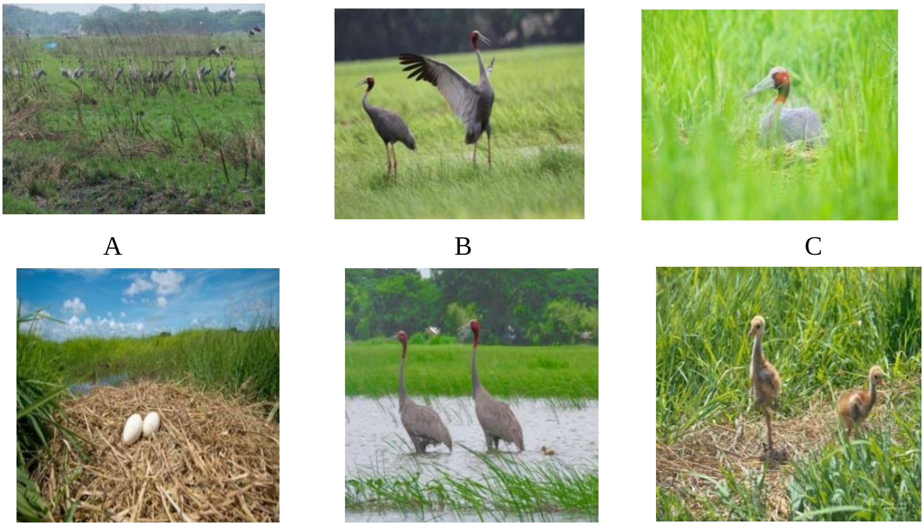
According to the survey result, agricultural expansion, fishponds, loss and degradation of wetlands, heavy use of pesticides, and human population growth have all contributed to the decline of Sarus Crane' population. The variety activities of human were also threats on biodiversity. The construction of fishponds was one of the significant threats to the habitats of Sarus Crane. Destruction of wetlands by agricultural expansion was a significant threat as well. Human interference, pollution, environmental contamination and lack of knowledge and public support were lesser threat for Sarus Crane.

Table 1. Recorded number of Sarus Crane' nests in Ayeyarwady Region

No	District	Township	No	Village name	No of Nest
1	Maubin	Maubin	1	Gone Min	1
			2	Nyaung Khar Shae	1
			3	Thae Phyu	2
			4	Yelegalay	2
			5	Ah Chaw	3
			6	Ah Lan Gyi	1
			7	Lain Kone	2
		Pantanaw	8	Kattiya	1
2	Myaung Mya	Einme	9	Pwesar	2
			10	Mayan Chaung	1
			11	Mezali	1
			12	Nyaung Wine	1
		Wakhema	13	Shwelaung	25
			14	Kan Gwin	2
			15	Kyon Kha Yaing	1
			16	Kyon Padi Kyoung Pa Daw	4
			17	Minlan	2
			18	Katsake	5
			19	Tarpat Ashae	2
			20	Kyon Hta Yeik	4
			21	Ka Leik Kyon Toke	2
			22	Kyon Ka Pyin	4
			23	Wea Gyi	2
			24	Sittan	1
			25	Ayun	1
3	Pyapon	Kyaiklat	26	Hlaingtar	2
			27	Kyaweku Khayaryoe	2
4	Labyutta	Mawlamyingkyun	28	Kyaw Zan	1
	Total				78

Table 2. Recorded number of Sarus Crane nest and individuals in four Districts

District	Township	Village	Nest	Number of individual
MaUbin	4	45	13	90
Myaung Mya	3	47	59	188
Pyarpon	1	3	4	13
Latbyutta	1	2	2	8
Total	9	97	78	299



A B C D E F

Plate I

A. A flock of Sarus Crane
B. Sarus Cranes in the paddyfield
C. Incubation of Sarus Crane
D. Eggs of Sarus Crane
E. Sarus Crane with chick
F. Sarus Crane chicks on the nest

Acknowledgements

We are deeply indebted to Dr Thida Lay Thwe, Professor (Head), Zoology Department, University of Yangon, who permitted to do this research. We would like to express our special thanks to Dr Tran Triet, Director of South East Asia Program, International Crane Foundation (ICF), USA for his invaluable guidance throughout this research. Thanks to Dr Gopi Sundar, Director of SarusScape, and International Crane Foundation (ICF) for technical supporting. Special thanks are due to U Tin Aung Tun, freelance conservationist for his invaluable technical supporting during the survey period.

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J. Myanmar Acad. Arts Sci. 2019 **Vol. XVII. No.3**

SPECIES COMPOSITION OF AVIFAUNA IN NORTHERN PART OF ALAUNGDAW KATHAPA NATIONAL PARK, SAGAING REGION

Myint Naung[1], Mie Mie Sein[2]

Abstract

The study was conducted on the occurrence of bird species in northern part of Alaungdaw Kathapa National Park during the study period from September 2012 to August 2015 with the objectives to identify the avifauna and to examine the species composition and relative abundance of bird species. Sampling sites were stratified based on the vegetation type and fixed radius point count technique was employed. A total of 109 species belonging to 76 genera, 38 families under 13 orders were recorded. The maximum percentage of family composition was observed in Picidae. Black-crested bulbul *Pycnonotus flaviventris* was found as very common species although Great Hornbill *Buceros bicornis*, Oriental-scops Owl *Otus sunia* and Daurian Redstart *Phoenicurus auroreus* were recorded as uncommon species. The species composition of bird in dry season was significantly different with other seasons. Globally threatened species such as Great Hornbill *Buceros bicornis* and Red-breasted Parakeet Psittacula alexandri as Near-threatened species, and Great Slaty Woodpecker *Mulleripicus pulverulentus* as vulnerable according to IUCN Redlist (2015) were recorded. This result is hoped to provide some data to attract more ecotourism in future.

Keywords: Species composition, Alaungdaw Kathapa National Park, Avifauna, Dry season, Globally threatened species

Introduction

Birds play an important role in forest ecosystem as potential pollinators, seed dispersers and scavengers. They are regarded as a viable indicator for biological biodiversity and changes in environmental conditions (Palei *et al.,* 2012). Many species of birds use different habitats at different time of the year. Many types of ecological habitats are woodland, grassland, cultivated agricultural areas, wetland, open water, sea coast, river bed and

[1.] Dr., Lecturer, Department of Zoology, Monywa University
[2.] Dr., Pro-Rector, Mawlamyine University

high mountains (Sibley, 2001).It is necessary to determine the total species richness of a bird community to make informed conservation decisions (Walther and Jean-Louis, 2001). The richness and composition of forest's birds can give an indication for the conservation of biological diversity, and environmental change and the impacts of habitat alternation (Bennun and Dranzoa, 1996).

Threats to birds are varied and complex, but most are linked to human interference in the ecosystem. Habitat destruction includes the deforestation of the world's tropical rainforests, which host a wide variety of birds (Alderton, 2007). For most globally threatened bird species characteristic of forest habitats, habitat loss is the main threat. Many forest birds are intolerant to habitat degradation, collection of fuel, wood and timber (Hicker and Roberts, 1994).

The conservation in Asia and globally also has to face up to the reality of tackling the underlying and indirect causes of biodiversity loss (BirdLife International, 2015). Myanmar may have retained on the highest levels of species richness and most extensive forest cover of any country in mainland South-east Asia due to its political and geographic isolation. While several new protected areas have been declared, much lack of the resources and infrastructure necessary to prevent biodiversity loss from poaching and habitat degradation (Rao *et al.,* 2002).

Son *et al.* (2011) stated that a large number of natural resources for tourism, such as the National Parks, Natural Reserves, and Aquatic/marine Conservation Areas have been put to use for development of tourism, in which bird watching travel included. Development of bird watching helps economic growth and environment conservation (Askari *et al.,* 2014). Moreover, birdwatching is a major component of wildlife tourism, and one of the most rapidly growing tourists to watch birds. The economic, recreational and social significance of bird watching has been recognized elsewhere (Jone and Buckley, 2001). According to the Forest Department, 43 protected areas were established in Myanmar, including Alaungdaw Kathapa National Park (AKNP) established at 1989. It is also an ASEAN Heritage Park. Several global threatened species were generally inhabited in these areas (Forest Department, 2009). In the present study, petroleum oil productions were observed in the study area. Illegal logging, barely-restricted hunting and destructive agriculture practices have spurred significant wildlife declines and rapid loss of natural habitats. In order to make inventory lists and assess the bird composition in Northern part of the Alaungdaw Kathapa National park, the present studies have conducted during three different seasons. This study provides up to date information about the bird species compositions in the park. The present study aims to identify the avian species and to examine the species composition and relative abundance in different habitats.

Materials and Methods

Study area

Northern part of AKNP in Kani Township is located along the Kalay-Monywa road between 22°26′44.0″ to 22°41′10.30″ North latitude and 094°29′22.98″ to 094°36′36.0″ East longitude (Plate 1). Elevation ranges from 171 m to 737 m above the seas level. Different habitats (shrubs, bamboo habitat, indaing habitat and dry mixed deciduous habitat) were assigned. Shrub and bush habitats were predominant with shrubs such as (Bizat) *Eupatorium odoratum*. Bamboo habitats were dominated by Tinwa *Cephalostachyum pergracile* Munro, and Hmyinwa *Dendrocalamus strictus* (Roxb.) Nee., Indaing (Dicterocarp) habitat was characterized by the prevalence of In *Dipterocarpus tuberculatus* Roxb., Ingyin *Shorea siamensis* Wall., and Thitya *S.obtusa* Wall. Mixed deciduous habitat was dominated by Kyune *Tectona grendis*, Pyinkado *Xylla dolabriformis*, Panga *Terminalla chebula* and Nyaung *Ficus* species.

Study period

The field surveys of this study were conducted from September 2012 to August 2015.

Bird surveys

Bird watching was conducted from 6:00 hrs to 11:30 hrs in the morning and from 15:00 hrs to 18:00 hrs in the afternoon depending on the light condition. Birds were censused using a fixed-radius point count method (Bibby *et al.*, 2000). Visual observation of avifauna was recorded using cameras and binoculars.

Identification of species

Birds were listed according to the classification systems proposed by Clements (2013). Species identification was made followings Smythies (2001), and Robson (2015). The plant species were checked by Kress *et al.* (2003).

Data analysis

$$\text{Relative abundance} = \frac{\text{number of individual of a species}}{\text{Total number of individual of all species in a particular site}}$$

Average relative abundances were categorized as uncommon

(uC < 0.01), common (0.01>=C < 0.05), very Common (vC >= 0.05) (Bisht *et al.*, 2004)

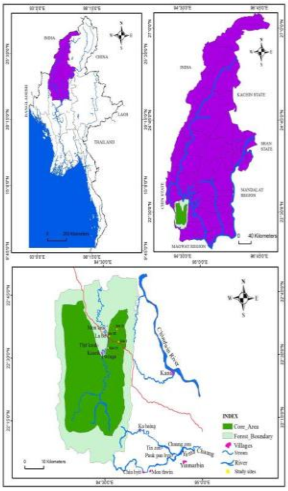

Plate 1 Location map of the study area

(Source: Geography department, Monywa University)

Results

A total of 5248 individuals representing 109 species and 38 families under 13 orders were recorded during September 2012 to August 2015. The maximum percentage of family composition was observed in Picidae. According to index of relative abundance, two very common species, 103 common species and four uncommon species were observed in all habitats. Among the record bird species, Red Jungle Fowl *Gallus gallus,* Black-crested Bulbul *Pycnonotus flaviventris* as very common species, Oriental Scops-owl *Otus sunia,* Great Hornbill *Buceros bicornis,* Common Iora *Aegithina tiphia,* Daurian Redstart *Phoenicurus auroreus* as uncommon species, and the remaining bird species (103) as common species were observed during the study period (Table 1, Plate 1). The relative abundance scores of bird species observed that 1, 0, 0, 1 were very common; 9, 33, 70, 93 were common; 1, 1, 0, 1 were uncommon at shrub and bush, bamboo, indaing and mixed deciduous habitat, respectively (Table 2). Red Jungle Fowl *Gallus gallus* as very common species, Daurian Redstart *Phoenicurus auroreus* as uncommon species were observed in shrub habitat. In bamboo habitat, Oriental Scops-owl *Otus sunia* was observed uncommon species. Very common species was not observed in that habitat. Black-crested Bulbul *Pycnonotus flaviventris* observed as very common species in mixed deciduous habitat although Great Hornbill *Buceros bicornis* and Common Iora *Aegithina tiphia* were observed as uncommon species.

The highest bird population was recorded in mixed deciduous habitat while the lowest population of the bird species was found in bamboo habitat. Among the different habitats, the number of bird species and individuals were most abundant during the dry season followed by rainy season and cold season (Fig. 1). Great Hornbill *Buceros bicornis* and Red-breasted Parakeet *Psittacula alexandri* were observed as Near-threatened and Great Slaty Woodpecker *Mulleripicus pulverulentus* as vulnerable although all bird species were least concern according to IUCN Redlist. Moreover, mammals such as Phayre's Langur (endangered) and Leopard (vulnerable) were observed in this study area.

Discussion

A total number of 109 species of birds were recorded in northern part of Alungdaw Kathapa National Park indicating rich with avifauna. Among the species recorded, Great Hornbill *Buceros bicornis* and Red-breasted Parakeet *Psittacula alexandri* as Near-threatened and Great Slaty Woodpecker *Mulleripicus pulverulentus,* as vulnerable were observed.

Mikusinski *et al.* (2001) stated that woodpeckers (family Picidae) are indicators of forest naturalness. Woodpeckers (Picidae) are extremely helpful for the health of forest trees to fight against harmful insects (Arslangundogdu, 2010). Many of the sensitive woodpeckers and other birds disappeared with increasing degrees of forest disturbance (Lammertink, 2004). In the present study, the number of species in family Picidae was higher than other families.

Most woodpeckers feed on variety of insects that attack healthy trees in forest. This indicted that AKNP was sustainable forest. Moreover, the presence of a large variety of woodpeckers enjoying the nature of mixed deciduous forest, it is also known as woodpecker land which attracted most bird watchers interested in tropical birds.

Red Junglefowl *Gallus gallus* was most abundant species in shrub and bush habitat. Collias and Collias (1966) stated that this species associated with the occurrences of bamboo, grass shoots, seed and fruits from a variety of small trees and bushes. The birds were preferred area covered with shrubs, bushes, small trees and euphorbias to the small, grassy clearings. In the present study, Red Junglefowl was mostly observed in shrubs and bushes habitat. Buffalo and cattle dungs and elephant dung were observed in this habitat. These dungs may contain seeds, various insects that provide some source of food to Junglefowl. Black-crested Bulbul *Pycnonotus flaviventris* was very common species which found in mixed deciduous habitat during the study period. Fruiting trees such as *Ficus* spp were more observed in mixed deciduous habitat. Black-crested Bulbul is frugi-/insectivorous bird. The population of frugi-/insectivorous bird fluctuated seasonally and associated with fruiting trees (Cueto and de Casenave, 2000). Theoretically, fruit maturation occurs during the dry season (Melo *et al.,* 2003). High number of bird species and individuals were recorded during cold and dry seasons when most flowering and fruiting trees were abundant during fruiting time, they fed mostly on fruits. At the time of scare of fruits, they forage on insects.

Oriental Scops-owl *Otus sunia* was observed in bamboo habitat as uncommon species. This species is a nocturnal bird with activity beginning at dusk. In the preset study, this species was roosted during the day singly. Great Hornbill *Buceros bicornis* in deciduous habitat was recorded as uncommon species in hot season. Johnsingh (2005) pointed out that this species feds on figs and more insects. The great hornbill's diet consist mainly fruits. Figs are particularly important as a food source. Moreover, this species forage along branches, moving along by hopping, looking for insects. In the present study, this species was observed in indaing tree in April. The breeding season is occurred in January to April. Great hornbill becomes very vocal. This indicated that the male to join the female. They prefer mature forests particularly emergent that rise above the canopy for nesting (Jame and Kanan, 2009). Common Iora *Aegithina tiphia* was observed as uncommon in mixed deciduous habitat in the study area. This species was uncommon in areas where forest but mostly found in scrubs, cultivated areas and garden (Hoyo *et al.,* 2005). In the present study, this species was found in deciduous forest in AKNP and only species that is foraged was recorded. Daurian Redstart *Phoenicurus auroreus* was observed in shrub and bush habitat as uncommon species. This species is a winter visitor. Insects and seeds are more plentiful in shrub and bush habitat. Daurian Redstart feeds on insects, berries and seeds. Insects are its main food especially during breeding season (Robson, 2015).

Rajpar and Zakaria (2013) revealed that bird population varied in different habitats depending on vegetation structure and composition, availability of food resources, occurrence of suitable foraging, and nesting. Li *et al.* (2009) revealed that the arthropods were more distributed in the mixed deciduous forest. In the present study, mixed deciduous habitat supported higher bird species and population as compared with other habitats. This is probably due to availability of abundant food sources such as insects, fruits. The distribution of food resources such as arthropods for insectivorous birds within forest changes drastically with the season (Murakami, 2002). Moreover, fruiting trees such as *Ficus lacor* (nyaung gyin) were more observed in this habitat.

Breeding birds may have been more easily detected in April to May in Myanmar (Bezuijen *et al.* 2010) and breeding season of birds in Myanmar is almost occurred in February to April (Smythies, 2001). In the present study, the population of bird species was the highest in dry season. Many species were more detectable in this season because most leaves of trees were shed. Winter visitors (common sandpiper *Actis hypoleucos*, Daurian Redstart *Phoenicurus auroreus* and Taiga Flycatcher *Ficeduala albicilla*) and local migrants (such as Shikra *Accipiter badius* and Indian Cuckoo *Cuculus micropterus*) were observed in this season. Thus the highest number of species and individuals were observed in dry season.

Characteristic of forest and habitat loss is the main threat for threatened species. The variety of species and their habitats play an important role in ecosystem function and in many services ecosystems provide (IUCN, 2015). Globally threatened species are more observed in Alaungdaw Kathapa National Park. Among the species observed, Great Hornbill *Buceros bicornis* and Red-breasted Parakeet *Psittacula alexandri* as Near-threatened species and Great Slaty Woodpecker *Mulleripicus pulverulentus* as vulnerable species were observed in the present study.

This result is hoped to provide some data to attract more ecotourism in future. However, there is a need for implementing necessary conservational measures for the stability and sustainability of the living assets. Thus the habitats of Alaungdaw Kathapa National Park should be more managed and conserved to provide rich biodiversity.

Gallus gallus

Otus sunia

Buceros bicornis

Mulleripicus pulverulentus

Pycnonotus flaviventrus

Phoenicurus auroreus

Plate 1. Some recorded bird species in the study area

Table 1. Lists of bird species recorded, and their relative abundance

Order/Family	Scientific name	Habitats			
		SB	B	I	MD
Galliformes					
Phasianidae (0.92%)	*Gallus gallus*	0.05	0.01	0.02	0.01
Columbiformes					
Columbidae (4.59%)	*Streptopelia orientalis*	-	-	0.01	0.01
	Streptopelia chinensis	-	-	-	0.01
	Treron curvirostra	-	-	-	0.02
	Treron phoenicopterus	-	-	-	0.02
	Ducula aenea	-	-	-	0.01
Cuculiformes					
Cuculidae (2.75%)	*Cuculus micropterus*	-	-	0.02	-
	Phaenicophaeus tristis	-	0.01	0.01	0.02
	Centropus sinensis	0.01	-	-	-
Charadriiformes					
Charadriidae (0.92%)	*Vanellus indicus*	0.01	-	-	0.01
Scolopacidae (0.92%)	*Actitis hypoleucos*	-	-	-	0.01
Strigiformes					
Strigidae (2.75%)	*Otus sunia*	-	0.00	-	-
	Athene brama	-	0.01	0.01	0.01
	Glaucidium cuculoides	-	-	0.01	-
Accipitriformes					
Accipitridae (1.83%)	*Spilornis cheela*	-	-	0.01	0.01
	Accipiter badius	-	-	0.01	0.01
Trogoniformes					
Trogonidae (0.92%)	*Harpactes erythrocephalus*	-	0.01	0.01	0.01
Bucerotiformes					
Upupidae (0.92%)	*Upupa epops*	-	-	001	-
Bucerotidae (1.83%)	*Anthracoceros albirostris*	-	-	-	0.03
	Buceros bicornis **NT**	-	-	-	0.00
Coraciformes					
Meropidae (2.75%)	*Nyctyornis athertoni*	-	-	0.02	0.01
	Merops philippinus	-	-	0.01	0.01
	Merops leschenaulti	-	-	0.02	0.01

Order/Family	Scientific name	Habitats			
		SB	B	I	MD
Coraciidae (1.83%)	*Coracias benghalensis*	0.01	0.02	0.01	0.02
	Eurystomus orientalis	-	-	0.01	0.01
Alcedinidae (0.92%)	*Halcyon smyrnensis*	-	-	0.01	0.01
Piciformes					
Megalaimidae (3.67%)	*Megalaima lineata*	-	-	0.01	0.02
	Megalaima asiatica	-	-	0.02	0.01
	Megalaima australis	-	-	-	0.01
	Megalaima haemacephala	-	-	-	0.01
Picidae (10.09%)	*Dendrocopos canicapillus*	-	0.01	0.01	0.02
	Dendrocopos analis	-	0.01	0.03	0.04
	Micropternus brachyurus	-	-	0.01	0.02
	Dryocopus javensis	-	-	0.01	0.01
	Chrysophlegma flavinucha	-	0.01	0.01	0.02
	Picus chlorolophus	-	0.01	0.01	0.01
	Picus canus	-	0.01	0.03	0.04
	Dinopium javanense	-	-	0.01	0.02
	Chrysocolaptes lucidus	-	-	0.01	0.01
	Blythipicus pyrrhotis	-	-	-	0.02
	Mulleripicus pulverulentus **VU**	-	-	0.01	0.02
Falconiformes					
Falconidae (2.75%)	*Microhierax caerulescens*	-	-	0.01	0.01
	Falco tinnunculus	-	-	0.01	0.01
	Falco severus	-	-	0.02	0.03
Psittaciformes					
Psittacidae (2.75%)	*Psittacula finschii*	-	-	0.01	-
	Psittacula alexandri **NT**	-	-	0.01	0.01
	Psittacula roseate	-	-	0.01	-
Passeriformes					
Aegithinidae (0.92%)	*Aegithina tiphia*	-	-	-	0.00
Campephagidae (4.59%)	*Tephrodornis gularis*	-	-	0.02	0.01
	Coracina macei	-	-	0.02	0.01
	Coracina javensis	-	-	0.01	0.01
	Hemipus picatus	-	0.01	0.01	0.02
	Pericrocotus speciosus	-	-	0.01	0.03
Laniidae (1.83%)	*Lanius schach*	-	0.01	0.01	0.01
	Lanius tephronotus	-	0.01	0.01	0.01
Oriolidae (3.63%)	*Oriolus chinensis*	-	-	-	0.03
	Oriolus tenuirostris	-	-	0.01	0.02
	Oriolus xanthornus	-	-	0.01	0.01
	Oriolus traillii	-	-	-	0.01

Order/Family	Scientific name	Habitats			
		SB	B	I	MD
Dicruridae (5.50%)	*Dicrurus leucophaeus*	-	0.01	0.03	0.03
	Dicrurus annectans	-	-	0.01	0.01
	Dicrurus aeneus	-	-	0.01	0.01
	Dicrurus remifer	-	-	0.02	0.03
	Dicrurus hottentottus	-	-	0.01	0.01
	Dicrurus paradiseus	-	-	0.01	0.03
Monarchidae (0.92%)	*Hypothymis azurea*	-	0.02	0.01	0.02
Corvidae (1.83%)	*Urocissa erythrorhyncha*	-	0.01	0.01	0.01
	Dendrocitta vagabunda	-	0.01	0.03	0.02
Paridae (0.92%)	*Parus cinereus*	-	0.02	0.02	0.02
Cisticolidae (1.83%)	*Prinia rufescens*	0.01	0.01	-	-
	Prinia inornata	0.01	0.01	-	-
Pycnonotidae (3.67%)	*Pycnonotus flaviventris*	-	0.01	0.03	0.05
	Pycnonotus cafer	-	-	0.01	0.02
	Iole virescens	-	-	-	0.02
Sylviidae (2.75%)	*Orthotomus sutorius*	0.01	-	0.01	-
	Phylloscopus claudiae	0.01	0.01	-	0.01
	Abroscopus superciliaris	-	0.02	0.01	-
Timaliidae (2.75%)	*Pellorneum ruficeps*	-	0.01	-	-
	Garrulax monileger	-	0.03	0.02	0.03
	Garrulax pectoralis	-	0.02	0.02	0.02
Sittidae (1.83%)	*Sitta cinnamoventris*	-	0.01	0.01	0.01
	Sitta frontalis	-	0.02	0.01	0.02
Sturnidae (0.92%)	*Gracula religiosa*	-	-	-	0.03
Turdidae (1.83%)	*Myophonus caeruleus*	-	-	-	0.01
	Turdus boulboul	-	-	0.01	0.01
Muscicapidae (9.17%)	*Copsychus saularis*	-	0.02	0.01	0.02
	Copsychus malabaricus	-	0.01	0.01	0.01
	Phoenicurus auroreus	0.00	-	-	-
	Enicurus immaculatus	-	-	-	0.01
	Saxicola caprata	-	0.01	-	0.01
	Saxicola ferreus	0.01	0.01	-	-
	Monticola gularis	-	-	0.01	0.01
	Monticola solitaries	-	-	-	0.01
	Ficedula albicilla	0.01	-	-	0.01
	Cyornis glaucicomans	-	-	-	0.01
	Culicicapa ceylonensis	-	-	-	0.01
Chloropseidae (1.83%)	*Chloropsis cochinchinensis*	-	-	-	0.01
	Chloropsis aurifrons	-	-	-	0.01
Dicaeidae (1.83%)	*Dicaeum chrysorrheum*	-	-	0.01	0.01

Order/Family	Scientific name	Habitats			
		SB	B	I	MD
	Dicaeum cruentatum	-	-	-	0.01
Nectariniidae(3.67%)	*Anthreptes malacensis*	-	-	-	0.01
	Cinnyris asiatica	-	-	-	0.01
	Aethopyga christinae	-	-	0.01	0.01
	Aethopyga siparaja	-	-	-	0.01
Passeridae (0.92%)	*Passer flaveolus*	-	-	0.01	0.01
Motacillidae (2.75%)	*Motacilla alba*	-	-	-	0.01
	Motacilla cinerea	-	-	-	0.01
	Anthus hodgsoni	-	001	0.02	0.01
	Total (species)	11	34	70	95
	Total (individuals)	524	660	1197	2867
	All total species	109			
	All total individuals	5248			

SB = Shrub and bush; B = Bamboo; I = Indaing; MD = Mixed deciduous; NT = Near-threatened species, VU = Vulnerable species

Table 2. Number of bird species in different relative abundance scores in different habitats during the study period

Relative abundance scores	Shrub and bush	Bamboo	Indaing	Mixed deciduous
vC	1	-	-	1
C	9	33	70	93
uC	1	1	-	1
Total	11	34	70	95

vC = very common; C = common; uC = uncommon

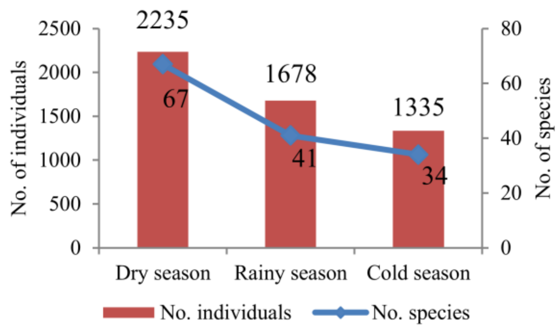

Figure 1. Number of species and individuals of recorded bird species in different seasons

Acknowledgements

I would like to thank Dr. Thura Oo, Rector, Dr. Thet Naing Oo and Dr. Sein Sein Aung, Pro-Rectors, Monywa University for their permission to present and publish this paper. Thanks are also due to Professor Dr. Khin Soe Win, Head of Department of Zoology, Monywa University, for her kind supports. I am grateful to Dr. Hla Hla Win, Professor, Department of Zoology, Monywa University, for her kind of suggestions. I am also thankful to Dr. Mie Mie Sein, Pro-Rector, Mawlamyine University for her close supervision and encouragements given to me during this research. I wish to particularly thank to U Ye Myint, Assistant Director, Alaungdaw Kathapa National Park, for helping me in various ways during the period of field trip, and also thanks to all staffs from National Park for their helps in field surveys.

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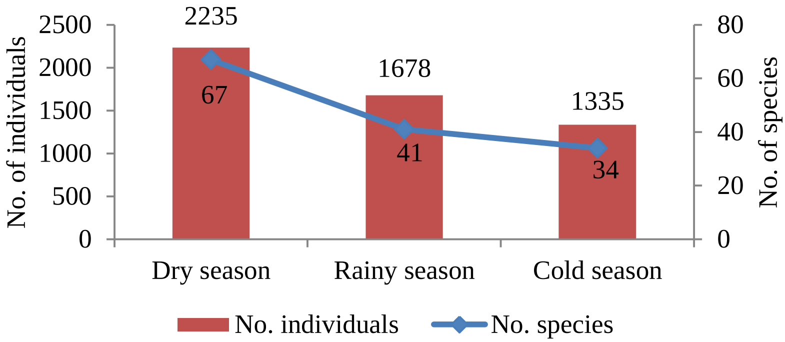

Fig. 1 Number of species and individuals of recorded bird species in different seasons

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J. Myanmar Acad. Arts Sci. 2019 **Vol. XVII. No.3**

SPECIES COMPOSITION AND THE EFFECTS OF WATER LEVEL FLUCTUATION ON WATERBIRD POPULATION AT INMAGYI WETLAND IN MYINMU TOWNSHIP, SAGAING REGION

Htay Khaing[1], Mie Mie Sein[2]

Abstract

The species composition and the effects of water level fluctuation on waterbird population was determined by using fixed radius point count method at Inmagyi Wetland in Myinmu Township, Sagaing Region. A total of 31 waterbird species belonging to 27 genera, 12 families and seven orders were recorded in study areas. The study was conducted from July 2016 to June 2017. Out of 31 species, 19 species were residents and 12 species were winter visitors. Three globally Near Threatened species, Spot-billed Pelican *Pelecanus philippensis*, Painted Stork *Mycteria leucocephala* and Black-headed Ibis *Threskiornis melanocephalus* were observed during the study period. The study area was divided into two habitats, that is, marshy swamp and open water body to study the effects of water level fluctuation on waterbird population. The result of the Pearson's test indicated that negative correlation ($r = -0.171$) was observed between water level and waterbird population in marshy swamp habitats and almost no correlation ($r = 0.005$) was observed in open water body habitat. However the population of waterbirds in marshy swamp were significantly different from open water body. This study revealed that Inmagyi Wetland has habitat heterogeneity and seasonal fluctuation of water level that had attracted residents and winter visitors of waterbird species to perform various activities such as feeding, roosting and breeding.

Keywords: Water level, Waterbirds, Wetlands, Marshy swamp, Open water body

Introduction

Wetlands are fringe habitats between terrestrial and aquatic ecosystems (Beury *et al.*, 2008). Wetlands are highly important habitat for

[1.] Dr., Lecturer, Department of Zoology, Monywa University
[2.] Dr., Prorector, Mawlamyine University

diverse fauna including mammals, birds, fishes, reptiles, amphibians, and aquatic invertebrates (Nelson *et al*., 2000).

Wetland is defined as area of marshes, fen, peat land and water, whether natural or artificial, permanent or temporary with water that is static or flowing, fresh, brackish or salt, including areas of marine water where the depth of water at low tide does not exceed six meter. Wetlands are major breeding, nesting, and migration staging areas for waterfowls and shorebirds (Ramsar convention, 2010).

"Waterbirds" refers to the bird species that entirely depend on wetlands for a variety of activites such as foraging, nesting, loafing, and moulting. Moreover, the term waterbird is used to refer to birds that live on or around water. Some water birds are more terrestrial or aquatic than others and their adaptations include webbed feet, bills and legs adapted to feed in water and the ability to dive from the surface or the air to catch prey in water (Rajpar and Zakaria, 2011).

Waterbirds not only constitute the most prominent group which attract people to wetlands, but also are good bioindicators and useful models for studying a variety of environmental problem (Urfi *et al*., 2005). The species richness and relative abundance of birds depend upon wetland characteristics such as size, water level, quality of water, availability and distribution of food resources, presence of suitable roosting and nursery sites (Wiens, 1989) (cited by Sharma and Manakshi, 2012).

Water level fluctuation in wetlands can be caused by seasonal flooding, tides, and agricultural irrigation or drawdown. The effect of water level fluctuation on water-birds varies among groups and seasons. Generally, water level fluctuation creates habitats with diverse water depths changing in time and space. This provides more foraging opportunities and consequently supports a high species richness and abundance of waterbirds. Water level fluctuation, however, may create "ecological traps" and be detrimental for the breeding, brood-rearing, and molting of waterbirds (Kaminski *et al.*, 2006).

Inmagyi Wetland is the natural agricultural wetland and one of the flooded plain in Myinmu Township, Sagaing Region. The water level in the catchment area of Inmagyi Wetland peaked during the rainy season but the water level decreased gradually in cold season and the lowest water level occurred in the dry season. Thus the water level fluctuated greatly seasonally in Inmagyi Wetland. Rajpar and Zakaria (2011) indicated that the water level is a major factor that influenced the waterbird species composition and relative abundance directly and indirectly. This study investigates the species composition and the effects of water level fluctuation on waterbird population at Inmagyi wetland.

Materials and Methods

Study Area and Study Sites

Inmagyi Wetland is located in Myinmu Township, Sagaing Region and situated between 21° 50' 48.85" and 21° 52' 36.81" N, and 95° 26' 48.25" to 95° 27' 53.83"E (Plate 1). The total surface area of Inmagyi Wetland is about (8.64) km^2, one of the flooded plains directly connected with the Ayeyarwady River. The water depth of Inmagyi Wetland is approximately 5 m in the rainy season and reduces with a water depth about 1 m in the dry season. The study area embodied two major aquatic habitats based on the fluctuation of water level namely, (i) marshy swamp and (ii) open water body (Plate 2).

Study period

Field surveys were conducted at Inmagyi Wetland from July 2016 to June 2017.

Field Technique

The field survey was conducted twice a month. Birds were observed by using a binocular. The bird photos were taken with digital camera. Bird watching was undertaken two times during the period from 6:30 am to 10:30 am in the morning and from 3:30 pm to 5:30 pm in the evening. Bird watching and counting were carried out using a boat and also walking along marshy swamp. Bird census was made by using point count method (Hutto *et al.,* 1986). Twenty four point count stations at 200 m interval apart from each other were established in marshy swamp (12 stations) and open water body (12 stations).

Foraging Behaviour

Foraging behaviour of the waterbird species was recorded on direct observations during every field surveys. The waterbirds were grouped based on foraging behaviour and habitats use such as dabbling duck (small or middle size of Anatidae species that require shallow wetland for feeding), diving waterbird (includes all kinds of waterbird species that can dive for feeding) and shorebird (wader species and are not good at swimming) according to Chan-Woo *et al.* (2006), and based on visual observations.

Water Level

The changes of water level in two habitats were measured by measuring tape with a heavy sinker attached and biweekly measurements were simultaneously taken at six sampling points. The water level was recorded in feet and inches, later on, were converted into meter scale.

Identification and classification

Identification of the bird species was made according to King and Dickinson (1975), Smythies (2001) and Robson (2015). Birds were listed in the classification systems proposed by Bird Life international checklist Version 8 (2015).

Analysis of data

The effects of water level fluctuation on waterbirds population were determined by direct observation. In addition, the correlation between water level and waterbird population was determined using Pearson's correlation coefficient in order to understand the effects of water level on waterbirds population.

Pearson Correlation

$$\mathbf{r} = \frac{\sum(\mathbf{X}-\bar{\mathbf{X}})(\mathbf{Y}-\bar{\mathbf{Y}})}{\sqrt{[\sum(\mathbf{X}-\bar{\mathbf{X}})^2][\sum(\mathbf{Y}-\bar{\mathbf{Y}})^2]}}$$

Comparison using ***t***-test to determine the significant difference of waterbird population between the two habitats (Zar, 2010).

$$t = \frac{(\bar{x}_1-\bar{x}_2)}{\sqrt{\left[\frac{(n_1-1)S_1^2+(n_2-1)S_2^2}{(n_1+n_2-2)}\right]\left(\frac{n_1+n_2}{n_1 n_2}\right)}}$$

(Source: Google Earth, 2016)

Plate 1. Location map of the study sites

A. Marshy Swamp Area

B. Open Water Body

Plate 2. Different habitats in the study

Results

A total number of 10,729 waterbird individuals accounted from 31 species, 27 genera, 12 families and seven orders were recorded from the two habitats, namely marshy swamp and open water body at Inmagyi Wetland. All the 31 species were recorded in the marshy swamp area and in which, 22 species included in open water body (Table 1).

Species Composition of Orders and Families in the Study Area

The seven orders of waterbirds were recorded in Inmagyi Wetland such as Anserifromes, Podicipediformes, Gruiformes, Ciconiiformes, Pelecaniformes, Suliformes and Charadriiformes throughout the study period. The four families were included in Charadriiformes, three families in Pelecaniformes, and one family in Anseriformes, Podicipediformes, Gruiformes, Ciconiiformes and Suliformes. Maximum number of species were observed in family Ardeidae (eight) followed by Charadriidae (four), Anatidae and Scolopacidae (three in each), Podicipedidae, Rallidae, Cioniidae, Threskiornithidae and Phalacrocoracidae (two in each) and Pelecanidae, Recurivirostridae and Jacanidae (one in each) (Table 1).

During the study period, the highest composition of species was found in order Pelecaniformes (35.48%), followed by Charadriiformes (29.03%), Anseriformes (9.68%), Podicipediformes, Gruiformes, Ciconiiformes and Suliformes (6.45%) (Figure. 1).

Out of the 31 species recorded, 19 species were residents and 12 species were winter visitors. A total of 19 species were found in all study sites. Spot-billed Pelican *Pelecanus philippensis*, Painted Stork *Mycteria leucocephala* and Black-headed Ibis *Threskiornis melanocephalus* happened to be Near Threatened species according to IUCN Red List (2015) (Table 1).

Foraging groups of waterbirds

The highest number of individuals recorded was shorebirds (7662 birds) followed by diving birds (1570 birds) and in dabbling ducks (1497 birds) in both study habitats (Table 2).

In marshy swamp area, 22 species were shore birds (Sb), six species were diving birds (Di) and three species dabbling duck (Db) according to foraging behavior of waterbirds. The highest number of individuals recorded also happened to be shorebirds (6719 birds) followed by dabbling ducks (903 birds) and in diving birds (363 birds) (Table 2, Fig. 2). However, in the open water body, only 22 species were recorded, including 13 species of shore birds (Sb), six species of diving birds (Di) and three species of dabbling duck (Db). The highest number of individuals recorded were diving birds (1207 birds) followed by shorebirds (943 birds) and dabbling ducks (594 birds) (Table 2, Fig. 3).

Water Level

The water level in the marshy swamp habitat varied due to the variation in the amount of rainfall and the entry of water from the catchment area of Inmagyi Wetland. The highest water level recorded was (1.829 m) in July 2016 and the lowest (0.025 m) in May 2017. In open water body, the highest water level 4.572 m was recorded in July 2016 and the lowest water level 0.305 m was observed in May 2017 due to low rainfall (Table 3).

Correlation between Water Level and Waterbird Population

The results of Pearson's test revealed negative correlation between water level and waterbird individuals in marshy swamp habitat ($r = -0.171$) and no correlation ($r = 0.005$) in open water body habitat.

Relationship between the Monthly Mean Water Level and the Number of Waterbirds in Marshy Swamp and Open Water Body

Lower population of waterbirds was found in July, August and September when the water level higher. The higher population of waterbirds were found in October, November, December and January with lower water level (Fig. 4 and 5)

According to ***t***-test, the population of waterbirds between marshy swamp and open water area was significantly different. Thus the highest waterbird population was observed in marshy swamp area than the open water area (Table 4).

Table 1. Status of the waterbird species recorded at Inmagyi Wetland

Order	Scientific name	Common name	IUCN Redlist	Status
Anseriformormes	**Anatidae**			
	Dendrocygna javanica	Lesser Whistling-duck	LC	R
	Tadorna ferruginea	Ruddy Shelduck	LC	WV
	Anas poecilorhyncha	Indian Spot-billed Duck	LC	R
Podicipediformes	**Podicipedidae**			
	Tachybaptus ruficollis	Little Grebe	LC	R
	Podiceps cristatus	Great Crested Grebe	LC	R
Gruiformes	**Rallidae**			
	Amaurornis phoenicurus	White-breasted Waterhen	LC	R
	Fulica atra	Common Coot	LC	WV
Ciconiiformes	**Ciconiidae**			
	Mycteria leucocephala	Painted Stork	NT	WV
	Anastomus oscitans	Asian Openbill	LC	WV
Pelecaniformes	**Threskiornithidae**			
	Threskiornis melanocephalus	Black-headed Ibis	NT	WV
	Plegadis falcinellus	Glossy Ibis	LC	WV
	Ardeidae			
	Nycticorax nycticorax	Black-crowned Night-heron	LC	R
	Ardeola grayii	Indian Pond-heron	LC	R
	Ardeola bacchus	Chinese Pond-heron	LC	R
	Bubulcus ibis	Cattle Egret	LC	R
	Ardea cinerea	Grey Heron	LC	R
	Ardea alba	Great White Egret	LC	R
	Ardea intermedia	Intermediate Egret	LC	R
	Egretta garzetta	Little Egret	LC	R
	Pelecanidae			

Order	Scientific name	Common name	IUCN Redlist	Status
	Pelecanus philippensis	Spot-billed Pelican	NT	R
Suliformes	**Phalacrocoracidae**			
	Microcarbo niger	Little Cormorant	LC	R
	Phalacrocorax carbo	Great Cormorant	LC	R
Charadriiformes	**Recurvirostridae**			
	Himantopus himantopus	Black-winged Stilt	LC	WV
	Charadriidae			
	Pluvialis fulva	Pacific Golden Plover	LC	WV
	Charadrius dubius	Little Ringed Plover	LC	WV
	Vanellus cinereus	Grey-headed Lapwing	LC	R
	Vanellus indicus	Red-wattled Lapwing	LC	R
	Jacanidae			
	Hydrophasianus chirurgus	Pheasant-tailed Jacana	LC	R
	Scolopacidae			
	Gallinago gallinago	Common Snipe	LC	WV
	Actitis hypoleucos	Common Sandpiper	LC	WV
	Tringa glareola	Wood Sandpiper	LC	WV

Table 2. Individuals of foraging groups of waterbird recorded at Inmagyi Wetland

Foraging group	Scientific name	No. of individuals		
		Open Water	Marsh Swamp	Total
Dabbling Ducks	*Dendrocygna javanica*	287	216	503
	Tadorna ferruginea	73	357	430
	Anas poecilorhyncha	234	330	564
		594	**903**	**1497**
Diving waterbirds	*Tachybaptus ruficollis*	31	18	49
	Podiceps cristatus	12	2	14
	Pelecanus philippensis	6	3	9
	Microcarbo niger	1014	270	1284
	Phalacrocorax carbo	41	25	66
	Fulica atra	103	45	148
		1207	**363**	**1570**
Shorebirds	*Mycteria leucocephala*	22	283	305
	Anastomus oscitans	96	1235	1331
	Threskiornis melanocephalus	75	627	702
	Plegadis falcinellus	0	685	685
	Nycticorax nycticorax	118	482	600

Foraging group	Scientific name	No. of individuals		
		Open Water	Marsh Swamp	Total
	Ardeola grayii	37	81	118
	Ardeola bacchus	42	63	105
	Bubulcus ibis	77	979	1056
	Ardea cinerea	44	63	107
	Ardea alba	56	134	190
	Ardea intermedia	38	74	112
	Egretta garzetta	170	232	402
	Himantopus himantopus	0	373	373
	Pluvialis fulva	0	79	79
	Charadrius dubius	0	181	181
	Vanellus cinereus	94	203	297
	Vanellus indicus	74	168	242
	Hydrophasianus chirurgus	0	104	104
	Gallinago gallinago	0	198	198
	Actitis hypoleucos	0	202	202
	Tringa glareola	0	149	149
	Amaurornis phoenicurus	0	124	124
		943	**6719**	**7662**

Table 3. Monthly water level (m) of the two study sites at Inmagyi Wetland

Months	Marsh Swamp			Open Water Body		
	Minimum water level (m)	Maximum water level (m)	Mean water level (m) (Mean ± SD)	Minimum water level (m)	Maximum water level (m)	Mean water level (m) (Mean ± SD)
July, 2016	1.219	1.829	1.448 ± 0.231	1.981	4.572	3.226 ± 1.031
August, 2016	0.914	1.676	1.168 ± 0.284	1.829	4.267	2.896 ± 0.978
September, 2016	0.610	1.524	0.991 ± 0.383	1.676	3.658	2.515 ± 0.816
October, 2016	0.305	1.372	0.686 ± 0.406	1.524	2.743	2.184 ± 0.559
November, 2016	0.152	0.914	0.457 ± 0.273	1.219	2.438	1.930 ± 0.498
December, 2016	0.127	0.610	0.326 ± 0.183	0.914	1.524	1.118 ± 0.249
January, 2017	0.102	0.457	0.220 ± 0.150	0.762	1.219	0.914 ± 0.161

Months	Marsh Swamp			Open Water Body		
	Minimum water level (m)	Maximum water level (m)	Mean water level (m) (Mean ± SD)	Minimum water level (m)	Maximum water level (m)	Mean water level (m) (Mean ± SD)
February, 2017	0.089	0.305	0.171 ± 0.088	0.610	0.914	0.754 ± 0.099
March, 2017	0.076	0.203	0.135 ± 0.047	0.508	0.762	0.669 ± 0.109
April, 2017	0.051	0.076	0.064 ± 0.014	0.457	0.610	0.533± 0.083
May, 2017	0.025	0.051	0.030 ± 0.010	0.305	0.457	0.432 ± 0.062
June, 2017	0.152	0.457	0.254 ± 0.124	0.610	1.372	1.041 ± 0.280

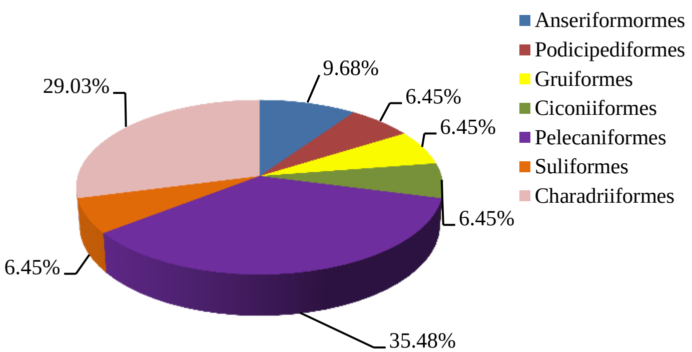

Figure 1. Species composition of waterbird species in different orders at Inmagyi

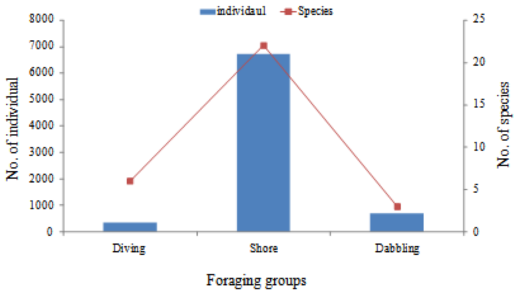

Figure 2. Comparison on the foraging groups of waterbird species and individual in Marshy Swamp at Inmagyi Wetland

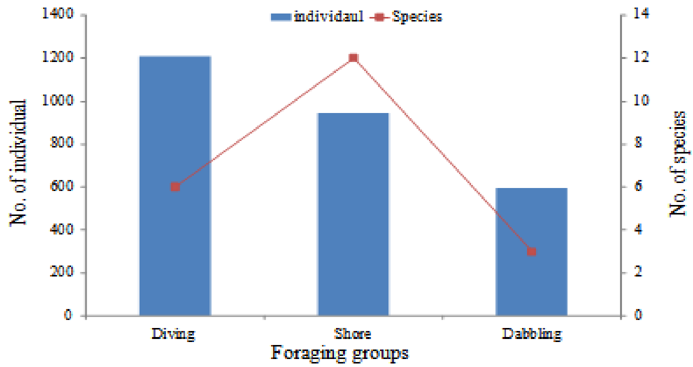

Figure 3. Comparison on the foraging groups of waterbird species and individual in Open water body at Inmagyi Wetland

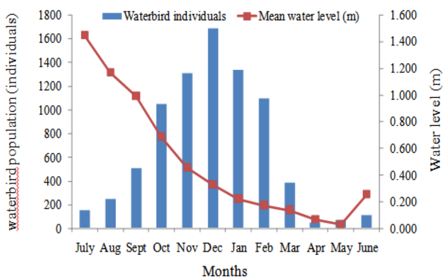

Figure 4. Relationship between the monthly mean water level and the number of waterbird individuals in Marshy Swamp at Inmagyi Wetland

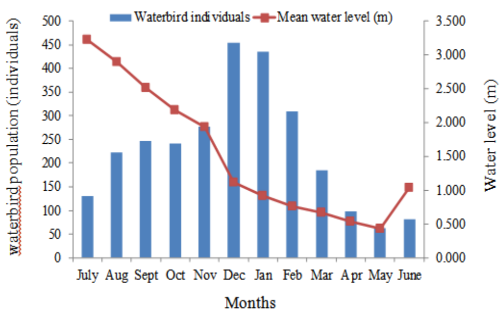

Figure 5. Relationship between the monthly mean water level and the number of waterbird individuals in Open Water Body at Inmagyi Wetland

Discussion

The water level of Inmagyi Wetland is dependent upon incoming water resource and precipitation. In present study, two habitats were allocated namely marshy swamp and open water habitats to study the waterbirds that thrive in the wetland.

Ma *et al.* (2007) stated that the difference in bird composition was related to habitat preferences. In the present study, all the total of 31 species of waterbirds recorded were encountered in marshy swamp and only 22 species in open water habitat. Therefore, marshy swamp harboured more waterbird species compared to open water, and is alluded to the foraging habit and habitat preference of these waterbirds.

Desgranges *et al.* (2006) examined wetland birds' response to water level fluctuation and revealed that wetland bird species were significantly associated with hydrological context. In the present study, the highest population of waterbird were found in October, November, December and January with lower water level while the lower population of waterbird were found in July, August and September when the water level higher. It indicated that the population of water birds and water levels were inversely proportional in both habitats except in dry season.

Bitterns and herons also preferred scattered emergent vegetation especially along the water body edges for foraging (Rajpar and Zakaria, 2014). In the present study, gregarious shore bird species like Asian Openbill and Cattle Egret, Glossy Ibis and Black-headed Ibis were highest number of individuals and are therefore relatively abundant in marshy swamp area. These waterbirds were carnivorous species and depend on marshy swamp area (Mistry and Mukherjee, 2015). It may be suggested that the shallow water offers crucial foraging sites for wading birds such as herons, egrets and storks because obtaining food is easily accessible.

In the present study, diving and dabbling ducks were found in both marshy swamp and open water body. The diving waterbirds such as Little Grebe, Great Crested Grebe, Common Coot, Little Cormorant and Great Cormorant preferred open water habitat for foraging sites. Especially, Cormorant species were mostly roosted on the trees which were sparsely composed in open water. Chan-Woo *et al.* (2006) revealed that diving birds were abundant in higher water level. Diving waterbirds feed in deeper water depth than other waterbirds. Saygili *et al.* (2011) reported that Cormorants, Grebe and Coots for feeding in open water, but mostly preferred the small “islands” in the lake.

Mortsch (1998) and Poiani *et al.* (1995) has reported that lower water level caused the changes in aquatic vegetation composition and structures and as a result, affected the grass and shrub communities. In the study period, the water level was gradually receded along the water body edges of Inmagyi Wetland to transform into marshy swamp, agricultural fields and small grasslands and shrubs appeared in these areas. The fluctuation in water level might alter the habitat characteristic that could cause prompt changes in fishes, amphibians, invertebrates and waterbird communities. Therefore, it indicated that the changes of water level influence the physical structure of habitat, the availability and accessibility of food and the presence of safe roosting or breeding sites for waterbirds.

Rajpar and Zakaria (2011) indicated that weak linear corelationship between water level and waterbird abundance in marshy swamp and negative linear correlation in open water body. Marshall (1997) also indicated that a negative relationship between lake water level and waterbird abundance, with highest waterbird numbers when water levels were lowest was found. In the present study, weak negative correlation was observed between the water level and population of waterbirds in marshy swamp and no correlation in open water habitats. It is assumed that this is due to the seasonal fluctuations of water level in both study habitats.

The results of ***t***-test revealed that, the population of waterbirds in marshy swamp was more significant difference than open water area. It may be suggested that shallow water area or marshy swamp area provided more food resources by the presence of fishes, amphibians, a variety of insects, invertebrate fauna and aquatic plants are the major component of waterbirds diet principally for cormorants, grebes, storks, ducks, plovers, sandpipers, stilts and egrets etc.

The present study indicated that the population of waterbirds was depended by their food supply and water level in habitat. The Inmagyi Wetland supports at least 12 migratory species of waterbirds and most of the shore birds used as foraging ground and breeding habitat in marshy swamp area. Therefore, the protection and monitoring of ecosystem of Inmagyi wetland should be carried out so as to control changes in the state of wetland especially on resident and migratory waterbird species.

Conclusion

The present study indicated that the marshy swamp habitat supported higher waterbird population as compared to open water body. The waterbird populations were directly influenced by the availability of foraging habitats and have preference to wetland habitat with surface water that supply suitable prey organisms for foraging. Therefore, the increase or decline of the waterbird species was found to be associated with the water level and the availability of the food resource changes in both marshy swamp and open water body habitats.

Acknowledgements

I am very grateful to Dr. Thura Oo, Rector, Dr. Sein Sein Aung and Dr. Thet Naing Oo, Prorectors, Monywa University for their permission to carry out the present research. I would like to thank Dr Khin Soe Win, Professor and Head, Department of Zoology, Monywa University for her encouragement and providing facilities for the research in the Department.

Thanks also due to Dr Mie Mie Sein, Prorector, Mawlamyine University for her suggestions, guidance, providing relevant literatures and critical reading of the manuscript.

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