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FOREWORD

On 26 February 2026, the Myanmar Academy of Arts and Science (MAAS) was reconstituted to accelerate the implementation of four different strategies:

(a) encouraging universities and colleges to engage in more research activities
(b) organizing domestic and international research conferences
(c) publishing research journals and proceedings, and
(d) fostering research collaboration among local and international universities

Since the year 2001, the MAAS has been making every effort to fulfil these important functions in collaboration with universities and colleges across the country. As a result, the MAAS has published research papers in 23 volumes on diverse academic disciplines in the Journal of the Myanmar Academy of Arts and Science. It is a great honor to say that the 24th research conference was successfully held at the University of Yangon on 9–11 March 2024. The theme of this conference is *'Research and Innovation for Peace, Prosperity and Sustainability'*. A total of 369 research papers presented by both faculty and postgraduates at the conference have been published in Volume XXIII as follows:

Vol. XXIII, No. 1A	Chemistry
Vol. XXIII, No. 1B	Chemistry, Industrial Chemistry
Vol. XXIII, No. 2	Physics, Mathematics and Computer Studies
Vol. XXIII, No. 3	Zoology, Marine Science, Environment and Water Studies
Vol. XXIII, No. 4	Botany, Geology
Vol. XXIII, No. 5	English, History, Philosophy, Geography, Psychology, International Relations, Oriental Studies
Vol. XXIII, No. 6	Myanmar
Vol. XXIII, No. 7	Law, Library and Information Studies, Archaeology, Anthropology, Language and Linguistics
Vol. XXIII, No. 8	Applied Statistics, Management Studies, Statistics, Commerce, Economics, Journalism and Tourism
Vol. XXIII, No. 9A	Educational Theory and Management, Curriculum and Methodology
Vol. XXIII, No. 9B	Educational Psychology

I firmly believe that the empirical research findings from these papers will provide practical assistance for research candidates and those who are interested in research work. I am also hopeful that these findings will significantly contribute to the well-being of the community at large as well as the State's socio-economic development.

On behalf of the members of the MAAS, I would like to offer my special gratitude and thanks to those who have contributed their research papers to this journal. I also do appreciate the editing work done by senior professors and scholars of high standing as well as the members of the MAAS Editorial Board. Finally, my heartfelt thanks go to the members of the Publication Committee and the Department of Higher Education for their great dedication to the success of this journal.

Dr. Soe Win
President
Myanmar Academy of Arts and Science

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LIFE CYCLE AND NUTRITIONAL COMPOSITION OF DUNG BEETLE, *HELIOCOPRIS BUCEPHALUS* (FABRICIUS, 1775), UNDER CAPTIVE CONDITION*

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Abstract

Larvae of *Heliocopris bucephalus*, commonly known as the dung beetle, are edible insects with high market value in Myanmar. The aim of the present study was to examine the life cycle of the cow dung beetle, *Heliocopris bucephalus* (Fabricius, 1775), under captive conditions and analyze the nutritional composition of its pupal stages. The rearing of dung beetles were conducted in two parts: Experiment I focused on the developmental stages from adults to the third instar larvae, while Experiment II covered the development from natural brood balls, fourth instar larvae, to the adult stages. For the first part of the study, thirty adult beetles were randomly collected from a three-feet-height dung hill in Pobba Thiri Township, Nay Pyi Taw Division, and reared in insect boxes (15.75 x 11.75 x 9.5 inches) filled with layers of cow dung at the Applied Entomology Laboratory, Zoology Department, University of Yangon, in November 2023, with a sex ratio of one male to three females. The adults built brood balls, but they were not as tightly packed as those in natural conditions. After about two and a half months, the third instar larvae in the brood balls were killed due to infection by entomopathogenic fungi and predation by mole crickets that had contaminated the dung. The rearing boxes were renovated to prevent fungal infection and predators and Experiment II was conducted. Experiment II started with the collection of wild dung brood balls containing fourth instar larvae which were then cultivated in the laboratory. The fourth instar larvae developed into fully grown adults after a study period of three months. The entire life cycle of *Heliocopris bucephalus*, combining the results from Experiments I and II, lasted approximately six months to complete. The nutritional values of the pupal stage, which is the edible stage, were analysed for protein, fat, carbohydrate, fibre, moisture and ash content. The pupae contained 45.40% protein, 29.16% fat, 13.92% carbohydrate, 3.86% fibre, 3.44% moisture, 4.22% ash content and 497 kcal/g energy value. The nutritional content of *H. bucephalus* is higher than that of livestocks, with the protein content being nearly double that of soybeans, which have the highest protein content among plant-based foods.

Keywords : Dung beetles, under captive condition and nutritional composition

Introduction

Edible insects are seen as a key solution to the challenge of meeting the increasing global demand for animal protein, which is highly valued for its nutritional benefits (Bruinsma *et al*., 2003). Insects should therefore be considered as an alternative source of animal protein, or "micro-livestock," especially since livestock already occupies over two-thirds (68%) of all agricultural land (FAOSTAT, consulted August, 2015). This is largely due to insects' high feed conversion efficiency and their ability to consume a wide range of feed sources (Halloran *et al*., 2016).

Entomophagy, the practice of eating insects, is traditionally followed in 113 countries worldwide (MacEvilly and Ms Claire, 2000). It is estimated that insects are part of the diet for at least two billion people, and over 1,900 insect species are currently consumed as food (FAO, 2013). Worldwide, the most commonly eaten insects are those that are easily accessible, such as

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beetles (Coleoptera: 31%), caterpillars (Lepidoptera: 18%), bees, wasps, and ants (Hymenoptera: 14%), grasshoppers, locusts, and crickets (Orthoptera: 13%), cicadas, leafhoppers, planthoppers, scale insects, and true bugs (Hemiptera: 10%), termites (Isoptera: 3%), dragonflies (Odonata: 3%), flies (Diptera: 2%), and various other species (5%) (Van Huis *et al.*, 2013). Insects are most frequently consumed in Africa, Asia, and Latin America (Jongema, 2015). Most edible insects have protein levels similar to those in beef, fish, or shrimp, ranging from 27 g to 54 g of protein per 100 g of edible portion on a fresh weight basis (FAO, 2003).

Most larval stages of insects, including certain species of dung beetles such as *Heliocopris bucephalus*, have been traditionally consumed in various parts of Myanmar where they are often considered as a delicacy. As researchers have discovered that the local people consume edible forest insects not because they are environmentally-friendly or nutritious but because they are cheap compared to meat or poultry that are widely available. Rather they choose to eat insects simply because the insects are good in taste (DeFoliart, 1995).

Since Myanmar is an agricultural country, there is a lot of cattle breeding and therefore beetles that eat dung from herbivores like cows and buffaloes can find anywhere. Through manipulating faeces during their feeding process, cow dung beetles initiate a series of ecosystem functions ranging from nutrient cycling to parasite suppression (Andresen, 2002). In which, *Heliocopris bucephalus* (Coleoptera, Scarabaeidae) plays an important role in nutrient cycling by decomposing large herbivore dung (Nichols *et al*., 2008). They also act as secondary seed dispersers by burying them in the brood ball, which also protects the seeds from rodent predators (Hanski & Cambefort, 1991).

In rural areas like Rakhine and Kayin States, people harvest *Heliocopris bucephalus* from the wild, particularly from agricultural fields and forested areas where these cow dung beetles are abundant. The beetles are typically collected by hand and prepared in various ways, such as frying, roasting, or boiling. According to the local people, adult beetles are not very suitable for human consumption because of the high chitin substances content (wings, exoskeleton, legs etc.).

There is potential for developing small-scale farming of edible cow dung beetles as a sustainable food source. This would involve breeding and raising *Heliocopris bucephalus* in controlled environments, which could reduce pressure on wild population while providing a reliable source of nutritious food. The present study aims to investigate the developmental stages and the duration of the life cycle of *Heliocopris bucephalus* under captive condition and to examine the nutritional composition regarding the edible pupal stages of *Heliocopris bucephalus.*

Materials and Methods

Study area and period

This study was conducted from November 2023 to October 2024 at the Applied Entomology Laboratory of the Department of Zoology, University of Yangon. The adult beetles used in Experiment I and brood balls for Experiment II were collected from a dung hill in Pobba Thiri Township, Nay Pyi Taw Division (19°57′16″N 96°10'19"E) (Fig.1). Fresh cow dung for feeding was sourced from Hmawbi Township, Yangon Division.

Life cycle of *Heliocopris bucephalus* under captive condition

The life cycle of *Heliocopris bucephalus* was studied in two separate experiments. The first involved rearing adult beetles to the third instar larval stages, while the second focused on rearing fourth instar larvae from natural habitats to the fully grown adult stages.

Experiment I: Rearing Adults to Third Instar Larvae

Collection of breeders

A total of 30 adult *Heliocopris bucephalus* individuals were collected in November 2023. During the sampling period, the temperature and humidity of the area were 26°C and 70% respectively. The samples were collected during their breeding season. The height of the selected dung hill, which is in the forest where most of the farmers are grazing their herds, was about three feet (91 cm) (Plate 1 A). At a depth of around 30 cm and below the surface, twenty to fifty adults *Heliocopris bucephalus* were found (Plate 1 B). The beetles were collected by digging dung hill with a spade and by using hand-picking method. Collected samples were placed into plastic boxes filled with a mixture of solid cow dung and soil from their natural habitats.

Additionally, five natural brood balls were taken from burrows which about 100 to 150 meters away from their habitat cow dunghills and each of the burrows are about 100 meters apart. The places of the burrows were marked and the brood balls were carefully brought to the laboratory for further observation.

Solid and fresh cow dung from the study sites were also brought to the Applied Entomology Laboratory of Zoology Department, University of Yangon.

Preparation for rearing *Heliocopris bucephalus*

Upon arrival to the laboratory, adult *Heliocopris bucephalus* were placed into rearing plastic boxes measuring (15.75 x 11.75 x 9.5 inches) (Plate 1 C).

Before putting the collected samples, the boxes were prepared with layers. Two inches of soil layer were added at the bottom of rearing containers. Two inches of solid cow dung in the middle layer and about one inch of fresh cow dung was added at the top twice a week. The twenty collected *Heliocopris bucephalus* were maintained in the five prepared plastic boxes with a sex-ratio of 1 male and 3 females (1:3) in each box. The boxes were sealed to the top with iron filter for ventilation and to effectively prevent the escape of collected samples. The rearing plastic boxes were carefully dug twice a week and checked whether there will be oviposition or egg hatching.

After oviposition, the eggs were put back into their rearing plastic containers to get parental care and the sizes of the eggs were also recorded. After hatching of eggs, the young larvae were placed again into 2500 ml plastic containers separately. Larvae developmental periods and morphological characteristics were also observed and recorded every week.

Fresh cow dung about 180 g to 200 g were fed every three days because adult beetles absorb only the liquid nutrients of the fresh dung as the attractiveness of fresh dung lasts only 72 hours for dung beetles. Fresh cow dung was taken once a month from Hmawbi Township (17°05′53″N 96°04'13"E), Yangon Division. The boxes used for rearing adult *Heliocopris bucephalus* were maintained under their ambient temperature of (26 °C - 28 °C) and relative humidity of (50% to 80%). All these experiments were made under the fixing laboratory condition.

Experiment II: Rearing from brood balls to adult beetles

Collection of natural brood balls

In February 2024, twenty-one brood balls were dug from Pobba Thiri Township (19°57′16″N 96°10'19"E), Nay Pyi Taw Division (Fig. 1). The brood balls were collected from the four marked burrows that can be found easily as the soils are different from the soil around them (Plate 2 B). In each burrow, there were at least three brood balls and eight the most (Plate 2 C). They were brought to the laboratory to rear from the brood balls, the fourth instar stage, to

the adult stage. In the laboratory, each brood ball was broken with an approximately 5 cm hole to check the larval stage development through the opening.

Preparation of rearing boxes for experiment II

To prevent fungal infection and predators, the rearing boxes were renovated by placing a layer of soil from brood balls' natural habitat at the bottom of the rearing plastic boxes. The half of the brood balls were buried in the soil and the other half with an exit hole were exposed for ventilation (Plate 2 D). The brood balls were also sprayed with water about twice a day to maintain humidity and moisture. Temperature was maintained between 25°C to 30°C by showing sunlight once a week to prevent contamination and fungal infection.

Preparation of the samples for nutritional analysis

For nutritional values analysis, another twenty-four brood balls with pupal stages were collected from the same area were used. The brood balls were cracked from their exit holes because the other parts were as hard as a stone (Plate 3 A). The edible fully grown *Heliocopris bucephalues*'s pupal stages were undergo physical termination by sun- drying and subsequent oven-drying in the oven at a specified temperature (200°C for five times – for 10 minutes each) until it became a crispy stage (Plate 3 B, C, D and E). The crispy stage larvae were grinded by a blender and measured the weight of the powder (Plate 3 F and G). The resulting powder (50g) were analysed at the "Food Industries Development Supporting Laboratory (FIDSL)" which is located in the Union of Myanmar Federation of Chambers of Commerce and Industry (UMFCCI) Yangon. Moreover, the nutritive values of commonly consumed nutrient groups such as chicken, pork, beef, soybeans and edible insects were claimed from the previous studies by different authors for comparison (Plate 3 H).

Chemical analysis

Each nutritional value was analysed chemically as a test method recommended by the Association of Official and Analytical Chemists (AOAC, 2019). The crude protein was determined by using Kjeldahl method. Determination of moisture (loss on drying) content by Air Oven method and ash content was performed by using loss of weight in ignition using temperature control oven, Muffle Furnace method. Next, fat content was determined by Buchisoxlet Extraction method and fibre by Fibre Cap method.

Data analysis

The recorded data were calculated by Mean ± Standard Deviation (SD) in Excel.

Figure. 1. Map of the study area (Source from Google Map)

A. Selected three feet height dung hill

B. Cluster of adults *Heliocopris bucephalus*

C. Measurements and Preparation design of rearing plastic box

Plate 1. Preparation for rearing *Heliocopris bucephalus*

A. Sample collection site

B. Soil showing unusual condition of burrowing insects

C. Brood balls found in natural condition

D. Brood balls with exit holes

Plate 2. Samples collection for Experiment II and renovation of rearing boxes

A. Breaking brood balls B. Pupal stages C. Sun-drying D. Oven-drying

E. Crispy stage larvae F. Grinding G. Measuring the weight of powder (50g) H. Ready to analyse

Plate 3. Preparation for the examination of the nutritional values of *Heliocopris bucephalus's* pupal stages

Results

Experiment I: Rearing of adult beetles under captive condition

In Experiment I, adult *Heliocopris bucephalus* were maintained in experimental plastic boxes, and their egg-laying and brood ball-making processes were examined.

Brood ball-making process

After 2 weeks of maintaining the adult *Heliocopris bucephalus* in the experimental plastic boxes, some part of the soil in the rearing plastic boxes were seen to be dried. The brood balls which have various sizes and shapes were seen but some were found exclusively as eggs. There were about 15 to 20 eggs, averaging four eggs per rearing box. However, there was only one egg per brood ball, and the measurements of the brood balls were 5 ± 3.5 mm.

Morphologies of the life stages of Experiment I

Eggs were pearl white colour and they were about 1.83 ± 0.29 mm in length and 1.6 ± 0.15 mm in width. When the ovoid shape egg was approached to hatch, the egg became yellowish colour egg. This stage lasted from 10 to 15 days.

First instar larva which has a translucent and cylindrical body with cephalic capsule was found. The first larva was about 5 ± 1 mm in length and 1.33 ± 0.29 mm in width. This stage lasted from 5 to 10 days.

The second instar shed its exoskeleton and its body segments became distinct and jaws became well developed. Spiracles and legs were also distinct. The body was crescent shape and pale translucent in colour. The larva was about 10 ± 2 mm in length and 4.3 ± 1.53 mm in width. This stage lasted from 15 to 20 days.

The head capsule was orange in colour and yellowish body colour with C shaped. Body length was 25 ± 5 mm and width was 7.6 ± 2.52 mm. The most active stage and the larvae eat solid dung only. A small short hair occurred along the body and the larvae were stay buried when they feed and grow. A single orange spots line was present on each side of the body and this stage lasted from 40 to 60 days.

However, under laboratory conditions, the larvae were killed by entomopathogenic fungi and mole crickets (Plate 4). Therefore, the life cycle of Experiment I started from collected individuals and ended in third instar larval stage. The measurements of both length and width are increasing along with their life stages (Table 2). The duration of the life cycle of *Heliocopris bucephalus* lasted for two and a half months.

Plate 4. Recorded fungal infection and predators of *Heliocorpris bucephalus* inside the rearing boxes

Experiment II

In Experiment II, brood balls of the *Heliocopris bucephalus* were collected from their natural habitats and reared in the experimental plastic boxes. The changes of larval stages were examined through the opening.

Morphologies of the life stages of Experiment II

Fourth instar larva which is elongated and yellowish colour body was found in one of the natural brood balls. They feed intensively on solid dung. Their body size became large and robust compared to earlier instars and reached its maximum size around 5.16 ± 0.76 cm in length and 3.67 ± 0.76 cm in width. Their whole body were full with cow dung. If their brood balls were accidently broken, they will close the hole with the dung which are coming out from their mouthparts. The fourth instar stage lasted from 20 to 30 days.

The prepupa stage is the edible stage. It is a grub with C-shaped and cream white to light yellow colour body. The prepupa body is full with fat although there was no more cow dung throughout its body. The body was 4.5 ± 0.5 cm in length and 3.5 ± 0.5 cm in width. During this stage, the larva was not found eating and less active as they are undergoing significant physiological changes, setting the stage for the transition to the pupal stage. When transforming to pupal stage, there was a cocoon like skin which is protecting the transforming pupa and it was stripped off when the pupa is ready to emerge. This stage lasted from 10 to 12 days.

Prepupa was moulted its exoskeleton and the pupa was emerged. This is the critical period of metamorphosis where the larva transformed into an adult beetle. Feeding was not observed in this stage. Although the pupa stayed quiet, but sometimes the pupa shakes and twists suddenly. This stage was completely free with cow dung throughout its body. Body length was 4.5 ± 0.5 cm and width was 3 ± 0.5 cm. The size of the pupa is generally proportionate to the size of the adult beetle. Initially, the pupa was yellowish in colour, but it darkens slightly as it matures and prepares for adult emergence. Wing form, legs and head appeared in this stage. This pupal stage lasted from 15 to 20 days.

The initial adult stage was emerged from the pupal chamber with reddish colour exoskeleton. It actively fed on fresh cow dung which they used it to help their body to fully growth stage. The legs and ventrum are covered with coarse rust-red hairs. They mostly stay beneath the soil and active at night that they are trying to escape from the rearing boxes by using their unfolded wings. This stage lasted from 30 to 40 days.

Table 1. Morphology of fully grown adult stage

No	Name	Description
1.	Body 	Robust, oval, and convex, with a shiny black exoskeleton.
2.	Head 	Equipped with a broad, shovel-like structure. Males have a prominent, forward-facing horn on their head while the absence of a horn in female is a key feature of sexual dimorphism in this species.
3.	Antennae 	Antennae are clubbed at the end with a segmented, fan-like structure, used for detecting scents (especially dung).
4.	Pronotum 	Broad and shield-like, often covered in fine punctuations. In males, this area is particularly robust.
5.	Abdomen 	Deep red with sex segmented and present rust red hairs.
6.	Legs 	Clothed with erect red hair. Forelegs are modified with spines and teeth for digging. The tibiae of the hind legs are flattened and adapted to aid in rolling dung balls.

Duration of the life cycle of Experiment II

The life cycle of Experiment II started from the fourth instars which are brought from four marked natural burrows and ended in fully grown adult stage (Table 1). As the fourth instar body is large and robust, they are highest in length and largest in width among the measurements of the life stages of Experiment II. Adult is the second highest in length and the prepupa and pupa shared the same lengths. Pupa is the smallest while prepupa and adult have the same width (Table 3). The duration of this life cycle lasted for three months.

Complete life cycle of dung beetle, *Heliocopris bucephalus* (Fabricius, 1775)

The completed life cycle is the combination of the life cycles of Experiment I and Experiment II (Plate 5). Among the measurements of the life stages, the fourth instars took the highest in both length and width. Following by the prepupa, pupa and adult stage was the second highest in both length and width (Fig 3). In durations, the adult stage was the longest and the third instar was in the second place. Followed by fourth instar, the pupa, the second instar, the egg, the prepupa and the first instar was the shortest respectively (Fig 4). The durations of the whole life cycle lasted for six months to complete.

Table 2. Biomorphometrics and life stages of dung beetles in Experiment I

No	Stages	Shape & Colour	Length (mm) Mean ± SD n = 3	Width (mm) Mean ± SD n = 3	Duration (Day) Mean ± SD n = 3
1	Egg	ovoid shape yellowish colour	1.83 ± 0.29	1.6 ± 0.15	12 ± 2
2	1st instar larva	cylindrical translucent	5.0 ± 1.0	1.33 ± 0.29	6 ± 1
3	2nd instar larva	Crescent Pale translucent	10.0 ± 2.0	4.3 ± 1.53	15 ± 3
4	3rd instar larva	C shaped orange in color (Head) yellowish color (Body)	25.0 ± 5.0	7.6 ± 2.52	35.33 ± 7.51

Table 3. Biomorphometrics and life stages of dung beetles in Experiment II

No	Stages	Shape & Colour	Length (mm) Mean ± SD n = 3	Width (mm) Mean ± SD n = 3	Duration (Day) Mean ± SD n = 3
1	4th instar stage	Elongated yellowish colour	5.16 ± 0.76	3.67 ± 0.76	20.67 ± 5.13
2	Prepupa edible stage	C-shaped grub Cream white to light yellow colour	4.5 ± 0.5	3.5 ± 0.5	6.6 ± 2.0
3	Pupa edible stage	Oval shaped Yellowish in color	4.5 ± 0.5	3 ± 0.5	17 ± 2.52
4	Initial adult stage	Robust, oval & convex Black and dark brown with deep red elytra	5 ± 0.5	3.5 ± 0.5	37.67 ± 2.52

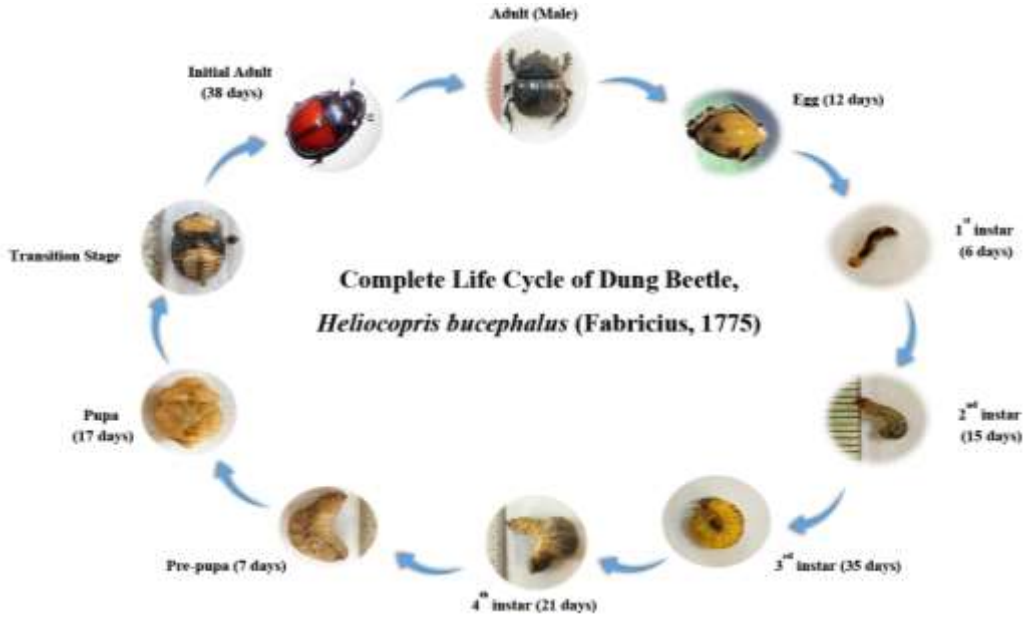

Plate 5. Complete Life Cycle of *Heliocopris bucephalus* (Fabricius, 1775)

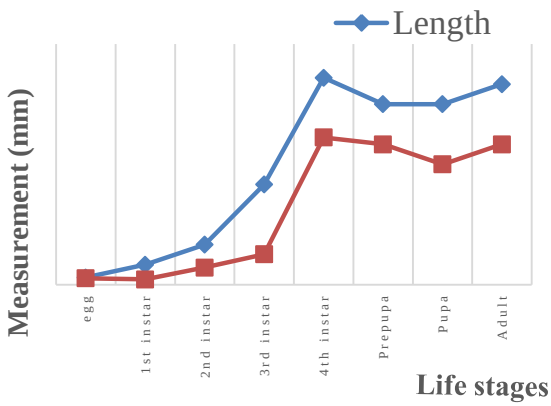

Figure. 3. Measurements of the life stages of *Heliocopris bucephalus*

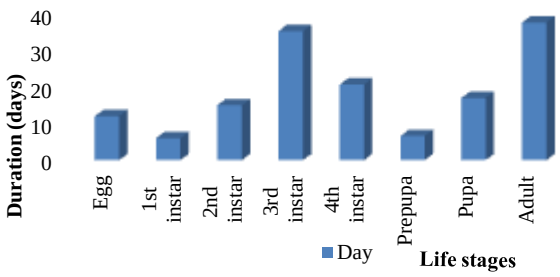

Figure. 4. Duration of the life sages of *Heliocoprris bucephalus*

Nutritional composition of *Heliocopris bucephalus*

In the analysed nutritional contents of the pupal stages of *H. bucephalus*, five kinds of nutritional values were recorded. Among them, the content of the protein has the highest with 45 g and fat is the second highest with 29 g. Carbohydrate took the last place with 14 g. The nutritional content of the analysed energy on 1 package per 100g is 497 kcal. As for fibre, the content was analysed as 3.86%. And this study also investigated the other extracts such as moisture and ash content of *Heliocopris bucephalus* with 3.44% and 4.22% respectively (Fig. 5).

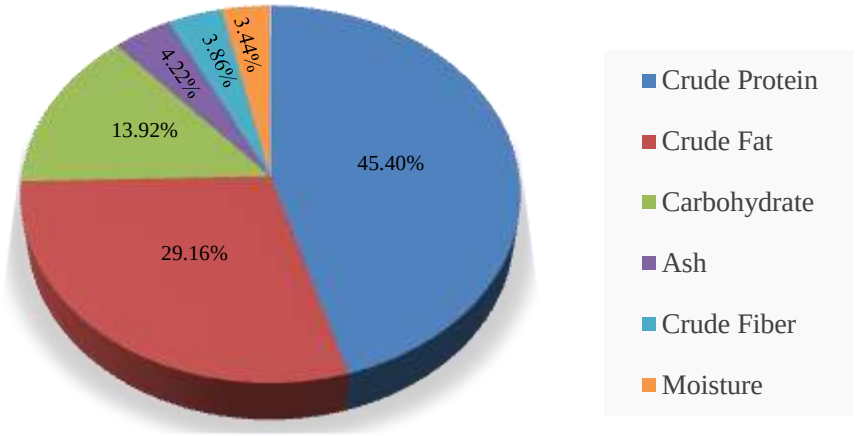

Figure. 5. Nutritional composition of *Heliocopris bucephalus* (Fabricius, 1775)

Discussion

Successful farming of edible insects, such as crickets and silkworm larvae, has been reported in previous studies (Zhou *et al*., 2007 and Fujita *et al*., 2013). However, the farming of *Heliocopris bucephalus* has not yet been fully developed. This study provided valuable insights into the reproductive behavior and growth stages of *H. bucephalus* under laboratory conditions, supporting its potential for future cultivation.

In Experiment I, *H. bucephalus* was unable to construct brood balls successfully. According to Halffter and Matthews (1966), larger brood balls are essential for larval survival, providing necessary nutrition and enhancing survival rates. In this study, *H. bucephalus* adults formed brood balls of variable sizes, likely due to the limited availability of cow dung in the rearing containers, which may have affected larval nutrition and subsequent survival rates.

High mortality among larvae was observed, likely due to entomopathogenic fungi and predation by mole crickets. Rath *et al*. (1992) and Viljoen (1997) found that *Beauveria bassiana* and *Metarhizium anisopliae* often exploit moist conditions, favoring fungal growth and spread. Mole crickets, which damaged brood balls, may also have contributed to reduced egg and larval survival. These observations indicate the necessity of regular monitoring and strict environmental management in captive conditions to reduce larval losses. Additionally, disinfection of cow dung before introducing it into the rearing boxes could help mitigate brood ball contamination.

The pupal stage of *H. bucephalus* in this study lasted between 17 and 25 days, aligning with observations by Halffter and Edmonds (1982), who noted similar durations under varying environmental conditions. Differences in temperature and humidity likely contributed to the variations between this study and others, underscoring the importance of environmental control in developmental timing. As environmental factors, particularly humidity and temperature, significantly influence the life cycle of insects (Bale *et al*., 2002, Hance *et al*., 2007 and Khaliq *et*

al., 2014). Further research should be carried out to investigate the optimal temperature and humidity conditions for *H. bucephalus* development.

The nutritional composition analysis demonstrated *H. bucephalus* as a rich protein source, providing 45 grams of protein per 100 grams, comparable to beef (FAO, 2003). This beetle's protein content is almost twice that of soybeans, highlighting its potential as a sustainable protein source (Food Data Central, 2019). These findings align with et.al(2013), who advocated for insect proteins as viable alternatives to traditional livestock sources.

In conclusion, *H. bucephalus* shows significant promise as a sustainable protein source, especially in regions where conventional protein sources are limited. However, additional research is essential to optimize captive breeding, control fungal infections, and assess the environmental parameters that influence growth and survival rates.

Conclusion

The present study successfully examined the life cycle and nutritional composition of *Heliocopris bucephalus* under captive conditions. Rearing *Heliocopris bucephalus* involves several steps to ensure their successful growth and reproduction. It was found that the dung beetle has a life cycle of approximately six months, with distinct developmental stages from egg to adult stage. Despite challenges faced during Experiment I, including fungal infections and predation, the modifications made in Experiment II allowed for successful rearing. Maintaining stable temperature and humidity levels throughout the larval development are the most important steps of the life cycle of the studied species. Although their optimal temperature is around 26°C, they thrived better in temperature above 30°C.

The nutritional analysis revealed that the pupal stage, which is edible, offers a significant source of protein, fat, and carbohydrates. With 45.40% protein content, the dung beetle surpasses many conventional food sources, highlighting its potential as an alternative protein source. These findings not only enhance our understanding of *H. bucephalus* but also underscore its importance for food security, particularly in regions where edible insects are a cultural and economic asset. Further research is encouraged to optimize captive breeding methods and explore the commercial viability of this species as a sustainable food source.

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EFFICACY OF DIFFERENT TRAP TYPES TO CONTROL BANANA ROOT WEEVIL *COSMOPOLITES SORDIDUS* (GERMER, 1823)*

Ya Min Thu Yein[1], Khin Mi Mi[2], Tha Zin Hlaing[3]

Abstract

The banana weevil (*Cosmopolites sordidus*) is an important pest for banana growers. The objective of this study was to evaluate the most effectiveness of different trapping methods to control C. sordidus in Aithonesint village, Taungoo Township, Bago Region. A field trial comparing four treatments (T1: Pseudostem trap, T2: Pitfall trap, T3: Pheromone trap, and T4: Control) was conducted using a Randomized Complete Block Design (RCBD) over four months (October 2023 to April 2024). A total of 576 traps (144 traps per treatment) were distributed across the study site (one acre farm). The data of monthly averages of weevils captured by trap were recorded. The averages of the treatments were compared by the Tukey's Studentized rang (HSD) test at 5% significant level. A total of 1,412 adult weevils were captured. The results showed that the Pheromone trap (T3) was significantly superior to all other traps, attracting the highest number of adults (n=611, or 43.27% of the total catch). Conversely, the Pseudostem trap (T1) was the most attractive to larvae, capturing n=415 (or 88.87% of the total larvae). No significant correlation was found between the captured adult population and weather parameters (average temperature and relative humidity). In conclusion, the study recommends the use of Pheromone traps for monitoring and mass trapping of C. sordidus adults and Pseudostem traps for monitoring larval populations in the Taungoo area.

Keyword: *Cosmopolite sordidus*,adult, larvae, pheromone trap, pseudostem.

Introduction

Banana is one of the main staple starchy foods in developing countries, with its domestication originating in Southeast Asia (Ortiz,1997). In banana cultivation, arthropod pest play as a major difficulty for banana growers. There are 490 species of significant arthropod pests worldwide. The most important pests were *Cosomoplites sordidus* (root borer weevil), *Odoiporous longicollis* (stem borer weevil), Fruit capterillar and aphids (Ostmark, 1974).

The banana plants are highly susceptible to banana weevil *C. sordidus* which is considered as the most economically important pest because of its damage and host plant specific (Gold *et al*., 2001). The adult weevils were nocturnal and hiding during the day under the leaf sheaths of the plants, and inside the pseudostems and rhizomes of harvested plants that makes difficult to observe for farmers (Vinatier and Vinatier, 2013).

The use of agrochemicals to control banana weevils was expensive for resource-poor farmers and these chemicals have been resistant to this weevil that has been recently reported in some countries (Collins *et al*. 1991).The population dynamic and phytopathogens of banana plants are highly relationship because this weevil was the causal agents of the Fusarium wilt Race 4 and bacterial wilt (Were *et al*. 2015). It is essential to know the population dynamic of banana weevil to carry out the suitable control of that pest (Tinzaara *et al* , 2007).

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Integrated pest management approach (IPM) has been recently developed for control of weevil. In this approach, cultural control practices including crop sanitation and trapping method appears to be developed for weevil control. Pseudostem traps for weevil trapping are the most commonly advocated method of IPM option (Gold 1998). However, this method has not been easily used because of labour-intensive.

Pheromone trap has been used a plausible alternative due to easy to use and effective method. The pheromone trapping method has been reported to reduce and increase yields in banana production (Alpizer and Fallas, 1997). Intense split pseudostem trapping has been reported significantly reduce *C. sordidus* damaged after one year (Gold *et al.*, 2002), Semiochemical- enhanced mass trapping has also been shown as an effective control (Alpizar *et al.*, 1998). Pitfall traps were been used to monitoring the weevil population in Uganda (Tinzaara *et al.*, 1999).

The control potential of different trapping methods may depend on climate, cultivation practices, proportion of the population attracted and monetary cost of trapping. The possible occurrence of weevil biotypes may influence above factors and these factors may vary according to local areas. No research comparing pheromones to other traps has been reported and the only research was found in tropical countries (Tinzaara *et al.*, 1999).Therefore, the present study was to evaluate the effective trapping methods for management of banana root weevil *Cosmopolites sordidus.*

Materials and Methods

Study Area and Period

The study was conducted at Ai Thone Sint Village, Taungoo Township, Bago Region (located between 19∘01′05.5″N and 96∘25′11.9″E) (Fig. 1 and Plate 1). The study site was a 2 acre (8093.71 m2) banana farm. The study period lasted seven months from October 2023 to April 2024.

Experimental Design

As experimental design, the randomized complete block design was chosen. In this method, it contains equal size blocks (8093.71$m^{2)}$ and in which all treatments were be carried out. The research was worked out in 8093.71m^2 with four treatments and six replications in 4 months duration of trapping. The four treatments were T_1 – Pseduostem trap, T_2 – Pitfall trap, T_3 – Pheromone trap and T_4 – Control.

The 8093.71$m^2$was divided into six equal blocks. Each block was again divided into six equal parts, each part was represented as one subplots. Six traps of each treatment were distributed in each block, overall, totally 36 number traps of each trap type were be distributed in one month. After four months, finally 144 traps for each trap type were carried out. Totally 576 traps for all treatments were done during the experiment.

Data Collection

The collections of the weevil were conducted in 2, 4, 6, 8 and 10 days after the distribution of the traps. The number of collected specimen was also recorded.

Data analysis

The data of monthly collected insects were summarized and stated in graphs, tables and other descriptive methods were calculated in Excel. The averages of treatments were compared

with the Tukey' Studentized range (HSD) test at 5% level. Statistical analysis was performed using the statistical software JMP 16 Pro.

Meteorological variables (average temperature and relative humidity) were obtained from Meteorological Department, Taungoo Township and correlated with the recorded weevil population for the four months study period using Microsoft Excel 2010.

Fig. 1 Map of study area (Google Earth,2018)

Plate 1. Study site

A. Pseudostem trap

B. Pitfall trap

C. Pheromone trap

D. Control

Plate 2. Different trap types used for sample collection

Results

Four treatments (T1 - Pseudostem trap, T2 - pitfall trap, T3 - Pheromone trap,T4-control) were conducted to collect the banana weevil *C. sordidus* at Ai Thone Sint Village, Bago region (Fig 1, Plate 1 and Plate 2).

During the study period, a total of 1412 adult populations were recorded from 576 traps. The mean numbers of adult in different traps were presented in Fig. 2.The highest population was

found in November (Table 1). There were significantly differences among treatment plots and the control. The pheromone traps attracted significantly more (n=611) weevils than the other treated plots and control.

There was no significant monthly difference in the number of adults of *C. sordidus* (Table 1).There was no significant relationship between adult population numbers with relative humidity and average temperature (Fig. 4 and Fig. 5).

The collected numbers of larvae of *C. sordidus* during sampling period was presented in Table 2. In the result observed that no significant monthly difference of total population. However, a significant effect between the types of traps was noticed. Among them, the pseudostem trap attracted more insect (n=415) than those of other trap types and control. The number of recorded larvae from pheromone trap was almost similar to the pitfall trap (Fig. 6).

There was observed slightly relationship between larvae populations and weather conditions of study area (Fig. 8 and Fig. 9). Their population fluctuation of both adult and larvae *C. sordidus* at different sampling dates was also shown in Fig. 3 and Fig. 7.

Table 1 Total number of adults of *C. sordidus* collected per type of trap and per month

Type of trap	No. of trap	Total no.of *C.sordidus* (adult)				Total	Percent
		Nov;	Dec;	Jan;	Feb;		
T1 (Pseudostem trap)	144	92	79	69	66	306	21.67%
T2 (Pitfall trap)	144	89	81	78	63	311	22.03%
T3 (Pheromone trap)	144	158	148	150	155	611	43.27%
T4 (Control)	144	60	49	41	34	184	13.03%
Total	576	399	357	338	318	1412	100%

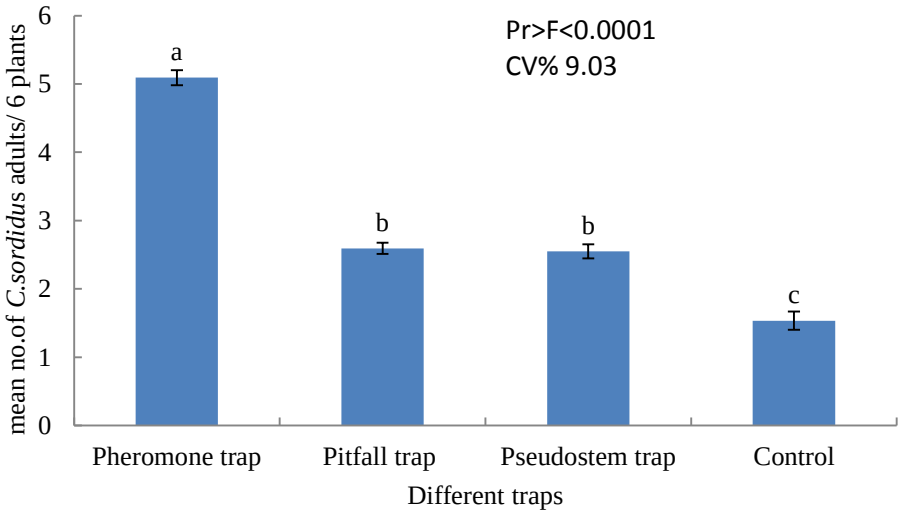

Figure. 2 Average number of adults *Cosmopolites sordidus* per 6 plants in different traps (averages followed by the same letters do not statistically differ from each other by Tukey' Studentized range test at 5% significant level)

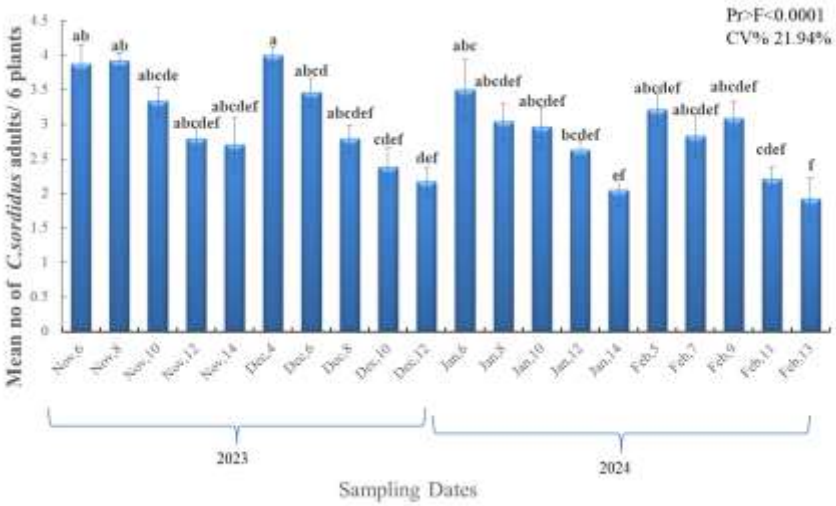

Figure. 3 Population fluctuation of *C. sordidus* adults at different sampling dates in November, 2023 to December 2024 in Taungoo Township (averages followed by the sameletters do not statistically differ from each other by Tukey' Studentized range test at 5% significant level)

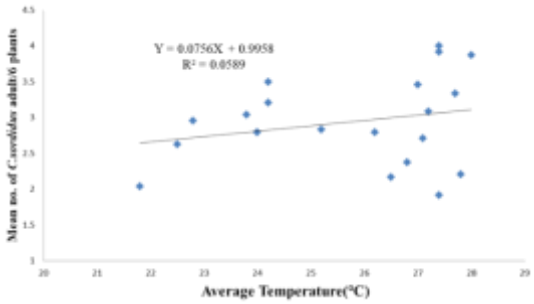

Figure. 4 Relationship between the mean numbers of *C. sordidus* adults/6 plants and average temperature at different sampling dates during study period

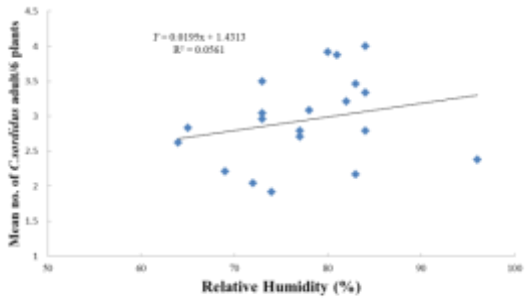

Figure. 5 Relationship between the mean numbers of *C. sordidus* adults/6 plants and relative humidity at different sampling dates during study period

Table 2 Total number of larvae of *C. sordidus* collected per type of trap and per month

Type of trap	No. of trap	Total no.of *C.sordidus* (larvae)				Total	Percent
		Nov;	Dec;	Jan;	Feb;		
T1 (Pseudostem trap)	144	128	99	96	92	415	88.87%
T2 (Pitfall trap)	144	3	1	2	1	7	1.49%
T3 (Pheromone trap)	144	2	1	1	2	6	1.28%
T4 (Control)	144	9	13	7	10	39	8.36%
Total	576	142	114	106	105	467	100%

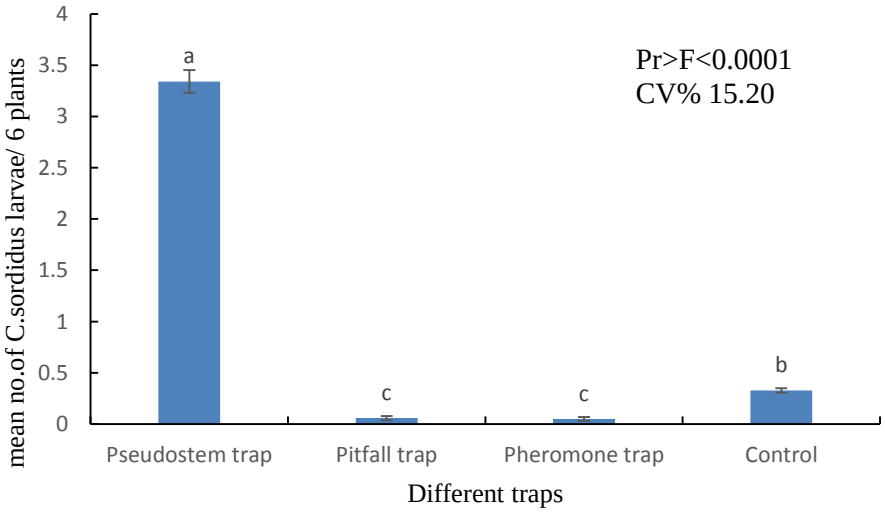

Figure. 6 Average number of larvae of *Cosmopolites sordidus* per 6 plants in different traps (averages followed by the same letters do not statistically differ from each other by Tukey' Studentized range test at 5% significant level)

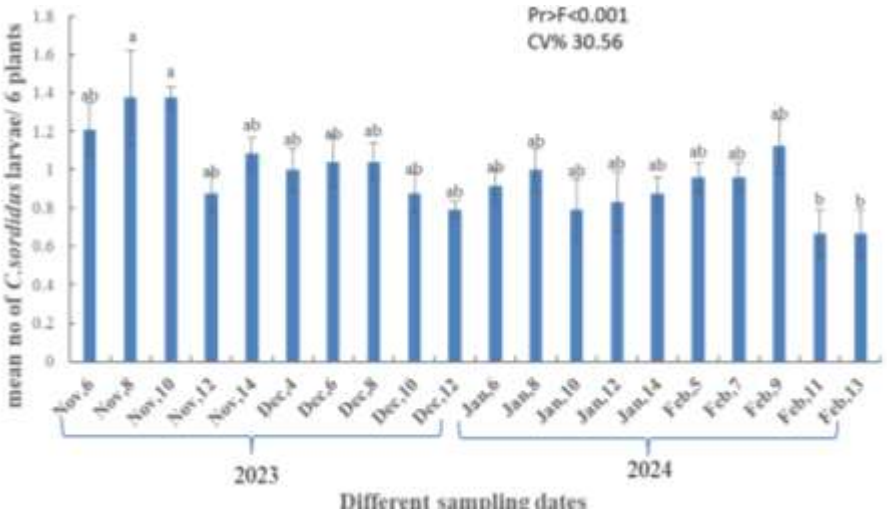

Figure. 7 Population fluctuation of *C. sordidus* larvae at different sampling dates in November, 2023 to December 2024 in Taungoo Township(averages followed by the same letters do not statistically differ from each other by Tukey' Studentized range test at 5% significant level)

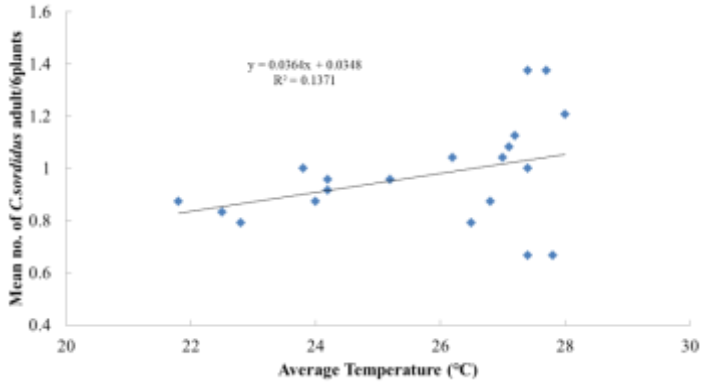

Figure. 8 Relationship between the mean numbers of *C. sordidus* larvae/6 plants and average temperature at different sampling dates during study period

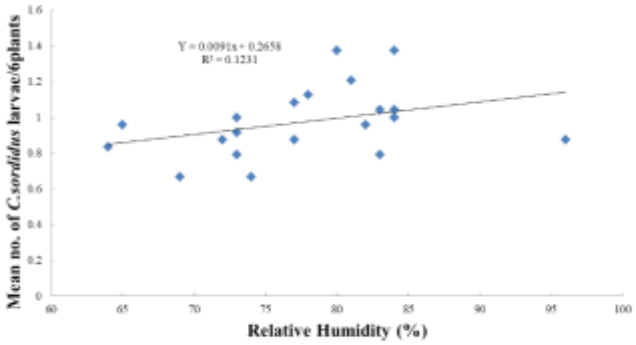

Figure. 9 Relationship between the mean numbers of *C. sordidus* larvae/6 plants and relative humidity at different sampling dates during study period

Discussion

In the present study, four treatments (T1 - Pseudostem trap, T2 - Pitfall trap, T3 - Pheromone trap, T4 - Control) were compared to examine the most effective trapping methods and to detect the incidence of *Cosmopolites sordidus*, banana root weevil population. During the present study, there was no significant difference in the numbers of both weevil adult and larva population considering between the month of evaluation and trap types. However, there was significantly difference between trap types was found. The pheromone trap type attracted more weevil adults (611) than those of other trap types, contributing about 43.27% of the total of insect captured. The average number of weevil captured in pheromone trap was statistically higher than the value of others recorded insect numbers from other traps. Tinzaara (2003) stated that pheromone trap was very effective to attract the *C. sordidus* adult. According to Jayaraman et al (1997), pheromones have been shown to attract significantly more weevil adults than plant material traps.

Especially, the larvae of *C. sordidus* were most significantly caught in pseudostem traps. The highest weevil larvae (n=415) were collected with 88.87% of total population than those of other trap types. Masonza (2003) reported that compared to pheromone traps, pseudostem traps were more attractive to *C. sordidus* larvae. Tinzaara (2003) mentioned that pseudostem traps were very effective to attract *C. sordidus* larvae.

Nevertheless, there was no significant influence of the month on the number of adults and larvae of *C. sordidus* captured. There was slightly different of catch in different sampling dates. The highest population number of adult was found in 2023, December 4 and the highest number of larvae was 2023, November 8 and 10. The lowest number of adults was observed in 2024 February 13 and the number of larvae were lowest in 2024, February 11 and 13. Juliana *et a*l (2017) reported that there was not relationship between the numbers of insect caught and the month.

There was no significant correlation between the number of adults captured in the traps with average ambient temperature and relative humidity at sampling dates during the study period. But, there was slightly relationship between the number of larvae collected in the traps with average temperature and relative humidity at different sampling dates was noticed. Tinzaara *et al* (2005) reported that the relationship between climate factors and the number of *C. sordidus* caught from traps was not related. Duyuk (2012) also stated that relative humidity and temperature was not significantly influence on the number of caught insects from the traps.

Analyzing the average of adults and larvae monthly captured, it was found that the collected insect numbers was closer to the inferior threshold for insect management. Moreover, it was also noticed that the traps of pheromone underestimated the number of adults captured and pseudostems traps was highest attractive to larvae. Fancelli *et al* (2013) mentioned that considering the threshold is based on the average number of insects collected from the traps, the suitable control measures may implement and it might contribute to reduce of weevil incidence.

The high attractiveness of pheromone traps and their durability attributed effectively to catch the weevils. Although, the reduction in pheromone efficacy for larvae was unclear. Pseudostems showed a high lava population. Pitfall traps and pseudostem traps were not significantly differ from each other in the number of adult caught. The collected larvae numbers was not also significantly differ between pitfall traps and pheromone traps.

Tinzaara *et al* (1999) reported that compare to pseudostem traps, pheromone traps generally attracted the highest total numbers of adult during all seasons. But in Costa Rica, pseudostem traps increased the attractiveness five to ten time and pitfall traps was two and a half time more effective than pheromone traps. The lower relative efficacy of pheromone may be weevil biotype, plant variety and/or climate factors (Alpizar and Tallas 1997).

In conclusion, the attractiveness of the pheromone traps for adults of *C. sordidus* was more effective to other traps. Consequently, the pseudostem traps were more attractive to larvae of *C. sordidus*. Thus, pheromone traps show potentials as an economical monitoring and mass trapping and pseudostem traps can be used preferably for the larval monitoring in plantains not yet harvested.

Conclusion

In the present study, four treatments (T1 - pseudostem trap, T2 - pitfall trap, T3 - pheromone trap, T4 - control) were conducted to examine the effectiveness for the monitoring of banana weevil *C. sordidus*. The experiment showed the pheromone traps were most effectiveness to monitor the adult weevils and pseudostem traps were most attracted to weevil larvae. The average numbers of weevil caught and the ambient weather parameters such as average temperature and relative humidity were not significantly related during the study period.

The findings provide localized data supporting the use of both trap types in an IPM strategy for *C. sordidus* control in Taungoo Township.

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MOLECULAR DETECTION OF MULTIPLE DRUG RESISTANT *KLEBSIELLA* SPECIES FROM URBAN RUNOFF*

Aye Chan Thu[1], Hla Htet Soe[2], Aye Aye Khine[3], Kay Lwin Tun [4]

Abstract

Wastewaters from community drainages may be reservoirs for various bacterial strains harboring drug-resistant genes associated among healthcare and community. Among pathogenic bacteria, *Klebsiella* is an important human pathogen causing opportunistic nosocomial and community acquired infections. In the present study, *Klebsiella pneumoniae* was recorded with the confirmation of phenotypic and molecular analyses. The 16s rRNA gene of the selected isolate (MHBD 10^{-2}(3)), collected from community drainage, Latha Township, was successfully amplified using PCR resulting in a sequence length of 996bp and comprehensive identification results using the phylogenetic analysis involved a comparison of the isolate's sequence with others obtained from GeneBank database. The studied isolate revealed a close clustering with *Klebsiella pneumoniae* species, supported by 100% nucleotide identities. This study may highlight a rapid and cost-effective diagnostic tool for detection of antibiotic-resistant pathogenic bacteria in the natural environment.

Keywords: phenotypic, molecular identification, phylogenetic, 16s rRNA, *Klebsiella pneumoniae*, community drainage

Introduction

Antibiotics are commonly used and easily available in pharmacies in Myanmar. The authority from pharmacists or doctors is not required to purchase the antibiotics in local markets. Inappropriate usage of antibiotics creates a selection pressure for bacteria to acquire antibiotic resistance. The increasing trends of antimicrobial agent (AMA) consumption are more likely to drive to the emergence and spread of antimicrobial resistant (AMR) microbes which cause the large burden of infectious diseases (Godinho, *et. al*., 2024 and Pwint, *et. al*., 2021). In 2019, the deaths of estimated 4.95 million people had associated with bacterial AMR. A global report estimated that without further action, by 2050, 10 million people per year will be dead and 100 trillion USD worth of global production will be lost due to AMR (Miyano, *et. al*., 2022). Thus, AMR has become a major threat to global health, food security and development today.

A research conducting national situational analyses from 11 countries of the South-East Asia, including Myanmar, reported high antibiotic use and poor implementation of policies to encourage appropriate use (Holloway., 2015). In Myanmar in 2019, there were 11,200 deaths attributable to AMR and 40,200 deaths associated with AMR. Myanmar has the 64th highest age-standardized mortality rate per 100,000 population associated with AMR across 204 countries (www.health-data.org). Hence, an ongoing challenge in Myanmar has been the high AMR-related mortality with a high burden of infectious diseases that effectively reach to about 55

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million population, most of whom battle tuberculosis, acute respiratory infections, diarrhea and malaria (national action plan, version 01, 2017 and www.health-data.org).

The widespread prevalence of antimicrobial resistant bacteria not only in humans and animals but also in the natural environment has resulted due to many common factors favoring AMR bacteria growth. In cases of developing countries like Myanmar, the drastically increasing rate of bacterial resistance to antibiotics has been linked with poor antibiotic quality, enormous amount of antibiotics use in healthcare, agriculture, aquaculture and livestock, easy purchase without prescription, misuse due to the negligence of careful consultation with professionals, lack of awareness and inadequate AMR surveillance (Figure 1). Besides, national policy on the scale or use of antimicrobials in humans and animals is not well established in Myanmar (Pwint, *et. al*., 2021, Mehanni, *et. al*., 2023 and www.health-data.org).

In addition, untreated or improperly treated discharged wastewater which contain hazardous pollutants such as radioactive, chemical and pharmaceutical wastes into the urban drainages and also free DNA encouraging pathogenic microorganisms to acquire multi-drug resistant genes by horizontal gene transfer is the main reservoir contributing to the inescapable consequence of environmental pollution by antimicrobials. Majority of runoff from hospitals, wastewater treatment plants, aquaculture run off and livestock breeding which are considered as hot spots for the dissemination antibiotic resistance, directly dispose to nearby water body via community drains (Thanda, *et. al*., 2021 and Mehanni, *et. al*., 2023).

Considerably, the current situation highlights that the suboptimal wastewater treatment infrastructures in the community, the hospital setting as well as aquaculture and livestock sectors in Myanmar may pose a significant biosafety risk, an emergence of multi-drug resistant bacteria and contamination of both surface and ground water. Moreover, according to the latest national AMR surveillance data and one study of Myanmar in 2017, the reported high levels AMR bacteria were *Stapholococcus aureus*, ESBL Enterobacteriaceae, *Pseudomonas* species and *Acinetobacter* species and *Enterococcus* species (Mehanni, *et. al*., 2023 and www.health-data.org).

On the other hand, there are very few and no evidence-based studies how much AMR bacteria cause contamination in the natural environment. Nowadays, factors influencing the abundance, distribution and potential transmission routes of antibiotic resistant bacteria remains poorly understood and are unclear as there is a limited data concerning resistant microbial profiles associated with wastewaters.

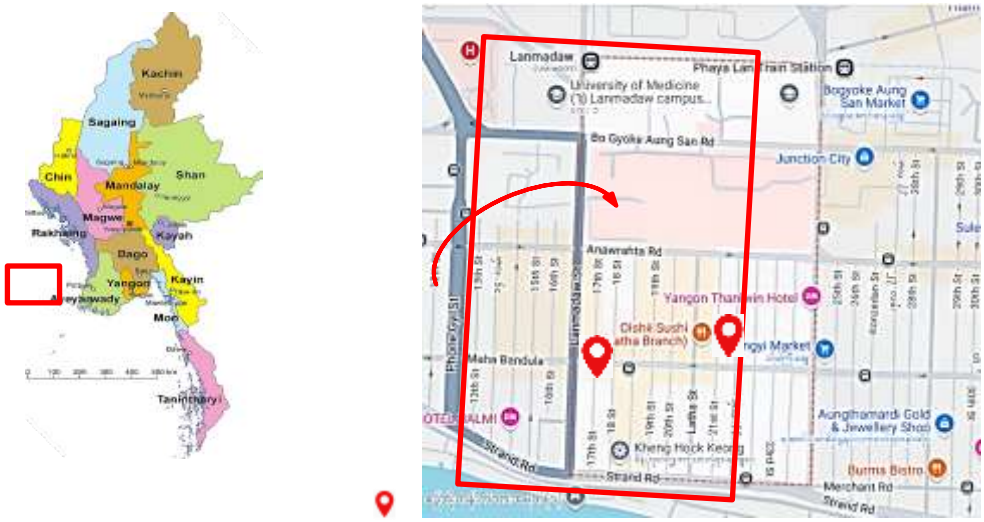
Therefore, this study aims to advocate insights on multi-drug resistant bacteria present in community drainage and improve awareness amongst general public and professionals by generating the evidence-based data to fulfill the research gaps for AMR organisms in Myanmar with One-Health perspective.

Materials and Methods

Sampling site and the study period

Three water samples were collected from three community drainages alongside of Maha Bandula Road, Shwedagon Pagoda Road and Anawrahta Road, Latha Township shown in the map (Figure - 1).

A volume of 500mL of water samples from community drainages were taken from each site with the use of sterile new plastic bottles (sterilized by shaking with 70% ethanol for three times then rinsing with sterile distilled water), preserved in cool box containing ice packs and transported to Microbiology Laboratory at Department of Zoology, University of Yangon. The research was conducted from May to September 2023.



Myanmar Latha Township, Yangon

Figure 1 Map showing location of Latha Township of Yangon, Myanmar (www.googlemap.com)

Sample preparation

One mL of each water sample was diluted into the 9mL of distilled water (1:10 concentration) then homogenized gently by pipetting. A 10 fold serial dilutions were made to the ninth level.

Isolation of bacterial isolates

0.1 mL of each prepared dilution mixture was aseptically inoculated on Nutrient agar medium by the spread plate method (Dubey and Mashwari, 2002). Then, all the plates were incubated at 37^{o}C for 24 hours. After incubation, separate colonies from each plate were selected and sub-cultured again on the same medium by streak plate method to obtain pure cultures. These plates were incubated at 37˚C for 48 hours. The pure collected bacterial isolates (8 isolates from MHBD, 8 isolates from ANYT and 4 isolates from SDG samples) were kept in nutrient agar slants (containing 20% glycerol) in sterile stock bottles with caps and stored at -20˚C for long term use (Bisen and Verma, 1998 and Dubey and Mashwari, 2002).

Determination of drug-resistant patterns by phenotypic confirmatory testing (Antibiotic Susceptibility Assay)

The Kirby-Bauer disk diffusion method according to the CLSI standards was performed to determine the antimicrobial susceptibility profiles of all 20 pure isolates. The test was carried out using Mueller-Hinton medium against five antibiotics; namely, Ampicillin AMP (10 µg), Erythromycin EM (15 µg), Tetracycline TE (30µg), Linezolid LZ (30µg) and Oxacillin (1µg). A total of five commercially available antibiotics was purposely chosen with the aim of targeting to

inhibit cell wall biosynthesis, protein synthesis and DNA replication (Pwint, *et. al*., 2021 and Godinho, *et. al*., 2024).

Bacterial inoculums were prepared by suspending the freshly grown bacteria in 4-5ml sterile nutrient broth. The suspensions were spread uniformly on Mueller-Hinton agar with a sterile swab. Antibiotic discs were then placed on the surface of the inoculated media, center to center at least 25mm apart. After that, the plates were incubated at 37^{o}C for 18-24 hours. After incubation, inhibition zone diameters were measured and interpreted as susceptible (S), intermediary (I) or resistant (R) with the standards of CLSI chart (Thanda, *et. al*., 2021, Mahmud, *et. al*., 2023, Gashaw, *et. al*., 2024, and Godinho, *et. al*., 2024).

Characterization of the selected bacteria

Among 20 bacterial isolates obtained, one strain ((MHBD 10^{-2}(3)) which demonstrated the highest level of antimicrobial resistance was selected as a suitable candidate for further morphological characterization and molecular analysis.

Morphological examination was carried out by determining on the characteristics features such as size, shape, color, edges of the colonies, texture and elevation and observing Gram-staining nature and shapes of the bacterial cells (Bisen and Verma, 1998).

The biochemical reactions of the isolate were examined by utilizing the tests such as (i) Citrate Utilization, (ii) Triple Sugar Iron, (iii) Methyl-Red, (iv) Voges-Proskauer, (v) Indole formation, (vi) H_2S production, (vii) Motility, (viii) Oxidase, (ix) Catalase and (x) Urease (Buchanan, *et al*., 1974).

Molecular Analysis

Molecular Identification

The selected bacterial isolate ((MHBD 10^{-2}(3)), shown resistant to five tested antibiotics, was identified at the species level based on the analysis of their 16S ribosomal RNA gene sequences.

DNA Extraction and PCR amplification of 16S rRNA gene

The total genomic DNA from the selected bacteria was extracted by using Total Nucleic Acid Extraction Kit (MAX DETECT BEYOND BOUNDARIES) according to the manufacturer's instructions. The quantity, purity and concentration of extracted DNA from the isolate was measured by NanoDrop spectrophotometer (Thermo Scientific, USA). Amplification of PCR assay was performed with a set of the primers; the forward primer U1 (5'- CCA GCA GCC GCG GTA ATA CG - 3') and the reverse primer U2 (5'- ATC GGY TAC CTT GTT ACG ACT - 3') by using GoTaq DNA polymerase (Promega, Madison, WI, USA) (Table 1). 20µL of PCR mixture was prepared containing 2µL of template DNA, 1µL of forward and revere primer, 7µL of distilled water and 10µL of Go PCR Master Mix (Promega, Madison, WI, USA). The reaction mixture was run through PCR conditions: 94^{o}C for 10 min, 35 cycles of 94 ^{o}C for 1min, 55 ^{o}C for 1min and 72 ^{o}C for 2min, with a final extension cycle of 72 ^{o}C for 10min (Lu. *et al*., 2000).

The PCR product (996 bp) was visualized and separated in agarose gel electrophoresis (1% agarose gel in 1X Tris-Acetate EDTA (TAE) buffer stained with Ethidium bromide and photographed by UV transilluminator,

Table 1 Primers utilized in PCR amplification

Primers	Sequence (5′-3′)	Target gene	PCR product (bp)	Reference
U1 Forward	CCA GCA GCC GCG GTA ATA CG	16s rRNA	996	Lu. *et al*., 2000
U2 Reverse	ATC GGY TAC CTT GTT ACG ACT	16s rRNA		

DNA sequencing of isolated bacteria

PCR amplication

50µL of PCR amplification was performed to proceed DNA sequence analysis. each 50µL PCR mixture contained 5µL of template DNA, 2.5µL of forward and revere primer, 17.5µL of distilled water and 25µL of Go PCR Master Mix (Promega, Madison, WI, USA). The conditions used for PCR amplification will be as follows: 94^{o}C for 10 min, 35 cycles of 94 ^{o}C for 1min, 55 ^{o}C for 1min and 72 ^{o}C for 2min, with a final extension cycle of 72 ^{o}C for 10min (Lu. *et al*., 2000). Subsequently, amplified PCR product (50µL) was separated 1% agarose gel containing SYBR Safe DNA gel stain (WizPure, Seongnam, South Korea) and visualized under LED transilluminator to observe approximate base pair from the resulted band.

Gel purification for sequencing

For DNA sequencing, DNA was then extracted and purified from distinct bands using the FastGene® Gel/PCR Extraction Kit (Nippon Genetics Europe GmbH, Bunkyo, Tokyo). To isolate DNA, first excise the fragment from an agarose gel using a clean scalpel. Transfer up to 300 mg of the gel slice into a microcentrifuge tube, add 500 µl of binding buffer GP1, vortex and incubate at 55°C for 10-15 minutes, inverting the tube every 2-3 minutes. Next, apply up to 800 µl of the sample mixture from previous step into the GP Column and centrifuge at 13,000 rpm for 30 seconds. Add 600 µl Wash Buffer GP2, and centrifuge at 13,000 rpm for 30 seconds. Discard the flow-through, return the column to the tube, and centrifuge again for 2 minutes to dry the column. Finally, add 20-50 µl elution buffer GP3 to the column, let it stand for 2 minutes and centrifuge at 13,000 rpm for 2 minutes to elute the purified DNA.

DNA sequencing

The DNA sequencing of the isolated amplicons was transmitted to Macrogen, Inc., Korea. Following the manufacturer's instructions, sequencing operations were carried out using the ABI Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the DNA Engine Tetrad 2 Peltier Thermal Cycler (BIO-RAD). Every template underwent single pass sequencing with the U1 (Forward) and U2 (Reverse) primers. One was sequenced to compared 16s rRNA genes.

Phylogenetic analysis

The obtained data of 16S rRNA gene sequences was edited, aligned and compared with Bioedit and NCBI Genbank database using NCBI's Standard Nucleotide BLAST search. MEGA 11 Program was utilized for constructing phylogenetic trees to determine the taxonomic position of the selected bacteria by applying a neighbor-joining method of 1,000 replicates used for bootstrapping (Kobayashi, *et. al.*, 2016, Kobayashi, *et. al.*, 2017, Mahmud, *et. al*., 2023, Mehanni, *et. al.*, 2023 and Godinho, *et. al*., 2024). The analysis confirmed the species belonging to *Klebsiella pneumoniae* and *Salmonella bongori* and *Salmonella enterica* subsup.*arizonae* were

included as outer groups to form a separate clade that confirms its divergence from *Klebsiella pneumoniae*.

Results

Antibiotic resistance pattern and identification of the bacterial isolate using phenotypic characters

Among 20 tested isolates, MHBD 10^{-2}(3) exhibited resistance to all antibiotics, showing no clear zones, whereas the other isolates were susceptible to at least one antibiotic (Table 2 and Figure 2).

The selected bacterial isolate (MHBD 10^{-2}(3)) showing the highest multi-drug resistance was tentatively identified by colony morphology, cell shape and biochemical reactions. The colony was creamy white, circular, raised, fluidal with 2 to 3mm diameter (Figure 3). Cell shapes of the isolate under light microscope appeared rod-shaped with gram negative reaction(Figure 3).

Biochemical characters of the isolate are depicted in the table 3. The isolate was able to ferment glucose, lactose, and sucrose, as indicated by the yellow coloration observed in the Triple Sugar Iron (TSI) test. Positive reactions were recorded in the Citrate Utilization, Urease, and Catalase tests, evidenced by a color change to blue, pink, and the formation of gas bubbles, respectively. The positive result in the Citrate Utilization test indicates that the bacterium can utilize citrate as its sole carbon and energy source. In the Catalase test, the production of oxygen gas bubbles confirmed the presence of the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen. Similarly, the pink coloration in the Urease test demonstrated that the isolate produces urease, an enzyme responsible for the hydrolysis of urea into ammonia and carbon dioxide. Conversely, the isolate exhibited negative results for the Oxidase, Methyl Red (MR), Voges-Proskauer (VP), hydrogen sulfide (H_2 S) production, Indole, and Motility tests (Table 3 and Figure 3).

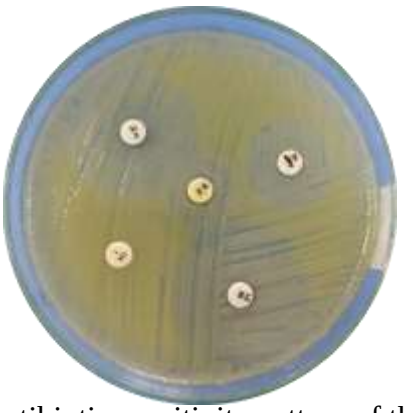


Figure 2 Antibiotic sensitivity pattern of the isolate

Colony on nutrient agar

Cell shape (1000x)

Biochemical reaction.
(1) TSI (A/A), (2) Citrate (+),
(3) MR (-), (4) VP (-), (5) H_2S (-),
Indole (-), Motility (-), (6) Urease (+)

Figure 3 Phenotypic Characteristics of the selected isolate

Table 2 Zone of inhibition (mm) of different antibiotics against the tested isolate

Isolate	Antibiotics	Zone of Inhibition (mm)	Inhibitory zone diameter to nearest millimeter (mm) (CLSI standards)		
			Sensitive (S) ≥	Intermediate (I)	Resistant (R) ≤
MHBD 10^{-2}(3)	EM (15 µg)	0 (R)	23	14-22	13
	TE (30µg)	0 (R)	15	12-14	11
	AMP (10 µg)	0 (R)	17	14-16	13
	LZ (30µg)	0 (R)	23	21-22	20
	OX (1µg)	0 (R)	20	-	-

Table 3 Biochemical characters of the selected isolate

Isolate	Biochemical tests												Tentative Genus
	TSI			Cit	MR	VP	SIM			Oxidase	Catalase	Urease	
	Slant	Butt	Gas				H_2S	Indole	Motility				
MHBD 10^{-2} (3)	A	A	+	+	-	-	-	-	-	-	+	+	*Klebsiella* spp.

DNA sequence analysis and phylogenetic tree construction

From the measurement of nanodrop spectrophotometry, DNA concentration was high with the range of 169.9ng/µL accompanying with the acceptable DNA purity with the range of 1.94 even though there was possible contamination with the absorbance ratio value of 1.06. Continuously, PCR analysis of MHBD 10^{-2} (3) isolate successfully amplified 16S rRNA gene using U1 (Forward) and U2 (Reverse) primers, showing the expected single band at specific size: approximately 996 base pairs (bp) in length (Figure 4). DNA sequences of a 1506 bp fragment obtained from the isolated were illustrated in figure 5.

NCBI BLAST analysis resulted the 14 sequences which are shown in Table 4. Query sequence of the isolate was evaluated and compared with the database sequences based on query coverage, e-value and percent identity. BLAST results revealed that the isolate is 99.64% similar to *Klebsiella pneumoniae* with the accession number AP018750 with sequence submissions originated in Thailand (Table 4). The phylogenetic tree was constructed with the examined isolate (MHBD 10^{-2} (3)) and the 14 similar sequences obtained from the GenBank database using the neighbor-joining method; the accession numbers are shown in Figure 6. The constructed phylogenetic tree clearly resolved the relationships among these sequences.

The 16S rRNA gene sequence of the isolate formed a well-defined cluster, aligning in a distinct clade with *Klebsiella pneumoniae* (AP018750). This clustering was strongly supported with 100% bootstrap value in the analysis. *Salmonella bongori* and *Salmonella enterica* subsp. *arizonae* constituted a distinct clade, affirming their separation from the *Klebsiella pneumoniae* species cluster.

Table 4 16s rRNA gene partial sequence identities of the studied bacteria to other species in the Gene Bank

No.	Description	Query Cover	E value	Percentage Identification	Accession	Origin	Country
1.	*Klebsiella pneumoniae* KP64	100%	0.0	99.64%	AP018750.1	Wound	Bangkok, Thailand
2.	*Klebsiella* sp. VITAJ23	100%	0.0	99.64%	KX770742.1	Contaminated soil	India
3.	*Klebsiella pneumoniae* F17KP0054	100%	0.0	99.64%	CP052136.1	Blood	South Korea
4.	*Klebsiella pneumoniae* 8695	100%	0.0	99.64%	CP085889.1	Patient's ascites	Wenzhou, China
5.	*Klebsiella pneumoniae* JX-CR-hvKP-10	100%	0.0	99.64%	CP064258.1	Blood	Jiangxi, South China
6.	*Klebsiella pneumoniae* KpWEA2 DNA	100%	0.0	99.64%	AP024570.1	Fecal sample	Fukuoka, Japan
7.	*Klebsiella pneumoniae* K56	100%	0.0	99.64%	MW843607.1	Ward	Saudi Arabia
8.	*Klebsiella pneumoniae* NK_H4_026	100%	0.0	99.64%	CP152609.1	Blood	Norway
9.	*Klebsiella pneumoniae* KSB1_7G	100%	0.0	99.64%	CP110723.1	Rectal swab	Australia
10.	*Klebsiella pneumoniae* UHD-50_PH	100%	0.0	99.64%	CP121131.1	Rectal swab	United Kingdom
11.	*Klebsiella pneumoniae* 15017	100%	0.0	99.64%	CP132688.1	Urine	Virginia, USA
12.	*Klebsiella pneumoniae* K014	100%	0.0	99.64%	CP128719.1	Blood	Chicago, USA
13.	*Klebsiella pneumoniae* KPN528	100%	0.0	99.64%	CP020853.1	Respiratory	Houston, USA

No.	Description	Query Cover	E value	Percentage Identification	Accession	Origin	Country
14.	*Klebsiella pneumoniae* 2017-45-134-04A	100%	0.0	99.64%	CP109720.1	Dispensary sink drain	Florida, USA

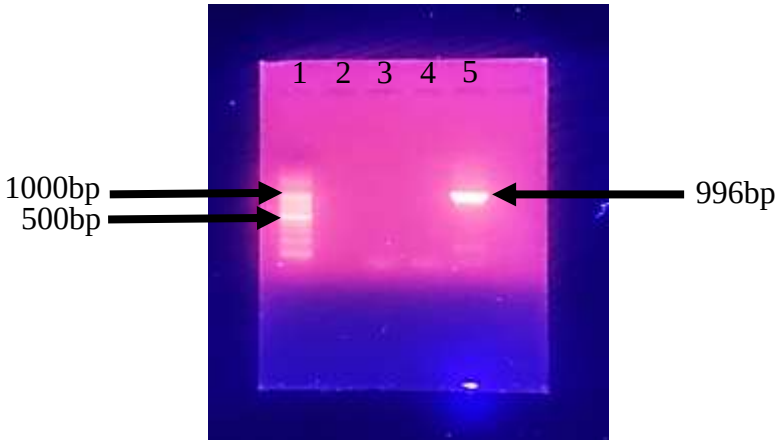


Figure 4 Agarose gel image of amplified fragment of the selected isolate 16s rRNA gene (996bp) using PCR. Lane 1: 1000bp DNA ladder, Lane 5: amplified PCR products from the bacterial isolate MHBD 10^{-2} (3)

GTCGAACGTTATCGGATTACTGGGCGTAAGCGCACGCAGGCGGTCTGTCA --- --- --- --- --- --- --- 50
AGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTG --- --- --- --- --- --- --- 100
GCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAA --- --- --- --- --- --- --- 150
ATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACA --- --- --- --- --- --- --- 200
AAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC --- --- --- --- --- --- --- 250
CCTGGTAGTCCACGCCGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGA --- --- --- --- --- --- --- 300
GGCGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGG --- --- --- --- --- --- --- 350
CCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGG --- --- --- --- --- --- --- 400
AGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGAC --- --- --- --- --- --- --- 450
ATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGAC --- --- --- --- --- --- --- 500
AGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGT --- --- --- --- --- --- --- 550
CCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTCGGCCGGGA --- --- --- --- --- --- --- 600
ACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTC --- --- --- --- --- --- --- 650
AAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCAT --- --- --- --- --- --- --- 700
ATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATATAGTATGT --- --- --- --- --- --- --- 750
CGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCT --- --- --- --- --- --- --- 800
AGTAATCGTAGATCAGAATGCTACGGTGAATACATTCCCGGGCCTTGTAC --- --- --- --- --- --- --- 850
ACACCGCCCGTCACACCATGAGAGTGGGTTGCATACAAATTAAGTAGCTT --- --- --- --- --- --- --- 900
AACCTTCGGGAGGGCGCTTACCACTTTGTGATTCTGACTGCGGTGAGCCC --- --- --- --- --- --- --- 950
TACATTTACGGGGGGGGGAGGAAAAAAAAGCAGCAGCCTGGAATGCAGTG --- --- --- --- --- --- --- 1000
TATTGGGAGATTATGTAGAGCTGAAGTTCTGGAAAAGCATCGATGCTTGT --- --- --- --- --- --- --- 1050
GATTAGAACGAAAGGCAACGTTAGAATAGTTGGATTTGAGTTGGTCACAT --- --- --- --- --- --- --- 1100
GCATTGCTAATTTAGTGGGCAATGGTAATGAAGTAAGCGCAGCTGGTGGT --- --- --- --- --- --- --- 1150
TATGTTGAAAGAGTAGCATCAGTGGGCGATGAAAGGAGGTGACGTTGTGA --- --- --- --- --- --- --- 1200
TAAGATGCTTTTGACGCCTTTGGTGAGCGTTCTTTGGATGGATGGAATCA --- --- --- --- --- --- --- 1250
TACAATGTAGTGCCCAAATGATTTAGCGATAAGTCGAAGGATGTCGCTAT --- --- --- --- --- --- --- 1300
AATAAACAAGTGAGATGTTGTGCCGATGAACATCTCTGTATAGTAATAGG --- --- --- --- --- --- --- 1350
CGATGGCTAACGACATGAGTATGGCTGTGAACTAGAGCAATGATGAATGG --- --- --- --- --- --- --- 1400
TAGAACATAAAACATTGTTTTTCCGTCTATCACTATGACTTGGTATAGCG --- --- --- --- --- --- --- 1450
ATGAGATGGGGCTGCATCGAGGACGCGTATACGCGGGTTGGTTACACTCC --- --- --- --- --- --- --- 1500
ATTGGA --- --- --- --- --- --- --- 1506

Figure 5 Partial sequences of 16s rRNA gene of the studied bacteria

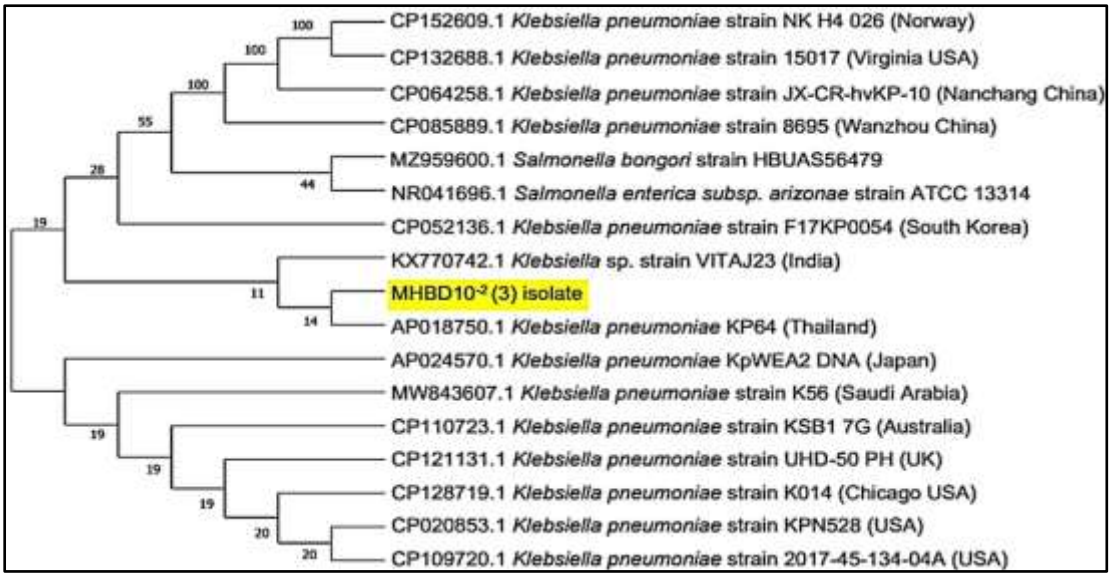


Figure 6 Phylogenetic tree of 16s rRNA gene of the studied bacterial isolate using the neighbor-joining method. *Salmonella bongori* and *Salmonella enterica* subsp. *Arizonae are used as out groups on phylogenetic analysis.*

Discussion

The present study aimed to isolate multi-drug resistant pathogenic bacteria from community drainage systems in urban areas. Biochemical and phenotypic characterization, along with molecular analysis through 16S rRNA gene sequencing, were performed to identify the selected bacterial isolate (MHBD 10^{-2} (3)) exhibiting the highest level of antibiotic resistance at the species level. Additionally, antimicrobial susceptibility test was carried out by the Kirby Bauer disc diffusion method to detect the pathogenic bacteria resistant to different classes of antibiotics. Based on phenotypic and genotypic analyses, the selected isolate, resistant to all tested antibiotics, was identified as *Klebsiella pneumoniae*.

On the nutrient agar medium, *Klebsiella pneumoniae* isolate were as large colonies with creamy white, circular, raised, fluidal appearance. The microscopic examination showed cells of gram-negative bacilli. Positive results were recorded in Citrate Utilization, Triple Sugar Iron, Urease and Catalase tests followed by gas production. Negative responses were observed in oxidase, Methyl-red, Voges Proskauer, H_2S production, Indole and Motility tests. These results were agreed with AI Jadar and Ibrahem, 2022. The present studied isolate was entirely resistant to Ampicillin AMP (10 µg), Erythromycin EM (15 µg), tetracycline TE (30µg), linezolid LZ (30µg) and oxacillin (1µg). Other researches has also reported these antibiotics to be among the least effective against *K.pneumoniae* isolates. (Badger-Emeka, *et. al*., 2021). Considerably, these results further highlight the global public health threat resulting MDR bacterial isolates.

In the present study, the PCR method was performed with the use of U1 (forward) and U2 (reverse) primers that amplify conserved regions (16S rRNA) in various bacteria for DNA sequencing (Lu, *et. al.,* 2000). DNA sequence analysis was carried out by NCBI BLAST search database. The isolate exhibited 100% query coverage and 99.64% sequence identity to the standard strain recorded in gene bank, *Klebsiella pneumoniae* KP64 (AP018750). A phylogenetic tree was constructed to determine the kinship relationship of MHBD 10^{-2}(3) isolate resistant to

multiple antibiotics. From the result, the close relationship is evidenced by their shared clade, indicating a high level of genetic similarity between the two strains. Moreover, the clustering pattern indicates that MHBD10-2(3) shares a more recent common ancestor with the Thailand strain than with other *Klebsiella pneumoniae* strains from different geographical locations, suggesting possible regional or environmental influences and diverse sources contributing to the observed genetic similarities. This finding may be relevant for understanding the spread of multi-drug resistant *K. pneumoniae*, highlighting the potential for cross-border transmission or similar selective pressures in these environments (AI Jadar and Ibrahem, 2022).

Klebsiella is the second most common cause of nosocomial infections among Enterobacteriaceae. *Klebsiella* spp. are opportunistic pathogens that cause severe diseases in hospital setting and are difficult to identify and are often misclassified in clinical microbiology laboratories. Majority of *Klebsiella* infections are caused by *K. pneumoniae*. This organism cause pneumonia, urinary tract infection, soft tissue infection and septicemia, which often leads to septic shock. Isolates from various sources are just as virulent as clinical isolates from hospitals and can produce important virulence factors. In this study, referenced isolates from various sources from different countries are carbapenem-resistant and ESBL (extended-spectrum-β-lactamase producing *K.pneumoniae*. From sequence alignment and phylogenetic analysis, the studied isolate had the closet genetic similarity with KP 64 (Thailand strain), resistant to carbapenems and causing disease outbreak in Thailand (Sakamoto, *et al*., 2018). So, the isolate may be considered as potentially harboring hypervirulent, multidrug- resistant and transferable genes because of its specific resistance-associated genes against different antibiotics. Therefore, precise confirmation of *Klebsiella* identification is very important for taxonomic and molecular characterization. Also, PCR analysis has been reported to be more accurate in the identification of *Klebsiella* species when compared with other preliminary identification tests (Rawy, *et. al*., 2020).

Conclusion

The identification of the studied isolate (MHBD 10^{-2}(3)), isolated from community drainage of Latha township was carried out through both morphological and molecular methods. The isolate showed highest resistance to multiple antibiotics. The PCR of 16S rRNA gene sequence gave an approximately 996bp amplicon for the isolate. Phylogenetic analysis showed that the isolate was *Klebsiella pneumoniae*, with 100% similarity to *Klebsiella pneumoniae* KP64 (AP018750). Further studies are needed to elucidate specific antibiotic resistance genes using genome sequencing.

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EFFECT OF FEED TYPES ON GROWTH PARAMETERS AND HATCHING RATE IN NATIVE CHICKEN, *GALLUS GALLUS DOMESTICUS* (LINNAEUS, 1758)*

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Abstract

The demand for chicken's meat and eggs has increased substantially due to its good quality and nutritive values. The low productivity of native chicken is primarily attributed to low genetic potential, poor nutrition and disease control. Nutrition is a major influence on growth rate and egg production for breeding. This study aimed to identify the low-cost chicken feed with high profit that can yield favorable growth parameters and hatching rates for the farmers in the rural areas. The study was carried out from December 2023 to September 2024 in Bagan Village, Waw Township, Bago Region. A total of 27 (3-month-old) native chickens with initial weight of 540.00 ± 11.06g were randomly selected for three treatments. One male and eight females (1:8) were reared in each poultry house. The treatments were denoted as the birds fed commercial diet (T1), commercial diet substituted by 50% of locally available ingredients – broken rice, rice bran, snail shell powder and shrimp meal powder (T2) and a formulated diet including maize, broken rice, rice bran, snail shell powder and shrimp meal powder (T3), respectively. Throughout the grower stage, the growth parameters (body weight and weight gain) of all treatments were not significantly different from each other. The results of feed intake and FCR were influenced by feed types among three groups. The result also showed that the fertility rate of the native chickens fed three different diets was similar although there were significant differences in the laying and hatching rate of the eggs. In terms of net return and hatching performance, the native hens fed T2 diet exhibited the highest profit and those fed T1 diet obtained the highest proportion of hatched chicks (62%). This study concludes that the T2 diet is the most viable option for rural farmers, as it effectively reduces feed costs while maintaining growth performance and providing a high profit margin.

Keywords: nutrition, growth parameters, hatching rate, net return

Introduction

Poultry makes a substantial contribution to food security and nutrition, providing energy, protein, and essential micro-nutrients to humans. Poultry meat and eggs are among the most common animal source food consumed at the global level, through a wide diversity of cultures, traditions and religions, making them key to food security and nutrition. It is particularly important for small holders and poor rural and urban communities producing in large scale and intensive operations (Mottet and Tempio, 2017). In recent years, the consumer demand for chicken's meat and eggs has increased substantially.

Native chickens are an affordable and important source of protein for the people of Myanmar. The consumption of chicken's meat and eggs increased substantially in Myanmar, up to 72% and 40% respectively, between 2010 and 2015 (Fang *et al*., 2021). They are reared throughout the country in small flocks for home consumption and for sell. They are well-known

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for their hardiness, disease resistance, with good foraging ability and free ranging. Their meat is more delicious than that of commercial chickens because it is delicious, low fat and cholesterol.

Typical growth rate of native chickens is much lower than that of commercial broilers. Native hens lay small sized eggs when compared to layer hens but are popular for good broodiness and mothering ability. They usually lay the first eggs at the age of 22-28 weeks. A native hen will typically lay a clutch of between 10 and 14 eggs depending on season and in particular availability of feed (Henning and Pym, 2019). Broodiness is a natural behavior to incubate a clutch of eggs for the purpose of getting offspring. The farmers in rural areas usually use broody hens without using artificial incubators to hatch the chicks at very low costs (Sarkar, 2022).

The supply of native chickens is often inadequate to meet consumer demand due to traditional production methods, such as natural mating and egg incubation under broody hens which results in high chick mortality rates. Nowadays, there are three types of poultry production system could be found in Myanmar: (1) free range production or traditional/backyard production system; (2) Semi-intensive (range plus intensive) production system of small-scale commercial production and (3) intensive production system (Win, 2012).

The productivity of native chickens under traditional production is generally considered low. For better production, they are offered water and balanced nutrients, improved shelter and vaccination. Zainudin (2005) said that local chicken breeding pattern in villages is more extensive way (80%) than intensively or semi-intensive (20%) and chicken performance was better maintenance in intensive and semi-intensive.

The feed is the largest cost component, which is about 70% of the total production cost in poultry (Teguia and Beynen, 2005). The price of commercial feed is considered very expensive for rural villagers. Therefore, an alternative way that can reduce the feed cost is using the local ingredients instead of commercial feed. The purpose of mixing commercial feed with other local feed is to lower the feed prices, while still taking into account the nutritional content.

Feeds are derived from two sources, agriculture and fisheries. Agricultural by-products such as broken rice, rice bran and maize are used as energy feed. These products come from the middle and upper parts of the country where crops are extensively grown. Fish and prawn meals are derived from the fisheries sector (Myat Kyaw, 2003).

Maize is one of the most crucial feed ingredients with easy digesting and palatable quality in poultry. Broken rice and rice bran are the by-product of rice milling used in poultry. Shrimp meal powder is high in nutritional value, palatable and pleasant aroma. Snail shell powder has only been used as a calcium source in the diets.

Several previous studies have shown that replacing some commercial rations with locally available feed ingredients gave similar growth performance of the chickens. Gulilat *et al.* (2022) carried out a study that the effects of substitution of different levels of commercial starters with a homemade ration on the growth performance of Sasso and indigenous chickens and indicated that one alternative can be done in an effort to reduce the cost. Erwan (2020) reported that corn

could be used at 30% to substitute commercial feed without significantly affecting growth performance and plasma total cholesterol (TCHO) concentration of KUB chickens in Indonesia.

It is imperative to find out cheaper source of protein and to reduce feed cost for poultry to formulate economic diet with conventional feed like maize, broken rice, rice bran, shrimp meal powder and snail shell powder. The present study aims to identify the low-cost chicken feed that can yield favorable growth parameters and hatching rates with the following objectives: to record the growth parameters of native chickens treated with different feed types, to evaluate the hatching and survival rate of native chickens and to determine the fertility of the eggs by candling.

Materials and Methods

Study area and period

The experiment was carried out in Bagan Village, Waw Township, Bago Region, locating about 93 km far from Yangon city between latitude 17° 28' 38" N and longitude 96° 40' 33" E (Fig. 1).The present study was conducted from December 2023 to September 2024.

Housing

In this study, three poultry houses were constructed. Each house has a thatched roof and bamboo slatted floor with an area of 180 cm length × 180 cm width and 60 cm above the ground. A green shade net roofing fence has an area of 270 cm length × 180 cm width with wire mesh for ventilation. A total length of 180 cm bamboo perches was provided and the distance between each house is 240 cm. A round feeder was placed to one side of the house and a drinker on the other side.

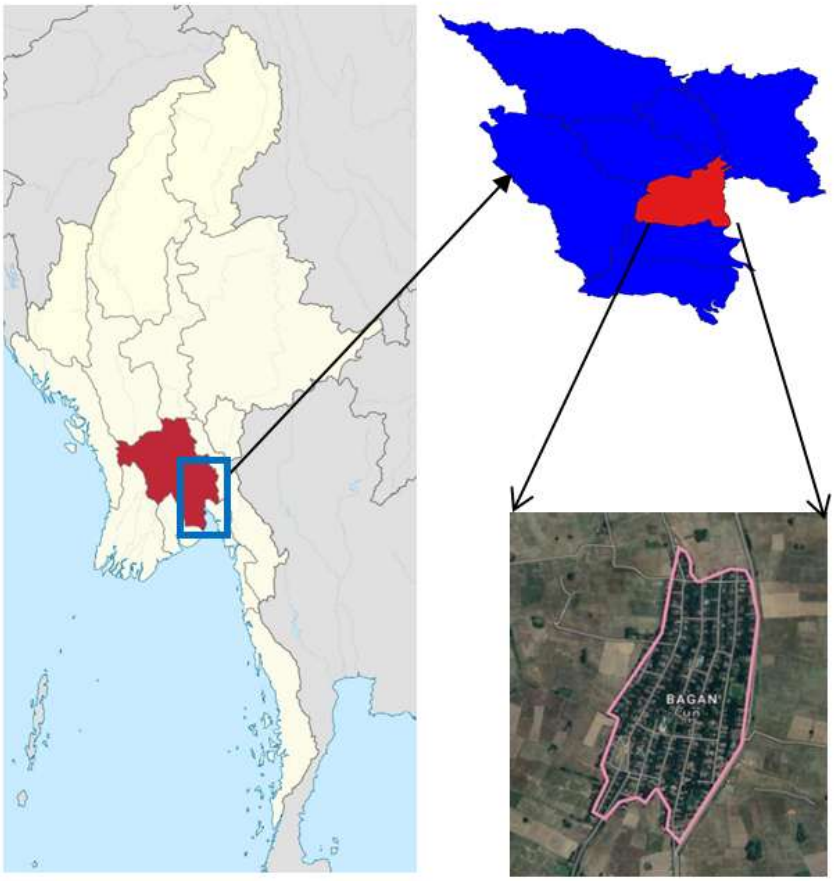


Figure 1. Map of Bagan Village, Waw Township in Bago District (Source: Google)

Experimental design and management

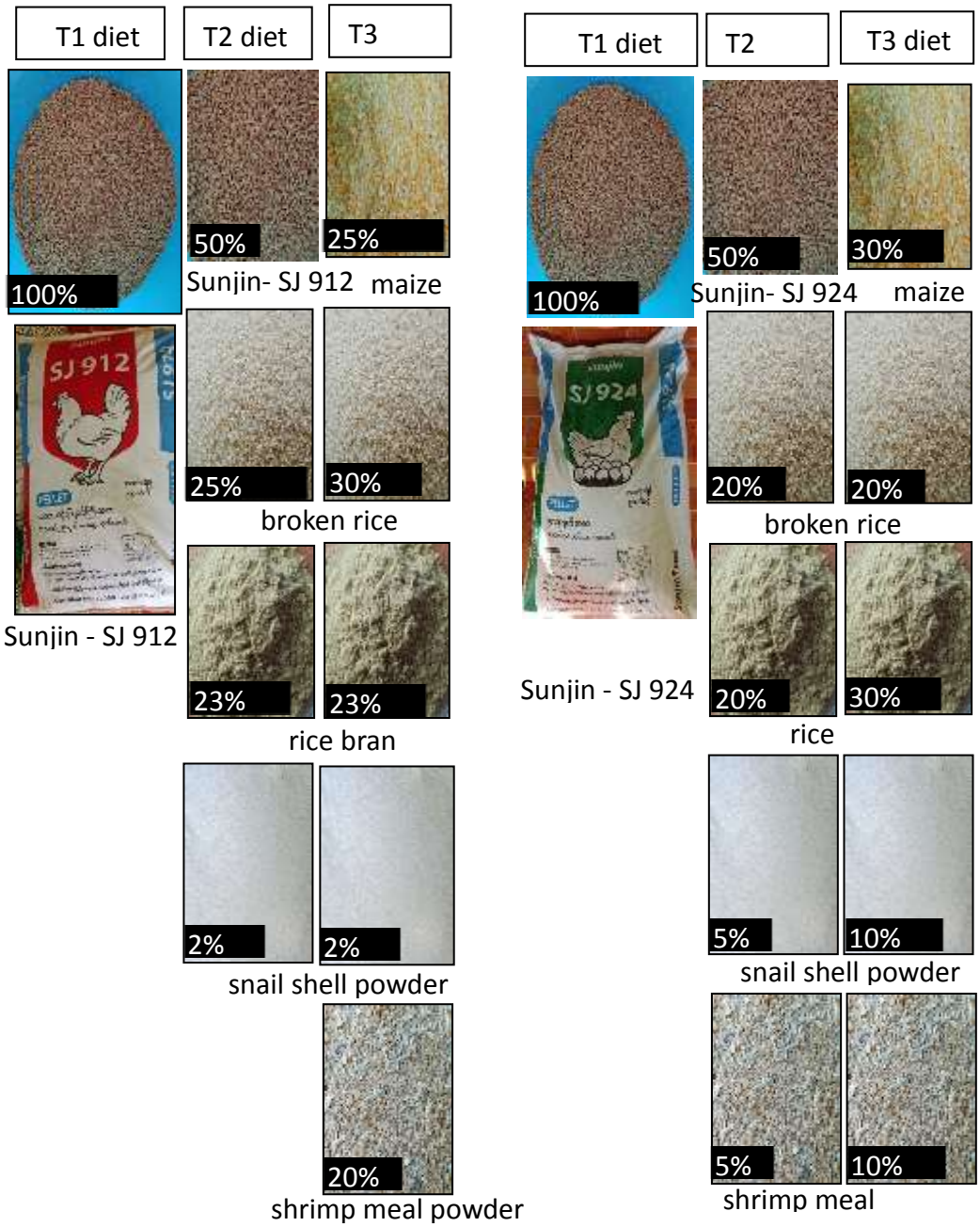
A total of 27 native chickens (3-month-old) were purchased from the villagers. On arrival, all chickens were individually weighed. They were vaccinated by eye drop with La Sota vaccine against new castle disease. Chickens were raised under natural light without using artificial bulbs during the nighttime. The manure of the chickens was swept twice a week.

The chickens having the initial weight (540.00 ± 11.06g) of three males and 24 females were randomly selected for three treatments. For each treatment, one male and eight females were reared in each poultry house.

The nutrient composition of the feed ingredients for T2 and T3 was analyzed in Assay Laboratory of Ministry of Livestock, Fisheries and Rural Development in Myanmar. During the grower stage, the chickens on T1 group were fed commercial diet-Sunjin (SJ 912-100%). For T2, commercial diet-Sunjin (50%), broken rice (25%), rice bran (23%) and snail shell powder (2%) were given. The birds of T3 were provided broken rice (30%), maize (25%), rice bran (23%), shrimp meal powder (20%) and snail shell powder (2%). The protein levels of three diets are 19%,14% and 12%, respectively (Table 1 and Plate 1 A).

During the laying stage, the chickens were provided with commercial diet-Sunjin (SJ 924-100%) for T1, commercial diet-Sunjin (50%), broken rice (20%), rice bran (20%), shrimp meal powder (5%) and snail shell powder (5%) for T2 and broken rice (30%), maize (30%), rice

bran (20%), shrimp meal powder (10%) and snail shell powder (10%) for T3. The protein levels are 17%, 13.85% and 9%, respectively (Table 1 and Plate 1 B).



A. The raw ingredients for grower stage(meat) B. The raw ingredients for layer stage(eggs)

Plate1. The percentage of raw materials for three dietary treatments

Feeding

The experimental diets were given three times a day of (6:00 am, 10:00 am and 2:00pm). Clean and fresh water was provided with vitamin powder diluted 1 gram/liter in drinking water. Before giving the diets to the chickens, the amount of feed was weighed using a 5kg digital balance every day. The left-over feed was weighed back at the end of the feeding time (6:00 pm)

before new feed was given to each group. The daily record sheet was used to note the feed issues on a treatment group. In this study, the chickens were fed a maximum of 10% of the body weight. The quantity of the feed given to the chickens was evaluated by multiplying the average body weight of the female with 10% (Table 2 and 3).

Preparation of brooding boxes

A total of 24 boxes with a length of 46 cm and width of 30 cm x breadth of 20 cm were made for the hens to be comfortable. All boxes were filled with clean and soft straws on top up to 2/3 of the depth after spraying 10g of anti-lice medicine powder around the boxes. Nesting straws were changed twice a week.

Egg candling method

On day 18, egg candling was performed to examine the development of embryos, infertile eggs and dead embryo in shell. All the eggs from the wooden brooding boxes were checked by using the flashlight in the dark room. If the fertile eggs were visible with a network of blood vessels indicating the growing embryo and the embryo was found at the center of the network. It was rather small or lacking embryo which showed nothing but the outline of the yolk or a dark floating mass were designated as infertile eggs. Infertile and other eggs with dead embryos were removed.

Data collection

Growth Parameters

Body weight: The body weight of the chickens was taken using a digital balance at resting time at night and recorded at weekly interval from 13 week to 20 week. The mean body weight of chickens was determined weekly by dividing the total weight per house by the number of birds.

Growth rate: The body weight gain was calculated by (Kumar *et al*., 2023),

Weight gain=Body weight in particular weight-Body weight of previous week

Feed Intake: Weekly feed intake of all the chickens was recorded up to 20 week of age and those of the native hens was done for laying stage by the following formula.

$$\text{Average daily feed intake} = \frac{\text{the given feed - the left - over feed}}{\text{Number of birds}}$$

Feed Conversion Ratio (FCR): FCR was calculated by dividing the total quantities of feed consumed by total gain in body weight during the same period. According to Aviagen (2018),

$$\text{FCR} = \frac{\text{Total feed intake}}{\text{Total weight gain}}$$

Fertility

Fertility of the eggs was calculated by using the following formula, (Islam *et al*., 2014)

$$\text{Fertility (\%)} = \frac{\text{Total number of fertile eggs}}{\text{Total number of eggs set}} \times 100$$

Hatchability

The chicks come out of the shell on 21st day of incubation were recorded as hatched chicks. Hatchability of eggs was calculated on the basic of eggs set and on fertile eggs was calculated by the following formulae (Islam *et al.*, 2014).

$$\text{Hatchability on eggs set (\%)} = \frac{\text{Total number of chicks hatched}}{\text{Total number of eggs set}} \times 100$$

$$\text{Hatchability of fertile eggs (\%)} = \frac{\text{Total number of chicks hatched}}{\text{Total number of fertile eggs}} \times 100$$

Statistical analysis

Microsoft Excel (Office, 2019) was used for data collection and the creation of the selected diagrams. Statistical analysis of data was undertaken by using IBM SPSS statistics version 26. All the data were analyzed to compare with the One-way Analysis of Variance (ANOVA) method. In all of the described inferential statistical analyses, differences were considered statistically significant for $p \leq 0.05$ and tended to be significant at $0.05 > p$. Significant differences among the means of the treatments for growth parameters (body weight, weight gain, feed intake and FCR) were performed at p value of 0.05 using Duncan's Multiple Range Test. The differences for all significant effects on egg characteristics and hatching parameters were analyzed by using Turkey's honestly significant difference test.

Table 1. Nutrient composition of experimental diets

Nutrient Composition	Grower diet			Layer diet		
	T1	T2	T3	T1	T2	T3
Crude protein (%)	19	14	12	17	13.85	9.50
Metabolizable energy (kcal/kg)	3200	3100	2950	2700	2740	2800
Calcium (%)	0.8	1.1	1	3.80	3.85	3.81
Available phosphorous (%)	0.4	0.25	0.28	0.45	0.36	0.26

Table 2. The formulation of the experimental grower diet

Age (week)	Total no. of chicken	ABW (g)	10% of ABW (g)	One day(g) for 9 birds	T1	T2				T3				
					SJ-912 (100%)	SJ-912 (50%)	BR (25%)	RB (23%)	SSP (2%)	BR (30%)	Maize (25%)	RB (23%)	SMP (20%)	SSP (2%)
13	9	617	62	558	558	279	140	128	11	167	140	128	112	11
14	9	740	74	666	666	333	167	153	13	200	167	153	133	13
15	9	814	81	729	729	365	182	168	14	219	182	168	146	14
16	9	896	90	810	810	405	203	186	16	243	203	186	162	16
17	9	973	97	873	873	437	218	201	17	262	218	201	175	17
18	9	1048	105	945	945	473	236	217	19	284	236	217	189	19
19	9	1133	113	1017	1017	509	254	234	20	305	254	234	204	20
20	9	1223	122	1098	1098	549	275	252	22	329	275	252	220	22

Note: ABW = Average Body Weight, g = gram, M = Maize, BR = Broken Rice, RB = Rice Bran, SMP = Shrimp Meal Powder, SP = Snail Shell Powder, SSP = Snail Shell Powder, SJ = Sunjin

Table 3. The formulation of the experimental layer diet

Age at first eggs laid (Week)	Total no. of chicken	ABW (g)	10% of ABW (g)	One day (g) for 8 birds	T1	T2					T3				
					SJ-924 (100%)	SJ-924 (50%)	BR (20%)	RB (20%)	SMP (5%)	SSP (5%)	BR (30%)	M (30%)	RB (20%)	SMP (10%)	SSP (10%)
21	8	1339	134	1072	1072	536	214	214	54	54	322	322	214	107	107

Note: ABW = Average Body Weight, g = gram, M = Maize, BR = Broken Rice, RB = Rice Bran, SMP = Shrimp Meal Powder, SP = Snail Shell Powder, SSP = Snail Shell Powder, SJ = Sunjin

Results

Effect of three diets on the body weight and of native chickens

The result showed the live body weight of chickens generally increased with age (Table 4). No significant differences ($p>0.05$) in body weights among three treatment groups. However, the birds fed the diet T1 (commercial feed-100%) gave the better growth performance in comparison with those fed other dietary treatments. At the age of 14 and 15 week, T2 group had the higher body weight than T3 group and vice versa. It was observed that the native chickens attained the body weight of 1.2kg in week 20 (Plate 2).

Effect of three diets on weekly feed intake of native chickens

Weekly feed intake of all treatment groups was gradually increased with increasing age. The result showed that the feed intake was significant different ($p<0.05$) among the chickens fed different diets (Fig. 2). But the birds under the three dietary treatments consumed similar quantities of feed at 15 weeks of age. The total amount of feed was significantly higher in the chickens fed T3 diet (5205.68g/bird). The chickens under T2 treatment (4499.97g/bird) consumed more feed than those under T1 treatment (4366.54g/bird) during the grower phase.

Effect of diets on body weight gain of native chickens

The body weight gain of experimental birds was not differed significantly ($p>0.05$) among the treatment groups (Table 5). However, the body weight gain of the chickens was significantly differed among three groups at the age of 19 week. The chickens fed T1 diet showed the best weight gain (723.30g) in comparison with other groups, followed by T3 (691.08g) and T2 (686.64g).

Effect of three diets on feed conversion ratio (FCR)

The result of weekly feed conversion ratio showed that there was significant difference ($p<0.05$) among the three groups of chickens (Fig. 3). The FCR value of the chickens under three groups ranged between 3.62-8.83 in T1, 3.93-8.05 in T2 and 4.54-10.36 in T3, respectively. The overall means were 6.41, 6.72 and 7.64 for T1, T2 and T3, respectively. Overall, the chickens fed T1 diet were obtained the best FCR and more efficient in utilizing feed compared to those fed other diets.

Effect of diets on egg parameters

The number of fertile and infertile eggs did not vary significant ($p>0.05$). There were significantly differences in the numbers of laid eggs, hatched eggs, dead embryos and survived

chicks after one month among three treatment groups. The mean number of eggs laid per hen in this study was 10.50 ± 0.76, 10.25 ± 0.46 and 9.75 ± 0.46 for T1, T2 and T3, respectively. The number of hatched eggs per hen was highest in T1 group (6.38±0.52), followed by T2 (5.75±0.71) and T3 (4.75±0.89), respectively. Moreover, the number of dead embryos per hen in this study was highest in T3 (2.63±0.52) and the lowest in T1 (1.88±0.64). The average number of survived chicks after one month per hen was maximum in T1 (4.00±0.53), followed by T2 (3.13±0.83) and T3 (2.38±0.52), respectively (Fig. 4).

Effect of diets on hatching rate

The results showed that the fertility rate of the native chickens fed three different diets was similar although there were significant differences ($p<0.05$) in the hatching rate of the hens (Fig.5). The proportion of fertility for the eggs obtained from three treatment groups was 78.50%, 76.75% and 75.75% for T1, T2 and T3, respectively. The lowest hatching rate was noted in the native hens from T3 group (48.75%) but the highest proportion of hatched chicks on egg sets was found in T1 (62%), followed by T2 (56.13%). There was a significant difference ($p<0.05$) in the percentage of embryonic mortality among three treatment groups. The embryonic mortality rate of T3 group (36.13%) was slightly higher than other treatment groups (22.50 %, 27.13% for T1 and T2, respectively) (Fig. 5).

Effect of diets on feed cost

Although the total feed cost was almost similar in all treatments, T2 diet was low-cost chicken feed. The results showed that the highest net return was obtained in chickens fed T2 diet with the mount of 43050MMK, followed by T3 with 41760 MMK and T1 with 41690 MMK. The highest net return was noted on T2 compared to other treatment groups (T1 and T3) (Table 6).

Table 4. Mean body weight (g) of native chicken on different feed types

Age (week)	N	Treatment			F-value	p-value
		T1	T2	T3		
13	9	641.11	624.44	620.00	0.321	$>0.512^{NS}$
14	9	777.77	744.44	735.55	0.924	$>0.411^{NS}$
15	9	841.11	815.55	820.00	0.545	$>0.587^{NS}$
16	9	913.33	906.66	911.11	0.016	$>0.984^{NS}$
17	9	1004.44	983.33	984.44	0.174	$>0.842^{NS}$
18	9	1100.00	1054.44	1057.77	0.394	$>0.679^{NS}$
19	9	1168.88	1137.77	1160.00	0.117	$>0.890^{NS}$
20	9	1268.88	1233.33	1237.77	0.191	$>0.827^{NS}$

NS = non-significance ($p>0.05$)

N = Total no. of chicken

Table 5. Weekly body weight gain (g/bird) of native chicken on different feed types

Age (week)	N	Treatment			F-value	p-value
		T1	T2	T3		
13	9	95.55	77.77	73.33	1.510	$>0.241^{NS}$
14	9	136.66	120	115.55	1.463	$>0.317^{NS}$
15	9	63.33	71.11	84.44	1.094	$>0.351^{NS}$
16	9	72.22	91.11	91.11	0.795	$>0.463^{NS}$
17	9	91.11	76.66	73.33	1.430	$>0.250^{NS}$
18	9	95.55	71.11	73.33	1.328	$>0.284^{NS}$
19	9	68.88^{a}	83.33^{ab}	102.22^{b}	4.737	<0.018
20	9	100	95.55	77.77	1.196	$>0.320^{NS}$
Total		723.30	686.64	691.08		

NS = non-significance ($p>0.05$) N = Total no. of chicken

$^{a, b, c}$ superscripts on the same row indicates significant difference ($p<0.05$).

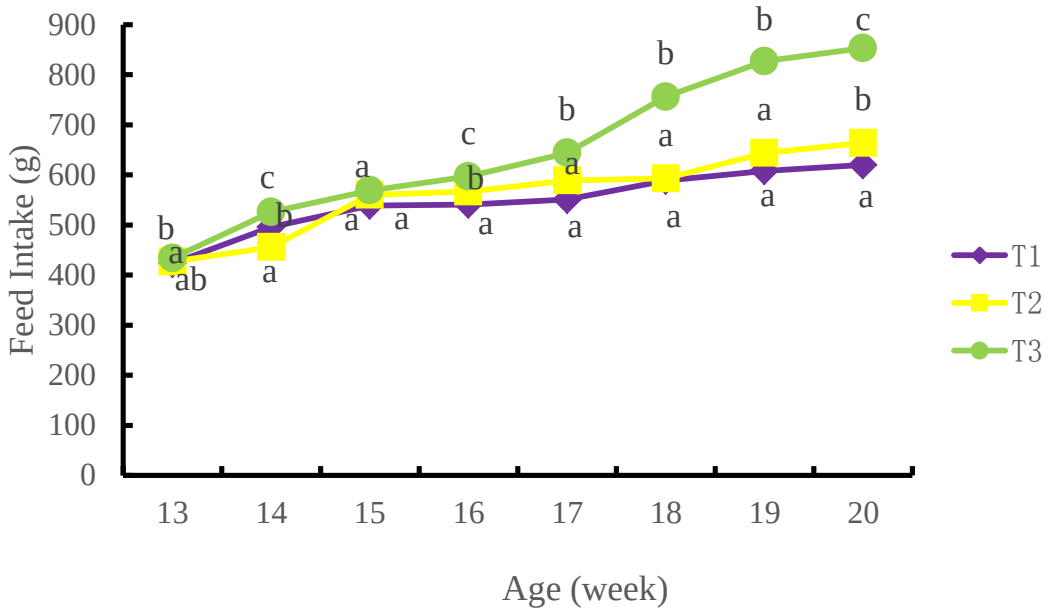
a = the lowest value, b = the highest value and ab = no significant difference between a and b

Table 6. Feed Cost for grower phase

Parameters	T1	T2	T3
Feed cost per viss (MMK)	2600	2390	2150
Total feed (viss)	24.18	24.92	28.86
Total feed cost (MMK)	62870	59600	62050
Final live weight (viss)	0.77	0.75	0.76
Sale revenue (MMK)	90090	87750	88920
Net return (MMK)	41690	43050	41760

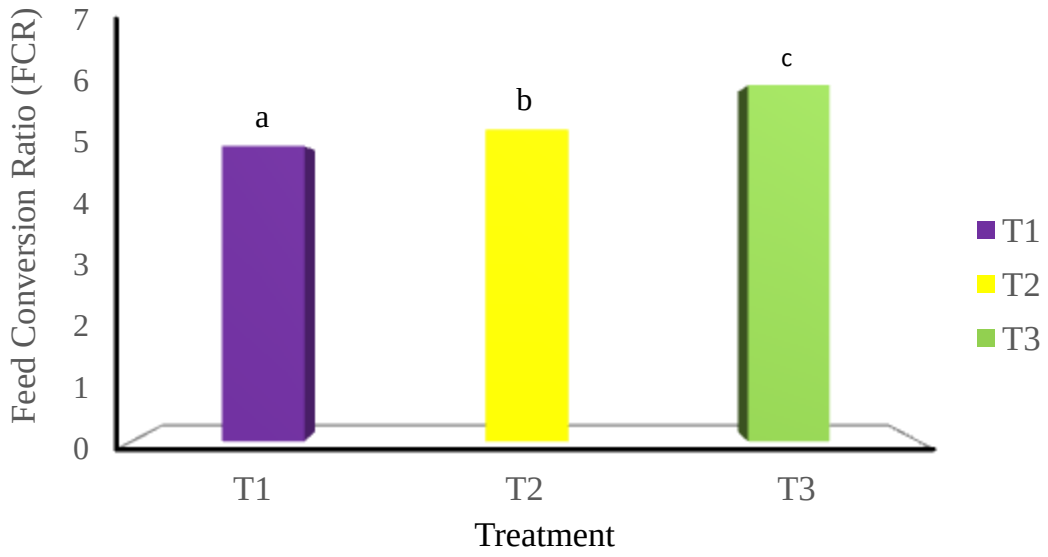
*Market price of live chicken = 13000 MMK per viss

MMK = Myanmar Kyat



a = the lowest value, b = the moderate value, c = the highest value and ab = no significant difference between a and b

Figure. 2 Effect of experimental diets on the weekly feed intake of native chickens



a = the lowest value, b = the moderate value and c = the highest value

Figure. 3 FCR of chickens fed on three different diets

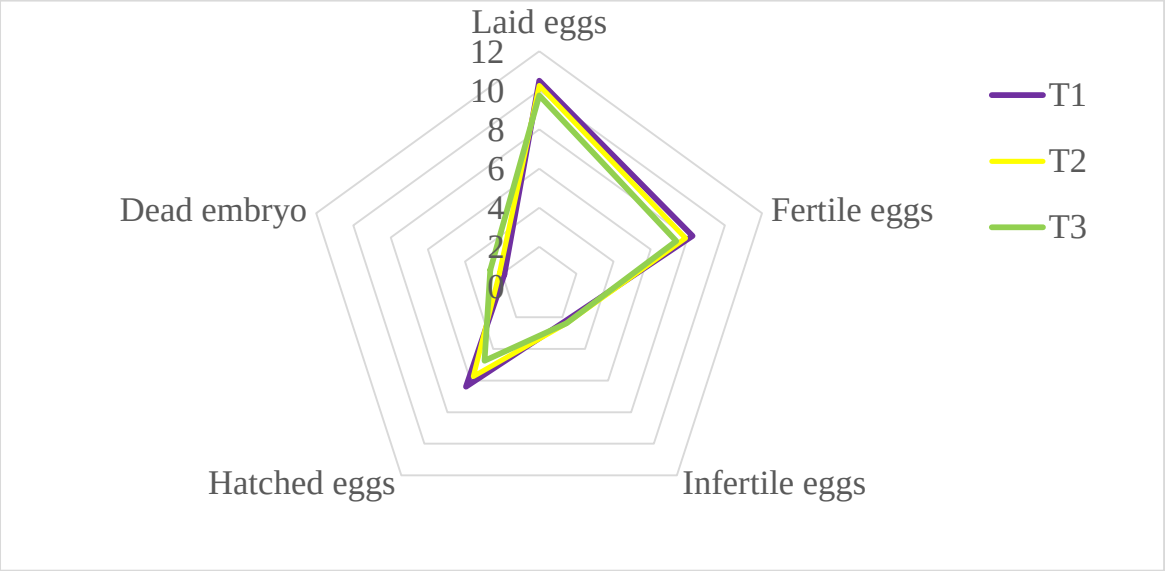
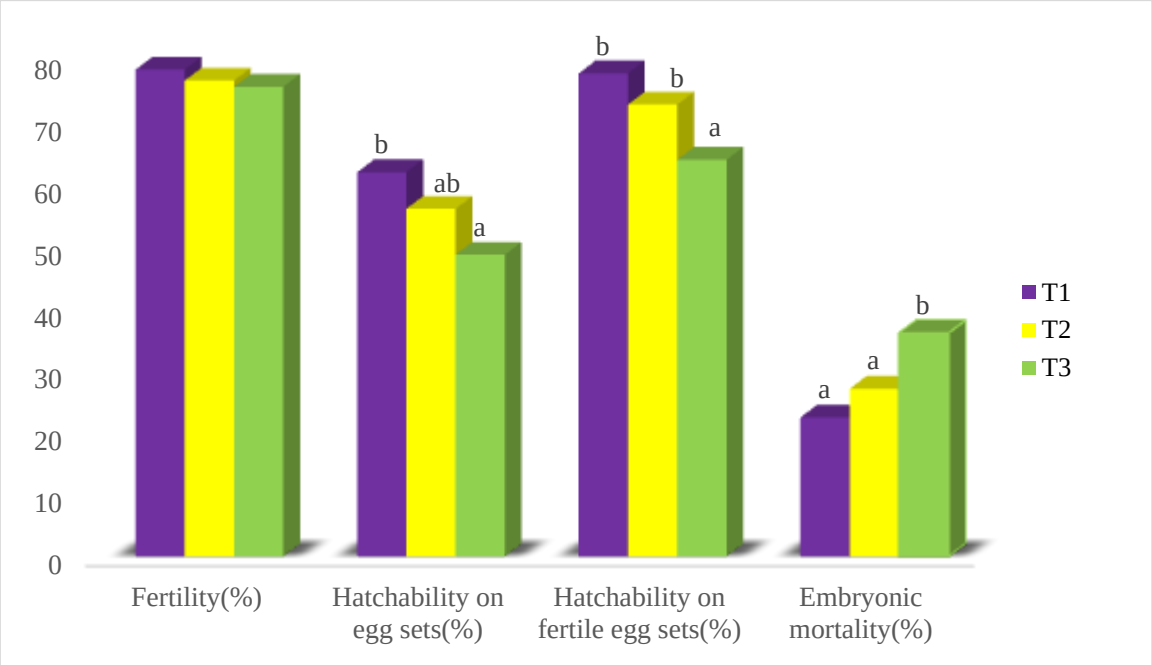


Figure. 4 The number of egg parameter



a = the lowest value, b = the highest value and ab = no significant difference between a and b

Figure. 5 Hatching performance of native chickens under three different diets

A. The chickens at the beginning of the experiment

B. The chickens fed T1 diet

C. The chickens fed T2 diet

D. The chickens fed T3 diet

Plate 2. Poultry farms by feeding different diets

Discussion

According to the results of the present study, the growth performance parameters (body weight and weight gain) of all the treatments during grower period were not significantly different from each other. The reason might be possible that all the chickens in three treatments may get the sufficient nutrients from all the diets which are formulated with not only isocaloric and isonitrogenous level but also the similar levels of calcium and phosphorous. This result is in agreement with the finding of Mutayoba *et al.* (2012) reported that differences in growth rate between the free-range local chickens fed with commercial feed (14.6% CP/2604 kcal ME kg-¹)

and homemade feed (15.5% CP/3620 kcal ME kg^{-1}) with maize 55%, maize husks 20%, cassava leaf meal 15%, soyabean meal 7%, egg shell 2% were not significant.

The results of feed intake and FCR were significantly different among three groups. However, the chickens fed T1 diet (Sunjin-100%, 19% CP and 3200 Kcal ME/kg) showed numerically lower value in feed intake and FCR than those fed T2 and T3 diet. The reason might be possible that the commercial feeds were formulated with appropriate levels of protein, carbohydrates, fat as well as vitamins and minerals. They are available in pellet form with larger particle sizes and more difficult to digest but they can reduce feed wastage and improve feed utilization. The feed with high quality allows the chickens to absorb all the nutrients they need without wasting it.

T3 diet is mash form with smaller size and easier to eat and digest but the chickens do not get the balanced nutrients due to higher wastage. Thus, T3 group fed the diet containing broken rice (30%), maize (25%), rice bran (23%), shrimp meal powder (20%) and snail shell powder (2%) is higher in feed intake and FCR than other groups.

In the present study, total feed cost was highest in T1 diet and lowest was in T2 diet containing Sunjin (50%), broken rice (25%), rice bran (23%) and snail shell powder (2%). The highest net profit was obtained from T2 due to the usage of high nutritional value of commercial diet and cost-effectiveness of local ingredients approaches saving feed cost and maintaining growth rate. The net return of T2 treatment is similar with Ramon and Zul (2016) reported that the treatment P1 (commercial feed 40% + corn 34.7% + rice bran 25% + minerals 0.3%) generates the highest profit.

In the present study, the number of eggs produced per hen was highest in T1 group (10.50 ± 0.53) and lowest in T3 group (9.75 ± 0.46) eggs. The better efficiency obtained from T1 and T2 could be due to its sufficient quantity of multivitamins, minerals and amino acids that promotes good health and egg production. Besides, natural ingredients such as maize, broken rice and rice bran that are used as dietary products in poultry but they may not directly provide egg improvement like other nutritional additives. The present study showed that the commercial diet was more efficient than natural ingredients for egg production. Wondu *et al.* (2013) reported that the chickens fed thrice a day produced 11.53 eggs is inconsistent with this study.

According to NRC (1994), calcium is the most abundant mineral element in snail shell. In the present study, 5% and 10 % of snail shell were added in laying stage of T2 and T3, respectively. Shell thickness of the eggs in this study was 0.51mm, 0.52mm and 0.50 mm in T1, T2 and T3, respectively. According to Yamak *et al.* (2015), the shell thickness of all the eggs in this study indicated that thick shelled eggs. The shell color of the present study was brown, light brown and whitish color for T1, T2 and T3, respectively.

The present result also indicated that there is no difference in fertility rate but the highest level of crude protein obtained the best hatchability percentage. The hatchability of the T3 (CP-9%) group was significantly lower than those of the T1 (CP- 17%) and T2 (CP- 13.5%) groups during the study period. This poor performance is linked to the high rate of embryonic mortality

in this group (Larbier and Leclercq, 1992). The reason might be due to higher protein diets can improve egg quality, leading to better hatchability.

During the study period, no mortality was occurred in all treatment groups. Therefore, the survivability was 100% in all grower chicken groups.

Conclusion

According to the findings of the present study, the feed types were not influenced on the growth parameters but affected on hatching rate of native chickens. The native chickens fed three different feed types gave similar growth parameters. Therefore, T3 diet containing locally available feed ingredients is said to be sufficient for rearing native chickens with low profitability in the grower phase. It was concluded that T2 diet containing broken rice (25%), rice bran (23%) and snail shell powder (2%) could be used up to 50% to substitute the commercial feed to reduce feed cost. It can be used to yield the favorable target weight and save the feed cost in rural areas. In term of laying and hatching performance, the native hens fed the highest T1 diet exhibited the best results in comparison with those fed other diets. It is suggested that this study is able to provide food security, the improvement of income by using the least-cost feed for the rural households.

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LANDMARK-BASED MORPHOMETRIC ANALYSIS ON BODY SHAPE OF SOME FISH SPECIES IN THAUNG PYIN "IN", THEGON TOWNSHIP, BAGO REGION

Kyawt Kay Khaing[1], Aye Aye Aung[2],

Abstract

The present research focused to examine landmark-based morphometric variations of *Notopterus notopterus*, *Macrognathus zebrinus*, *Anabas testudineus* and *Trichogaster pectoralis* from Thaung Pyin "In", Thegon Township, Bago Region. A total four of 20 specimens of each species were collected from the study area. The 10 morphological landmarks were selected along the outline of the specimens. The number of landmarks based on the respective species and the truss network were also changed on morphology. In the present study 8 to 10 points in landmark and 16 to 21 trust networks were recorded. Multivariate analysis of variance (ANOVA) was carried out to test the significance of morphological differences among the study species. The principal component analysis (PCA) indicated shape variation and explained the total variance (66.03%, 79.44%, 64.78% and 69.66 %) of *N. notopterus*, *M. zebrinus*, *A. testudineus* and *T. pectoralis* respectively. Discriminant function analysis (DFA) was accounted for three morphological indices in *M. zebrinus* , four morphological indices in *N. notopterus* and *A. testudineus*, and six morphological indices in *T. pectoralis* of the within group variability. Individuals into their original group were correctly classified and the result showed 100% in *N. notopterus*, *M. zebrinus* and *T. pectoralis* and 95% in *A. testudineus*. All statistically analyses were carried out using SPSS Software Package Version 25.

Keywords: Landmarks, Truss network, Morphometric variations and Some fishes

Introduction

The estimation of morphological variation within species population could be performed using conventional and truss morphometric approaches (Marcus *et al*,1996). Morphometric is defined as the study of quantitative analysis such as size and shape of living organisms, which for understanding the taxonomy as well (Park, *et. al.,* 2013). Image analysis systems have clearly played an important role in the advancement of morphometric research. Morphometric methods are now more powerful for stock identification through improved data collection, better description of shape, and the potential of new analytical techniques (Walker, 1997). Landmark-based morphometric was carried out to determine the variation of population structure and develop breeding program for sustainable production and conservation (Hossain *et al.,* 2015). The technique using morphometric imposes no restrictions on the direction of variation and localization of shape change and is highly effective in capturing information about the shape of an organism (Cavalcanti *et al*., 1999). Truss network systems constructed with the help of landmark points are powerful tools for stock identification (Turan *et al.*, 2004). Data points were arranged in a "Trusses" around the fish which layout minimize the numbers of measurements and increases the sensitivity of the analysis (Hossain *et al.*, 2010).

The relationships between various body parts of fish can be used to assess the well-being of individuals. Several techniques have been proposed for stock identification, an essential aspect of

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fisheries science management, which focuses on recognizing self-sustaining components within natural populations (Cadrin *et al*., 2004). In Myanmar, numerous studies have examined the taxonomy, distribution, and habitats of freshwater and marine fish across various river systems and floodplains (Day, 1978). Key rivers, such as the Ayeyarwady, Sittaung, and Thanlwin, flow into the coastal zones, supporting diverse aquatic ecosystems (Myint Pe, 2002). Among the fish species in these areas, *Notopterus notopterus* (Pallas, 1769) (Bronze featherback ,locally known as Nga-pe), *Macrognathus zebrinus* (Blyth, 1858)(Zebrafish spiny eel , Nga-mwedo) , *Anabas testudineus*, (Bloch, 1792) (Climbing Perch, Nga-bye-ma) and *Trichogaster pectoralis* Regan, 1910 (Snakeskin gourami , Belar) are significant food sources and play important ecological roles. The study was conducted at Thaung Pyin "In" in Thegon Township, Bago Region, which is fed by several chaungs originating from the Bago Yoma and Pyay Mountain ranges. This research aims to explore and assess their morphometric variations in the same stock. The objectives of this study are to examine and compare the morphometric differences in within species and between species of studied fish.

Materials and Methods

Study Area and Period

The study was conducted at Thaung Pyin “In,” part of the Inma “In” complex in Thegon Township, Bago Region , located around latitude 18∘32′2.26″ N and longitude 95∘21′16.55″ E (Plate 1). A total of 80 individuals (20 for each species) were collected directly from fishermen during December 2023 to August 2024. The collected specimens were immediately transferred to a cold box for transport to the laboratory for identification and morphometric measurements, following the classification systems of Talwar and Jhingran (1991) and Froese and Pauly (2018).

Truss network system

The variation in fish shape and size was assessed using the truss network system. The specimen was laid on the sandwich sheet into right body posture and fins were into a natural position. For example, ten of the morphological landmarks were chosen along the outline of the specimen and pinned down with long different color pins. Then removed specimen, the pinning positions were marked by using the permanent marker pen. Measurements were taken on 21 inter-landmark distances between 10 homologous landmarks using a standard truss network protocol (Fig. 1 and Plate 2) (Strauss and Bookstein, 1982). When the number of landmarks change, the number of truss network measurements known as inter- landmark distances may also change. The identification and classification of the collected fish species were followed after Talwar and Jhingran, 1991 and Froese and Pauly, 2018.

Data analysis

Necessary to remove size-dependent variations from all of the measured morphometric characters, use allometric formula as proposed by Elliott *et al*., 1995.

$M_{adj} = M\,(L_s / L_o)^b$, where M is the original measurement, M_{adj} = The size-adjusted measurement, L_s is the overall mean of the total length samples in each analysis, L_o is the total length and b is the slope of the regression coefficient, was calculated for each character from the observed data as the slope of log M on log L_o. One way ANOVA was used to test the significance of 21

morphological differences. Wilk's lambda (λ) was used to assimilate the variation between and among the entire population. PCA (Principal component analysis) were used decreasing redundancy among the morphometric variables and removing plentiful autonomous variables was carried out Veasey *et al.,* 2001 and Samaee *et al.,* 2006. SPSS (version 25) was used for statistical analyses.

Calculation of Truss measurement

The number of truss measurements was calculated by equation based on landmarks.

If n = 10, truss measurement number = $\frac{5n}{2} - 4$

$= \frac{5\ x\ 10}{2} - 4 = 21$ (Strauss & Bookstein, 1928)

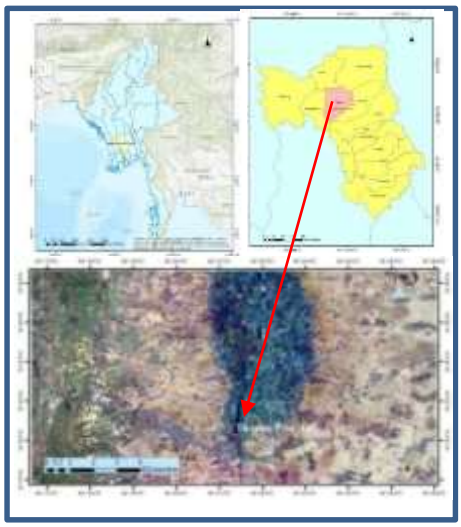


Plate 1 Map of Study Area

A.*Notopterus notopterus* B.*Macronagthus zebrinus* C. *Anabas testudineus* D. *Trichogaster pectoralis*

Plate 2 Location of landmarks on the fish

A.*N. notopterus* B.*M. zebrinus* C. *A. testudineus* D. *T. pectoralis*

Figure. 1 Constructing truss network between landmarks

Table 1 Location of landmarks and data sheet for Morphometric measurements

Point	***Notopterus notopterus***	***Macronagthus zebrinus***	***Anabas testudineus* / *Trichogaster pectoralis***
1	Tip of the snout	Tip of the snout	Tip of the snout
2	Upper end of the operculum	Origin of the eye	Upper end of the operculum/ End of the eye

Point	***Notopterus notopterus***	***Macronagthus zebrinus***	***Anabas testudineus / Trichogaster pectoralis***
3	Beginning of the scaled nape	Origin of the dorsal fin base	Original of the dorsal spine fin / End of the skull
4	Origin of the dorsal fin	End of the dorsal fin base	End of the dorsal spine fin / Origin of the dorsal fin
5	Middle of the caudal fin	Upper origin of the caudal fin	End of the dorsal soft fin/ End of the dorsal fin
6	Opposite of origin of the dorsal fin	Lower origin of the caudal fin	Middle of the caudal fin/ Upper of caudal fin
7	Origin of the pelvic fin	Origin of the anal fin base	End of the anal soft fin /Middle of caudal fin
8	Origin of the pectoral fin	Origin of the pectoral fin base	End of the anal spine fin /Lower of caudal fin
9	End of the maxilla		Origin of the anal spine fin/ Anterior origin of the anal fin
10			Origin of the pelvic fin base/ Anterior origin of the pelvic fin

Results

Four different species of fish, *Notopteurus notopterus, Macrognathus zebrinus, Anabas testudineus* and *Trichogaster pectoralis* belonging to four families, four genera under three orders were studied. A total of 20 specimens of each species were examined, making 80 specimens in total.

Table 2 Total length of fish species (n= 20)

Sr. No	Species	Total length(mm)		Weight (g)	
		Range	Mean ± SD	Range	Mean ± SD
1	*Notopterus notopterus*	236 – 269	249.30 ±10.04	94-128	109.7 ± 10.01
2	*Macronagthus zebrinus*	130 – 167	149.10 ± 9.58	10-17	13.0 ± 2.03
3	*Anabas testudineus*	114-138	125.35 ± 6.69	26-39	32.05 ± 4.08
4	*Trichogaster pectoralis*	174 – 207	193.55 ± 7.49	77-144	121.1 ± 16.12

The length range and mean with standard deviation for each specimen were shown in table 2. Among the morphometric measurements, one way ANOVA test showed that all truss measurements for two species were highly significant ($P < 0.001$) with Wilk's lambda value $0 < \lambda < 1$. The eigen values of principal component analysis of was *N. notopterus* three factors (6.57 for PC1, 3.47 for PC2 and 1.85 for PC3), *M. zebrinus* was three factors (9.11 for PC1, 2.08 for PC2, 1.52 for PC3), *A. testudineus* (8.44 for PC1, 3.08 for PC2 and 2.09 for PC3) and *T. pectoralis* (10.36 for PC1, 2.41 for PC2 and 1.86 for PC3) in table 3. The principal component analysis (PCA) of 18 morphometric measurements of *N. notopterus* yielded three principal components explaining 66.03 % of the total variance (36.52 % for PC1, 19.26% for PC2 and 10.25 % for PC3), 16 morphometric measurements of *M. zebrinus* yielded three principal components explaining 79.44 % of the total variance (56.96 % for PC1, 12.98% for PC2 and 9.51% for PC3), 21 morphometric measurements of *A. testudineus* yielded three principal components explaining 64.78 % of the total variance (40.17 % for PC1, 14.68% for PC2 and

9.93% for PC3) and 21 morphometric measurements of *T. pectoralis* yielded three principal components explaining 69.66 % of the total variance (49.33 % for PC1, 11.49% for PC2 and 8.84% for PC3)in the entire dataset (Table 3).

Table 3 Eigen-value, percentage of variance and percentage of Cumulative for the study species

Species	Factor	Extraction sums of square		
		Eigen-value	% of variance	Cumulative%
Notopterus notopterus	1	6.57	36.52	36.52
	2	3.47	19.26	55.78
	3	1.85	10.25	66.03
Macrognathus zebrinus	1	9.11	56.96	56.96
	2	2.08	12.98	69.93
	3	1.52	9.51	79.44
Anabas testudineus	1	8.44	40.17	40.17
	2	3.08	14.68	54.85
	3	2.09	9.93	64.78
Trichogaster pectoralis	1	10.36	49.33	49.33
	2	2.41	11.49	60.82
	3	1.86	8.84	69.66

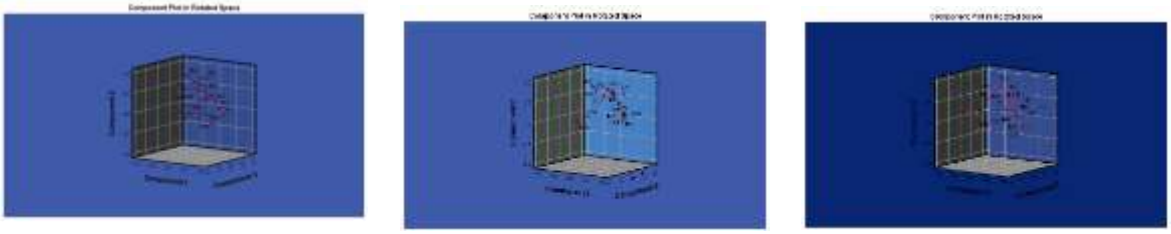
The distance codes of *N. notopterus* are HL2, Head length 2, landmarks1 to 9, HL3, Head length 3, landmarks 2 to 3, HD1, Head diagonal 1, landmark 2 to 8 and BL4, Body length 4, landmarks 6 to 7. The distance codes of *M. zebrinus* are BW2, Body width 2, landmarks 3 to 8 and BD5 and Body diagonal 5, landmarks 1-8. The distance codes of *A. testudineus* BL2, Body length 2, landmarks 4 to 5, BW3, Body width 3, landmarks 5 to 7, BD6, Body diagonal 6, landmarks 5 to 8 and BL5, Body length 5, landmarks 7 to 8. The distance codes of *T.pectoralis* HL3, Head length 3, landmarks 2 to3 and CFL1, Caudal fin length 1, landmarks 6 to 7(Table 4).

As the extraction in PC analysis, PC1 showed that the low standardized score was HL3 (-0.21) and PC2 also showed that the low standardized score was BL 4 (-0.20) component plot in rotated space also revealed the low neuroticism (HL2 and HD1) of *N. notopterus*. PC1 showed that the low standardized score was BW2 and BD5 (0.02) and PC2 also showed that the low standardized score was BW2 (0.08) component plot in rotated space, also revealed the low neuroticism (BW2) of *M.zebrinus*. PC1 showed that the low standardized score was BL2 (-0.19) and PC2 also showed that the low standardized score was BW 3 (-0.34) component plot in rotated space also revealed the low neuroticism (BD6 and BL5) of *A. testudineus*. PC1 showed that the low standardized score was HL3 (-0.05) and PC2 also showed that the low standardized score was CFL1 (-0.26) component plot in rotated space also revealed the low neuroticism (HL3 and CFL1) of *T. pectoralis* (Table 4 & Fig.2).

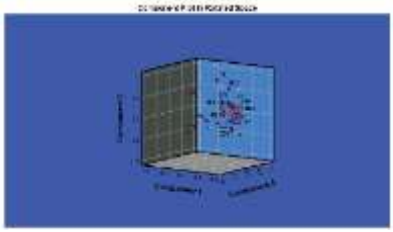
Table 4 Result of factors extraction in PC analysis after varimax normalized rotation in the study fish species

Species	Distance code	PC1	PC2	PC3
Notopterus notopterus	HL2 *	0.16	0.14	0.73
	HL3	-0.21	0.87	-0.09
	HD1*	-0.15	0.74	0.35
	BL4	0.77	-0.20	0.28
Macrognathus zebrinus	BW2*	0.02	0.08	0.76
	BD5	0.02	0.87	0.16
Anabas testudineus	BL2	-0.19	0.54	-0.01
	BW3	0.62	-0.34	0.39
	BD6*	0.09	-0.08	0.89
	BL5*	-0.01	0.26	0.83
Trichogaster pectoralis	HL3*	-0.05	0.65	0.56
	CFL1*	-0.00	-0.26	0.82

The discriminant function analysis of *N. notopterus* showed two morphological indices defining 100.0% (DF1),0.0%(DF2),0.0%(DF3) and 0.0%(DF4) as the morphological differences. The truss distances with important loading on DF1 were shown with total variance of 100.0%. The discriminant function analysis of *M. zebrinus* showed two morphological indices defining 97.4% (DF1),2.3%(DF2) and 0.3%(DF3) as the morphological differences. The truss distances with important loading on DF1 were shown with total variance of 97.4%. The discriminant function analysis of *A. testudineus* showed two morphological indices defining 91.8% (DF1),5.2% (DF2),2.2% (DF3) and 0.8% (DF4) as the morphological differences. The truss distances with important loading on DF1 were shown with total variance of 91.8%. The discriminant function analysis of *T. pectoralis* showed two morphological indices defining 93.8% (DF1),4.2% (DF2),1.2% (DF3), 0.4% (DF4), 0.3% (DF5) and 0.1% (DF6) as the morphological differences. The truss distances with important loading on DF1 were shown with total variance of 93.8% (Table 4).



A. *Notopterus notopterus* B. *Macronagthus zebrinus* C. *Anabas testudineus*



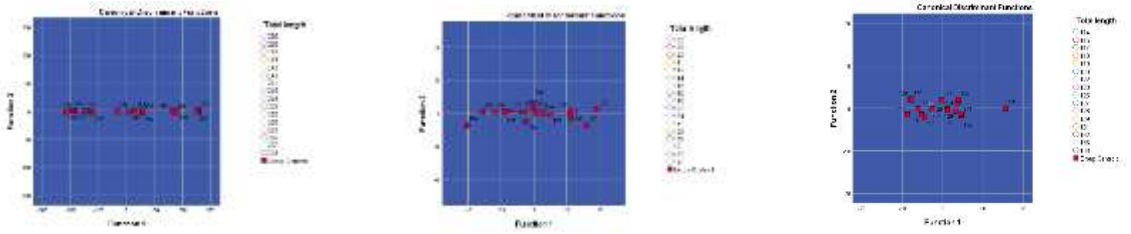
D. *Trichogaster pectoralis*

Figure. 2 Showing of score plots of the four fish species

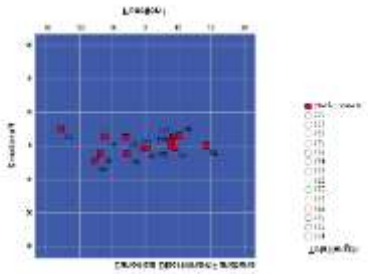
Table 5 Pooled within-groups corrections between discriminating variables and standardized canonical discriminant function of landmark distances of the four fish species

Species	DF1	DF2	DF3	DF4	DF5	DF6
Notopterus notopterus	100.0%	0.0%	0.0%	0.0%	-	-
Macrognathus zebrinus	97.4%	2.3%	0.3%	-	-	-
Anabas testudineus	91.8%	5.2%	2.%	0.80%	-	-
Trichogaster pectoralis	93.8%	4.2%	1.2%	0.4%	0.3%	0.1%

Plotting of canonical discriminant functions of *N. notopterus* showed more or less overlapping among the 20 individuals of for both morphometric (Total length) and 18 truss measurements in discriminant space. All total lengths were intermingling among population. Plotting of canonical discriminant functions of *M. zebrinus* showed a clear overlapping among the 20 individuals for both morphometric (total length) and 16 truss measurements in discriminant space. All total lengths were intermingling among populations. Plotting of canonical discriminant functions of *A. testudineus* showed more or less overlapping among the 20 individuals for both morphometric (total length) and 21 truss measurements in discriminant space. More sample total length was intermingling among each total length and some of these were less missing. Plotting of canonical discriminant functions of *T. pectoralis* showed more or less overlapping among the 20 individuals for both morphometric (total length) and 21 truss measurements in discriminant space. All total lengths were intermingling among population (Fig.3).



A. *Notopterus notopterus* B. *Macronagthus zebrinus* C. *Anabas testudineus*



D. *Trichogaster pectoralis*

Figure.3 Centroid of discriminant function score plot of the four fish species

A correct classification of individuals into their original population of *N. notopterus* varied 100% by discriminant analysis (DF1 & DF2) and 100% of individuals group cases could be classified in their correct prior grouping. A correct classification of individuals into their original population of *M. zebrinus* varied 99.7% by discriminant analysis (DF1 & DF2) and 100% of individuals group cases could be classified in their correct priori grouping. A correct classification of individuals into their original population of *A. testudineus* varied 97.0% by discriminant analysis (DF1 & DF2) and 95% of individuals group cases could be classified in their correct prior grouping. A correct classification of individuals into their original population of *T. pectoralis* varied 98.0% by discriminant analysis (DF1 & DF2) and 100% of individuals group cases could be classified in their correct prior grouping.

Discussion

Islam and Mollah (2012) expressed the efficiency of the allometric formula in removing the size effect from the data was justified by using correlation between total length and adjusted characters. That is why total length was excluded first and not transformed because using this parameter as standard all other parameters were standardized. Sathianandan (1999) also explained morphometric studies by statistical methods were based on a set of traditional measurements which were providing uneven and biased aerial coverage of the entire body form of the specimen. Truss network provides a more systematic and geometric characterization of fish shape. According to above knowledge, the present study of fish measurements was done by truss network system. Analysis of truss network measurements in the present study was done by principal component analysis (PCA). Joseph and Godwin (2000) explained that PCA does not require any prior information about the groups in the analysis of truss data. In the present study univariate analyses showed that morphometric characteristics of the study four species were significantly different among the certain group ($P < 0.05$). Wilk's lambda values of all network morphometric measurements were $0 < \lambda < 1$ so they were shown significantly differences. All 18 landmark measurements of *N. notopterus* ,16 landmark measurements of *M. zebrinus,* 21 landmark measurements of *A. testudineus* and *T. pectoralis* were found highly correlated with total length. Strauss and Bookstein (1982) reported that the factor analysis is related to principal component analysis but the two are not identical. Eigen-values of factor analysis are contaminated with error variances. Multivariate techniques simultaneously consider the variations in several characters and there by assess the similarities among the samples. The key characters used for the discrimination of the body form were those that have high eigenvalues. Thit Thit Lwin (2023) showed that the eigen-values of factor analysis are study in morphometric variation of some fish species from Weadaunt Village group of Sittaung River Basin, Bago Region based on truss network analysis. In this study, variation of eigen-values of factor analysis (FA) was found the study species. Eigen-values of two factor analysis *Mystus pulcher, Oreochromis mossambicus* and *Channa panaw* and three factor analysis *Mystus microthalmus.* In the present study, variation of Eigen-value of three factor analyses were *N. notopterus, M. zebrinus, A. testudineus* and *T. pectoralis.* The Eigen- value and factor analysis were missed the prior and present study because of the different study sites and commanded facts (value and factor). Stearns, 1983 stated that the phenotypic plasticity of fish is very high. They adapt quickly by modifying their physiology and behavior to environmental changes. These

modifications ultimately change their morphology. Analysis of truss network measurements in the present study was done by principal component analysis (PCA) to know the variation of fish size and shape withing the group of fish species. In the present study, principal component analysis (PCA) was used to assess or observe the variation of within species by plotting the Principal Component scores (PC). The PCs which revealed significant differences were used for XY plots (PC1 and PC2). In the present study, the score on the scatter plot of *N. notopterus*, *M. zebrinus*, *A. testudineus* and *T. pectoralis* were indicated the size variation of network measurements in morphological and no shape effect variation in this population. Karakousis *et al.* (1991) stated that discriminant function analysis (DFA) could be a useful method to distinguish different stocks of the same species. Hossain *et al*. (2015) stated that in the discriminant functional analysis (DFA), the first DF accounted for much more of the within group variability than did the remaining other DFs. It was obvious that the other DFs explained much less of the variance than the first DF. Therefore, the other DFs were much less informative in explaining difference within the population.The present study, although the results of discriminant function analysis (DFA) of the study species were correctly classified individuals for three species 100% (*N. notopterus, M. zebrinus and T. pectoralis)* except *A. testudineus* 95% into their original population in discriminant space and from the principal component analyst's perspective, the morphological structure of only species was size variation and other three species had no size and shape variation with less missing variables in their respective populations. In the present study, *A. testudineus* was the size differentiation of the original group. They were collected and examined from the same site, Thaung Pyin "In". Therefore, observed morphological variations of study species might be due to environmental factors and genetic differences within these populations.

Conclusion

An allometric formula was used for size - adjustment transformation for all characters and multivariate analysis. including Factor Analysis, Principal Component Analysis and Discriminant Functional Analysis were utilized. Truss network analysis, in combination with these multivariate statistical methods, effectively tested and displayed morphological differences in both size and shape. The current findings provide valuable preliminary baseline information on the morphometric variation within each species in Thaung Pyin "In". Future studies should investigate whether the observed morphological differences in fish are due to genetic factors or environmental conditions. Studying the same species in different stocks in the future will offer more comprehensive insights for comparing morphometric variation across fish populations.

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ANALYSIS OF HEAVY METAL IN SOME FISH SPECIES FROM RICE-FISH FARM OF WEST TARPET VILLAGE, MAUBIN TOWNSHIP, AYEYARWADY REGION

Soe Myint Khine[1], Cho Cho Thin[2], Kay Lwin Tun[3]

Abstract

Rice-fish systems can be broadly defined as the cultivation of rice alongside the simultaneous or rotational presence of naturally occurring fish and other aquatic species that are harvested through fisheries, as well as introduced fish populations that are cultured. Fish species are vital for maintaining ecological health and are also economically important for human consumption. In this study, the seasonal variation of heavy metal concentrations (As , Cd and Pb) which bio-accumulated in muscle tissues of three fish species (*Catla catla, Labeo rohita, Puntius gonionotous*) cultured in the rice-fish farm located at West Tarpet Village, Maubin Township were analyzed. Seasonal variation of metal concentrations in water and soil from rice- fish farm were also investigated during the study period. The concentrations of heavy metals in collected specimens were analyzed in the Universities' Research Center (URC) at University of Yangon. The levels of metal concentrations varied depending on the seasons and the different fish species. The highest concentration of As was found in *Catla catla* in the cold season and Cd in *Puntius gonionotus* in the hot season but the results did not exceed the WHO/FAO maximum permissible limits. Pb concentration of *Catla catla* in the rainy season was found to be the highest and over-established by WHO/FAO. The Cd and Pb concentrations of river water were highest in the hot season and in the rainy season; the results were higher than WHO/FAO. The average concentration of Pb in the rainy season of soil sample was the highest and observed to be beyond the WHO/ FAO limit. Based on the results of the present study, the concentration levels of Pb in soil, water samples and, *Catla catla*, as well as Cd in river water exceeded the acceptable limits recommended by WHO/FAO. Significant relations between As and Cd concentrations in the muscle tissue of the fish species and their size (total length) and (body weight) were observed. According to the results of the transfer factor, all tested metals accumulated in muscle tissues of studied fish species came from their abiotic media. The concentration of heavy metals in muscle tissues of *Labeo rohita* and *Puntius gonionotus* were not exceed the permissible limit of WHO/FAO, 2011 and there was no a risk for the public by eating these species.

Keywords: rice- fish farm, heavy metal, muscle tissue, soil, water, fertilizer, transfer factor

Introduction

Rice-fish farming system experimental trials in Myanmar's Ayeyarwady Delta and Letpadan Township, Tharyarwady District, Bago Region were implemented in 2017. Rice-fish systems are helping the people of Myanmar to increase their income from rice fields and enrich the supply of aquatic products while only having minimum paddy modifications (Kalyoncu *et al*., 2009). Traditionally, farmers and neighboring households in South and Southeast Asian countries caught wild fish from water bodies in and around rice fields for household consumption and as an alternate source of income (Halwart and Gupta 2004; Dey *et a*l. 2013). Not only the adequate supply of carbohydrate, but also the supply of animal protein is significant through rice-fish farming. Fish are rich in micronutrients and vitamins, and thus human nutrition can be greatly improved through fish consumption (Larsen *et al*., 2000; Roos *et al*., 2003). Fish accumulate contaminants directly from water and/or through the food chain. Since fish are at higher trophic levels in aquatic ecosystems, metal concentrations in fish muscle could be several orders of magnitude higher than in the environment, resulting in a cumulative impact of toxic metal accumulation in humans through chronic consumption (Chen *et al*., 2018). The exposures of

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metals through animal-derived food like fish intake are normally higher than plant-derived food, and it would be necessary to clarify the exposure level of heavy metals during the consumption of rice - fish farming system fish, especially for the rice fields that have been polluted. In addition, Soil contamination by heavy metals has been accelerated in many parts of the world in the past few decades, due to anthropogenic activities (Facchinelli *et al*., 2001; Wei and Yang, 2010). With the increasing threat of heavy metals in soil, the RFS is facing increasing risks (Grant *et al*., 2008)

Fish acts as the primary source of animal protein within the diet of the Myanmar population; with individual consumption rates estimated at 30 kg per person per year (WorldFish, 2017). Bioaccumulation of heavy metals in fish causes serious threats to human when they are consumed. Heavy metals have attracted attention due to their ubiquity in materials, resistance to biodegradability, and causations of numerous illnesses like gastrointestinal, hematological, hepatic, renal, and neurological problems, as well as carcinogenic effects (Nogawa, K., *et al*, 2017; Rahman, M.S., *et al.*, 2012).

The economy of the study area is predominantly agricultural, the farmers use chicken manure, combined with NPK fertilizers, other kinds of fertilizers, and pesticides to achieve high yields of their crop which means rice-fish farms could be at risk of heavy metals. Therefore, the risk assessment of heavy metal concentration in fish for human health is important and necessary. Due to above- mentioned reasons, the present study was carried out to assess the content of heavy metals residues that bio-accumulated in muscle tissues of three fish species from rice-fish farm.

Materials and Methods

Study Area

Study area is situated at West Tarpet Village, Maubin Township, Ayeyarwady Region, between 16° 43′ N and 95° 39′ E (Fig. 1 and Plate 1). A total area of 4046.85m^2 of rice - fish farm was chosen as study site.

Study Period

Study period lasted from June 2023 to May 2024.

Collection of Samples

Three fish species (*Catla catla*, *Labeo rohita*, *Puntius gonionotus*) were seasonally collected from rice-fish farm. Collected fish specimens were identified followed by Talwar and Jhingran (1991). Water samples were collected seasonally from the rice-fish farm, reservoir, and river. Seasonal sampling was conducted during the rainy (August), and cold (December) and hot (April) seasons of 2023-2024. Fertilizer samples (compound (fertilizer 1) and nitrogenous fertilizers (fertilizer 2)) used in the study area and soil from this area were also collected to assess heavy metal content.

Sample preparation

The collected fish specimens were washed with tap water, and their total length (cm) and body weight (g) of specimens were measured. The muscles were then removed and weighted, dried in an oven at 90˚C until a constant weight was achieved, and stored in airtight containers. Digestion of the samples was carried out according to the dry method by using a furnace. Water samples were filtered through a 0.45µm Whatman filter and analyzed directly by AAS. The soil sample was sun-dried, grounded and sieved to obtain a fine powder. Fertilizer samples were also ground into a fine powder to facilitate further analysis. Digestion of the samples was conducted according to the dry method using a furnace.

Data Analysis

The heavy metal content in the muscles of the collected fish species, as well as in the water, soil, and fertilizers samples were analyzed in triplicate using a Flame Atomic Absorption Spectrometer (FAAS) (Perkin Elmer AA analyst 800 and Winlab-32 software) in Universities' Research Centre (URC) at Yangon University. Seasonal variations in the test results were compared with the maximum permissible limits set by WHO/FAO in 2011 (MPL). The transfer factor (TF) in muscle tissues from the abiotic media, which include water and soil was calculated according to Kalfakakour and Akrida- Demertzi (2000) and Rashed (2001) and Irenosen and Ishmael (2019) as follows:

$$TF = \frac{\text{concentration of metal in fish tissue}}{\text{concentration of metal in abiotic media (water/soil)}}$$

TF value is greater than 1 indicates that there is the bioaccumulation of metals in fish tissue. The relationship between heavy metal concentration (As, Cd and Pb) in muscle tissues and size of fish species were assessed by using the linear regression method (Le Cren, 1951).

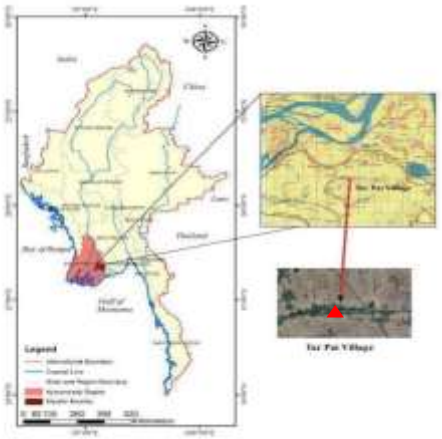

Figure. 1 Mape of study area, West Tarpet village (Source: Google)

Results

Three fish species (*Puntius gonionotus*, *Catla catla*, *Labeo rohita*) were collected seasonally from rice - fish farm in West Tarpet Village, Ayeyarwady Region (Fig. 1). The total length and total weight of the collected fish species were presented in table 1. The seasonal variation in the mean concentration of heavy metals (arsenic, cadmium and lead) in different fish samples were presented in fig. 2, 3 and 4 respectively. In the cold season, the highest value of arsenic concentration in muscle tissues of *Catla catla* was 0.00308mg/L (Fig. 2).

In the present study, the seasonal variation of cadmium concentration in study fish species was shown in Figure 3. In the rainy season, the highest average value of cadmium in the muscle tissue of *Puntius gonionotus* was 0.031 mg/L. In the present study, the highest value of lead concentration in the muscle of *Labeo rohita* was 0.334 mg/L in the hot season (Fig. 4).

In the result of the current finding, the metal concentration levels of arsenic, cadmium and lead in water samples for seasonal variations were presented in Figs. 5, 6 and 7 receptively. In the cold season, the highest value of arsenic concentration in the rice-fish farm water was 0.00145mg/L (Fig. 5). Among the water samples, only the river water was observed with the

highest concentrations of cadmium (0.038mg/L) and lead (0.295mg/L) in the rainy season (Fig. 6 and 7). In addition, the seasonal variation of metal concentrations in the soil sample was also determined during the study period (Fig. 8). The highest value of arsenic, cadmium and lead concentrations in the soil sample were found 0.00665 mg/L, 0.006mg/L and 0.2mg/L in the rainy season followed by hot season while those were the lowest in cold season. The concentrations of fertilizers used in rice-fish farm were also detected in the study period (Fig.9). The highest values of arsenic, cadmium and lead concentrations in fertilizers were 0.01315mg/l, 0.027mg/l and 0.391mg/l respectively.

Regression coefficient values of the relationship between arsenic and cadmium concentrations in muscle tissue and total length of all studied fish species were found 0.5416 to 0.9998 and 0.1049 to 0.2966. Positive correlation between arsenic and cadmium concentrations and total length and the body sizes of studied fish species were observed. In the present study, linear regression analysis between heavy metal concentrations in muscle tissues and body sizes of fish species was conducted. Regression coefficient values of the relationship between lead concentrations in muscle tissue and total length of all studied fish species were found 0.898 to 0.9983. Linear relation between lead concentration and the body sizes of studied fish species were negatively related (Table 4). Index values of the transfer factor of three heavy metals from water and soil of abiotic-media to muscle tissue of the studied fish species were present in table 5. It is found that the transfer factor of heavy metal in muscle tissue from water and soil was observed to be greater than that of the limitation value of 1.

Table 1 Mean total length (cm) and body weight (g) of studied fish species to test metal concentration

Species	Rainy		Cold		Hot	
	Total length(cm)	**Weight (g)**	**Total length(cm)**	**Weight(g)**	**Total length(cm)**	**Weight(g)**
Catla catla (n=10)	6.72 ± 0.59	11.64 ± 1.25	25.23 ± 13.2	325 ± 115.3	28.23 ± 11.25	445 ± 22.15
Labeo rohita (n=10)	10.28 ± 0.74	17.29 ± 4.83	23.2 ± 11.23	300.5 ± 12.35	30.45 ± 12.3	405 ± 26.47
Puntius gonionotus (n=10)	8.37 ± 1	11.83 ± 1.31	25.32 ± 2.19	261 ± 122.38	27 ± 0.7	300.61 ± 43.65

Table 2 Maximum permissible limit of three metals in fish and water samples (WHO/FAO, 2011)

Sr.no	Types of sample	Metals		
		Arsenic	**Cadmium**	**Lead**
1	Fish	0.5	0.5	0.3
2	Water	0.01	0.01	0.01

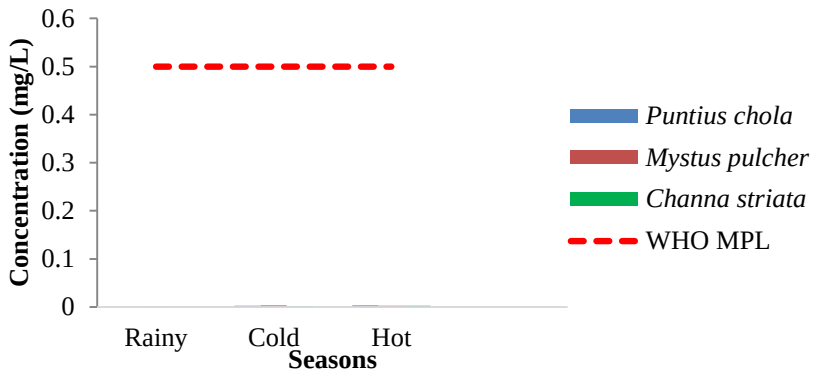

Figure. 2 Seasonal variation of Arsenic concentration in studied fish species

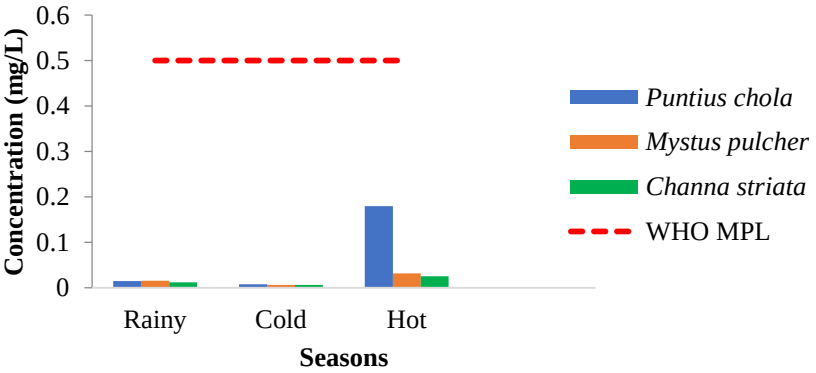

Figure. 3 Seasonal variation of Cadmium concentration in studied fish species

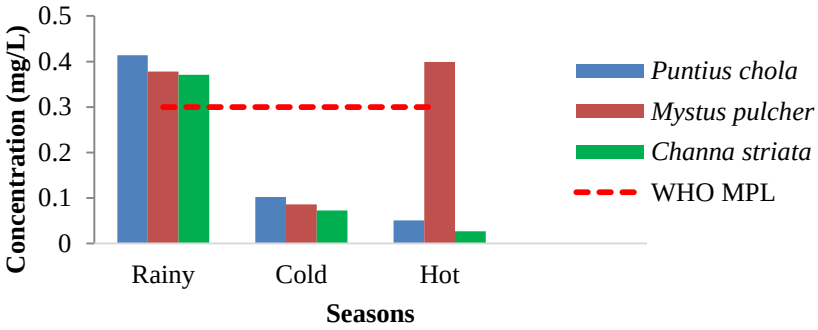

Figure. 4 Seasonal variation of Lead concentration in studied fish species

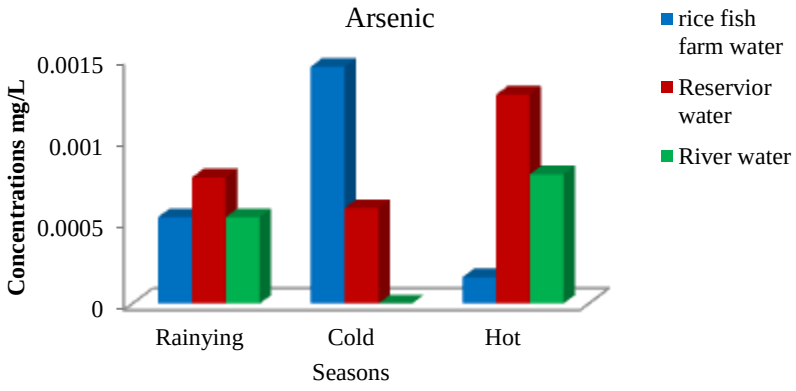

Figure. 5 Seasonal variation of Arsenic concentration in water samples of study area

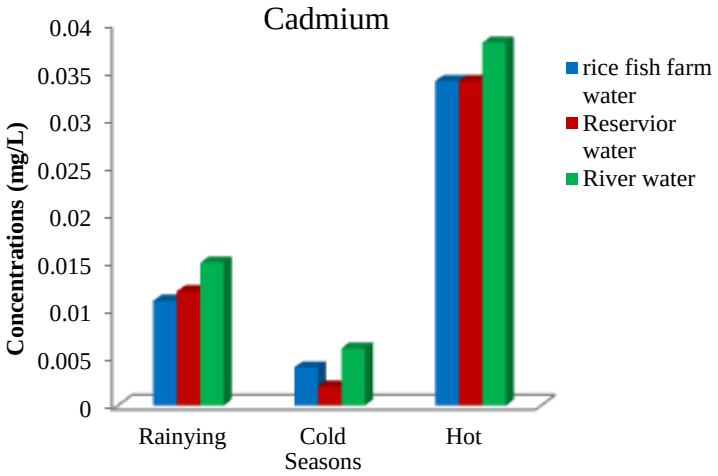

Figure. 6 Seasonal variation of cadmium concentration in water samples of study area

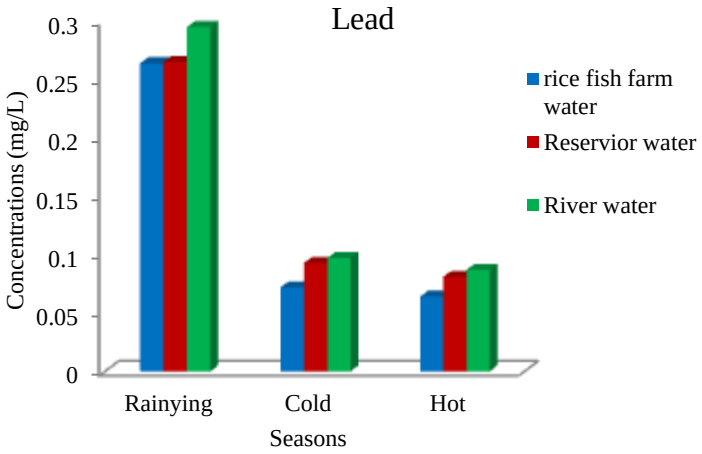

Figure. 7 Seasonal variation of lead concentration in water samples of study area

Table 3 Maximum permissible limit and effects of metals concentration in soil sample

No.	Metals	WHO,2007 MPL	TEC	MEC	PEC
1	Arsenic	0.01	9.8	21.4	33
2	Cadmium	0.01	0.99	3	5
3	Lead	0.05	36	83	130

TEC= Threshold effect concentration, MEC= Midpoint effect concentration,
PEC= Probable effect concentration

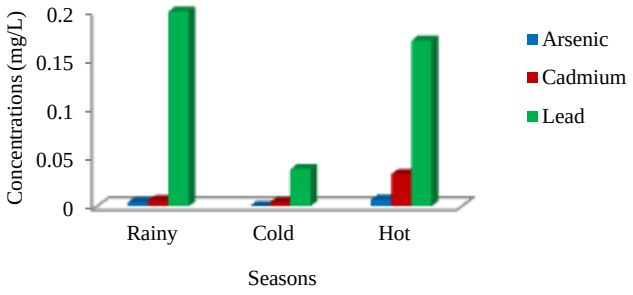

Figure. 8 Seasonal variation of metals concentration in soil samples

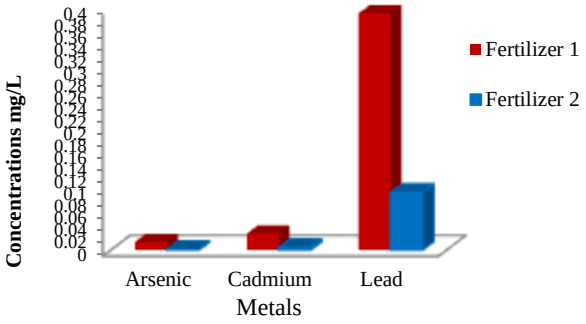

Figure. 9 Concentration of heavy metal in fertilizers

Table 4. Relationship between body length (cm) and body weight (g) of fish and heavy metal concentrations in muscle tissue of studied fish species

Species	Heavy metals	Length	Remarks	Weight	Remark
		Linear equation		Linear equation	
Catla catla	As	y = 0.0003x-0.0025 R^2 = 0.7207	Positive relation	y = 2E-05x+0.0008 R^2 = 0.8378	Positive relation
	Cd	y = 0.0006x+0.0051 R^2 = 0.1721	Positive relation	y = 4E-05x+0.0065 R^2 = 0.2914	Positive relation
	Pb	y = -0.0142x+0.4283 R^2 = 0.9983	Negative relation	y = - 0.0007x+0.3331 R^2 = 0.9666	Negative relation
Labeo rohita	As	y = 0.0001x+0.001 R^2 = 0.9927	Positive relation	y = 5E-06x-9E-05 R^2 = 0.9998	Positive relation
	Cd	y = 0.0001x+0.0134 R^2 = 0.0413	Positive relation	y = 4E-05x+0.007 R^2 = 0.2106	Positive relation
	Pb	y = -0.0475x+1.5187 R^2 = 0.4432	Negative relation	y = -0.0007x+0.2894 R^2 = 0.9498	Negative relation
Puntius gonionotus	As	y = 9E-05x+0.0007 R^2 = 0.5865	Positive relation	y = 6E-06x+6E-05 R^2 = 0.5416	Positive relation
	Cd	y = 8E-05x+0.0117 R^2 = 0.0782	Positive relation	y = 4E-05x+0.0123 R^2 = 0.1337	Positive relation
	Pb	y = 0.0063x+0.1421 R^2 = 0.8966	Negative relation	y = -0.0009x+0.2885 R^2 = 0.9926	Negative relation

Table 5 The TF of heavy metals from water and soil in muscle tissues of studied fish species in all season

Species	Metals	Rainy		Cold		Hot	
		TF from water	TF from soil	TF from water	TF from soil	TF from water	TF from soil
Catla catla	As	0	0	2.12	0	5.32	0.13
	Cd	0.91	1.67	2.5	2.5	0.74	0.76
	Pb	1.27	1.67	0.88	1.66	0.53	0.2
Labeo rohita	As	0	0	0	0	12.55	0.3
	Cd	0.45	0.83	2.75	2.75	0.65	0.67
	Pb	1.1	1.44	0.54	1.03	0.44	0.16

Species	Metals	Rainy		Cold		Hot	
		TF from water	TF from soil	TF from water	TF from soil	TF from water	TF from soil
Puntinus gonionotus	As	0	0	1.74	0	6.58	0.16
	Cd	0.36	0.67	2.25	2.25	0.91	0.94
	Pb	1.06	1.4	0.71	1.34	0.63	0.24

Discussion

Heavy metals (As, Cd and Pb) in muscle tissue of three fish species (*Catla catla*, *Labeo rohita* and *Puntius gonionotus*) collected from the rice-fish farm in West Tarpet Village of Maubin Township, Ayeyarwady region were analyzed seasonally during the study period. Metal concentrations in the water and soil of the aquatic environment at the study site, as well as those in the fertilizers used in the rice-fish farm, were also assessed.

In the present study, the highest concentration of arsenic (As) was found in *Catla catla* (0.00308 mg/L) among the three species in the cold season. However concentration levels of arsenic were found to be lower than the WHO/FAO maximum permissible limits. Khin Thida Kyaw (2008) and Aye Aye Mu (2011) reported that arsenic concentrations of all studied fish species in all seasons in Daydaye and Hinthada Township, Ayeyarwady region did not exceed the WHO/FAO maximum permissible limit.

In the present study, cadmium (Cd) content was found to be highest in *Puntius gonionotus* (0.031mg/L) in the hot season. But concentration level of this metal was found to be lower than WHO/FAO maximum permissible limits. Yee Yee Win (2021) observed that Cd concentrations of all studied fish species from Thanlwin River Segment were found to be lower than the WHO/FAO maximum permissible limit during all three seasons.

In this investigation, the lead (Pb) concentration of *Catla catla* (0.334 mg/L) in the rainy season was found to be the highest and it showed over the recommended maximum permissible limit established by WHO/FAO. Cho Cho Thin (2017) revealed that lead concentration in the muscle tissue of herbivorous, carnivorous, and omnivorous fish was highest during the rainy season in the Ayeyarwady River segment of the Salay environs, which aligns with the findings of the present study. Kargin (1996) reported that multiple factors, including season and the physical and chemical properties of water, can significantly influence metal accumulation in various fish tissues.

The concentration of lead was found to be higher in water, fertilizer and soil samples in the present study. Similarly, the valuesobserved for lead concentration was the highest in fish species *Catla catla* in the present study. Ming Ho (2005) stated that food is one of the major sources of lead exposure; the others are air and drinking water. Plant food may be contaminated with lead through its uptake from ambient and soil; animals may then ingest the lead-contaminated vegetation. Therefore, lead concentrations in muscle tissues of the herbivores, *Catla catla* may transfer from water, fertilizer and soil due to bioaccumulation and may attain higher concentrations in such manners.

In all detected heavy metals, the concentrations of Pb in all studied fish species were found to be highest in the rainy season in this investigation. Lead is a non-essential and toxic metal that is released into the aquatic environment through the use of fertilizers (Pascoe and Mattery, 1997). In the study area, the farmer would use fertilizers to obtain high yields for their crops. In the rainy season, the concentration of heavy metals from fertilizers may come from the paddy fields and accumulate in the river and aquatic fish species. Arsenic (As) concentrations in

rice-fish farm water (0.00145mg/L) showed the highest in the cold season. But the result did not exceed the maximum permissible limit established by WHO/FAO. Seinn Moh Moh Paing, (2019) observed that arsenic concentrations in the water of Panhlaing River segment of Nyaung Done township were the highest in the cold season, which is similar to the present study's findings as well.

In this study, cadmium (Cd) concentration of river water (0.038mg/L) was highest in the hot season and the result was higher than the WHO/FAO maximum permissible limit. Lead (Pb) concentration of river water (0.295mg/L) was highest in the rainy season and followed by reservoir water (0.265 mg /L) and rice fish farm water (0.264 mg/L) and the obtained results were higher than the WHO/ FAO maximum permissible limit. Cogun *et a*l., (2006) stated that industrial discharge and agricultural runoff are forms of human activities that are released into rivers caused into water heavy metal pollution in water seasonally. In the present study, average concentration values of arsenic (0.00665mg/L) in the hot season, and cadmium (0.006 mg/L) in the rainy season of soil samples were the highest but did not exceed the permissible limit. Lead concentration (0.2 mg/L) in rainy season of the soil sample was the highest and observed to be beyond the WHO/ FAO maximum permissible limit.

According to the recorded data, all metals concentrations (As, Cd and Pb) in the sediment during the study period were observed to be lower than the "threshold effect concentration (TEC)", midpoint effect concentration (MEC)" and probable effect concentration (PEC)" (MacDonald *et al*., 2000). This is in agreement with the previous finding by Cho Cho Thin *et al*., (2022) described that Fe, Zn, Pb, Cd and As concentrations in the sediment of Kyet Mauk Taung Dam were observed lower than TEC, MEC and PEC.

In the present study, the concentration of lead (Pb) in compound fertilizer was observed to be the highest at 0.391 mg/L, followed by cadmium at 0.027 mg/L and arsenic at 0.01315 mg/L, respectively. In Nitrogenous fertilizer, the lead concentration (0.095mg/L), cadmium (0.004mg/L) and arsenic (0.00657mg/L) were detected. Edogbo *et al*., (2020) observed that organic fertilizers may also contain a different concentration of heavy metals applied to fishponds and soil. Base on the all recorded data of the present study, the concentration levels of Pb in soil, water samples and *Catla catla*, as well as Cd in river water exceeded the acceptable limits recommended by WHO/FAO.

In the present study, linear regression analysis between heavy metal concentrations in muscle tissues and body sizes of fish species was conducted. Significant relations between As and Cd (positive relation) concentrations in muscle tissues of all studied fish species and their body size (body length and body weight) were observed. Cho Cho Thin (2017) observed that the relationship between As concentration in muscle tissues of some carnivorous and omnivorous fishes and their body size were significant. Yee Yee Win (2021) stated that the length and weight of *Polynemus paradiseus* and As concentration were positively correlated in the study site. The result showed that significant decline in lead (Pb) concentrations with the increase in size in all studied fish species. Many researchers reported that Pb concentration were inversely proportional to the total length and body weight of fish (De Wet *et a*l., 1994; Canil and Atli., 2003. None of the samples recorded increasing Pb levels with increasing body weight as reported by Farkas *et al*., (2003) and Namin *et al*., (2001). Liang *et al*. (1999) studied metal (Zn, Cu, Cd, Cr, Pb, Ni) concentrations in various polycultured fish species and found that metals in fish viscera were negatively correlated (except Zn in viscera of common carp). One explanation for the negative relationships between metal concentrations and size found in this study may be the difference in metabolic activity between younger and older fish. The net accumulation of heavy metals in an

organism is a result of the difference between uptake and depuration and this is the most important factor in metal accumulation. The dilution of tissue metal concentrations due to growth and/or lower metabolic activity in older individuals may not be seen if metal concentrations in water are higher than the capacity of these factors. Body size has been shown to have a potentially strong effect on concentrations in muscle tissues and organs (Canli and Atli, 2003; Farkas *et al*., 2003; Yi and Zhang, 2012; Noël *et al*., 2013).

Rased (2001) reported that when the calculated transfer factor was higher than 1 it means that fishes bioaccumulate metals from the environment in all cases (eg. water, soil and plants). In the present study, the transfer factor (TF) of lead from water to muscle tissue of all studied fish species was observed in the rainy season and cadmium from soil in *Catla catla*. the lead from soil environment to muscle tissues of *Catla catla*, *Labeo rohita* and *Puntius gonionotus* was observed in the rainy season. In the cold season, the transfer factor of arsenic from water environment to muscle tissue of *Catla catla* and *Puntius gonionotus* was observed. Next, the transfer factors of Cd from the water environment to muscle tissues of all studied fish species were observed. And the transfer factor of Cd and Pb from the soil to muscle tissues of all fish species was also observed in the cold season. In this investigation, the transfer factor of As from water environment to muscle tissues of all studied fish species was found in the hot season. The enrichment of heavy metals in the environment can result from both anthropogenic activities and natural processes (Forstner and Whitman, 1979; Nriagu, 1989; Veena *et al*., 1997). In this study, most of transfer factor for water to fish and soil to fish showed greater than 1. This means that the fish undergo bioaccumulation of these tested metals from the abiotic media (water, soil and fertilizers) (Kalfakakour and Akrida- Demertzi, 2000). According to the index value of transfer factors, it may be assumed that Pb concentration in muscle tissues of *Catla catla* came from their abiotic media. The result revealed that the lead concentrations in *Catla catla* in studied fish species were higher than the permissible limit. However, other fish species from the study area were generally safe for human consumption, although the bioaccumulation of metals in fish tissue was slightly elevated. Therefore, regular monitoring of heavy metal levels in fish is necessary, and the public should be made aware of the environmental damage caused by human activities.

Conclusion

In the present study, the concentrations of heavy metals (As and Cd) in all examined fish species were found to be below the maximum permissible limits set by WHO/FAO in all three seasons. The concentration of heavy metal (Pb) in *Catla catla* was found to exceed the maximum permissible limit set by WHO/FAO during the rainy season, likely due to agricultural runoff of fertilizers and pesticides. Significant relations between As and Cd concentration in the muscle tissue of the fish species and their size (total length) and (body weight) were also observed. The results revealed that the concentration of Pb in all water samples, as well as in soil and the concentration of Cd in river water from the study area during the study period were higher than the maximum permissible limits set by WHO/FAO. All the study fish species, except for *Catla catla* from the study site, were below the maximum permissible limits set by WHO/FAO (2011) and were generally considered safe for human consumption. According to the results of the transfer factors, heavy metals accumulated in different tissues of the studied fish species primarily came from water and soil, and the level of bioaccumulation was found to be slightly elevated.

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BREEDING SUCCESS AND EFFECTS OF DIFFERENT DIETS ON THE GROWTH OF SIAMESE FIGHTING FISH, *BETTA SPLENDENS* REGAN, 1910

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Abstract

This study investigated the reproductive performance and subsequent growth rates of the Siamese fighting fish (*Betta splendens*). The breeding experiment compared the effects of two water types (tap water and 40% almond leaf extract water) and three nesting materials (Regiform , Plastic, and Banana leaf) on hatching and survival rates. Statistical analysis showed that the highest hatching rate (70.00%) occurred in tap water with a Plastic nest, while the highest survival rate (53.85%) was achieved in almond extract water with a Plastic nest, indicating the benefit of almond extract for fry survival. Additionally, a four-month growth trial was conducted on B. splendens fingerlings using five different diets: Daphnia (Diet I), Mosquito larvae (Diet II), Pellet feed (Diet III), Mixed feed (Diet IV), and Artemia cysts (Diet V). ANOVA results revealed that live and mixed feed sources—Mosquito larvae (83.72% weight gain), Mixed feed (80.49%), and Daphnia (74.42%)—resulted in significantly higher mean body weights compared to Artemia (P-values < 0.05). Conversely, Artemia showed the highest survival rate (100%), despite the lowest weight gain (41.46%). The findings offer valuable insights for optimizing breeding and feeding protocols in ornamental fish aquaculture.

Keywords: *Betta splendens*, breeding performance, growth rates, survival rates

Introduction

The genus *Betta* is a large group of small, colorful freshwater ray-finned fishes belonging to the gourami family, Osphronemidae. The best known *Betta* species is *Betta splendens*, the Siamese fighting fish, appeared to be small in size but considerable variation in size was noted. *Betta splendens*, Siamese fighting fish is native to tropical Southeast Asia including the northern Malay Peninsula, central and eastern Thailand, Cambodia, southern Vietnam and Myanmar (U.S. Fish and Wildlife Service, 2019).

They are cultured varieties produced through generations of selective breeding of the *B. splendens*. Therefore, selected breeding methods and captive breeding of Bettas are popular practices in the ornamental fish production market. During the breeding, Betta shows high parental care animal species. Bettas are bubble nest builders and nest guarders. The males build bubble nests at the surface of the water; these can be very large in mass, incorporation pieces of vegetation (Sandar Aung, 2017). Males behaviorally build bubble nests and care for developing eggs until they reach the larval stage.

Male *Bettas* may fight to claim territory, or to protect their eggs or offspring from rival males. The fish will spread his fins, shudder his body, extend his gill opercula and membranes, and generally appear much larger than his resting size (Krempels, 2015).

Nutrition plays an important role in maintaining good health and normal behavior, enhancing the external appearance and reproduction performance of ornamental fish (Keshavanath and Patil, 2006). For Siamese fighting fish, live diets include zooplankton, tubifex

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worms, *Daphnia*, mosquito larvae, and small aquatic insects, which are commonly used in rearing throughout its life span (Mejia *et al*., 2021). Since Siamese fighting fish is naturally carnivorous, they prefer a live diet over an artificial pellet diet (Sermwatanakul, 2019).

The knowledge of quantitative aspects such as length-weight relationship, condition factor, growth and mortality of fishes are important tools for studying fishing biology (Lizama, *et al*., 2002). Length-weight relationship study of a fish is a pre-requisite for assessing the population health status of the species (Le Cren, 1951). Fish can attain either isometric growth, negative allometric growth and positive allometric growth (Nehemia and Justin 2012). Condition factor (K-value) is also useful index for monitoring of feeding intensity, age and growth rates of fish (Anene, 2005). Fishes sufficiently fed would have K greater than equal to 1 (One) but when undernourished K is less than 1 (Aye Aye Aung *et al*., 2022).

Good nutrition is essential for promoting growth, health, and breeding performance in fish. To assess these phenomena, the readily available aquarium species Siamese fighting fish, Betta splendens, was chosen for the present study. Therefore this research aimed to: (1) investigate and compare the hatchability and fry survivability of *B. splendens* across different water types and nesting materials , (2) evaluate the overall breeding success under controlled conditions and (3) examine the effect of different diets on the subsequent growth performance of B. splendens fingerlings.

Materials and Methods

Experimental Site and Study Period

This study was conducted in the museum of Department of Zoology, Pyay University. This study was conducted during December, 2023 to August, 2024. Before the experiment, identification of fish was made according to Tawlar and Jhingarn (1991), and Jayaram (2013).

Breeding Performance of Siamese Fighting Fish

Maintenance of broodstock

Six pairs of healthy, seven-month-old Siamese fighting fish were sourced from local aquarium shops in Pyay Township, averaging 3.94±0.28 cm in total length and 0.95±0.12 g in body weight. Broodstock were individually maintained in 12×12×20 cm3 glass tanks filled with 0.8 L of water and fed dry pellets and Daphnia at 6% of their body weight, twice daily.

Experimental Design for Assessing Breeding Performance in Two Water Types and Three Nesting Materials

The breeding pairs were stocked at a ratio of one male to one female in aquarium tanks (30 x 30 x 45 cm^3). To compare the effects of water types, two conditions were used: tap water and 40% almond water. In each type of water, equal pieces of regiform, plastic, and banana leaf (8 x 8 cm^2) were placed on the water surface in each tank to facilitate bubble nest formation by the male.

Nursery of fry

The fry was fed with boiled egg yolk ten days after hatching and later, commercial pellet and artemia cysts. Care was taken not to over feed fry to avoid the pollution of water and water surface must be kept stable.

Breeding Performance in Different Batches

The breeders were given a rest period of 15 days after mating and parental care. Following this, they were prepared for the next breeding batch. The above-mentioned procedure was applied for every batch, with each pair being used as breeders for four consecutive batches.

Effect of Different Diets on the Growth Rate of Siamese Fighting Fish

Experimental Fish and Experimental Design

The offspring of Siamese fighting fish (50 individuals) (2.91±0.33cm) were obtained from the previous breeding performance experiment. The fishes were placed into glass-tank (24 x 12 x 18 cm3) filled with tap water. Five groups of glass-tank were prepared stocking each tank with 10 fish fingerlings of Siamese fighting fish. The fish in each tank were fed with each kind of diets. To avoid water pollution, food remained at the bottom of each aquarium was siphoned off after feeding. Total length and body weight of sample fish were measure the twice a month. The survival rate of fish was checked every day during the experiment. Total length and body weight of fish were measured by using Digital Calipers and Digital Balance respectively. The growth of body length and weight was estimated based on the sample fish randomly taken from the rearing aquarium to include all size groups of fish (small, medium and large fish).

Larvae Feed Preparation

During the experimental periods, the fishes were fed with different diets amounting 6% of body weight of fishes in each experimental tank and they were feed twice a day at 3% each. The fishes were fed with Daphnia (Diet I), Mosquito larvae (Diet II), Pellet feed (Diet III), Mixed feed (50% of Daphnia and 50% of pellet) (Diet IV) and Artemia (Diet V) for this experiment.

Assessment of water quality

Water temperature and pH were measured weekly using thermometer and pH meter respectively.

Statistical Analysis

Hatching rate (%) = $100 \text{ x } \frac{\text{Number of fry hatched}}{\text{Number of egg laid}}$ (Ricker, 1975)

Survival rate (%) = $100 \text{ x } \frac{\text{Final number of fish}}{\text{Initial number of fish}}$ (Ricker, 1975)

Length Gain (LG) = {(FL-IL) / IL x 100} (Bagenal, 1978)

Weight Gain (WG) = {(FW-IW) / IW x 100} (Bagenal, 1978)

Condition factor (K) = $\frac{W}{L^3} \times 100$ (Fulton, 1904)

The water types and multiple comparison of nest places (regiform, plastic and banana leaf) and the significant differences between the treatments of different diets on weight the data were subjected to ANOVA test (R software version 3.4).

Results

Nest Building of *Betta splendens*

Once the male Betta fish becomes interested in the female, it will begin constructing a bubble nest. The nest is made of air bubbles coated in saliva and is typically located under regiform, plastic, banana leaf. The nest is considered complete when the male stops working on it. As the female lays her eggs, the male collects them in his mouth and spits them into the nest. The bubble nest serves to protect the eggs until they hatch.

Hatching rate and survival rate of *Betta splendens* in different nesting sites in Almond water and Tap water

In the present study, the first and second batches of *Betta splendens* were observed in December and temperature parameter was 25-27˚C, while the third and fourth batches were observed in January and the temperature parameter was 24-27˚C.This study on *Betta splendens* demonstrated significant differences in hatching and survival rates based on nesting type and water conditions. In almond water, regiform nests yielded hatching rates ranging from 45.45% to 60.00%, with survival rates peaking at 46.67%. In contrast, plastic nests showed more consistent success, achieving hatching rates between 50.00% and 64.29%, along with survival rates reaching up to 53.85%. Banana leaf nests, however, generally exhibited lower performance, with hatching rates between 40.91% and 51.85% and survival rates peaking at 42.86%. When considering tap water, hatching rates increased across all nest types, with plastic nests achieving the highest rate of 70.00% in Batch 4, while survival rates remained lower, particularly in regiform nests at 31.82 %(Table1, Fig.1,2 and Plate1).

Table 1 Hatching and Survival rates of different nesting places in Almond water and Tap water conditions

Nesting sites		Almond water		Tap water	
	Batch	Hatching Rate	Survival Rate	Hatching Rate	Survival Rate
Regiform nest	Batch 1	50.00	46.67	55.00	31.82
	Batch 2	45.45	46.00	60.00	38.89
	Batch 3	50.00	45.45	54.55	33.33
	Batch 4	60.00	46.67	66.67	41.67
Plastic nest	Batch 1	50.00	53.85	59.09	38.46
	Batch 2	45.83	45.45	64.29	42.22
	Batch 3	54.17	46.15	62.50	37.33
	Batch 4	64.29	50.00	70.00	42.86
Banana leaf nest	Batch 1	46.15	41.67	52.94	31.11
	Batch 2	40.91	40.00	50.00	36.36
	Batch 3	45.00	40.00	50.00	32.73
	Batch 4	51.85	42.86	58.82	35.00

In almond water, no significant differences were found among the groups for hatching rates, while a significant difference emerged among tap water groups. In tap water, a significant difference was observed, particularly between the "Plastic and Banana" groups (Fig.3). For survival rates, almond water demonstrated significant differences among groups, particularly between "Plastic and Banana" and "Regiform and Banana" groups (Fig.4). While tap water showed a potential difference among groups.

Body length and weight of different diets on the experimental period (four to seven month old fish)

During this study period, April-July, 2024, water temperature ranges from 25-34˚C and pH value range from 6.5-7.5.The study reveals that fish length and weight varied across different diets over experimental period. For Diet I (Daphnia), fish length ranged from 3.08 ± 0.37cm to 3.83 ± 0.23cm, with weight increasing from 0.43 ± 0.12g to 0.75 ± 0.13g. Similarly, Diet II (Mosquito larvae) saw length ranging from 3.15 ± 0.37cm to 3.94 ± 0.28cm and weight from 0.43 ± 0.13g to 0.79 ± 0.16g. Diet III (Pellet feed) exhibited length changes from 3.20 ± 0.32cm to 3.74 ± 0.21cm and weight from 0.44 ± 0.09g to 0.71 ± 0.11g. In Diet IV (Mixed feed), length increased from 3.09 ± 0.36cm to 3.82 ± 0.38cm, and weight from 0.41 ± 0.14g to 0.74 ± 0.16g. Finally, Diet V (Artemia cysts) showed length variations from 3.24 ± 0.32cm to 3.80 ± 0.24cm and weight from 0.41 ± 0.09g to 0.58 ± 0.10g (Plate 2).

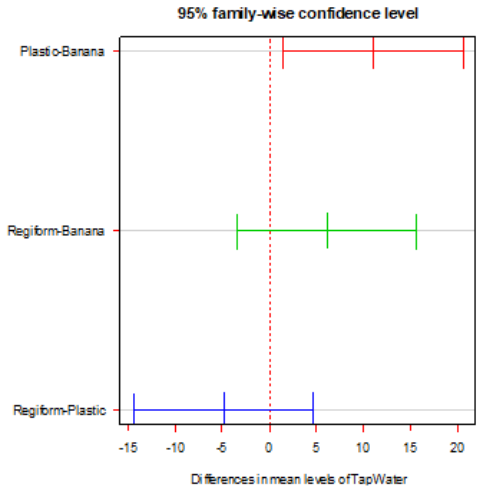


Figure.3 Multiple comparisons of mean differences between plastic, regiform, and banana nesting sites of hatching rate of tap water

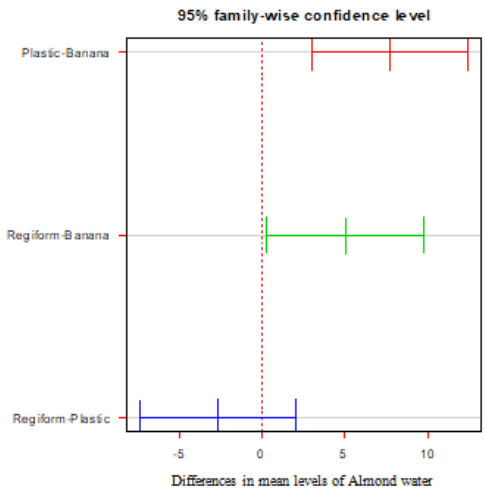
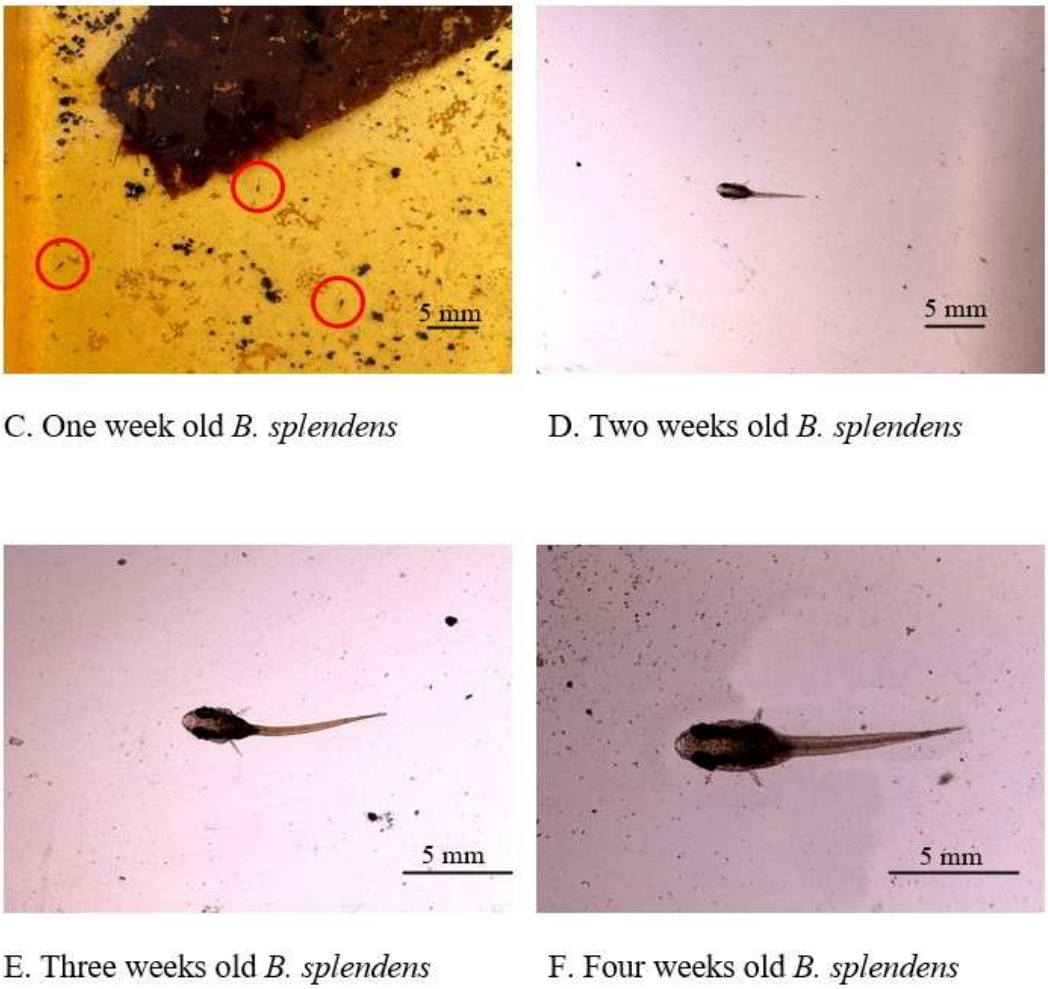


Figure.4 Multiple comparisons of mean differences between plastic, regiform, and banana nesting sites of survival rate of almond water



C. One week old *B. splendens* D. Two weeks old *B. splendens*

E. Three weeks old *B. splendens* F. Four weeks old *B. splendens*

Plate 1 Different age of *Betta splendens* in the tank

Table 2 Comparison of Weight Gain, Length Gain, Survival rate, K, r and b values of across different diets

Diet	Mean Weight Gain	Mean Length Gain	Survival rate	K-value	r-value	b-value
Diet I	74.42	24.35	80%	1.38	0.91	2.62
Diet II	83.72	25.08	80%	1.32	0.94	2.68
Diet III	61.36	16.88	90%	1.33	0.91	2.49
Diet IV	80.49	23.62	80%	1.38	0.91	2.56
Diet V	41.46	17.28	100%	1.11	0.86	2.07

Different diets based on weight gain, length gain, survival rates, K, r and b. Diet II has the highest weight gain at 83.72 and a mean length gain of 25.08. Diet III has the lowest length gain at 16.88. Diet V has the lowest weight gain of 41.46. Diet I and Diet II both had an 80% survival rate, with K values of 1.38 and 1.32. Diet III had a slightly better survival rate of 90% with a K value of 1.33. Diet IV also had an 80% survival rate, with K values of 1.38. However, Diet V

was the best, achieving a 100% survival rate, even though its growth potential (K value of 1.11) was lower. (Table 2, Fig.5).

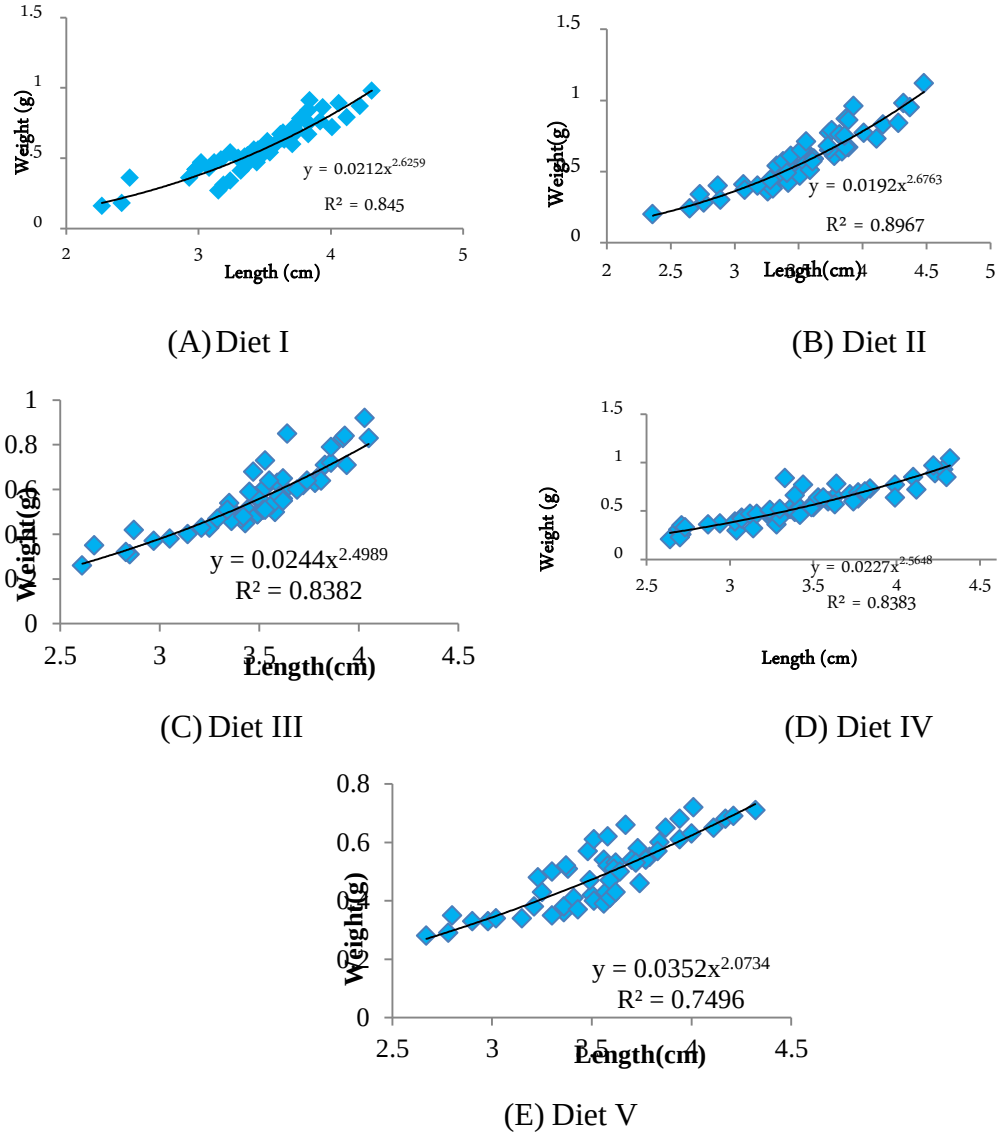
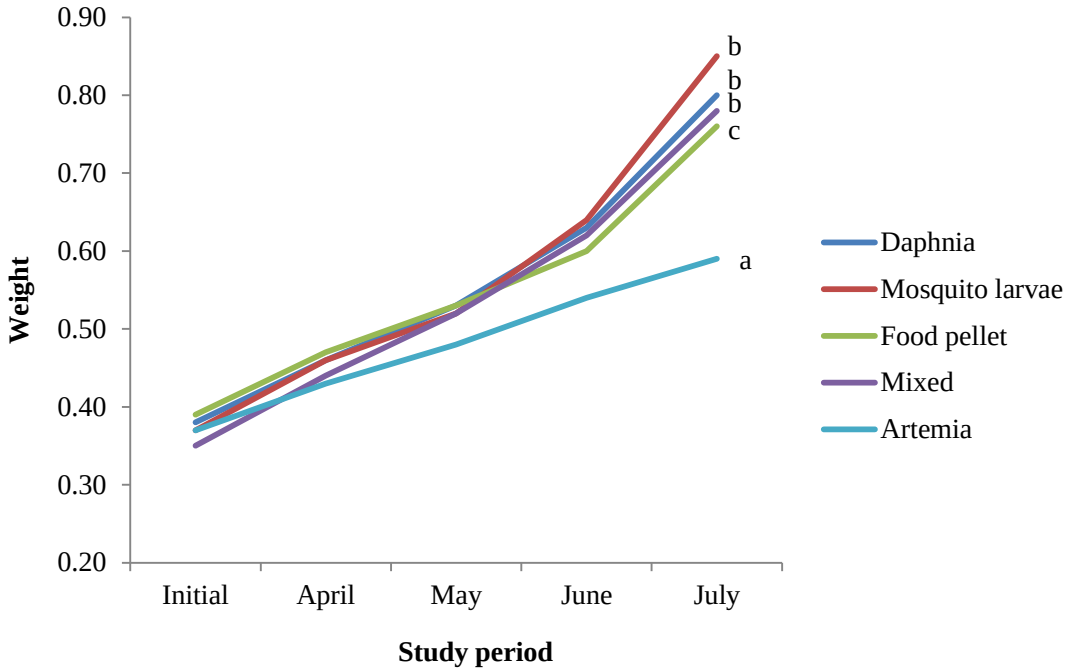


Figure.5 Relationship between body length and weight of *Betta splendens* fed with different diet

The ANOVA analysis assessed the variation in body weights of Betta splendens across different diet groups, revealing significant differences. Specifically, Daphnia, mosquito larvae, and a mixed diet showed significantly higher mean weights compared to Artemia, with p-values of 0.027, 0.007, and 0.045, respectively. Other diet comparisons did not yield statistically significant differences (Fig.6).



(a significant difference between b, a no significance different between c)

Figure.6 Comparison of body weight of *Betta splendens* fed with different diets

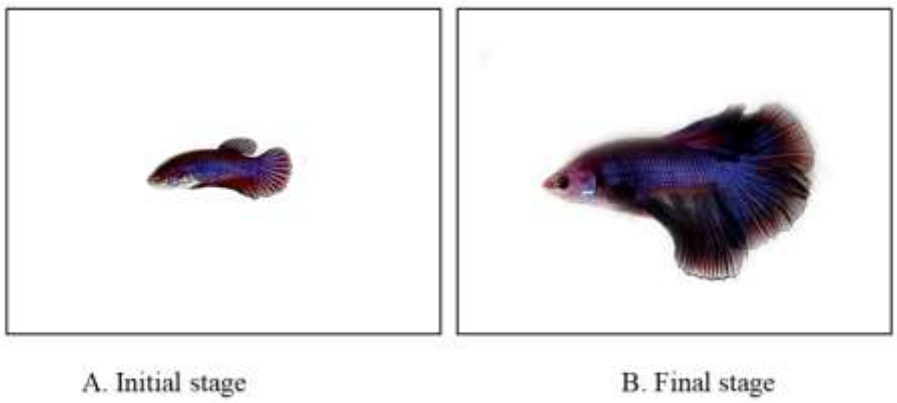


Plate 2 Initial stage and final stage of *Betta splendens* fish

Discussion

The Siamese fighting fish or Betta (*Betta splendens* Regan, 1910) is a popular aquarium fish that is commercially produced and exported throughout the world. Selective breeding of this species provides various brilliant colors, tail types and fin shapes (Thongprajukaew *et al*., 2018).

Patidar, (2022) reported, it's often best to purchase Betta fish from a reputable breeder depend on the age of fish. Breeding Betta fish works best when both mates are between four months and one year old. In the present study, pair of *Betta splendens* at the age of seven months old and in optimal health was chosen for breeding. So that, this results was similar to the above author.

Patidar, (2022) reported that Betta fish prefer water that's between 24˚C-28˚C, so unless they live in an area that has a warm climate all year round, they will need to install a heater. Warm water also aids in breeding Betta fish as it stimulates their urge to reproduce. *Betta splendens* were observed in four batches from December to January. It also specifies that the temperature during December and February ranged from 24 to 27 °C. So that, this results was similar to Patidar, 2022.

Srikrishnan *et al*., (2017) also reported that, females spawn under the nest, where the male waits to fertilize the eggs and typically 400-500 eggs are spawned by female fish. In Plastic nest, tap water reached a peak hatching rate of 70.00%, while almond water was lower at 64.29%. However, almond water sometimes had higher survival rates, particularly in the Plastic nest, where it reached 53.85%. In Banana leaf nest, hatching rates were lower for almond water compared to tap water. Tap water seems more effective for hatching, but almond water may support survival in certain conditions. Because of the chemical composition of almond water effect on embryonic growth and metabolic processes. The almond water did not significantly impact hatching rates among groups. In contrast, tap water showed significant differences, particularly between the "Plastic-Banana" nesting sites, indicating the influence of nesting sites on hatching success. For survival rates, almond water displayed significant differences, especially between the "Plastic-Banana" and "Regiform-Banana" nesting sites. In tap water, a potential different is noted between the "Plastic-Banana" nesting sites.

The effect of different diets on the growth performance of Siamese fighting fish was evaluated on the basis of weight gain (WG), length gain (LG), survival rate, condition factor (K) and length-weight relationship (LWR) using different diets. Sandar Aung, 2017 reported that the optimal water temperature of Siamese fighting fish keeping in 23-29°C and the pH value was 8. In the present study, water temperature recorded ranged from 25-34°C and pH value was 6.5-7.5 during the experimental period.

Zakes *et al*., (2006) reported that generally growth and survival rate increased with feeding frequency. In this experiment, the feeding frequency of two times per day was adopted during the experimental period. Mosquito larvae are the most common live diet for rearing Siamese fighting fish. They contain high amount of proteins and lipids that are notably suitable for feeding carnivorous fish, like Siamese fighting fish (Thongprajukaew *et al*., 2018). In this study, the highest weight gain of *Betta splendens* fed with mosquito larvae and lowest weight gain fed with Artemia. Length gain in *Betta splendens* fed mosquito larvae statistically was obtained better results than other diets. Lowest length gain was found in pellet feed. Therefore, this result was similar to above authors.

The condition factor (K) of a fish reflects physical and biological circumstances and fluctuations by interaction among feeding conditions, parasitic infections and physiological factors (Le Cren, 1951). Diet I (Daphnia) and Diet II (Mosquito larvae) both provided an 80% survival rate, with condition factors (K) of 1.38 and 1.32, respectively. Diet III (Pellet feed) achieved a 90% survival rate and a K value of 1.33. Diet IV (Mixed feed) also achieved an 80% survival rate and a K value of 1.38. Notably, Diet V (Artemia) excelled with a 100% survival

rate, although its K value of 1.11 indicates lower growth potential. Artemia may be best for survival, but other diets could support better growth.

Length-weight relationship is of great importance in fishery management. The correlation coefficient (r) was also estimated to determine the degree of linear relationship between the length and weight of samples (Sandar Aung, 2017). Costa and Araujo (2003) reported that r value of 0.99 was the highest value associated to the individuals that present highest weight for a given length. In this experiment, the highest values of correlation coefficient r = 0.94 was observed in Diet II (Mosquito larvae) and the lowest values of correlation coefficient r = 0.86 in Diet V (Artemia).

The significantly higher mean weights observed with Daphnia, mosquito larvae and the mixed diet suggest that these food sources provide essential nutrients, leading to better growth outcomes compared to Artemia. Because of the higher protein content and nutritional quality of these diets. Pellet feed did not show significant difference compare to Artemia.

The study investigated how different factors affect the breeding success and the growth of *Betta splendens*, a popular aquarium fish. This study show that the choice of nest material and water type significantly influenced reproductive outcomes, highlighting the importance of environmental conditions. . It found that diet plays a crucial role, with live food sources like Daphnia and mosquito larvae leading to better growth compared to Artemia. These results provide valuable insights for fish keepers and aquaculture enthusiasts, helping them understand the factors that contribute to the well-being and reproductive success of *Betta splendens* in captivity.

Conclusion

In conclusion, this study on the Siamese fighting fish (*Betta splendens*) highlighted important factors that affect their breeding success and growth. This study confirmed that fish aged between four months to one year are ideal for breeding and highlighted the importance of maintaining water temperatures between 24˚C and 28˚C. Additionally, the impact of different nesting sites and water types on reproductive success was explored, revealing distinct variations across batches and environments. For tap water, in plastic nesting sites produced the highest hatching rates, while almond water led to better survival rates. Live food sources such as Daphnia, mosquito larvae and mixed feed were found significant different compared to Artemia and pellet feed, although Artemia resulted in the highest survival rates. These results provide valuable insights for aquaculture practices and conservation efforts, emphasizing the multifaceted nature of factors influencing the health and reproductive success of *Betta splendens*.

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SEASONAL OCCURRENCE OF ZOOPLANKTON SPECIES IN INYA LAKE WITH EMPHASIS ON ROTIFER SPECIES

Moh Moh Myint[1], July Maung Maung[2], Kay Lwin Tun[3]

Abstract

The present study investigated the occurrence and abundance of zooplankton in Inya Lake, Kamayut Township, Yangon, from June 2023 to May 2024. The zooplanktons were collected monthly from the five study sites in Inya Lake; Site I (Approximately 90 m from the Restaurants), Site II (Approximately 240 m from Kan Thar Yar hospital), Site III (Approximately 500 m from Sailing and Rowing Club), Site IV (Approximately 120 m from U.S Embassy) and Site V (Approximately 240 m from Mya Kyune Thar Golf club). They were immediately preserved in 5% formalin after collection and identified based on their morphology and morphometric measurements. The results revealed that a total of 37 species of zooplankton comprising 24 species of rotifers belonging to 8 families and 10 genera, Cladocerans 8 species belonging to 4 families and 6 genera and Copepods 5 species, 4 families and 4 genera were recorded. The collected rotifers were identified and categorized into three sizes: large, small, and super small, based on their body length. The most abundant rotifer species were of small size. Among them, *Brachionus calyciflorus* was the most abundantly occurring species in the freshwater environment. The findings confirm that Inya Lake supports a diverse zooplankton community whose seasonal peaks (wet and cold seasons) and species composition are characteristic of productive freshwater bodies.

Keywords : Inya Lake, Zooplankton, seasonal occurrence, abundance

Introduction

Inya Lake offers essential services to the residents of Yangon. Efforts are now underway to conserve and protect the lake through a biological equilibrium approach to potentially utilize its water during unforeseen emergencies. The lake was originally constructed in 1885 to supplement the water supply to Kandawgyi Lake, which at the time was the primary water source for Yangon (Kalaya-Htun, 2005). Today, Inya Lake is a popular recreational spot for locals and tourists, offering boating, fishing, and relaxation. In addition to its recreational value, the lake plays an important role in the city's ecosystem, regulating temperature and acting as a buffer against flooding during the monsoon season. The food chain is a system in which organisms derive energy from their environment and pass it. Phytoplankton, as primary producers, form the base of the food chain (Treece, 2000). In the lake's ecosystem, zooplankton species, which form the second level of the food chain, are crucial food sources for various invertebrates and fish. Copepods, cladocerans, and rotifers are the main groups of zooplankton found in Inya Lake (Aung Kyaw Zaw, 2017). However, Inya Lake faces threats from pollution and human activities, making it important to balance conservation efforts with the needs of a growing city.

Zooplankton, which are small drifting organisms moved by water currents, play a crucial role in freshwater and marine ecosystems. They are the primary food source for many aquatic

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animals in both flowing and still-water ecosystems, transferring energy from algae to higher trophic levels like fish (Sebastian *et al*., 2014). Zooplankton, particularly rotifers, are vital in maintaining the ecological balance of freshwater systems like Inya Lake. They play a critical role in the food chain between primary producers and higher-level consumers.

Understanding the seasonal dynamics of zooplankton populations is important for ecological conservation and practical applications such as aquaculture. While Inya Lake supports a variety of zooplankton species, the impact of seasonal environmental changes on these populations remains understudied. Zooplankton are highly sensitive to environmental changes and serve as excellent indicators of water quality due to their short life cycles, allowing them to respond rapidly to changes in water conditions. They play a key role in indicating eutrophication status and overall productivity of freshwater bodies.

Among the zooplankton species, rotifers are well known due to their value in aquaculture. Particularly, rotifers are of interest due to their potential as live feed in aquaculture, especially in the early developmental stages of fish larvae. The present study focused on identifying the dominant zooplankton species in different areas of Inya Lake by seasons, with a particular emphasis on domestic rotifer species suitable for aquaculture. The aim was to study the occurrence of zooplankton species and the abundance of native rotifer species in Inya Lake to assess potential candidates for live food production.

Materials and Methods

Sampling site and the study period

The five different sites were selected as the sampling sites for water collection in Inya Lake. There were Site I (Approximately 90 m from the Restaurants by the lake's banks), Site II (Approximately 240 m from Kan Thar Yar hospital), Site III (Approximately 500 m from Sailing and Rowing Club), Site IV (Approximately 120 m from U.S Embassy) and Site V (Approximately 240 m from Mya Kyune Thar Golf club) Fig.(1).

Figure. 1. Map of the Study Area (Source: Google)

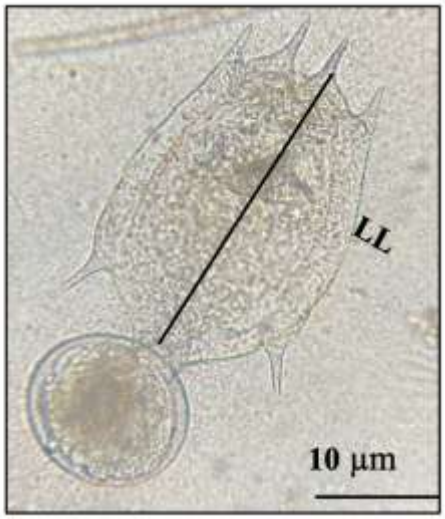
Collection of zooplankton in Inya Lake

Zooplankton were monthly collected from five study sites of Inya Lake. The collection was done between 6:00 – 9:00 am in a morning at approximately 0.5-1 m depth of water. A plankton net with a mesh size of 50 µm and a diameter of 40 cm was used to collect the zooplankton (Plate 1). The plankton samples were collected by filtering 30 mL of water through the plankton net. After collection, the plankton samples were filtered into the plastic bottles. Collection samples of 30 mL were mixed and preserved in 0.04 mL of 5% formalin in the plastic bottles. The preserved plankton specimens were examined under an inverted microscope (Olympus CKX 41). Identification of the zooplankton was followed according to Witty (2004), Shiel (1995), and Islam *et al*. (2022).

Classification of rotifer species

The collected rotifers were identified under an inverted microscope. Totally 100 individuals of each identified rotifer species were measured (Lavens and Sorgeloos, 1996). Based on the Lorica Length, the rotifers were classified as Large type (L-type) (lorica length ranging from 130 to 340 µm), Small type (S-type) (lorica length ranging from 100 to 210 µm), and Super small type (SS-type) (lorica length ranging from > 100 µm) (Plate 2).

Plate 1. Drifting water through the plankton net.



LL - Lorica Length

Plate 2. *Brachionus calyciflorus* (Palls, 1766)

Water quality analysis

The physico-chemical parameters Dissolved Oxygen (DO), Ammonia, Total Dissolved Solids (TDS), and pH of water samples collected from each study site were monthly recorded using the Test kit.

Data calculation

Species richness, abundance, and species diversity indices were calculated as follows:

(a) Species richness = Number of recorded species in the study area.
(b) Abundance = Number of individuals in study sites.
(c) Shannon-Weiner index of Diversity (1949)

$$H' = -\sum_{i=1}^{s}(P_i)\ (log_2 P_i)$$

$$P_i = S/N$$

H´ = Shannon-Weiner index of species diversity
S = Number of individuals of each species in the sample
N = Total number of individuals of all species in the sample
Pi = Proportion of individuals belonging to species

(d) Pielou's Evenness or Equitability (Pielou's, 1966).

$$J = \frac{H'}{log2S}$$

H´ = Shannon Weiner index
S = Total number of species
J = Distribution of individuals among species

Results

Occurrence and abundance of zooplankton species in Inya Lake

The 37 zooplankton species were recorded from five study sites (Plates 3) under three major groups of zooplankton: Rotifers, Cladocerans, and Copepods in the study lake (Table 1).

Rotifers belonged to two classes, two orders, and eight families: Brachionidae, Testudinellidae, Philodinidae, Lecanidae, Synchaetidae, Asplanchnidae, Trichocercidae, and Conochilidae. The family Brachionidae had the highest abundance, with 6,012 individuals. The second most abundant family was Testudinellidae, comprising 3,056 individuals, followed by Asplanchnidae with 1,499 individuals. Synchaetidae accounted for 243 individuals, Conochilidae for 123 individuals, Trichocercidae for 107 individuals, Philodinidae for 72 individuals, and Lecanidae for just one individual.

Cladocerans belonged to one class, three orders, and four families: Daphniidae, Moinidae, Sididae, and Chydoridae. The family Daphniidae had the highest number of individuals, with 2,338 recorded. Moinidae followed with 2,244 individuals, while Chydoridae and Sididae had 50 and 67 individuals, respectively.

Copepods belonged to three classes, three orders, and four families: Cyclopidae, Calanidae, Harpacticidae, and Centropagidae. Cyclopidae was the most abundant, with 12,855

individuals, followed by Calanidae with 2,483 individuals. Harpacticidae and Centropagidae were the least abundant, with 39 and 12 individuals, respectively.

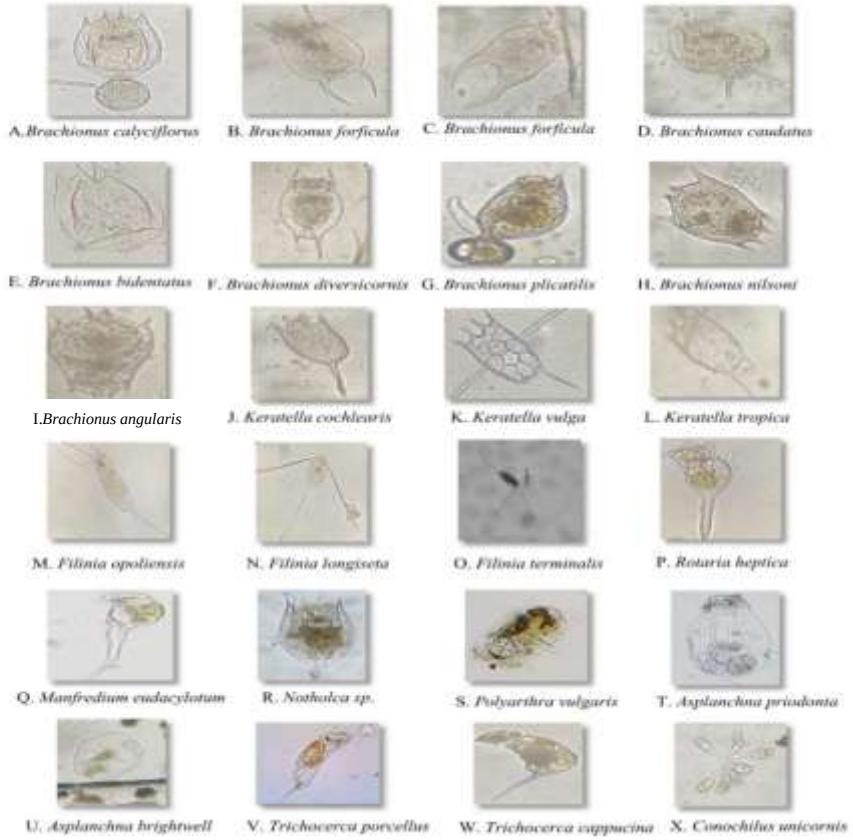


Plate 3. Recorded zooplankton species in Inya Lake (40×)

Table 1. Abundance of zooplankton in Inya Lake from June 2023 to May 2024

Phylum	Order	Family	Site I	Site II	Site III	Site IV	Site V
Rotifera	Ploima	Brachionidae	919	1298	1129	1256	1410
		Testudinellidae	537	613	525	652	729
		Philonidae	3	21	23	13	12
		Lecanidae	-	-	-	-	1
		Synchaetidae	58	48	43	47	47
		Asplanchnidae	283	365	351	245	255
		Trichocercidae	32	10	12	28	25
	Flosculariaceae	Conochilidae	67	36	4	9	7
Cladocerans	Anomopeda	Daphniidae	532	553	487	427	339
	Cladocera	Moinidae	554	549	474	351	316
		Sididae	20	4	11	3	12
	Diplostraca	Chydoridae	14	15	16	17	5
Copepods	Cyclopoida	Cyclopidae	2708	2898	2404	2243	2602
	Calanoida	Calanidae	675	566	422	420	400
		Centropagidae	7	5	-	-	-
	Harpacticoida	Harpacticidae	5	12	22	-	-
Total			6414	6993	5923	5711	6160

Monthly occurrence of zooplankton species in Inya Lake

At site I, the abundance of rotifer species reached its peak in January 2024, with the lowest abundance observed in June 2023. Cladocerans observed with a peak in October 2023, with their lowest levels also recorded in June 2023, whereas copepods peaked in August 2023 and reached their minimum in April 2024.

At Site II, rotifer species peaked in February 2024, with a minimum abundance in June 2023. A peak of cladocerans was found in August 2023, and the lowest abundance was in June 2023. Copepods peaked in August 2023 and reached their lowest abundance in April 2024. Rotifers also reached their peak abundance in February 2024, with the lowest levels in June 2023 at site III. Cladocerans peaked in October 2023 and showed their lowest abundance in June 2023, while copepods peaked in August 2023 and a minimum in April 2024.

At Site IV, the peak abundance of rotifer species occurred in February 2024, with the lowest abundance recorded in May 2024. Cladocerans reached a peak in November 2023 and were at their lowest in June 2023. Copepods peaked in July 2023 and displayed their minimum abundance in April 2024.

At Site V, rotifers reached their peak abundance in February 2024, with the lowest levels recorded in May 2024. Cladocerans peaked in November 2023, with their minimum abundance observed in July 2023. Copepods exhibited the highest abundance in June 2023 and reached their lowest levels in April 2024, as shown in Fig. 1.

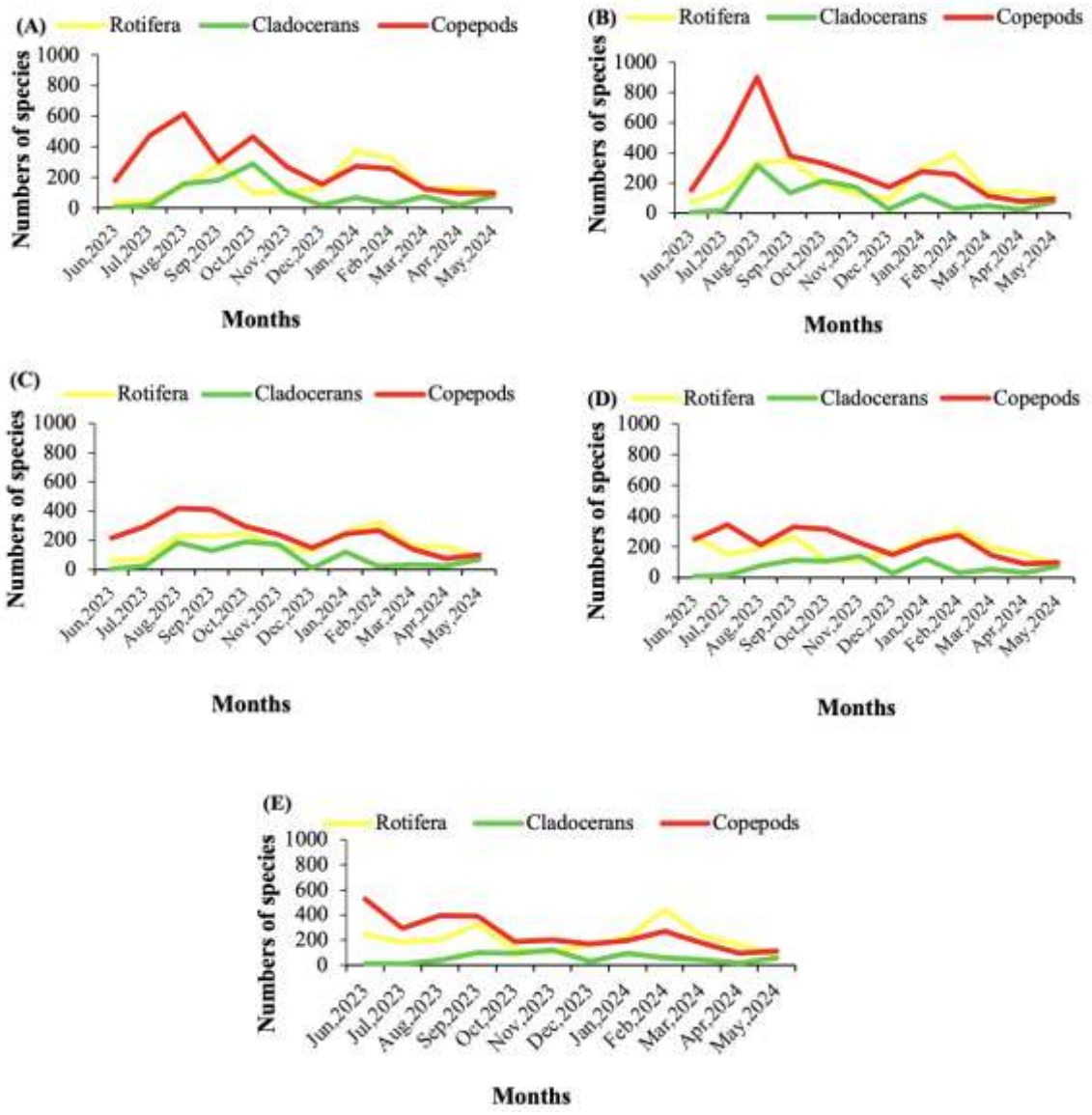


Figure. 1. Abundance of zooplankton species in five study sites during the study period (A)Site I, (B) Site II, (C) Site III, (D) Site IV and (E) SiteV

Species richness, population, diversity, and evenness of zooplankton species

The analysis of diversity indices across the five study sites reveals notable variation in species composition and distribution. Species richness was highest at Site III (32 species) and lowest at Site V (26 species), indicating slight differences in the number of species present. Abundance peaked at Site II (6993 individuals), while Site IV recorded the lowest count (5711), suggesting differences in population density. The Shannon-Weiner diversity index (H') was relatively consistent across sites, ranging narrowly from 2.419 (Site V) to 2.499 (Site III), reflecting a moderate and fairly uniform level of species diversity. Evenness values were also closely aligned, with Sites IV and V showing slightly higher evenness (0.741 and 0.743, respectively), indicating a more equitable distribution of individuals among species in those locations. Overall, while species counts and abundance varied, diversity and evenness remained fairly stable across all sites in Table 2.

Table 2. Comparison of species richness, diversity indices and evenness of zooplankton in five study sites.

No	Diversity indices	Site I	Site II	Site III	Site IV	Site V
1	Species Richness	29	31	32	28	26
2	Population	6414	6993	5923	5711	6160
3	Shannon-Weiner index (H')	2.43	2.464	2.499	2.468	2.419
4	Evenness (E)	0.722	0.718	0.721	0.741	0.743

Size classes of identified rotifers

In the present work, to select the possible zooplankton species for livefood production, rotifer species were categorized into three size classes: large (L), small (S), and super small (SS), based on lorica Length (Table 3). Four species were classified as large rotifers, including *Rotaria heptica* (608±130.11µm), *Notholca* sp. (250±353.55µm), *Asplanchna priodonta* (416±282.84µm), and *Asplanchna brightwell* (341±176.78µm). Twelve species were categorized as small rotifers, with *Brachionus calyciflorus* (200±70.71µm) and *B. falcatus* (115±21.21µm) being the most abundant. Other notable small species included *Keratella cochlearis* (90±42.43µm), *Filinia opoliensis* (110±70.71µm), and *Polyarthra vulgaris* (108±59.40µm). The super small rotifer group comprised eight species, with *Keratella vulga* (90±14.14µm) and *K. tropica* (83.5±23.33µm). Among the recorded rotifer species, *Brachionus calyciflorus* was the most abundant, which was classified as "S" size rotifer.

Table 3. Recorded rotifer species with the respective "Lorica Length" in Inya Lake during the study period

Sr. no	Species	Lorica Length (µm)	Remark	Numbers of recorded individuals during study period in all study sites
1	*Brachionus calyciflorus*	(200±70.71)	S	2,572
2	*Brachionus falcatus*	(115±21.21)	S	2,079
3	*Brachionus forficula*	(98±25.46)	S	977
4	*Brachionus caudatus*	(150±70.71)	S	79
5	*Brachionus bidentatus*	(91±35.36)	S	8
6	*Brachionus diversicornis*	(150±70.71)	S	264

Sr. no	Species	Lorica Length (µm)	Remark	Numbers of recorded individuals during study period in all study sites
7	*Brachionus plicatilis*	(174.5±58.69)	S	10
8	*Brachionus nilsoni*	(116.5±47.38)	S	8
9	*Brachionus angularis*	(75±21.21)	SS	14
10	*Keratella cochlearis*	(90±42.43)	S	1,086
11	*Keratella valga*	(90±14.14)	SS	691
12	*Keratella tropica*	(83.5±23.33)	SS	743
13	*Filinia opoliensis*	(110±70.71)	S	501
14	*Filinia longiseta*	(41.5±58.69)	SS	2
15	*Filinia terminalis*	(91.5±12.02)	SS	33
16	*Rotaria heptica*	(608±130.11)	L	72
17	*Manfredium eudacylotum*	(107.5±152.03)	S	1
18	*Notholca* sp.	(250±353.55)	L	1
19	*Polyarthra vulgaris*	(108±59.40)	S	243
20	*Asplanchna priodonta*	(416±282.84)	L	1,044
21	*Asplanchna brightwell*	(341±176.78)	L	455
22	*Trichocerca porcellus*	(58±35.36)	SS	55
23	*Trichocerca capucina*	(58±35.36)	SS	52
24	*Conochilus unicornis*	(52.5±24.75)	SS	123

Discussion

The structure of the zooplankton community in Inya Lake was studied through monthly surveys over a year. The result revealed 37 zooplankton species belonging to three groups: rotifers, cladocerans, and copepods. Among these rotifers was the most dominant group, followed by cladocerans and copepods. Specifically, 24 species of rotifers from 8 families and 10 genera were recorded. There were also 8 species of cladocerans from 4 families and 6 genera, and 5 species of copepods from 4 families and 4 genera. The family Brachionidae was the most dominant and diverse among the rotifers throughout the study period. These species are commonly found in eutrophic waters (Berzins and Pejler, 1989; Sampaio and Slopez, 2000). This pattern is common in lakes, ponds, reservoirs, and rivers (Neves *et al.*, 2003).

In different study sites, 15 families in Site I, and Site II, 14 families in Site III and Site V, and 13 families in Site IV.

In Site I, 7 families of rotifers, 4 families of cladocera, and 4 families of copepods were recorded. In Site II, 7 families of rotifers, 4 families of cladocera, and 4 families of copepods were recorded. In Site III, 7 families of rotifers, 4 families of cladocera, and 3 families of copepods were recorded. In Site IV, 7 families of rotifers, 4 families of cladocera, and 2 families of copepods were recorded. In Site V, 8 families of rotifers, 4 families of cladocera, and 2 families of copepods were recorded. Thus, the result noted that rotifers were the dominant species at all study sites. Similarly, Aung Kyaw Zaw (2017) observed three groups of zooplankton rotifers, cladocerans, and copepods. The rotifers were the dominant group, showing 65% in Inya Lake and 71% in Kandawgyi Lake.

The highest diversity was observed in August and September 2023, and the lowest in April 2024. Aung Kyaw Zaw (2017) reported that the more number of zooplankton species were observed in the wet season, followed by the cool season and the dry season. Chandraseker (1996) showed that the water temperature, turbidity and transparency, and dissolved oxygen were favorable for rotifer population. Also, Sukumaran and Das (2001) studied plankton abundance with the physico-chemical features of Manchribele reservoir in Bangalore, India. They reported that high chloride content and optimal temperature were favorable for zooplankton in different seasons. The low population was encountered in the hot season (April and May). Among them, the population density of rotifers is high in the cold season and less in summer. In cold seasons with low temperature and normal DO content, some factors influence the growth, reproduction, and mortality of zooplankton, such as depth, pH, nutrients, sunlight, and temperature (Gyllstrom and Hansson, 2004). The present study observed that the copepods (cyclopoid, calanoids) contributed the highest abundance and biomass of the three major groups almost every month in Inya Lake, which was similar to the result from Perez *et al*. (2008) and Papa and Zafaralla (2011). They are found to be dominant in oligotrophic lakes.

In the present study, three groups of zooplankton-rotifers, cladocerans, and copepods were analyzed, focusing on the 24 rotifer species identified based on lorical Length (Lavens and Sorgeloos, 1996), classified into three size categories: Large, Small, and Super Small.

Among the Large-sized rotifers (608±130.11µm), four species were recorded, including one species from the Brachionidae family, one from Philodinidae, and two from Asplanchnidae. In the Small-sized group (200±70.71µm), twelve species were identified, including eight from Brachionidae, two from Testudinellidae, one from Lecanidae, and one from Synchaetidae. In the Super Small-sized group (83.5±23.33µm), eight species were recorded, consisting of one from Brachionidae, four from Testudinellidae, two from Trichocercidae, and one from Conochilidae.

In this study, *Asplanchna priodonta* (Asplanchnidae) was the most frequently recorded species among the Large-sized group. In the Small-sized group, *Branchionus calyciflorus* (Brachionidae) had the highest number of individuals, while *Keratella tropica* (Testudinellidae) was the most abundant species in the Super Small-sized group, respectively. This study highlights the variation in size and abundance of rotifers across the study sites, offering important insights into the composition of rotifer communities.

Conclusion

The present study documented the species composition and seasonal variation of zooplankton in Inya Lake. Totally 37 zooplankton species of 16 families under seven orders across six classes, were recorded. The highest seasonal variation occurred during the wet season, while the lowest was observed in the hot season. The family Brachionidae showed a peak in abundance during the cold season. The greatest number of individuals was found in August and September, with the lowest in April. A notable abundance of the small-sized species *Brachionus calyciflorus* was recorded at Site V. These findings suggest that the nutrient availability, habitat conditions, and overall environmental quality of Inya Lake remain favorable for supporting zooplankton populations. Further studies are necessary to understand the community structure and ecological dynamics of the zooplankton in Inya Lake.

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GROWTH RESPONSE IN HATCHLINGS OF BURMESE ROOFED TURTLE, *BATAGUR TRIVITTATA* (DUMERIL AND BIBRON, 1835) AT YADANABON ZOOLOGICAL GARDENS, MANDALAY

Moe Thandar Khaing[1]

Abstract

This study investigated the growth performance of 30 Burmese Roofed Turtle (*Batagur trivittata*) hatchlings housed in three groups (n=10 per tank) at Yadanabon Zoological Gardens, Mandalay, from December 2019 to August 2020. Hatchlings were fed three different kinds of protein sources, such as the shrimp, *Macrobachium* sp. (Tank 1), dog food (Tank 2) and fish, *Oreochromis* sp. (Tank 3) biweekly combining with *Ipomoea* sp. (water spinach or "kazun-ywet") at the designated day. The results revealed that the negative allometric growth pattern in relationship of body weight and carapace length $b < 3$ with strong correlation $r > 0.95$ at the level of significant at $p < 0.05$ observed in all trials. Similarly, the monthly increments of body weight $b < 3$ with determination of coefficient $R^2 > 0.6655$ and carapace length $b < 0.05$ with determination of coefficient $R^2 > 0.7416$ were investigated. Pearson's correlation (r) generated the perfect association in various parameters of Burmese roofed turtles at the level of significance $p < 0.05$. In addition, t-test revealed that the significant difference in all trials at $p < 0.01$. The best absolute growth (AG), absolute growth rate (AGR) and relative growth rate (RGR) revealed in Tank 1, however, the moderate relative growth rate (RGR) disclosed in Tank 3 followed by Tank 2. Thus, providing the protein richness of various diets and fresh food has a great impact on the growth assessments of Burmese roofed turtles in captivity.

Keywords: turtles, feed, body weight, carapace length, Pearson correlation, t-values

Introduction

Turtles have long been associated with human either in myths, as food sources, as ornaments or as traditional medical ingredient (Rashid & Khan, 2000). The Burmese roofed turtle (*Batagur trivittata*) is one of six species of turtle in the genus *Batagur* of the family Geoemydidae.

The Burmese Roofed Turtle (*Batagur trivittata*) is endemic to the larger rivers of Myanmar. The females of *Batagur trivittata* are larger than males. The hatchlings are somewhat spherical, greenish yellow in color. The Burmese Roofed Turtle is one of the rarest turtle species on earth, and its biological conservation has been successively ongoing for more than 10 years (Cilingir, 2017).

In captivity, aquatic turtles should be fed a variety of foods. A few of the herbivores feed predominantly on plant matter and fruit in nature. Most herbivores will eagerly consume commercial turtle food which is high in protein and fat (Gurley, 2003). Diet containing a mix of protein, carbohydrates, vitamins and essential nutrient promoted faster turtle growth than a single food type. The high amount of animal protein in the diet should facilitate the rapid growth of animals.

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Recently, feeding of nutritional benefits on different diets in Burmese roofed turtles in captive breeding at Yadanabon Zoological Gardens is limited information concerning with their size structure of the turtle population. Therefore, the present study was carried out to investigate the growth performance of Burmese roofed turtle in captivity and to evaluate the efficacy of substituting a diet in these turtles.

Materials and Methods

Study area and period

The research was conducted at the captive breeding facility within the Yadanabon Zoological Gardens is located north of the Mandalay Royal Palace and at the foot of Mandalay Hill, Aungmyaythazan, Mandalay Region. It is situated at North Latitude 21° 58′ 30″ N and East Longitude 96° 5′ 0″ E, and sea level has above 80 meters (22 feet) (Fig. 1.). The study period spanned nine months, from December 2019 to August 2020.

Figure.1. Map of study area (Yadanabon Zoological Gardens). Source: Geography Department, University of Mandalay

Experimental design

Thirty *B. trivittata* hatchlings were randomly divided into three experimental groups (n=10 individuals per tank). Each group was housed in a separate concrete tank (5′×5′×4′), maintained with approximately 0.10 m of water, which was changed bimonthly (Plate 1.A). Individual identification was ensured by serially marking the carapace with enamel paint.

Feeding procedure

The shrimp, *Macrobachium* sp. (24 g) were fed on Burmese roofed turtles on Monday (Tank 1), dog food (24 g) on Tuesday (Tank 2) and fish, *Oreochromis* sp. on Saturday (Tank 3). These supplementary feeds were provided once per week. The *Ipomoea* sp. (Kazun-ywet) (24 g) was supplied every day in all tanks (Plate 1.A) and (Plate 3).

A. Turtle hatchlings tanks B. Measuring the weight C. Measuring the carapace length

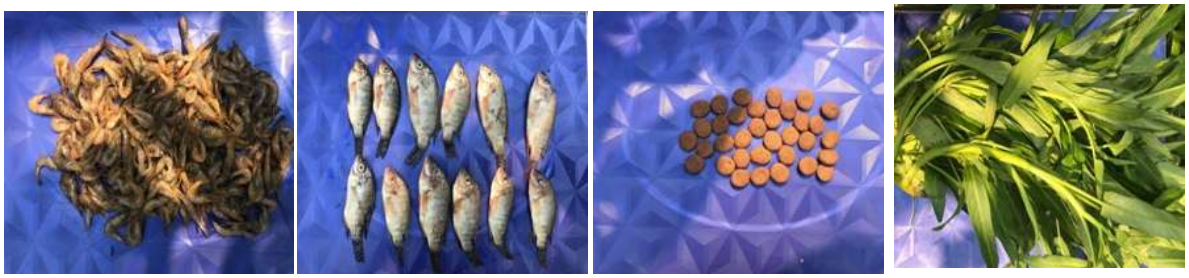
Plate 1 Materials for requirements of Burmese roofed turtles

Morphometric measurements

The body weight of Burmese roofed turtles was recorded with Digital kitchen scales in the nearest 0.01g (Plate 1.B). The carapace length for each turtle was measured with caliper in the nearest 0.01cm (Plate1.C).

(A) Carapace (B) Plastron

Plate 2 Dorsal (Left) and ventral (Right) view of juveniles *Batagur trivittata*



(A) Shrimp (B) Fish (C) Dog food (D) Kazun-ywet

Plate 3 Different types of diet for Burmese roofed turtles

Identification of the studied specimen

Identification and classification of specimen were carried out according to Shi *et al*. (2013) (Plate 2.).

Calculation of growth

The absolute growth (AG), absolute growth rate (AGR) and relative growth rate (RGR) were calculated by following Ricker (1979):

$$\text{Absolute growth rate (AGR)} = \frac{(W_t - W_i)}{t}$$

$$\text{Relative growth rate (RGR)} = \frac{(W_t - W_i)}{t} \times 100$$

Where, W_i is the initial weight or length, W_t is the weight at time, t is the length of the culture period

Regression analysis

The estimation of relationships between the carapace length and body weight of Burmese roofed turtles was investigated followed by LeCren (1951) using the linear equation: Y = a + b X. Where Y is the length (mm), and parameters a is the intercept, b is the slope, and X is the body weight (g). The logarithmic regression equation was used as followed Log Y=Log a + Log b X.

Data analysis

All data for growth performance of Burmese roofed turtles were analysed with t-tests and Pearson's correlation (r) at the level of significance $p < 0.01$ by using Microsoft Excel 2020.

Results

Thirty hatchlings Burmese roofed turtles *B. trivittata* were investigated by feeding on different diets (shrimps *Macrobachium* sp. in Tank 1, dog food in Tank 2 and fish *Oreochromis* sp. in Tank 3) combining with kanzun-ywet at Yadanabon Zoological Gardens, during December 2019 to August 2020.

The various parameters on growth performances of *B. trivittata* in each trial were shown in Table 1, Fig. 2, 3 and 4. The mean carapace length was presented in Table 2. The effect of different diets on their growth performance in each tank was presented in Table 3 and Fig. 4. And the relationships of their growth development in captivity breeding were shown in Table 4. The relationship of growth coefficient on these turtles was shown in Fig.5.

Table 2 Carapace length of Burmese roofed turtles

Month	Carapace length (cm)		
	Tank 1	Tank 2	Tank 3
December	10.20	10.21	10.60
January	10.38	10.40	10.77
February	10.57	10.40	10.93
March	11.36	10.89	11.76
April	12.10	11.47	12.44
May	13.20	12.58	13.21
June	14.70	13.78	14.24
July	15.70	14.76	15.30
August	16.00	13.30	16.00
Mean	12.10	11.47	12.44

Table 1 Growth performance of Burmese roofed turtles *Batagur trivittata* during December 2019 to August 2020

Months	Tank 1				Tank 2				Tank 3			
	BW (g)	AG (g)	AGR (g/day)	RGR (%)	BW (g)	AG (g)	AGR (g/day)	RGR (%)	BW (g)	AG (g)	AGR (g/day)	RGR (%)
December	188	-	-		191	-	-	-	204	-	-	-
January	194	6	0.2	3.19	191	0	0	0.00	213	17	0.3	4.41
February	216	28	0.47	14.89	203	12	0.2	6.28	239	35	0.58	17.16
March	254	66	0.73	35.11	227	36	0.4	18.85	281	77	0.86	37.75
April	309	121	1.01	64.36	261	70	0.58	3.65	318	114	0.95	55.88
May	388	200	1.33	106.38	339	148	0.99	77.49	389	185	1.23	90.69
June	501	313	1.74	166.50	423	232	1.29	121.47	475	271	1.51	132.84
July	581	393	1.87	209.04	499	308	1.47	161.26	551	347	1.65	170.10
August	598	410	1.71	218.09	523	332	1.38	173.82	598	394	1.64	193.14
Mean	309	160.5	1.17	85.37	261	109	0.785	48.17	318	149.5	1.09	73.285

BW = body weight, AG = absolute growth, AGR = absolute growth rate, and
RGR = relative growth rate

Table 3 Descriptive statistics on growth parameter of Burmese roofed turtles ($p < 0.01$)

Trials	Body weight (g)	Carapace length (cm)	Pearson correlation (r)	Degree of freedom	t-Critical	t-Statistics	p-value
Tank 1	358.778	12.690	0.999	8	2.306	6.381	0.00021
Tank 2	317.444	11.977	0.950	8	2.306	6.931	0.00012
Tank 3	363.111	12.806	0.998	8	2.306	7.189	0.00009

Table 4 Pearson correlation on various growth parameters in Burmese roofed turtles during December 2019 to August 2020

Parameters		Body weight (g)			Absolute growth (AG)			Absolute growth rate (AGR, g/day)			Relative growth rate (RGR, %)		
		Tank 1	Tank 2	Tank 3	Tank 1	Tank 2	Tank 3	Tank 1	Tank 2	Tank 3	Tank 1	Tank 2	Tank 3
Body weight (g)	Tank 1	1											
	Tank 2	0.9978	1										
	Tank 3	0.9968	0.9971	1									
Absolute growth (AG)	Tank 1	1	0.9978	0.9968	1								
	Tank 2	0.9978	1	0.9971	0.9978	1							
	Tank 3	0.9969	0.9978	0.9998	0.9969	0.9978	1						
Absolute growth rate (AGR, g/day)	Tank 1	0.9646	0.9471	0.9521	0.9646	0.9471	0.9490	1					
	Tank 2	0.9803	0.9688	0.9707	0.9803	0.9688	0.9685	0.9954	1				
	Tank 3	0.9682	0.9539	0.9644	0.9682	0.9539	0.9607	0.9927	0.9921	1			
Relative growth rate (RGR, %)	Tank 1	1	0.9978	0.9968	1	0.9978	0.9969	0.9646	0.9803	0.9682	1		
	Tank 2	0.9793	0.9879	0.9792	0.9793	0.9879	0.9806	0.9154	0.9471	0.9272	0.9793	1	
	Tank 3	0.9968	0.9971	1	0.9968	0.9971	0.9998	0.9521	0.9707	0.9644	0.9968	0.9792	1

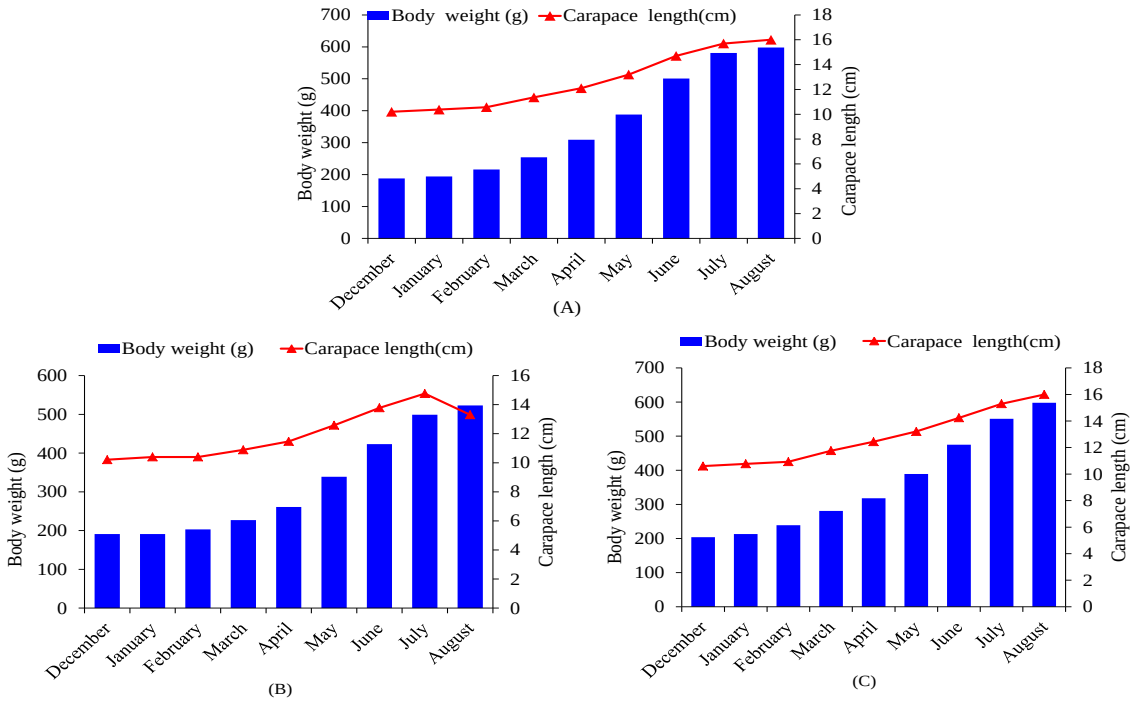


Figure.2. Relationship of body weight and carapace length of Burmese roofed turtle in Tank 1 (A), Tank 2 (B) and Tank 3 (C)

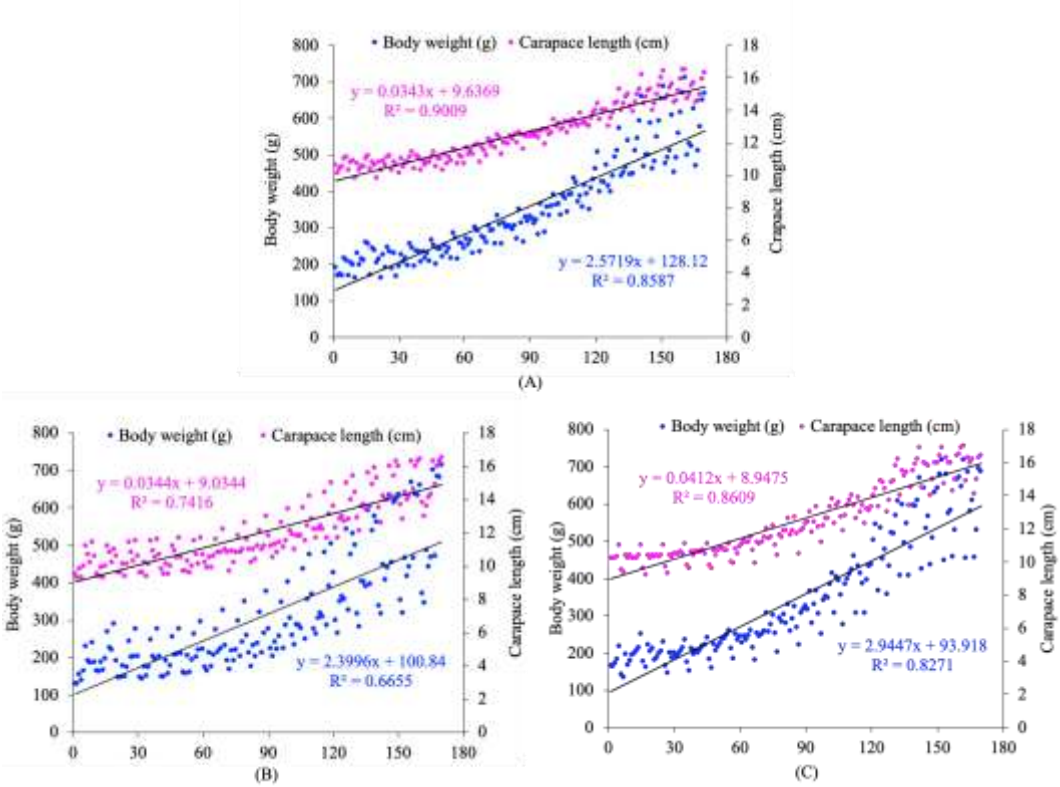


Figure 3. Relationship of body weight and carapace length of Burmese roofed turtle in Tank 1 (A), Tank 2 (B) and Tank 3 (C)

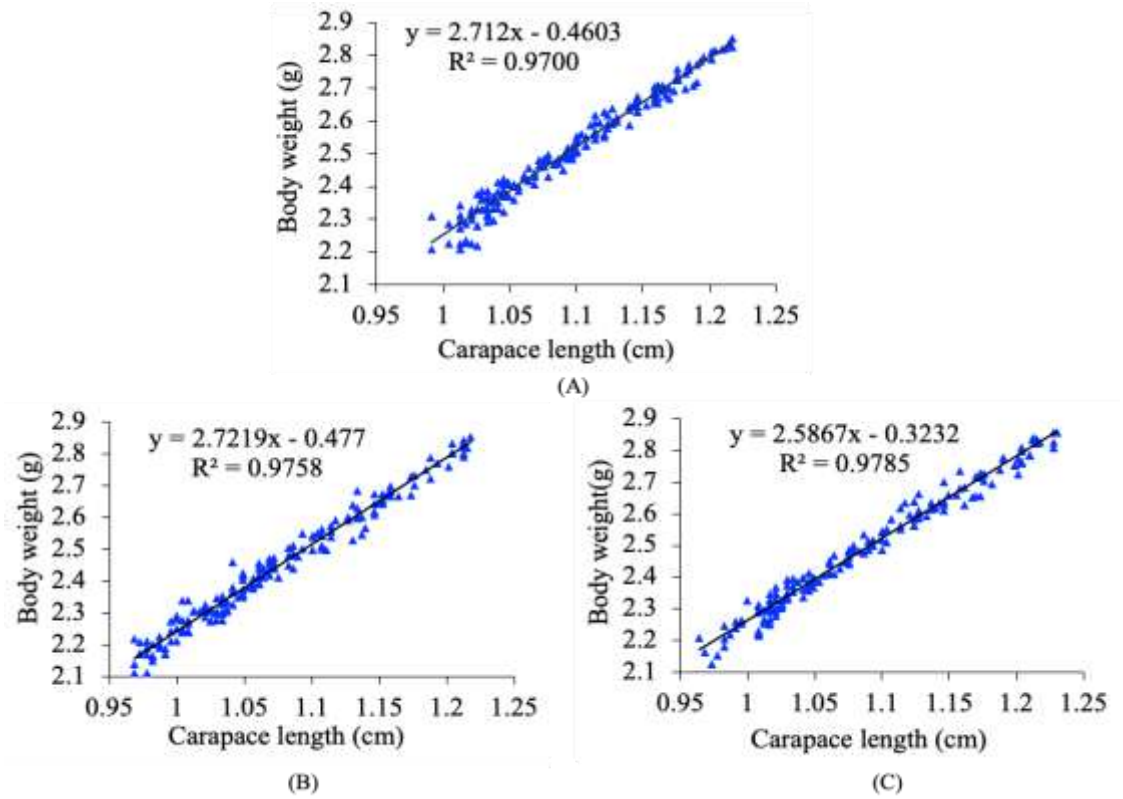


Figure.4. Relationship of body weight and carapace length of Burmese roofed turtle in Tank 1 (A), Tank 2 (B) and Tank 3 (C)

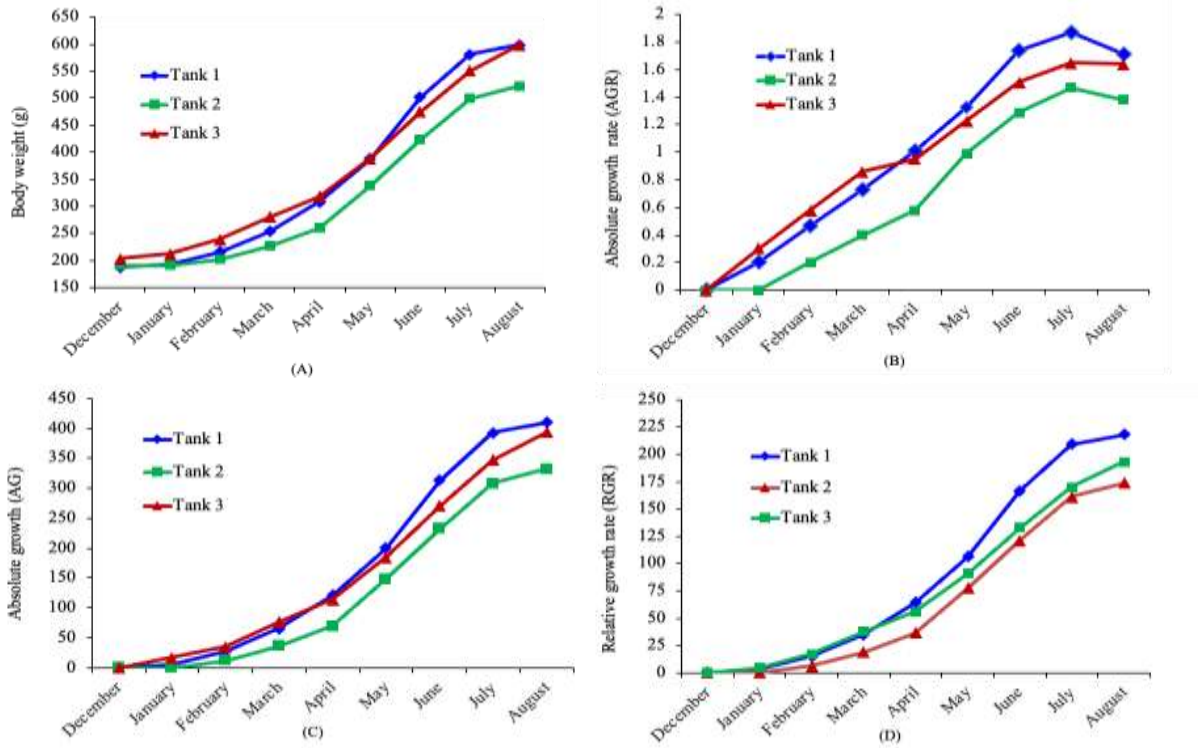


Figure.5. Comparison of different diets on growth performance of Burmese roofed turtle in each trial during December 2019 to August 2020. (A) Body weight (BW), (B) Absolute growth rate (AGR), (C) Absolute growth (AG) and (D) Relative growth rate (RGR)

Discussion

The Burmese roofed turtle *Batagur trivittata* is an endemic species and inhabited into the large rivers of Myanmar. These species are valuable in ecological approach serves as scavengers in the tanks, rivers and stagnant water and thus keep the aquatic ecosystems free from pollution.

In the experiment, the hatchlings are altered to juvenile stage in the months of May to August observed during the study period. All of 30 *Batagur trivittata* hatchlings are healthy and survived throughout the study period.

The nutrient composition of turtle diets are different depend on feeding of the animal and plant materials in which proteins are the major nitrogen source. According to the reference of the value of protein content was 76.44% in shrimp, 71.88 % in fish, 20% in dog food and 2.6 % in kazun-ywet (USDA Nutrient Database, 2019). Thus, the efficacy of different diets on growth performance of Burmese roofed turtles were investigated by feeding on different diets rearing in captivity, each group contains ten individuals per tank and the experimental duration for nine months.

After the experimental trials, the highest mean body weight of Burmese roofed turtles was 309 g (188g-598g) observed in Tank 1 followed by 318g (204g-598g) in Tank 3 and 261g (191g-523g) in Tank 2 in this study. Therefore, the diet of shrimp, *Macrobachium* sp. generated the highest mean body weight (g) of Burmese roofed turtles compared with other trials feeding on dog food in Tank 2 and fish *Oreochromis* sp. in Tank 3, in which turtle from Tank 2 showed the lowest growth performance. However, the growth increment of carapace length in Tank 3 was 12.44 cm (10.60 cm – 16.00 cm) followed by Tank 1 with 12.10 cm (10.20 cm – 16.00 cm) and Tank 2 with 11.47 cm (10.21cm – 13.30 cm) observed. Thus, the different diets of these turtles were not simultaneously provided the growth performance of Burmese roofed turtles in captivity.

The initial growth performance of turtles in Tank 1 and Tank 2 were found similar growth patterns in December and January, however the same growth development revealed in Tank 1 and Tank 3 during the April and May, furthermore, the similar growth increment was also observed in August at the end of the experiment. The significance difference of growth performance in all tanks was investigated at $p < 0.01$ with the Pearson correlation showed the perfect positive relationship $r > 0.9154$.

Concerning with the strength of the relationship between the carapace length and body weight of Burmese roofed turtles were investigated by using regression analysis indicated that the value of growth increment in carapace length was very low association $b = 0.0343$ with $R^2 = 0.9009$ in Tank 1; $b = 0.0344$ with $R^2 = 0.7416$ in Tank 2 and $b = 0.0412$ with $R^2 = 0.8609$ in Tank 3. In addition, the best growth performance of body weight in these turtles was observed in Tank 2 having the value of $b = 2.7219$ with $R^2 = 0.9758$ followed by $b = 2.712$ with $R^2 = 0.9700$ in Tank 1 and $b = 2.5867$ with $R^2 = 0.9785$ in Tank 3, respectively. Therefore, the efficacy of different diets generated the negative allometric growth patterns of Burmese roofed turtles in captivity.

Many researchers have been conducted the growth developmental pattern of turtles by feeding on various diets in situ and ex situ. Because growth can be quantitatively expressed as

the absolute growth (AG) on the basis of changes in length and / or weight in a determined duration. Absolute growth rate (AGR) was expressed in term of some intervals of time, e.g. day, week, month or year. The relative growth rate is given as percentage (Ricker, 1979).

In this study, the absolute growth (AG), absolute growth rate (AGR) and relative growth rate (RGR) of Burmese roofed turtles started at April indicated that the turtles from Tank 1 showed higher growth development than Tank 2 and Tank 3. The accelerated growth pattern of absolute growth rate (AGR) was also found in Tank 1, and the April is the unit development of their growth performance, and halts in July and then decreased in August. Thus, the growth developmental patterns were not influenced by feeding on different diets observed in this study.

Although the remarkable stunted relative growth rate (RGR) was found in April (Tank 2) after the July, their growth pattern was increased at the end of the trial. Therefore, feeding on the kazun-ywet and dog food generated the delay growth pattern in Burmese roofed turtles because they preferred more fresh meals and vegetables than dog food. Thus, among the different diets, the shrimp is more suitable for Burmese roofed turtles than fish and dog food in captivity.

Conclusion

The growth performances of Burmese roofed turtles were greatly influenced by providing the various diets, however, their growth pattern revealed the negative allometric growth pattern with determination of coefficient $R^2 > 0.90$ observed in all trials. Among the diets, the shrimp *Macrobachium* sp. was the better food for growth development of these turtles than fish *Oreochromis* sp. and dog. The good shape of body conformation revealed in Tank 1 than Tank 3 and Tank 2. The significant difference on various diets observed in all trials at the significant level $p < 0.01$. Therefore, the nutrients values of different diets are supplemental foods for growth development of these turtles in captivity.

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MOLECULAR AND MORPHOLOGICAL IDENTIFICATION OF NGA MYIN YINN, *SILONIA SILONDIA* (HAMILTON, 1822) IN TWANTAY CANAL

Aye Myat Chel[1], Yu Yu Aye[2], July Maung Maung[3], Kay Lwin Tun[4]

Abstract

The Silond Catfish (*Silonia silondia*), locally known as Nga Myin Yinn, is an economically significant freshwater species in Myanmar facing threats from overexploitation and habitat degradation. Genomic sequencing, alongside morphological analysis, is essential to support accurate identification and conservation efforts for this species. A total of 50 fingerling Silond catfish were collected from the Twantay Canal (a connection to the Ayeyarwady River) was obtained in May 2023. Species identification was confirmed using morphological characteristics, the FishBase application, and molecular analysis through cytochrome c oxidase I (coxI) gene sequencing. Two primer sets—Fish F2/R2 and LCO 1490 / HCO 2198—were used to amplify the *coxI* region of mitochondrial DNA successfully, yielding 525 bp sequences with the BigDye terminator sequencing method. Analysis of sequence data in the NCBI database showed a high homology (>93% sequence identity) between *S. silondia* populations from Myanmar, India and Bangladesh. Phylogenetic analysis using neighbor-joining (NJ) trees placed *S. silondia* from Myanmar in the same clade as *S. silondia* populations from Bangladesh, indicating strong genetic similarity across regions.

Keywords: *Silonia silondia*, Twantay canal, and Ayeyarwady River, Phylogenetic tree

Introduction

The Silond Catfish (*Silonia silondia*), locally known as Nga Myin Yinn in Myanmar, is one of the most valuable freshwater fish in the country, with market prices reaching up to 30,000 kyats/kg—making it more expensive than beef or pork. This species inhabits the Ayeyarwady River and its associated canals. *Silonia silondia* (Hamilton, 1822) belongs to the order Siluriformes (Nelson *et al*., 2016) and is distributed throughout South and Southeast Asia, including Bangladesh, India, Myanmar, Nepal, and Pakistan (Talwar and Jhingran, 1991; Gupta, 2015). It serves as a crucial target species for local fisheries, providing significant income and food security to communities in Myanmar.

Traditional fishing methods, such as the Nga Myin Yinn Tan long-line technique, are still widely practiced, particularly in the Katha, Mandalay, and Twantay townships. Despite its commercial importance, the population of *Silonia silondia* has been declining across its native range due to overfishing and habitat degradation, with local fishermen reporting decreasing catch rates. Although *S. silondia* is currently classified as "Least Concern" by the IUCN (2017), it is considered near-threatened in Pakistan (CAMB) and India (NBFRG) (Lakra *et al*., 2010) and has been listed as endangered in Bangladesh (IUCN, 2000). In Myanmar, reports from fishermen also indicate a marked decline in this species' abundance.

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To ensure the sustainability of *Silonia silondia*, targeted conservation measures and sustainable fishing practices are critical. Conservation strategies should include establishing protected areas, enforcing fishing quotas, and implementing habitat restoration efforts. Additionally, developing artificial propagation techniques could support sustainable production of this species. Unlike other freshwater fish already cultured in Myanmar, no artificial breeding methods have been established for *Silonia silondia*. The Department of Fisheries (DoF) has initiated preliminary studies to assess the feasibility of artificial propagation, focusing on fish behavior, nutritional requirements, and growth rates at the Twantay Township and Ta Loat Hla Fisheries Station in Maubin Township (Aye Aye Min, personal communication).

However, scientific research on *Silonia silondia* in Myanmar remains limited. Understanding the population structure, migration patterns, and species diversity requires reliable identification through both morphological and molecular methods, especially for the early life stages. Fingerlings of *S. silondia* are occasionally caught in the Twantay Canal, Yangon Region; however, they closely resemble fingerlings of the *Pangasius* species, making identification challenging. Therefore, in the present study, fingerling Silond catfish were collected from natural populations, and their species identity was confirmed through both morphological and molecular analyses.

Materials and Methods

Specimen collection

A total of 50 fish samples were collected from the Twantay Canal, Yangon region (16.74833°N, and 96.08643°E) using a net fence (Pike-Bawoon), with permission from the Department of Fisheries and the license owner (Plate 1 A and B) in May 2024. All samples were transported in oxygen-filled plastic bags with water (Plate 1 C) to the Laboratory of Aquatic Animal Diseases at the University of Yangon. In the laboratory, the fish were temporarily placed in cement tanks with aeration. These tanks were disinfected with 20% NaOH for 48 hours prior to introducing the fish.

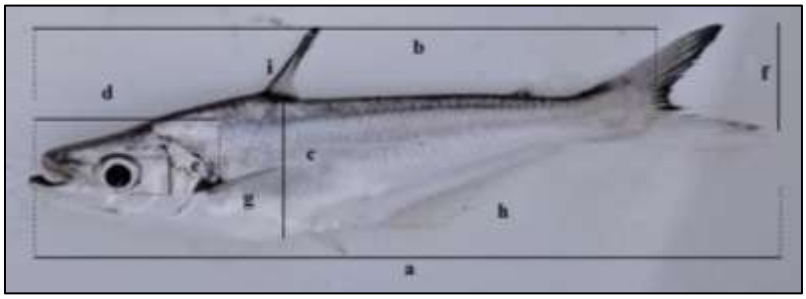
A. Department of fisheries station located at Twantay Canal B. Sample collection C. Transferred of fish using oxygen filled plastic bags

Plate 1 Sample collection at Twantay Canal

Morphological Identification of Fish Species

For the morphological identification of fish species, a total of 3 individuals were collected for morphometric measurements. Detailed measurements included total length, weight, standard length, body depth, head length, eye length, pectoral fin length, dorsal fin height, and the number of fin rays. Additionally, the number of scales along the lateral line, the number of gill rakers on

each arch, the number of teeth in the upper and lower jaws, and the structure of the adipose fin were recorded in Plate 2. Photographic records were taken while the fish were in fresh condition. Measurements were obtained from the sampled fish to assist in species identification, utilizing the identification keys provided by Day (1878), Lagler (1977), Talwar and Jhingran (1991) and Ferraris Jr. (1997).



Measuring of fingerling *Silonia silondia*

a. Total length, b. Standard length, c. Body depth, d. Head length, e. Eye length, f. Caudal fins, g. Pectoral fins, h. Dorsal fins and i. Pelvic fins

Plate 2 Morphometric measurements of fish

Molecular Identification of Fish Species

The muscle near the caudal fins was collected for DNA extraction for species identification of fish. A total of six individuals including three individuals conducted for morphology identification were collected for muscle samples. The samples were named Sample SS1, SS2, SS3, SS4, SS5 and SS6. Among them, three fish samples (SS1, SS2 and SS3) were prepared for the cytochrome c oxidase (*coxI*) region of mitochondrial DNA (mtDNA) amplification using two pairs of primers: forward primer F1 and reverse primer R1, and forward primer F2 and reverse primer R2, as some freshwater fish do not amplify well with the F1 and R1 primers (Ward *et. al.*, 2005).

In addition, other three fish samples (SS4, SS5 and SS6) were amplified for the *coxI* region of mtDNA using forward primer LCO 1490 and reverse primer HCO 2198 (Folmer *et al.*, 1994). Primers were selected based on the availability of the primer bank in the department. The amplified of the *coxI* using different primers sets are shown in Fig. (1).

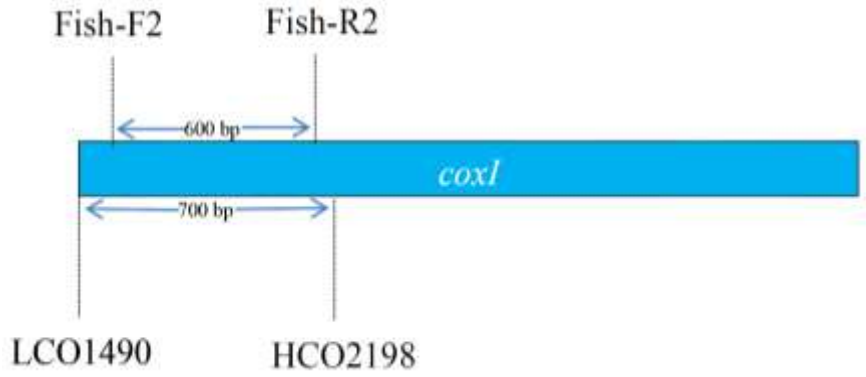


Figure. 1 The amplified regions of the *coxI* using two different primers sets

Genomic DNA Extraction of Fish Species

Fish muscle tissues were preserved in 70% ethanol in tissue sample bottle with labelled. The QIAGEN DNeasy Tissue Kit (QIAGEN Inc., Valencia, CA) was used for DNA extraction. Briefly, approximately 5 mg of tissue was digested in an extraction buffer solution (100 mM Tris, pH 8.0; 100 mM EDTA, pH 8.0; 100 mM NaCl) with Proteinase K (20 mg/ml) at 72°C for 4 to 24 hours. Remaining proteins and polysaccharides were removed using a DNeasy Mini spin column. The precipitated DNA was washed with 100% ethanol, resuspended in buffer AE, and stored in a freezer until utilization. The concentration of nucleic acid fish samples was determined by using NanoDrop one 2000 Spectrophotometer.

PCR Amplification and Gel Electrophosis of Fish Species

For PCR, 5 µl of extracted DNA was combined with 15 µl of water, 2.5 µl of each 25 µM primer, and 25 µl of AMPIGENE® HS Taq DNA Polymerase. The PCR profile for the Fish F1/Fish R1 and Fish F2/Fish R2 primers included an initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 1 minute, finishing with a final extension at 72°C for 10 minutes. For the LCO 1490/HCO 2198 primers, the PCR profile started with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 40°C for 1 minute, and extension at 72°C for 30 seconds, concluding with a final extension at 72°C for 7 minutes. The primer sequence and amplify regions were described in Table. (1).

The PCR products were separated and visualized using electrophoresis on a 2.0% agarose gel stained with GR Green loading dye (Wiz Bio Solution, Republic of Korea). A 100 bp DNA marker (Wizbio Solution Co. Ltd., Korea) was included for size comparison. Gel bands were visualized with a blue light transilluminator (GeneDireX, Inc.).

Table 1 Primers for the PCR amplification of fish species

Primers	Primer Sequences	Expected amplicon size	Reference
Fish-F1	5’TCAACCAACCACAAAGACATTGGCAC3’	600	Ward *et al.*, 2005
Fish-R1	5’TAGACTTCTGGGTGGCCAAAGAATCA3’		
Fish-F2	5’TCGACTAATCATAAAGATATCGGCAC3’	655	
Fish-R2	5’ACTTCAGGGTGACCGAAGAATCAGAA3’		
LCO1490	5’GGTCAACAAATCATAAAGATATTGG3’	700	Folmer *et al*., 1994
HCO2198	5’TAAACTTCAGGGTGACCAAAAAATCA3’		

Gel Purification and DNA Sequencing of Fish Species

Distant DNA bands were excised using a blade and transferred to 1.5 ml tubes (Plate 3). DNA extraction and purification from the bands were performed using the FastGene Gel/PCR Extraction Kit (NIPPON Genetics Co., Ltd.), following the manufacturer's instructions. The purified amplicons were then submitted to Macrogen, Inc. (Geumchun-gu, Seoul, Korea) for DNA sequencing. Single-pass sequencing was conducted on the template using forward primers. The sequencing reactions were carried out with the DNA Engine Tetrad 2 Peltier Thermal Cycler (BIO-RAD) and the ABI BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), adhering to the manufacturer's protocols.

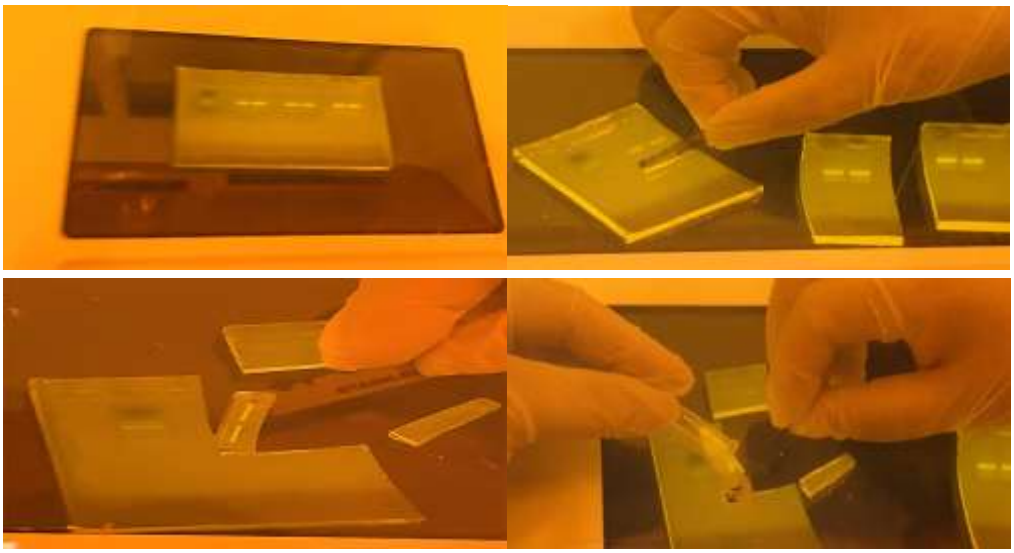


Plate 3 Cutting Distinct Bands of PCR Amplified Fish Samples for Gel purification

Sequence Alignment and Phylogenetic Analysis of Fish Species

Among the six samples five sequences were qualify for the alignment. The sequences from the five individual fish were aligned using MEGA 11 software. Manual editing was performed to remove mismatched sites and gaps. The ten most similar reference sequences, retrieved from the NCBI database, were compared to those obtained in this study. For a comprehensive analysis, the acquired sequences and reference sequences underwent multiple sequence alignment using the Muscle alignment tool in MEGA 11. The aligned sequences were then used to construct a phylogenetic tree, employing the Neighbor-Joining method with 1000 bootstrap replicates for validation.

Results

Morphological Description of *Silonia silondia*

A total of 50 individuals (13.86 ± 2.02 cm and 17.92 ± 10.09 g) were collected and their morphological identification was carried out. The species was confirmed as Silond catfish, *Silonia silondia* (Hamilton, 1822), through morphological characteristics using the FishBase software.

The fin formula of collected fish samples are is D1.I/7; D.0; P.I/11-13; V.6; A.40-46 (4/35-44); C.17.

D1. I/7

D1 refers to the first dorsal fin. **I** indicate the number of spines in that fin, which is 1.**7** refers to the number of soft rays in the first dorsal fin.

D. 0:

D refers to second dorsal fin was absent.

P. I/11-13:

P refers to the pectoral fins. **I** indicate 1 spine in the pectoral fin. **11-13** refers to the range of soft rays in the pectoral fin of collected samples.

V. 6:

V refers to the ventral (pelvic) fins. The number **6** indicates that there are 6 soft rays in the ventral fins selected fish.

A. 40-46 (4/35-44):

A refers to the anal fin. The range **40-46** indicates the number of soft rays in the anal fin of sampled fish. The notation **(4/35-44)** provides additional information that there are 4 spines in the anal fin, with a range of 35 to 44 soft rays.

C. 17:

C refers to the caudal fin. The number **17** indicates that there are 17 rays in the caudal fin.

According to the morphological identification, the fish is identified as *Silonia silondia*.

DNA Concentration of *Silonia silondia*

The extracted DNA concentrations of fish of fish samples was determined by using NanoDrop one 2000 Spectrophotometer. DNA concentration ranged from 32.8 to 329.6 ng/µl. The purity ratios (A260/A280) of the samples varied between 1.39 - 2.07 (Plate 4).

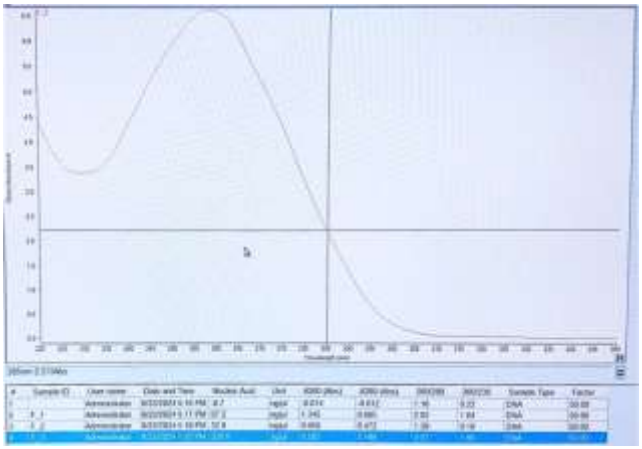
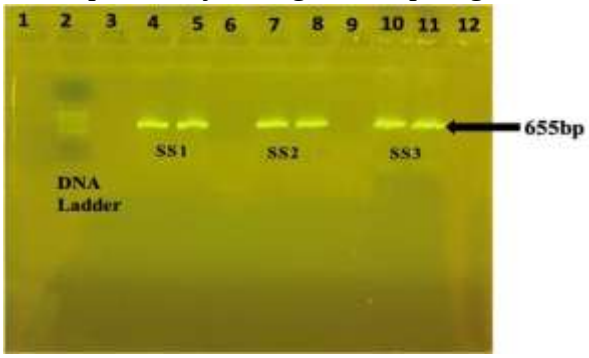


Plate 4 Graph of genomic DNA concentration of extracted from fish samples

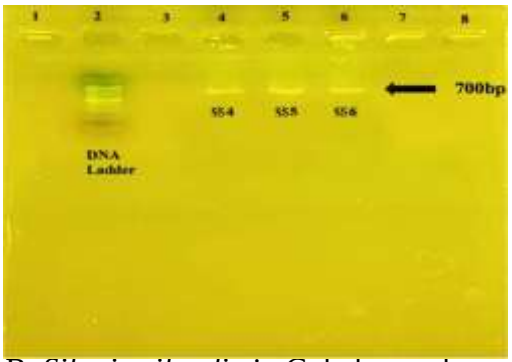
Amplification of *coxI* region of *Silonia silondia*

The *coxI* region of *Silonia silondia* samples was amplified using the FishF2/R2 primer set, with results shown in Plate 5 A. Lane 2 contains a 100 bp DNA ladder as a reference for fragment size. Lanes 4, 5, 7, 8, 10, and 11 display PCR results from three *Silonia silondia* samples (SS1, SS2 and SS3), showing successful amplification of a 655 bp fragment. In contrast, the FishF1/R1 primers could not amplified the fish samples.

For SS4, SS5, SS6, the *coxI* region of tissue samples was also successfully amplified with the LCO/HCO primers, yielding a 700 bp fragment as seen in Lanes 4, 5, and 6 (Plate 5 B).



A. *Silonia silondia* in Gel electrophoresis using Fish F_2 /R_2 primers



B. *Silonia silondia* in Gel electrophoresis using LCO1490/ HCO 2198 primers

Plate 5 PCR amplification of *Silonia silondia* using different primer pairs

Cytochrome c oxidase I (*coxI*) gene sequence analysis of *Silonia silondia* with the Fish-F2/ Fish-R2 primer pair

Among the three samples amplified with the Fish-F2/ Fish-R2 primers, two samples (SS1 and SS2) were successfully sequenced for their *coxI* regions. The third sample (SS3) showed contamination and noise, and thus was excluded from the data analysis.

The analysis indicated that the two *Silonia silondia* specimens form a slightly diverged clade with strong bootstrap support, reflecting a well-resolved evolutionary relationship with *Silonia silondia* and *Silonia childreni*. These species, collected from distinct geographic locations (Bangladesh and India, respectively), exhibited well-supported clades with bootstrap values of 94% and 93%, indicating considerable divergence from one another and from other groups in the tree. Notably, *Clupisoma bastari* from India clustered more closely with *Silonia silondia* than with other *Clupisoma* species (*C. prateri*, *C. garua*, and *C. sinense*), suggesting an unexpected phylogenetic relationship that warrants further investigation. The outgroup species, *Tenulosa ilisha* and *Monopterus javanensis*, formed a separate clade, which is characteristic of taxa outside the Siluriformes group, thereby contextualizing the relationships within the catfish lineage (Fig. 2, Table 2 and 3).

Table 2 Comparison of Cytochrome c oxidase 1 gene (*coxI*) sequence of the present sample *Silonia silondia* (SS1) and other species registered in NCBI database (The sequencing primer is Fish-F2/ R2)

Species	Locality	Percent Identity (%)	Assessor number in NCBI
Silonia silondia	Bangladesh	94.84	MK572595
Silonia childreni	India	93.98	MT654654
Clupisoma bastari	India	91.33	OP493140
Eutropiichthys vacha	India	90.89	KX946624
Proeutropiichthys taakree	India	90.74	OQ607735
Clupisoma sinense	China	90.45	JN020077
Clupisoma prateri	China	92.15	PP778663
Clupisoma garua	Pakistan	91.90	OP575582

Source: NCBI database.

Table 3 Comparison of Cytochrome c oxidase 1 gene (*coxI*) sequence of the present sample *Silonia silondia* (SS2) and other species registered in NCBI database (The sequencing primer is Fish-F2/R2)

Species	Locality	Percent Identity (%)	Assessor number in NCBI
Silonia silondia	Bangladesh	94.79	MK572595
Silonia childreni	India	93.92	MT654654
Clupisoma bastari	India	91.33	OP493140
Eutropiichthys vacha	India	90.79	KX946624
Proeutropiichthys taakree	India	90.68	OQ607735
Clupisoma sinense	China	90.51	JN020077
Clupisoma prateri	China	92.07	PP778663
Clupisoma garua	Pakistan	91.90	OP575582

Source: NCBI database.

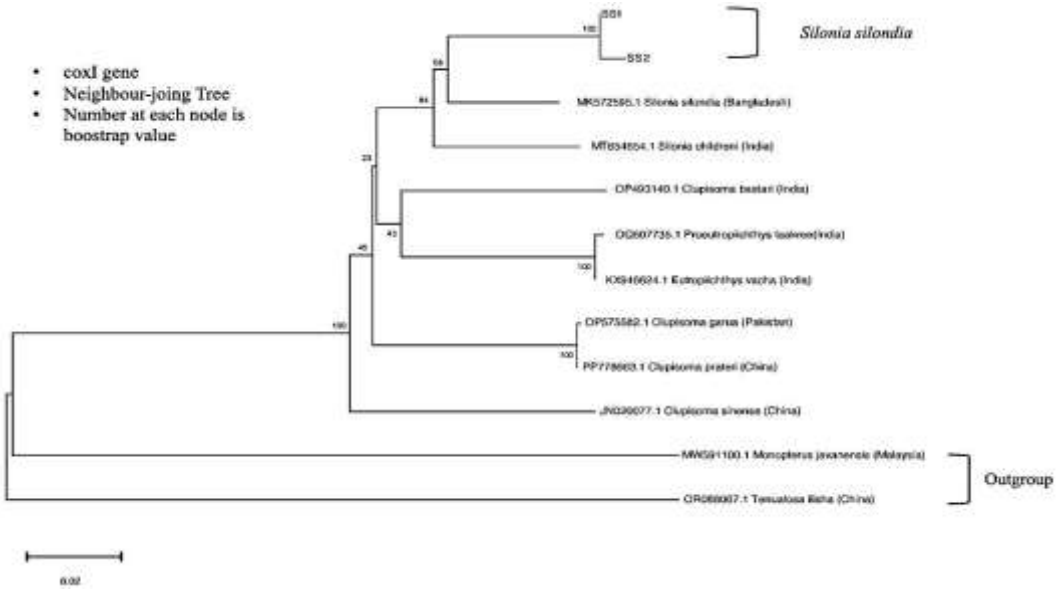


Figure. 2 Phylogenetic analysis of Species using Neighbor-Joining tree Based on *coxI* Gene Sequences amplified with Fish F2/R2 Primers

Cytochrome c oxidase I (*coxI*) gene sequence analysis of *Silonia silondia* with the LCO1490/ HCO 2198 primer pair

The resulting NJ tree indicated that the three *Silonia silondia* specimens formed a slightly diverged clade with strong bootstrap support, suggesting a well-defined evolutionary relationship with *Silonia silondia* and *Silonia childreni*. Specimens from geographically distinct regions—Bangladesh and India—exhibited well-supported clades with bootstrap values of 93% and 92%, respectively, highlighting significant divergence from each other and from other species in the analysis. Furthermore, species such as *Proeutropiichthys taakree* and *Eutropiichthys vacha* from the same region demonstrated a closer phylogenetic relationship with *Silonia silondia*, while the marine species *Tenulosa ilisha* and *Monopterus javanensis* clustered in a separate clade, reinforcing their role as outgroups in the phylogenetic analysis of these catfish species (Fig. 3, Table 4, 5 and 6).

Table 4 Comparison of Cytochrome c oxidase 1 gene (*coxI*) sequence of the present sample *Silonia silondia* (SS4) and other species registered in NCBI database (The sequencing primer is LCO1490 /HCO 2198)

Species	Locality	Percent Identity (%)	Assessor number in NCBI
Silonia silondia	Bangladesh	93.98	MK572595
Silonia childreni	India	92.81	MT654654
Eutropiichthys vacha	India	91.05	KX946624
Proeutropiichthys taakree	India	91.27	OQ607735
Clupisoma sinense	China	90.61	JN020077
Clupisoma prateri	China	92.25	PP778663
Clupisoma garua	Pakistan	92.06	OP575582

Source: NCBI database.

Table 5 Comparison of Cytochrome c oxidase 1 gene (*coxI*) sequence of the present sample *Silonia silondia* (SS5) and other species registered in NCBI database (The sequencing primer is LCO1490 /HCO 2198)

Species	Locality	Percent Identity (%)	Assessor number in NCBI
Silonia silondia	Bangladesh	93.71	MK572595
Silonia childreni	India	92.72	MT654654
Eutropiichthys vacha	India	89.89	KX946624
Proeutropiichthys taakree	India	90.10	OQ607735
Clupisoma sinense	China	89.41	JN020077
Clupisoma prateri	China	90.95	PP778663
Clupisoma garua	Pakistan	90.95	OP575582

Source: NCBI database.

Table 6 Comparison of Cytochrome c oxidase 1 gene (*coxI*) sequence of the present sample *Silonia silondia* (SS6) and other species registered in NCBI database (The sequencing primer is LCO1490 /HCO 2198)

Species	Locality	Percent Identity (%)	Assessor number in NCBI
Silonia silondia	Bangladesh	93.99	MK572595
Silonia childreni	India	92.00	MT654654
Eutropiichthys vacha	India	91.04	KX946624
Proeutropiichthys taakree	India	91.26	OQ607735
Clupisoma sinense	China	90.60	JN020077
Clupisoma prateri	China	92.24	PP778663
Clupisoma garua	Pakistan	92.06	OP575582

Source: NCBI database.

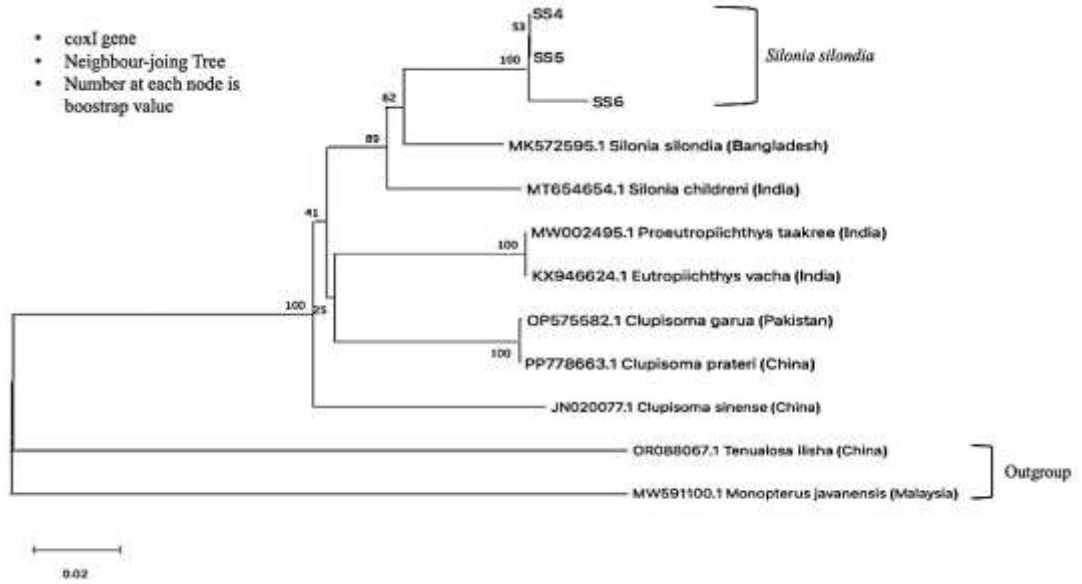


Figure. 3 Phylogenetic analysis of Species using Neighbor-Joining tree Based on *coxI* Gene m Sequences amplified with LCO1490 /HCO 2198 Primers

Discussion

In this study, two methods, morphological identification and DNA sequencing of the *coxI* region, were used to identify the species. The morphological analysis confirmed that the fish collected were *Silonia silondia*. Additionally, the phylogeny of *S. silondia* was analyzed using sequences from the Cytochrome c oxidase subunit 1 (*coxI*) gene. The *coxI* gene is often used for DNA barcoding, as it is crucial for cellular energy production and is highly conserved across species while still varying enough to differentiate them. Since 2003, studies have shown that the *coxI* gene is highly conserved within the same species but highly unique across different species (Ward *et al*., 2005).

In the present study, *coxI* regions of *Silonia silondia* were sequenced using three primer sets. Of these, the Fish F1/R1 primer set did not successfully amplify the *S. silondia* samples. Liu *et al*. (2020) used Fish F1/R1 and Fish F2/R2 primers to amplify *COI* regions in 179 fish samples collected from Xinxiang and Zhengzhou in Henan Province, China. They reported that Fish F1/R1 amplified the *COI* gene in 63 (35.20%) samples, while F2/R2 amplified 132 (73.74%) samples. This finding suggests that certain fish species may not reliably amplify with the Fish F1/R1 primer, aligning with our result that Fish F1/R1 could not amplify *S. silondia*.

The LCO/HCO primer pair, a widely-used set in cytochrome c oxidase subunit I (*COI*) amplification studies, has varying annealing temperatures, ranging from 42 °C to 55 °C (Dahlgren *et al*., 2000; Kelly *et al*., 2007; Margam *et al*., 2010; Muller and Beheregaray, 2010). These primers, LCO1490 and HCO2198, are universal DNA primers applicable across vertebrates and various animals (Sharma and Konayashi, 2014). In this study, three samples were successfully amplified using this primer set.

The *coxI* sequence from this study was compared with available genetic data of *Silonia silondia*. However, data from Myanmar were absent. Toe Toe Soe *et al*. (2021) analyzed the genetic data of *S. silondia* in the Ayeyarwady River, Myanmar, using mitochondrial DNA markers *cytb* and *ATPase* genes, but they did not include the *COI* region. Consequently, the present *COI* sequence could not be compared to the sequences of *S. silondia* reported by Toe Toe Soe *et al*. (2021).

Additionally, Theint Sandar Saing (2018) studied molecular variation and phylogenetics of *Silonia silondia* using *COI* and *cytb* datasets. However, their *COI* data are not registered in the NCBI database, preventing direct comparison. Mandal *et al*. (2016) reported sequence analysis of *S. silondia* from four Indian rivers using *cytb* (1140 bp) and *ATPase 6/8* (842 bp) markers, but did not use the *COI* region. *S. silondia* is an economically important food fish, and analyzing molecular markers can reveal genetic differentiation within populations, as shown in Mandal *et al*. (2016). Using *COI* is advantageous because it is short enough for rapid sequencing and cost-efficiency while still distinguishing species-level variations (IUCN Bangladesh, 2014).

In this study, the *coxI* region of *Silonia silondia* was compared to sequences in the NCBI database. The closest match was with samples from Bangladesh, showing 94% similarity, indicating a high level of genetic homology. Ng and Tan (1999) discuss how freshwater fish distributions across historically connected river basins, particularly during periods of lower sea levels, facilitated gene flow between populations in Southeast Asia and the Indian subcontinent. This historical connectivity likely contributed to the observed genetic homology.

This study highlights the genetic variation of *Silonia silondia* in Myanmar relative to other countries. Such findings are essential for understanding genetic resources in freshwater species across historically connected landscapes.

Conclusion

This study successfully confirmed the species identity of Silonia silondia (Nga Myin Yinn) fingerlings from the Twantay Canal using both morphological and molecular coxI sequencing methods. Morphological and molecular analyses confirmed the species identity as *Silonia silondia*. Molecular identification was performed through cytochrome c oxidase I (*coxI*) gene sequencing using multiple primers. Phylogenetic analysis based on neighbor-joining (NJ) trees, constructed from *coxI* sequences with Fish F2/R2 and LCO1490 /HCO 2198 primers, included sequences from *Silonia silondia* specimens, respectively, along with reference sequences from GenBank. The coxI region of *S. silondia* collected in Myanmar is highly homology to *S. silondia* collected from Bangladesh rather than the India and China. These findings are essential for future conservation, genetic resource management, and the development of artificial propagation programs for this commercially vital but increasingly vulnerable fish species.

Acknowledgements

We are especially grateful to Associate Professor Dr. Terahara Takeshi of the Department of Marine Biosciences, Tokyo University of Marine Science and Technology, for his invaluable guidance throughout this study. We would also like to thank Daw Aye Aye Min at the Ta Loat Hla Fisheries Station, Department of Fisheries (DoF), Maubin Township, for providing essential facilities and support for this study.

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EFFECTS OF DIFFERENT CONTROL STRATEGIES FOR INSECT PEST MANAGEMENT OF MANGO

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Abstract

Mango is one of the most economically significant agricultural products in Myanmar, but its production is severely constrained by insect pests. The effective pest management is important for enhancing mango production. The present study aimed to evaluate methods for reducing mango damage by investigating the effectiveness of insect pest control strategies in a mango orchard located at the Vegetable and Fruit Research and Development Center (VFRDC), Ye Mon Village, Hlegu Township, Yangon Region. Insect pests were collected from February to September 2024 using the three trap methods: methyl eugenol traps, yellow sticky traps, and pineapple fruit juice traps. A total of 21 insect species, representing 16 genera across 15 families and six orders, were recorded. Among them, 5722 individuals from 17 pest species and four natural enemy species were observed. The mango infection prevalence at the VFRDC was 13.3%. Of the control strategies tested, yellow sticky traps captured the highest number of pests. Visual inspection of fruit damage was more significant in the control group, where fruit size was reduced. Larvae were not found in fruits associated with methyl eugenol or yellow sticky traps. Statistically significant differences in insect capture were observed between traps ($P < 0.05$). The study concludes that the strategic deployment of Yellow Sticky and Methyl Eugenol traps is a highly effective, low-cost, and sustainable component of an IPM strategy for enhancing mango quality and yield in Myanmar.

Keywords: mango, pests, management, control

Introduction

The cultivation of mangoes is growing throughout the world. Its origin is Southern Asia, originating from the border area of Northwest Myanmar and Eastern India. Mangoes are commercially cultivated in the central Mandalay region, Ayeyarwady region, the Southern regions, and Shan State in the east over a size of some 80,000 hectares (Hirano *et al*., 2008). Among the cultivated mango species in Myanmar, Sein Ta Lone, *Mangifera indica* L., belongs to the family Anacardiaceae and is the most popular mango verity in Myanmar. Mango is attacked by a variety of insect pests, these insect pests attack roots, stems, leaves, flowers, and fruits thereby, causing a decline in quantity or quality of potentially harvestable and unmarketable (Brower, 1997). Nearly 440 species of fruit flies are distributed primarily in tropical Asia, the South Pacific, and Australia (White and Elson-Harris, 1992).

Adult female fruit flies attacked the fruits including mango by oviposition and maggots polluted and destroyed the fruit by feeding on the pulp (White and Elson-Harris, 1992). The main hosts of *Bactrocera dorsalis* are guava, mango, citrus, banana, avocado, papaya, etc. It is a serious pest of all fleshy fruits and vegetables in the general Southeast Asia region, Hawaii (Hill, 1983). *Bactrocera dorsalis* is a serious pest of mangoes and other soft fruits in Myanmar and in several parts of the world (Jayanthi and Verghese, 2002).

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Infested fruits are unmarketable and sometimes they cause total losses if it is not controlled by suitable insecticide, or the fruits are not bagged. Therefore, the fruit fly plays an important role in fruit production for domestic consumption and export as well (Pedigo, 2002). Therefore, pesticides are widely used to protect crops against insect pests. Agricultural insect pest management is largely dependent on the use of chemical insecticides. However, sticky traps are used as one of the effective Integrated Pest Management (IPM) strategies for different insect pests in most parts of the world (Stern *et al*., 1959).

The yellow sticky traps can be utilized for controlling and monitoring the population of many pests that are more effective in capturing adult insects than the other color sticky traps. Yellow sticky traps can capture a great number of adult pests therefore reducing the adults on the host (Mi Zin Mar Khaing, 2023). Methyl eugenol can attract male fruit flies at a distance of 0.6 km (Steiner, 1952). Methyl eugenol is a very significant sex pheromone for the control of male fruit flies. If the male fruit flies are absent and only females remain in the field then the population of fruit flies is automatically reduced without causing any harm to the crops (Steiner *et al*., 1965). The fruit juice trap that comprises a solution attractive to the insect. These traps are broadly applied in commercial crops and are low-cost to make. The rate of insect populations may depend on several factors including food, predators, host plants, and competitors. The aim of the present study is to provide information on the damage caused by pests. Thus, the study was conducted with the following objectives:

- to identify the insect pests in mango trees at the Vegetable and Fruit Research and Development Center (VFRDC)
- to calculate the prevalence of infestation rate on mango trees in study area
- to compare the effects of different methods in insect pests control on mangoes

Materials and Methods

Study site and period

The investigation on the insect pests of mango, Mangifera indica Linnaeus (1753) was conducted at the Vegetable and Fruit Research and Development Center (VFRDC), Ye Mon Village (N 16∘49′36″, E 96∘7′50″), (56 km from Northeast of Yangon), Hlegu Township, Yangon Region (Fig. 1 and Plate 1). The study area comprised 180 mango plants across 0.74 acres. Data collection spanned from January to September 2024.

Experimental Design and Trapping Methods

The experiment was set up as a Randomized Complete Block Design (RCBD) with four treatments, (1) Plot with methyl eugenol traps (2) Plot with yellow sticky traps (3) Plot with pineapple fruit juice traps and (4) Plot without traps (control). Each treatment had four replications. Traps were placed between plots. Insect collection was performed twice a month (every two weeks) between 7:00 am and 11:00 am. Collected specimens from each trap were transferred to plastic containers to the laboratory to count the numbers, identification of species, and further investigation. **Insect Identification**

Specimens were identified using dissecting and stereo microscopes at the VFRDC. Identification keys and published literature were used to classify insects into pests and natural

enemies (Hill, 1983; White Elson-Harris, 1992; Drew and Hancock, 1994; Nair, 1995 ; Morris and Waterhouse , 2001).

Fruit Quality and Damage Assessment

For each of the four treatments with four replications, ten mango fruits were randomly harvested for assessment. Each mango harvested from the field was weighed with a weight scale from the laboratory. Then, the width, length, and thickness of the mangoes were measured by using an vernier clipper. Then, the sweetness was measured using a refractometer. The number of oviposition marks, number of pest infestation marks, and presence of larvae on the mangoes were observed and counted. The number of oviposition marks, number of pest infestation marks, and presence of larvae in the pulp of mangoes were searched with hand lenses.

Data Analysis

Analysis of variance (ANOVA) test was used to analyze obtained data for comparison between the different trapping methods using the Statistical Package for Social Science (SPSS) software version. Mean and Standard deviation values were calculated by Microsoft Excel.
To calculate the prevalence of mango infestations, the following formula was used.

$$\text{Prevalence (\%)} = \frac{\text{Number of inspected trees}}{\text{Total number of mangoes}} \times 100$$

Where:

Number of inspected trees - the number of trapped trees in this study

Total number of mangoes - the total number of studied mango trees

(Source from Google Maps, 2024)

Figure. 1 Map of Myanmar showing study site, VFRDC in Hlegu Township, Yangon Region

A. Location of study site B. Mango trees in study site

Plate 1 Study area

Results

A total of 21 insect species belonging to 16 genera under 15 families and 6 orders were collected and identified in the study site of Ye Mon Village, Hlegu Township, Yangon Region. The percentage of species occurred according to the order (Fig. 2).

Descriptive account of insect pests

1. *Batocera rufomaculata* (De Geer, 1775) (Plate 2 A)

Its body is about 20 to 70 mm in length. The antennae are very long, and the adult beetles are grayish with two pink dots having a lateral spine. The spots are on the hardened forewings, having the bright red prothorax.

2. *Aulacophora foveicollis* (Lucas, 1849) (Plate 2 B)

The body is about 6 to 8 mm in length. It is reddish yellow, oval-shaped, and the antennae are about half of the body length. Its head is small, and the color is brown. It has dark-colored eyes.

3. *Aulacophora lewisii* (Baly, 1886) (Plate 2 C)

The body of adult beetles is 6 to 6.5 mm in length. Its body is slender, and it has black colored wings. Its head is golden yellow color, having black-colored eyes.

4. *Monolepta australis* (Jacoby, 1882) (Plate 2 D)

The body is about 6 to 12 mm in length. The color is yellow with a red spot on the base of each wing cover, having a red mark across the top of the shoulders.

5. *Odontotermes* sp. (Holmgren, 1912) (Plate 2 E)

The body is about 8.5 to 9.7 mm in length, including wings. The color of the body varies from light to dark brown. It has equal two large and translucent wings. The body shape is straight, having two straight antennae with a slight curve.

6. *Oecophylla smaragdina* (Fabricius, 1775) (Plate 2 F)

The body is about 6 to 10 mm in length. The adults are red-colored.

7. *Ricania* sp. (Germar, 1818) (Plate 2 G)

The body is about 10 to 14 mm in length. The color is dark brown with pale bands. A notch is included on each side of the forewings, and the rest of the margin appears with many bands.

8. *Leptocentrus taurus* (Fabricius, 1775) (Plate 2 H)

The body is about 9 to 13 mm in length. The color of the dorsal surface on the conical body is black, having white spots on the sides of the body.

9. *Dictyophara* sp. (Germar, 1830) (Plate 2 I)

The body is about 12 to 14 mm in length. The color of the body is light green at the end of the rostrum, and it is rounded in shape. It has slender legs, and the wing is membranous, the large, extending behind the abdomen.

10. *Idioscopus clypealis* (Lethierry, 1889) (Plate 2 J)

The body is about 8 to 9 mm in length. The color is light brown with dark spots on the vertex, having two spots on the scutellum.

11. *Idioscopus nitidulus* (Walker, 1870) (Plate 2 K)

The body is about 4 to 5mm in length. The color is dark brown, and it is wedge-shaped with wavy lines on the wings and three spots on the scutellum.

12. *Bemisia tabaci* (Gennadius, 1889) (Plate 2 L)

The body is about 1 mm in length, and has two pairs of white wings. The color of the body is light yellow.

13. *Drosophila melanogaster* (Meigen, 1830) (Plate 2 M)

The body is only about 3 mm in length and yellow-brown color. It has a rounded head and the antennae are short. Black stripes appear on the dorsal surface of its abdomen. Larvae are minute white maggots.

14. *Bactrocera dorsalis* (Hendel, 1912) (Plate 2 N)

The body is about 5.2 mm in length. It has a yellowish abdomen and a black T-shaped mark. A brown band appears along the edge of its wings. It has a prominent dark thorax with two yellow stripes on top and yellow marks on each side.

15. *Bactrocera correcta* (Bezzi, 1916) (Plate 2 O)

The body is about 5.6 mm in length. The thorax is black colored with yellow patches, while the color of the abdomen is also yellow-orange with a dark T-shaped mark, having black spots at the tip of the wings.

16. *Bactrocera zonata* (Saunders, 1841) (Plate 2 P)

The body is about 6.7 mm in length. The color is orange to brownish. It has a yellow dorsal shield having a dark spot on the tip of the wing.

17. *Bactrocera cucurbitae* (Coquillet, 1849) (Plate 2 Q)

The body is about 6.5 mm in length. The color of the body is light brown. It has bright yellow marks on the thorax and a black ‘T’ pattern appears at the base of the abdomen. The wings are clear having “a melon seed” shaped spot at the tip with a dark coastal vein.

Descriptive account of natural enemies

18. *Cheilomenes sexmaculatus* (Fabricius, 1781) (Plate 2 R)

The body is 3 to 6 mm in length. The shape is oval, and the color is shiny, light red. Six black spots appear on the wing covers including two zigzag lines, having a rear black spot.

19. *Chrysoperla* sp. (Steinmann, 1964) (Plate 2 S)

The body is about 12 to 20 mm in length. It is pale green with long antennae, having bright, and golden eyes. It has large, transparent and pale green wings and the body is delicate.

20. *Condylostylus* sp. (Bigot, 1859) (Plate 2 T)

The body is about 5 to 6 mm in length and slender. The colors of the head, eyes, thorax, and abdomen are shiny and metallic green. It has short black antennae. It has three segments, and the wings have two smoky-brown bands on the outer half, connected along the leading edge.

21. *Lucilia sericata* (Robineau-Desvoidu, 1830) (Plate 2 U)

The body is about 8 to 10 mm in length. The color varies from metallic green to copper green color. It has a hairy back and the wings are clear with light brown veins, and the colors of the legs and antennae are black.

Species composition and variation of insects during the study period

Among them, 5722 individuals from 17 pest species and four natural enemy species were observed. The highest population (1808 individuals) occurred in May, while the lowest (1260 individuals) was recorded in March 2024. *Bactrocera dorsalis* was the most abundant species (1365 individuals), followed by *Idioscopus nitidulus* (1139 individuals), while *Ricania* sp. and *Dictyophara* sp. were the least abundant (2 individuals each) (Fig. 2 and Table 1, 2).

Prevalence of insect pest infestations on mango trees

A total of (24) out of (180) plants were found to be infected, resulting in a prevalence rate of 13.3% (Table 3).

Insect population observed on different traps

The yellow sticky treatment is the highest capturing pests among other treatments. The methyl eugenol treatment is higher than the pineapple fruit juice treatment and lower than yellow sticky treatment in catching pests. Yellow sticky traps were found most attractive for insects (371.25^{b}±46.90). Pineapple fruit juice traps were less attractive than recorded traps (22.88^{a}±2.48). Statistically significant differences were found in the number of recorded insects captured on different traps at $P < 0.05$ (Table 4).

In methyl eugenol traps, a total of (2342) individuals with four species of pests were caught in mango orchards during the study period (Fig. 3). In yellow sticker traps, the total population of pests was (2895) individuals, and the population of natural enemies was (302) individuals. Totally (15) species of pests and four species of natural enemies were collected (Fig. 4 and 5). In the pineapple fruit juice trap, a total of (183) individuals with seven species of the pest were collected (Fig. 6).

Effectiveness of fruit quality and damage status of infestations caused by pests

The mangoes without treatment and with treatment were found significant to the insect pests. The lowest number of pest infestation marks and the highest number of fruit length and fruit width were in ME traps. The fruit weight and thickness in the FJ trap were the highest. The lowest average number of oviposition marks was recorded in YS traps (Table 5).

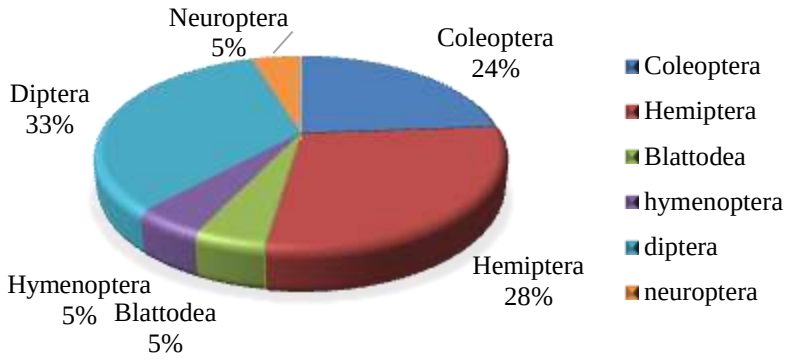
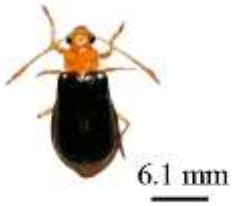


Figure. 2 Species composition of recorded insects according to order

(A) *Batocera rufomaculata*

(B) *Aulacophora foveicollis*



(C) *Aulacophora lewisii*

(D) *Monolepta australis*

(E) *Odontotermes* sp.

(F) *Oecophylla smaragdina*

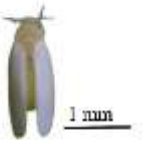
(G) *Ricania* sp.

(H) *Leptocentrus taurus*

(I) *Dictyophara* sp.

(J) *Idioscopus clypealis*

(K) *Idioscopus nitidulus*



(L) *Bemisia tabaci*

(M) *Drosophila melanogaster*

(N) *Bactrocera dorsalis*

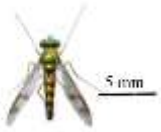
(O) *Bactrocera correcta*

(P) *Bactrocera zonata*

(Q) *Bactrocera cucurbitae*

(R) *Cheilomenes sexmaculatus*

(S) *Chrysoperla* sp.



(T) *Condylostylus* sp.

(U) *Lucilia sericata*

Plate 2 Pests and Natural enemies of Order Coleoptera, Hemiptera, Blattodea, Hymenoptera, Diptera and Neuroptera on Mango

Table 1 The recorded insect pests and natural enemies of mango

Sr. No.	Order	Family	Scientific Name	Common Name
1.	Coleoptera	Cerambycidae	*Batocera rufomaculata*	Mango stem borer
2.		chrysomelidae	*Aulacophora foveicollis*	Red pumpkin beetle
3.			*Aulacophora lewisii*	Pumpkin beetle
4.			*Monolepta australis*	Red-shouldered leaf beetle
5.	Hemiptera	Ricaniidae	*Ricania* sp.	Ricaniid planthopper
6.		Membracidae	*Leptocentrus taurus*	Treehopper
7.		Dictyopharidae	*Dictyophara* sp.	Planthopper
8.		Cicadeliidae	*Idioscopus clypealis*	Mango leafhopper
9.			*Idioscopus nitidulus*	Mango flower jassid
10.		Aleyrodidae	*Bemisia tabaci*	Silverleaf white fly
11.	Blattodea	Termitidae	*Odontotermes*	Flying termite
12.	Hymenoptera	Formicidae	*Oecophylla smaragdina*	Red ant
13.	Diptera	Drosophilidae	*Drosophila melanogaster*	Fruit fly
14.		Tephritidae	*Bactrocera dorsalis*	Oriental fruit fly
15.			*Bactrocera correcta*	Guava fruit fly
16.			*Bactrocera zonata*	Peach fruit fly
17.			*Bactrocera cucurbitae*	Melon fruit fly
18.	Coleoptera	Coccinellidae	*Chilomenes sexmaculatus*	Ladybird beetle
19.	Neuroptera	Chrysopidae	*Chrysoplera* sp.	Green lacewing
20.	Diptera	Dolichopodidae	*Condylostylus* sp.	Long-legged fly
21.		Calliphoridae	*Lucilia sericata*	Blowfly

Table 2 Species composition and variation of insects at different mango fruit development stages in the study period

Sr. No.	Species	Numbers of collecting insect time per observation												Total No. of individuals
		February			March			April			May			
		1st week	2nd week	Sub total	3rd week	4th week	Sub total	5th week	6th week	Sub total	7th week	8th week	Sub total	
1.	*Batocera rufomaculata*	1	0	1	1	0	1	0	0	0	0	0	0	2
2.	*Aulacophora foveicollis*	5	12	17	6	7	13	9	6	15	2	1	3	48
3.	*Aulacophora lewisii*	1	5	6	1	0	1	0	0	0	0	0	0	7
4.	*Monolepta australis*	0	0	0	0	0	0	0	1	1	2	0	2	3
5.	*Ricania* sp.	1	1	2	0	0	0	0	0	0	0	0	0	2
6.	*Leptocentrus taurus*	2	1	3	0	0	0	3	0	3	3	0	3	9
7.	*Dictyophara* sp.	0	0	0	1	0	1	1	0	1	0	0	0	2
8.	*Idioscopus nitidulus*	202	233	435	102	156	258	75	87	162	139	145	284	1139
9.	*Idioscopus clypealis*	126	200	326	107	153	260	122	44	166	154	98	252	1004
10.	*Bemisia tabaci*	7	5	12	10	6	16	4	3	7	5	2	7	42
11.	*Odontotermes*	1	2	3	1	17	18	43	15	58	8	32	40	119
12.	*Oecophylla smaragdina*	1	3	4	5	2	7	2	4	6	8	1	9	26
13.	*Drosophila melanogaster*	21	7	28	8	16	24	11	10	21	14	11	25	98
14.	*Bactrocera dorsalis*	74	82	156	136	141	177	240	157	397	282	253	535	*1365
15.	*Bactrocera correcta*	70	67	137	149	113	162	175	115	290	217	221	438	1127
16.	*Bactrocera zonata*	18	29	47	36	47	83	111	39	150	58	62	120	400
17.	*Bactrocera cucurbitae*	0	0	0	0	0	0	0	0	0	9	18	27	27
18.	*Cheilomenes sexmaculatus*	1	5	6	1	0	1	0	0	0	0	0	0	7
19.	*Chrysoperla* sp.	11	12	23	4	0	4	2	1	3	0	0	0	30
20.	*Condylostylus* sp.	16	5	21	5	26	31	34	16	50	23	23	46	148
21.	*Lucilia sericata*	43	50	93	1	2	3	4	0	4	4	13	17	117
	Total number of individuals	601	719	1310	574	686	1260	836	498	1334	928*	880	1808	5722

* maximum individuals

Table 3 Prevalence of mango infection caused by insect pests

Location	Number of inspected trees	Total number of mangoes	Prevalence (%)
VFRDC, Hlegu Township, Yangon Region	24	180	13.3

Table 4 Mean number (±SEM) of trapped insects on different traps

	Treatments			P-value
	ME	FJ	YS	
$\bar{X}$±SEM	$292.75^{b} \pm 45.12$	$22.88^{a} \pm 2.48$	$371.25^{b} \pm 46.90$	0.00

ME - Methyl Eugenol, FJ - Fruit Juice, YS - Yellow Sticky

$^{a, b}$ superscripts on the same column indicate significant differences ($P < 0.05$)

a – The minimum value, b – The maximum value

Table 5 Quality index of fruits and damage status of pest infestations

Treatment (Total)	No. of oviposition marks	No. of pest infestation marks	Presence of larva (cut)	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Thickness (cm)	Sweetness (%)
ME	0.83	0.13	0	406	14.4*	7.9*	7	14.2
FJ	2.67	0.67	1.3	408*	13.8	7.8	7.1*	16.2
YS	0.54	0.63	0	378	14	7.6	6.8	14
Control (No Trap)	7.93*	1.53*	7.3*	299	13	7.4	6.5	16.4*

* Maximum quality index

ME - Methyl Eugenol

FJ - Fruit Juice

YS - Yellow Sticky

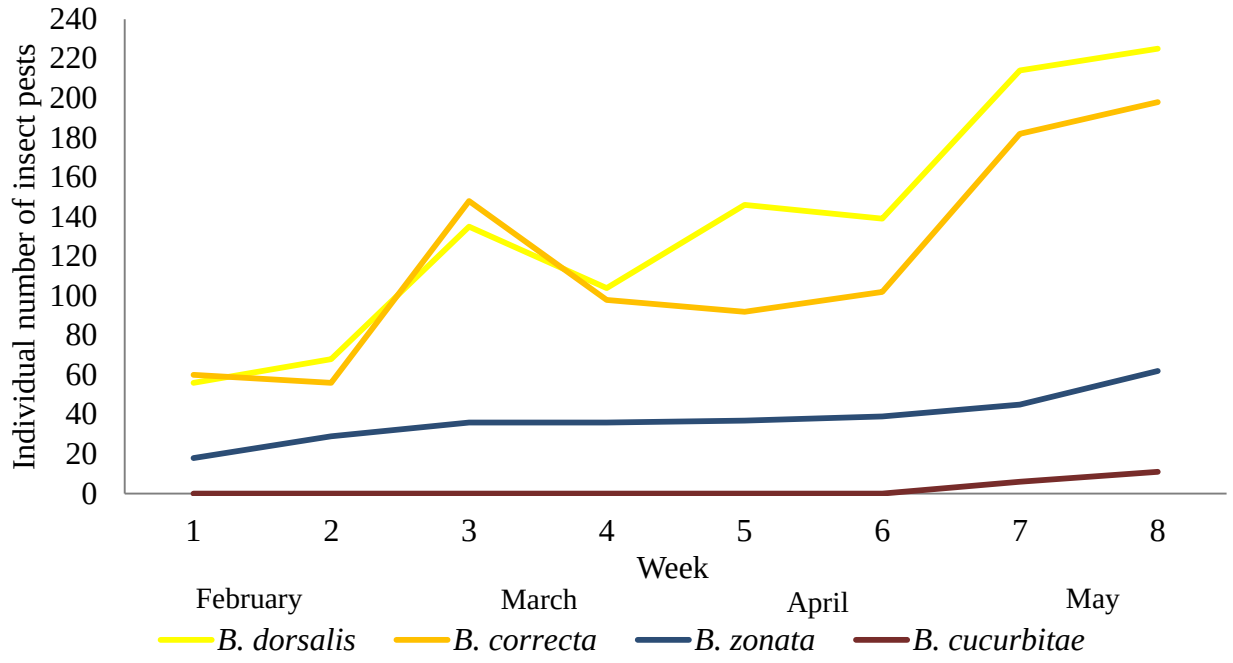


Figure. 3 Number of trapped insect pests on methyl eugenol trap

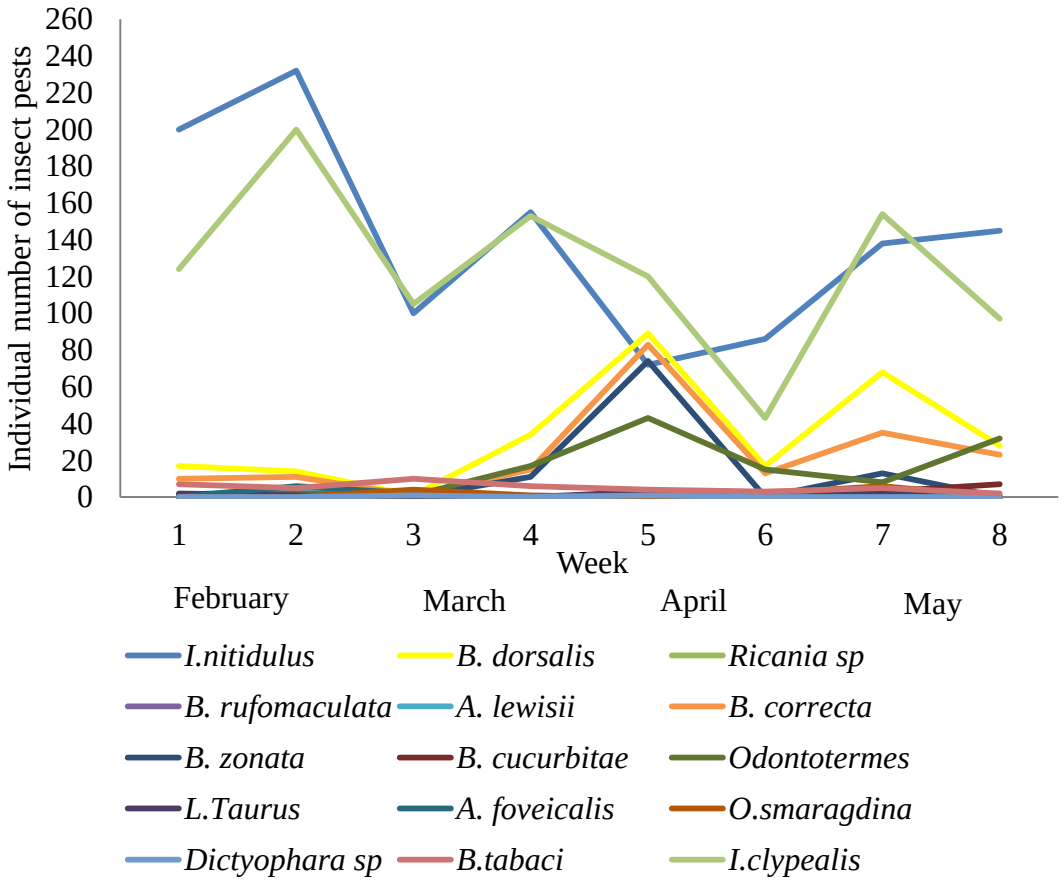


Figure. 4 Number of trapped insect pests on the yellow sticky trap

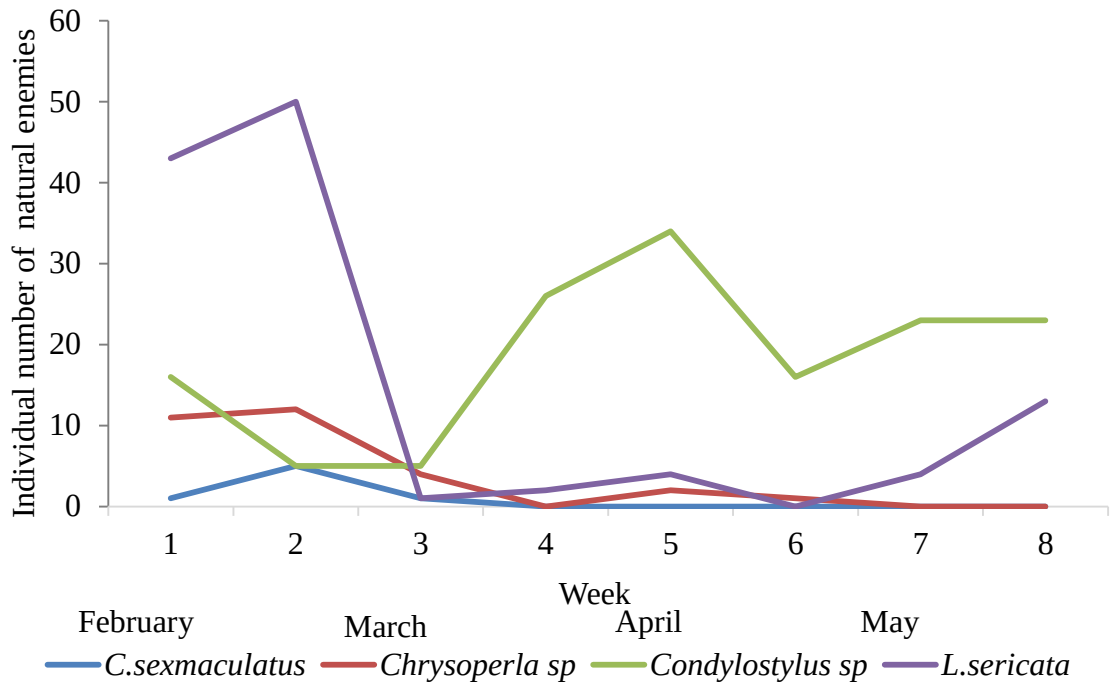


Figure. 5 Number of trapped natural enemies on the yellow sticky trap

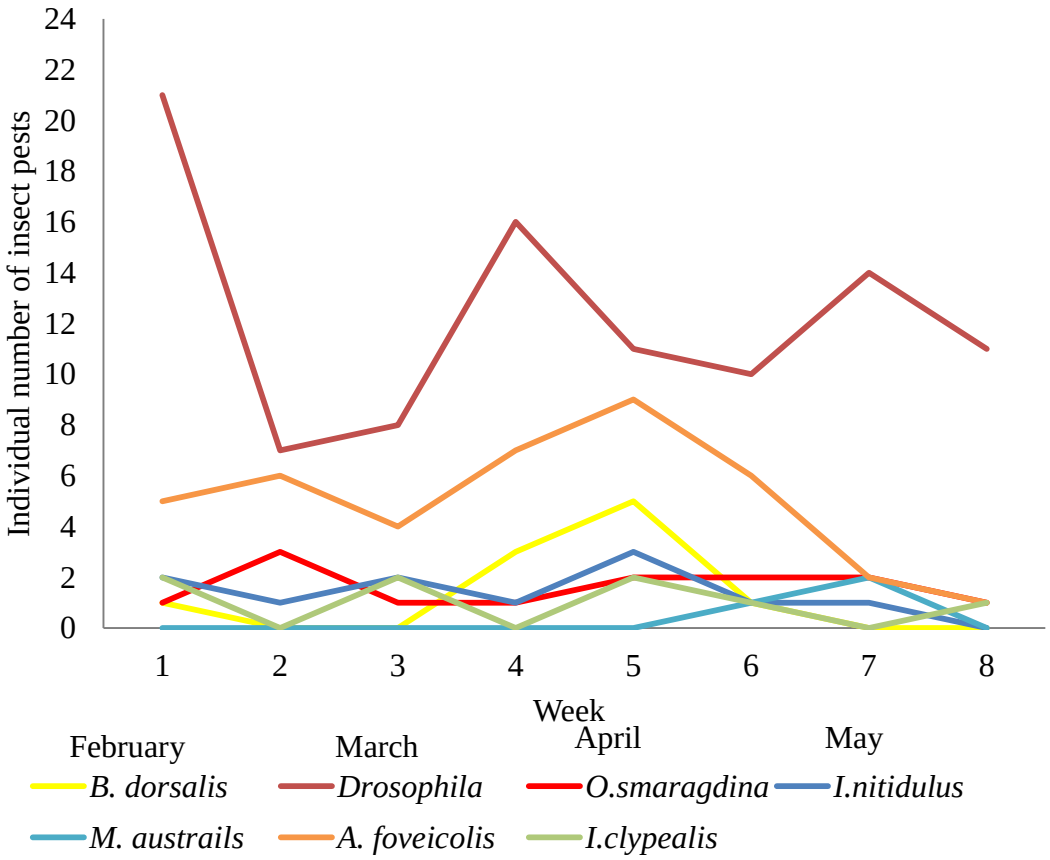


Figure. 6 Number of trapped insect pests on fruit juice trap

Discussion

The present study was done to assess the status of the pest infestations and their damage level on economically important mango cultivated at VFRDC, Ye Mon village, Hlegu Township of Yangon Region.

A total of 21 insect species representing 16 genera across 15 families and 6 orders were observed in this study site. In the present study, *Idioscopus clypealis*, *Idioscopus nitidulus*, *Bactrocera correcta*, and *Bactrocera dorsalis* occurred all through the study period. The minimum occurrence of insect pests was *Monolepta australis*, *Ricania* sp., *Dictyophara* sp. and *Batocera rufomaculata* in the present study. *Idioscopus* and *Bactrocera* species are dominant at study site in the present study. They occurred mostly during the harvesting period.

Nan Zarchi Win *et al*. (2014) assessed that *B. dorsalis* and *B. correcta* were most abundant from different fruit samples in Yezin, Myanmar.

According to the results, *Bactrocera dorsalis* and *Bactrocera correcta* were found to be the most dominant at mango in the study site. Therefore, the finding of the present study agrees with Nan Zarchi Win *et al*. (2014). Drew and Roming (1996) reported that the species *Bactrocera dorsalis* plays an important role in fruit production for domestic consumption and export as well.

Bana *et al*. (2018) studied that *Idioscopus clypealis* and *I. nitidulus* were the dominating species during the full bloom period.

In the present study, mango hopper *I. clypealis* and *I. nitidulus* were found in flowering and fruiting stages were considered as serious pests. Therefore, the finding of the present study is the same as Bana *et al*. (2018).

Among the natural enemies, 10 species of coccinellids and two species of chrysopids were observed in the mango ecosystem (Gundappa, *et al*., 2019). In the present study, *Condylostylus* sp., *Lucilia sericata* and *Chrysoperla* sp. can help reduce pest insect populations including aphids, thrips, white flies, spider mites, caterpillars and insect eggs.

A total of 24 out of 180 plants were found to be infected, resulting in a prevalence rate of 13.3%. Statistically significant differences were found in the number of recorded insects captured on different traps at $P < 0.05$.

In control, the visual inspection of fruit damage was more significant than the other three traps and also the sizes of fruit in control decreased in this study site. The lowest infestation occurred in ME and YS traps in the present study.

The yellow sticky traps were more effective than any other traps. These traps were effective not only to control the pest but also to improve the quality and yield of mango trees. The number of damaged fruits was the lowest in yellow trap. But the outcomes of plants without traps showed the lowest marketable fruits, the low quality of fruit size, the highest number of damaged fruits and the suffered stress in mangoes caused by the pests. Therefore, physical control is mostly controlled using pesticides, which harm on human health and beneficial insects.

The result of this study suggests important information on effective monitoring, and management of major pests in mango plants for farmers who should use a reduction to pesticides which further leads to less cost, less workers' exposure to pesticides, and ultimately less

pesticides-induced phyto-toxicity and less expenses. Therefore, most effective control methods should be used for local and exports markets.

Conclusion

This study found that Diptera was the dominant pest in *Mangifera indica* plantation at VFRDC, Hlegu, Yangon. Among the recorded species, *Bactrocera dorsalis* and *Idioscopus nitidulus* were major pests that whose populations exploded as the mango fruits near the harvest stage. Among control methods, methyl eugenol and yellow sticky traps were most effective in reducing infestations. However, in untreated control, visual inspection revealed greater fruit damage and smaller, less healthy fruits. The study suggests that the utilization of yellow sticky and methyl eugenol traps were cost-effective methods to control the pests with reduced labor requirements for sustainable mango production and quality maintenance. The economic importance of mangoes is essential to maintain quality and yield in both export and local markets.

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EFFECT OF THREE FEEDING DIETS ON OVIPOSITION PERFORMANCE OF COCKROACHES *PERIPLANETA AMERICANA* (LINNAEUS,1785) AND *PARCOBLATTA LATA* (BRUNNER VON WATTENWYL, 1865) REARED IN LABORATORY CONDITION

Myint Thandar Cho[1] , Tin Lay Mon[2], San San Hmwg[3]

Abstract

This study investigated the nymphal period of adulthood, maturation, and the reproductive performance of *Pariplaneta. americana* and *Pacoblatta lata,* which were reared with three types of diets. The results revealed the durations of nymphal development were varied with the different food diets. A shorter duration for the development of cockroaches from the 2nd stage to the 3rd stage by feeding rice bran and sawdust followed by cat food and dog food in American cockroaches, whereas from the 3rd stage to the adult shorter duration by feeding dog food. Similarly, it has a shorter duration of development by feeding rice bran and sawdust, followed by dog food and cat food in wood cockroaches. A similar duration was recorded in *P. lata* for the development from the 3rd stage to the adult. The pre-ovulation period among the different diet treatments showed that dog food was the most effective with a shorter duration for maturity case in both cockroach species. In *P. americana,* their feeding preference was a highly significant difference among the diets. The feeding on different diets was not changed in different life stages, and their developmental stages in wood cockroaches. Therefore, suitable diets for survival and development were different between *P. americana* and *P. lata.*

Keywords: *Pariplaneta. americana* and *Pacoblatta lata,* nymphal stage, pre-ovulation, feeding habit

Introduction

The world's population is predicted to increase by more than a third, arrival over 9 billion persons in 2050, with the key implication that the planet will have to generate 70% more food. As a result, livestock production would increase exponentially to double the current level. The biggest challenge is to ensure. There is a global capacity to provide adequate animal feed to prevent as much competition as possible with the demand for human food (FAO, 2009). In many regions, a large biomass of insects can be obtained as food, in part, this is because of the high diversity of insects associated with different environments, but it is also because of the ability of many species to breed quickly. The cockroach species remain economically, medically, and aesthetically important pests. The American cockroach has been studied extensively in various disciplines, it has been used primarily as a laboratory animal or model for basic research in physiology, biochemistry, pharmacology, toxicology, and ethology (Schal *et al.,* 1984). Only about 0.3% of the 4,000 cockroach species have adapted to human habitats (Bell, 1984).

However, cockroaches are a suitable ingredient from a nutritional and palatability perspective to replace fishmeal and other high-protein ingredients in animal feed. Among them, cockroaches are popular insects for experimental studies, for they are readily available, of a convenient size, and grow at all seasons of the year. They are generalist omnivores able to survive on a variety of foods, an ideal experimental animals for comparative quantitative studies. There are numerous studies with laboratory-reared cockroaches focused on the effects of diets on

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development and reproduction (Copper and Schal 1992, Raubenheimer and Jones 2006, Jensen *et al* 2015).

Periplaneta species are primarily adapted to live outdoors and invade residential and commercial properties seasonally in the southern temperate regions (Cornwell 1968, Ebeling 1975). The adult *P. americana* is long-lived, averaging one year and probably longer. Under adverse conditions, adults may live only about 100 days, but an adult life span of 2 to 3 years has been documented under ideal conditions (Gould and Deay, 1938., Rau 1940, Griffiths and Tauber 1942). Cornwell (1968) noted that nutrition and temperature affect development and reproduction.

Therefore, the present study was undertaken to assess the effect of three different food diets on the reproductive performance of two species, *Periplaneta americana* (Linnaeus,1785) and *Parcoblatta lata* (Brunner von Wattenwyl, 1865), under laboratory stock cultures (Plate 1). The result will provide information on reproductive biology for mass-rearing systems in the future. The objective was to compare studies of different food diets on developmental stages and reproductive performance of study cockroach species.

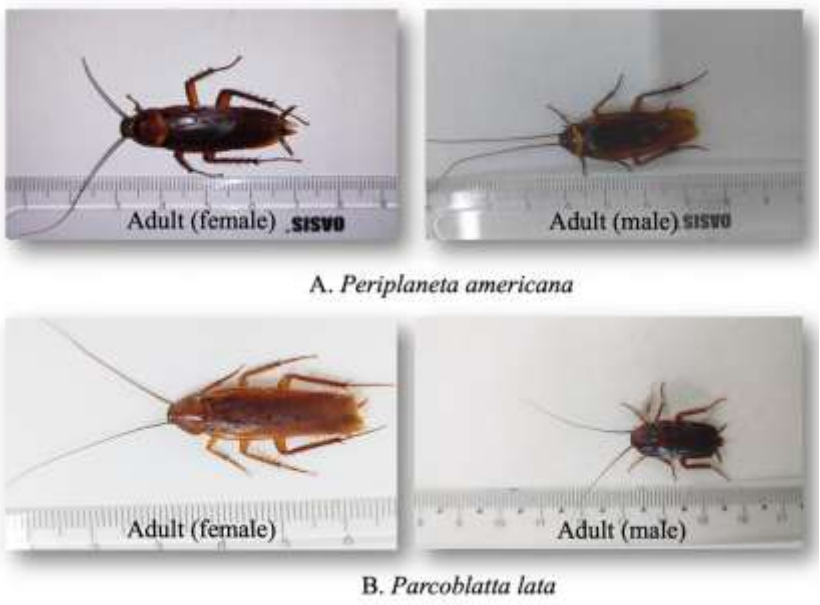

Plate 1. Two species of cockroaches *Periplaneta americana* and *Parcoblatta lata*

Materials and Methods

Study area and period

The research was conducted at the Laboratory of Zoology Department, University of Yangon from October 2023 to September 2024.

Rearing of cockroaches in the laboratory

The 2nd nymphal period of instars of the 3rd generation was used for all experiments. A total of 20 individuals (10 males and 10 females) of nymphs for both species *Periplaneta*

americana and *Parcoblatta lata* were reared separately in plastic bottles (1.5 L). The containers were covered with cloth at the top and sealed with rubber rings to prevent cockroaches from escaping and other animals from getting in. A layer of paper is placed in the bottom of rearing containers to absorb excess moisture emitted by the cockroaches. The rearing containers were cleaned every ten days intervals, and the paper sheets were replaced. The newly laid ootheca from each treatment was kept separately in another rearing plastic bottle to reduce cannibalism. The new nymphs were fed with enough food and supplied with water (Plate 2).

Rearing diets

Cockroaches were fed with each rearing diet, such as cat food and dog food purchased from pet shops and rice bran and sawdust (1:2) for giving days. A weight of *ca.* 2g of each diet was placed in a concentric circle at random at the bottom of each container. All foods were heat-treated in the oven with the lowest temperature (90-100˚C) before feeding them, and the nutritional composition of each diet as dry weight was examined. The composition of food diets was tested at the Analytical Laboratory Unit, Department of Fisheries, Yangon, as shown in Table 1.

Plate 2. Rearing condition of two species of cockroaches in laboratory condition

Data collection and analysis

The nymphal period to adult and their laying was recorded for both cockroach species to study the life cycle of certain species. The ootheca was checked daily and the date when it hatched. Dying insects were counted and removed. The numbers of instars and their survival were recorded for each treatment. The calculation on developmental stages on different nymphal periods, and the fecundity of adults (No. of oothecae, hatchability (%), cumulative number of hatched nymphs, number of hatched nymphs per ootheca), and the survival rate of both cockroach species. The statistical data analyses will be done by using a one-way ANOVA Test under the significant level $p < 0.05$ (0.5%).

Table 1. Composition in different food diets

Contents	Type of diet		
	Dog food	**Cat food**	**Rice bran and sawdust**
Crude Protein (%)	30	35	41.58
Crude Fat (%)	12	18	2
Fiber (%)	4.5	4	20.5
Moisture (%)	10	10	12.04

Results

The present results revealed the duration of the developmental stages and oviposition performance of cockroaches *P. americana* and *P. lata* by feeding them different diets such as cat food, dog food, and rice bran and sawdust under the rearing laboratory conditions.

Developmental stages of cockroaches in American cockroach *P. americana*

The cockroaches have a shorter duration for the development of cockroaches from the 2nd stage to the 3rd stage by feeding rice bran and sawdust (71 days) followed by cat food (94 days) and dog food (126 days). In the development of cockroaches from the 3rd stage to the adult stage, the feeding of dog food was shorter (25 days), followed by cat food (55 days) and rice bran and sawdust (71 days).

Oviposition performance in American cockroach *P. americana*

During the study period, the highest total number of egg cases were obtained from the cockroaches that feed on cat food (36 eggs), followed by rice bran and sawdust (18 eggs), and dog food (16 eggs). Of the total no. of hatched nymphs and the hatchability rate, 108 nymphs were hatched from nine hatched egg cases by feeding cat food (12.00%), 65 nymphs from six hatched egg cases by feeding dog food (10.83%), and 27 nymphs from two egg cases by feeding rice bran and sawdust (13.50%). The total number of deaths was 34 nymphs fed on cat food, 10 nymphs fed on dog food, and non-death nymphs fed on rice bran and sawdust (Table 2, Fig. 1).

Table 2. Duration of developmental stages and reproductive performance of *P. Americana*

Parameters	*P. americana*		
	Cat food	**Dog food**	**Rice bran & Saw-dust**
2nd-3rd stage (Days)	94	126	71
3rd-adult stage (Days)	55	25	71
Total egg case (oothecae)	36	16	18
Total no. of hatched nymphs/Total hatched egg case	108/9	65/6	27/2
Total no. of hatched nymphs per ootheca (%)	12.00	10.83	13.50
Total no. of death	34	10	Nil
Death rate (%)	31.48	15.38	0.00

Developmental stages oviposition performance in Wood cockroach *P. lata*

The cockroaches have a shorter duration for the development of cockroaches from the 2nd stage to the 3rd stage by feeding rice bran and sawdust (40 days) followed by dog food (69 days) and cat food (70 days). In the development of cockroaches from the 3rd stage to the adult, all feeding diets were similar in duration (20 days) The highest total number of egg cases (Oothecae) were obtained from the cockroaches that feed on dog food (42 eggs), followed by cat food (20 eggs), and rice bran and sawdust (12 eggs) during the study period. Of the total number of hatched nymphs and the hatchability rate, 53 nymphs were hatched from four hatched egg cases by feeding cat food (13.25%), 105 nymphs from seven hatched egg cases by feeding dog food (15.00%), and 43 nymphs from three egg cases by feeding rice bran and sawdust (14.33%). The total number of deaths was 15 nymphs fed on dog food, 15 on rice bran and sawdust, and non-death nymphs fed on cat food (Table 3, Fig. 2).

Table 3. Duration of developmental stages and reproductive performance of *P. lata*

Parameters	*P. lata*		
	Cat food	**Dog food**	**Rice bran & Sawdust**
2nd-3rd stage (Days)	70	69	40
3rd-adult stage (Days)	20	20	20
Total eggcase (oothecae)	20	42	12
Total no. of hatched nymphs/Total hatched egg case	53/4	105/7	43/3
Total no. of nymphs per ootheca (%)	13.25	15.00	14.33
Total no. of death	Nil	15	15
Death rate	Nil	14.29	34.88

Comparison of pre-ovulation period of cockroaches

In American cockroach *P. americana*, the longer duration of the pre-ovulation period from adult to the egg-laying stage was 168 days for the cockroaches that feed on dog food, followed by feeding dog food 25 days, and 10 days for the cockroaches that feed on rice bran and sawdust (Fig. 3, A). In the wood cockroaches *P. lata*, the pre-ovulation period from adult to the egg-laying stage was 207 days for the cockroaches that feed on cat food, followed by feeding rice bran and sawdust 64 days, and the shorter duration of development was 31 days for the cockroaches that feed on dog food (Fig.3, B).

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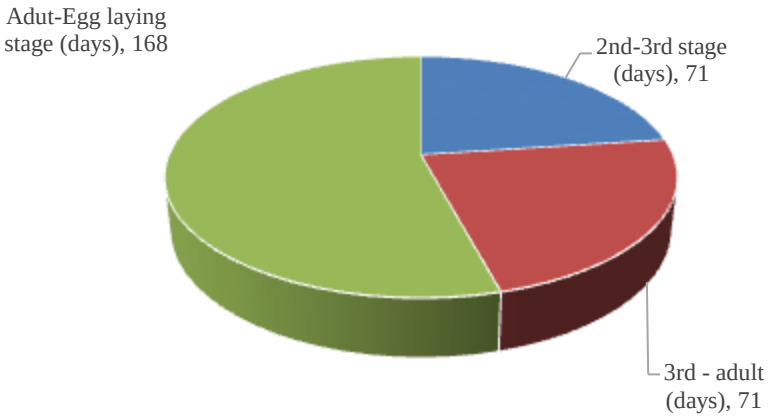
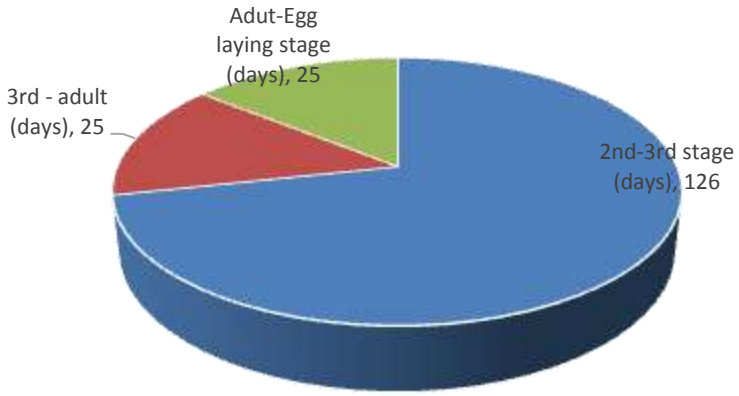
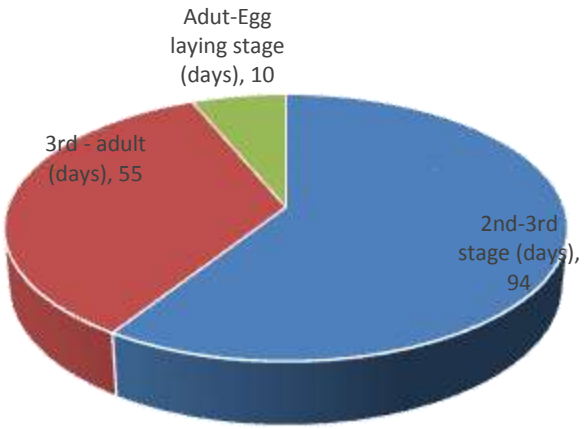


A. Rice bran and Saw dust treatment



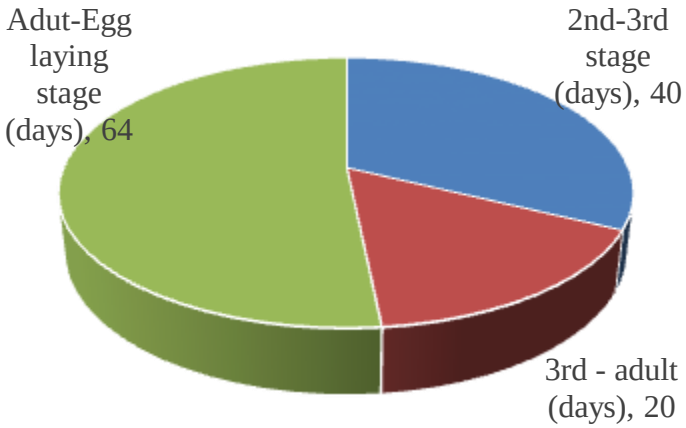
B. Dog food treatment



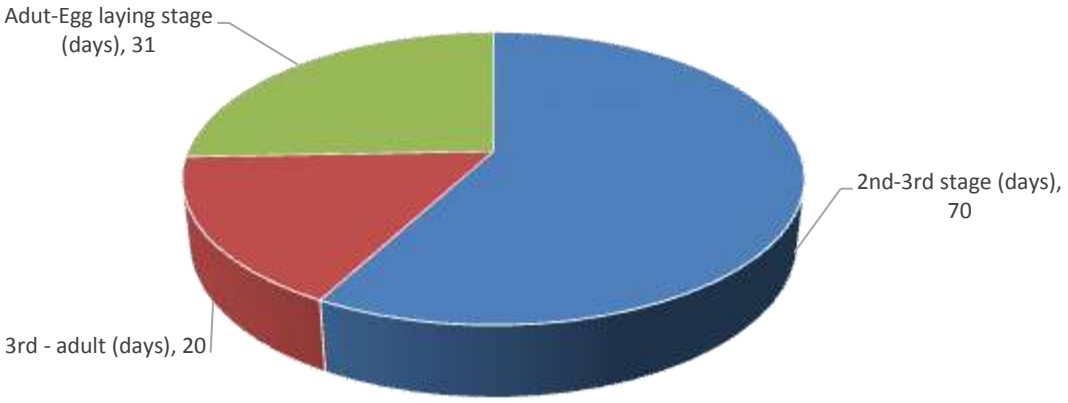
C. C

D. at food treatment

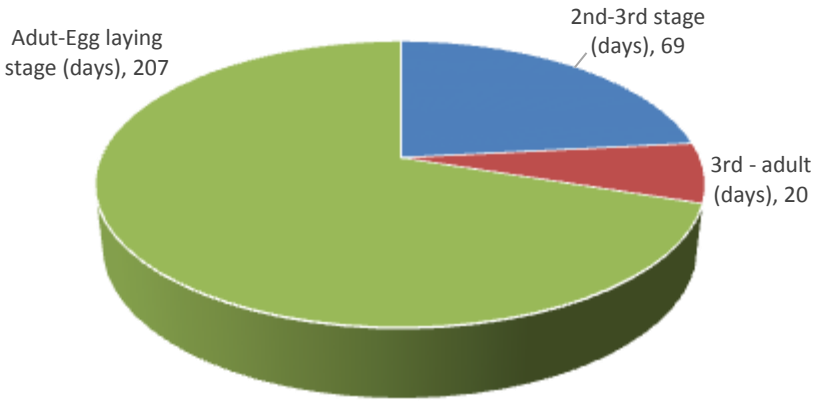
Figure. 1 Duration of developmental stages of *P. americana* by feeding different diets



A. Rice bran and Saw dust treatment



B. Dog food treatment



C. Cat food treatment

Figure. 2 Duration of developmental stages of *P. lata* by feeding different diets

Food preference of cockroaches

Both cockroach species' feeding rates increased due to their development during the study period (44 weeks of observation). The mean weight of the feed was highest at 1.54 ±0.39 gm on rice bran and sawdust followed by 1.42±0.49 gm on cat food and 1.25±0.45 gm on dog food. In particular, cat food was most preferable at the adult stage while feeding on dog food was decreased, and rice-bran and sawdust feeding steadily increased from the young age to the adult stage in *P. americana.* Their feeding preference was a highly significant difference among the diets (F = 4.911, P < 0.01) (Fig. 4, A).

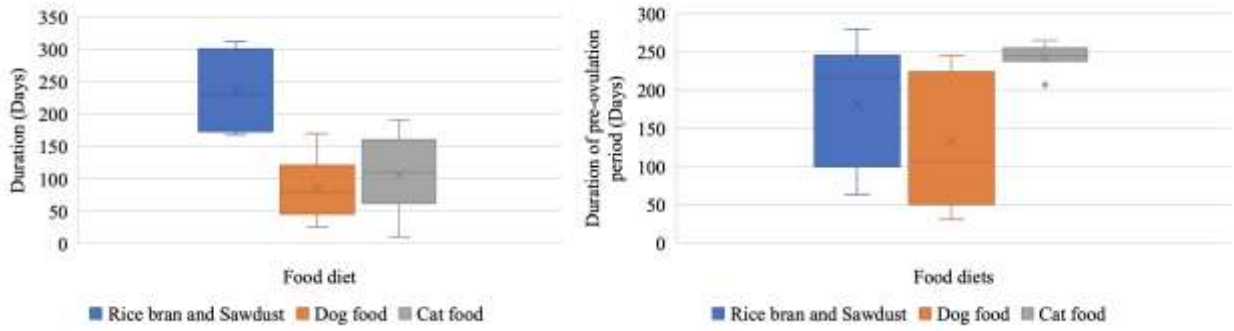


Figure. 3 Comparison of the duration of pre-ovulation among different feeding diets

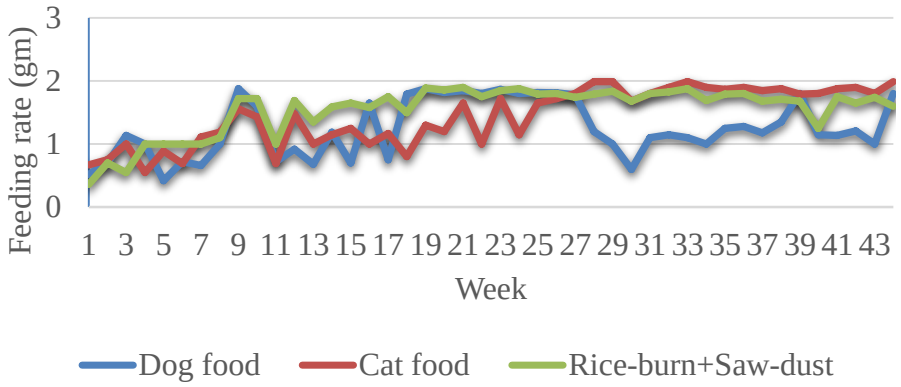
In *P. lata*, the mean weight of the feed was 1.35±0.35 gm on dog food followed by 1.34±0.30 gm on rice bran and sawdust, and the lowest feeding rate was 1.17±0.39 gm on dog food. In particular, cat food was most preferable at the adult stage while feeding on dog food was decreased, and rice-bran and sawdust feeding steadily increased from the young age to the adult stage. Their feeding preference was a significant difference among the diets (F = 3.709, P < 0.05) (Fig. 4, B).

Discussion

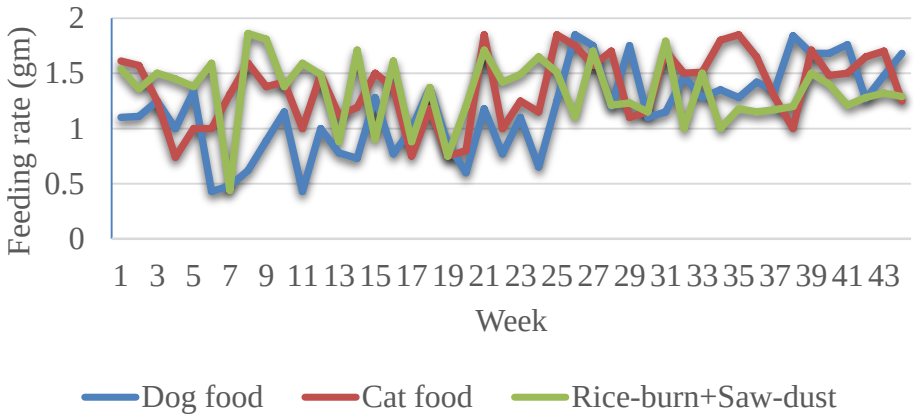
In the results of food experiments, the durations of nymphal development varied with the different food diets. The mixture of rice bran and sawdust was the most effective diet for the development with a shorter duration (71 days) in *P. americana*, whereas in *P. lata* (40 days). For the development from 3rd stage to adult, the shorter duration was recorded by feeding of dog food (25 days) followed by cat food (55 days) and rice bran and sawdust (71 days) in *P. americana*, whereas a similar duration was recorded in *P. lata* (20 days) by feeding all treatments.

According to the diet components, crude protein was highest in rice bran and sawdust diet rather than other cat food and dog food. It means that in the early nymphal development, the crude protein was the most essential for cockroaches in both species. The crude fat was highest in cat food (18 mg/kg) and the least in rice bran and sawdust (2mg/kg). The present result highlights that the suitable amount of crude fat concentration in the diet was vital for the development of cockroaches to the adult stage in *P. americana.* However, the crude fat contained in the diets was not an essential factor in *P. lata*.

The present experiment noted that in *P. americana* the highest number of oothecae were obtained from the treatment of feeding cat food (n = 36), followed by rice bran and sawdust (n =18), and dog food (n = 16) in contrast to wood cockroach *P. lata* the highest oothecae were obtained from the treatment of feeding dog food (n = 42), followed by cat food (n = 20), rice bran and sawdust (n=12). Thus, cat food was the most effective for laying eggs in American cockroaches, while dog food in wood cockroaches.



A. American cockroach *P. americana*



B. Wood cockroach *P. lata*

Figure. 4 Food preferences of study cockroach species

Nevertheless, the total number of hatched nymphs per ootheca was higher in feeding rice bran and sawdust treatment (13.50%) than that of cat food and dog food treatments. The death rate was also high in the treatment of feeding cat food (31.48%), while the nondeath rate in the treatment of feeding rice bran and sawdust in American cockroaches. In wood cockroaches, the highest total number of hatched nymphs per ootheca was also recorded in the treatment of feeding dog food accordingly, with the highest number of laid oothecae and death rate in this treatment as compared to other treatments.

The comparison of the pre-ovulation period among the different diet treatments showed that dog food was the most effective with a shorter duration for maturity case in both cockroach species, especially when it was observed in American cockroaches. The mixture of rice bran and sawdust was less effective for the development of pre-ovulation in this species. In wood cockroaches, the feeding of cat food was a prolonged duration. This means that less effective for the maturity of egg cases. The suitable diets for survival and development were different between *P. americana* and *P. lata*.

The result of the present work observed that their food preferences, the American cockroaches, were reduced feeding rate on dog food when they became an adult stage. Although the cat food and the rice bran and sawdust mixture were stable for their feeding until they became an adult stage. It supposes that the American cockroaches varied their food preference upon their life stages and development. In wood cockroaches, the feeding habits of different diets were not changed in different life stages and developmental stages. In conclusion, the present result highlights that feeding on varied diets should be considered for the mass rearing of varying cockroach species in the future.

Conclusion

This study successfully determined the distinct impacts of three diets on the life history of P. americana and P. lata. The optimal diet for development, reproduction, and survival varies significantly between the two cockroach species. The Dog Food (DF) diet promotes the fastest reproductive maturity (shortest pre-ovulation period) in both species. The Rice Bran/Sawdust (RB/SD) mixture offers the best survival rate for P. americana (0.00% death rate). In conclusion, the present result highlights that feeding on varied diets should be considered for the mass rearing of varying cockroach species in the future.The results underscore the need for species-specific and potentially life-stage-specific diets when establishing efficient mass-rearing systems for these cockroach species in the future.

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COMPARISON OF THREE DNA EXTRACTION PROTOCOLS BY ASSESSING DNA YIELD AND PURITY ON CATFISH, *CLARIAS* BATRACHUS (LINNAEUS, 1758)

Mya Thein Nwe[1], Aye Chan Thu[2], July Maung Maung[3], Kay Lwin Tun[4]

Abstract

There are various DNA extraction kits available on the market for animal tissue. In the present study, three DNA extraction kits, utilizing the silica solution-based extraction method, the GS solution-based method, and the Proteinase K solution-based method, were employed to extract total genomic DNA from *Clarias batrachus*, and their extraction efficiencies were compared. The quantity and quality of the extracted DNA were evaluated using a Nanodrop spectrometer. The Silica solution-based method yielded DNA concentrations of 19.1 ng/µl, 20.8 ng/µl, and 25.9 ng/µl, with A260/280 purity ratios of 2.05, 2.06, and 1.99, and A260/A230 contamination ratios of 0.06, 0.05, and 0.06, respectively, for three specimens of catfish. The GS solution-based method produced DNA concentrations of 167.2 ng/µl, 75.6 ng/µl, and 372.0 ng/µl, with purity ratios of 1.92, 2.09, and 1.99, and contamination ratios of 0.80, 0.85, and 1.30. The proteinase K solution-based method resulted in DNA concentrations of 124.2 ng/µl, 164.0 ng/µl, and 85.6 ng/µl, with purity ratios of 2.14, 2.09, and 2.14, and contamination ratios of 1.67, 1.73, and 1.35. Time analysis showed that the Proteinase K solution-based method was the fastest, requiring 22 minutes, while the Silica solution-based method and the GS solution-based method took 93 minutes and 82 minutes, respectively. This study highlights the differences in extraction efficiency, quality, and time consumption among the three methods.

Keywords: Three DNA extraction methods, catfish, DNA yield and purity, time consumption

Introduction

DNA extraction is a cornerstone technique in molecular biology, essential for diverse applications such as genetic research, species identification, and biodiversity studies (Alberb *et al.*, 2002). The efficiency and accuracy of DNA extraction directly affect the quality of genetic material, which is critical for downstream applications like PCR, sequencing, and genotyping, which in turn impact the reliability of subsequent analyses (Reddy & Nakano, 2012).

The method chosen for DNA extraction plays a significant role in determining the quality and quantity of DNA obtained, thereby influencing the reliability of subsequent analyses (Miller *et al.,* 1988). Effective DNA extraction is crucial not only for understanding genetic diseases and developing diagnostics and therapeutics but also for forensic science, genome sequencing, pathogen detection, and paternity testing (Wang and Wang, 2018)

Typically, DNA extraction involves several fundamental steps: disruption of cellular structure to create a lysate, separation of DNA from cellular debris, purification of DNA using a matrix, removal of contaminants through washing, and elution of the DNA (Promega product guides, 2024). Various techniques, including physical, enzymatic, and chemical methods, are

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employed for lysing cells and releasing nucleic acids (Wilson, 2001). The effectiveness of DNA extraction methods can vary depending on the species and the specific kit used (Kreader, 1996).

Nowadays, DNA sequencing for species identification of fish is widely used. The fundamental requirement for DNA sequencing is the extraction of DNA from the tissue of the investigated species. Different extraction kits may provide different concentrations and purities of nucleic acid.

The present study aimed to evaluate and compare modified versions of three different DNA extraction methods, focusing on their yield (DNA concentration) and quality (purity and contamination levels), with the following objectives to assess how well each method performs concerning the unique requirements of catfish *Clarias* species tissue samples, considering factors such as time efficiency and ease of use, and to identify the most suitable technique for optimizing DNA extraction in this context.

Materials and Methods

Sample collection

Three individuals of catfish, *Clarias batrachus,* were purchased available at Hledan market, Kamaryut Township in Yangon in May 2024, and immediately carried in frozen condition to the molecular laboratory of the Department of Zoology, University of Yangon. In the laboratory, the fish was confirmed as *Clarias batrachus* using FishBase software.

Preparation of the DNA extraction

For DNA extraction, the muscle tissue was excised from each collected fish specimen and preserved in 70% ethanol to maintain DNA integrity. The samples were labeled as MAF 1, MAF 2, and MAF 3 (Fig.1). Each tissue sample was processed using three distinct DNA extraction methods for comparative analysis of their efficiency. A 25 mg portion of tissue was carefully weighed from each sample and assigned to one of three extraction methods such as (i) Silica-based extraction method product of HiMedia Laboratories Pvt.Ltd, (India): Samples were labeled MAF 1 Sili, MAF 2 Sili , and MAF 3Sili , (ii) GS solution-based extraction method product of Selangor Darul Ehsan, (Malaysia, MD-TNA-100): Samples were labeled MAF 1 GS,MAF 2 GS, and MAF 3 GS, and (iii) Proteinase K-based extraction method product of Qiagen, (Hilden, Germany): Samples were labeled MAF 1 QG,MAF 2 QG, andMAF 3 QG.

The DNA extraction procedures for catfish tissue were carried out according to the protocols specific to each method.

(A) (B)

Figure. 1 (A) Collected catfish species. (B) Fixation and labeling of specimens

Silica solution-based extraction method

Firstly, a new 1.5 ml microcentrifuge tube was prepared and labeled. In that tube, 25 mg of fish tissue sample was suspended in 100 µl of GT buffer, ground by a grinder to be homogenized, and then 800 µl of GT Buffer was added and centrifuged at 12000 rpm for three minutes. Secondly, a new 1.5 ml microcentrifuge tube was prepared with 40 µl of Silica solution. After adding Silica, 600 µl of upper clear solution was transferred from the mixture tube, vortexed for 20 seconds, and then centrifuged at 12000 rpm for three minutes. The supernatant was decanted, and 500 µL of GT Buffer was added to the tube. The mixture was then vortexed for 20 seconds and centrifuged for 3 minutes at 12000 rpm.

The supernatant was decanted, and 1000 µl of 70% Ethanol was added and mixed by vortex for 20 seconds, and centrifuged for three minutes. Furthermore, decanted the supernatant and then pipetted the remaining ethanol along with dried the pellet to ensure no ethanol remains in the tube. In addition to 1000 µl of DEPC ddH_2O was added, mixed by vortex, incubated at 55 ºC for ten min in the incubator, and 12000 rpm for three min. Finally, 500 µl of supernatant was transferred into a new 1.5 ml microcentrifuge tube.

GS solution-based extraction method

A total of 300 µl of GS solution was added into a 1.5 ml microcentrifuge tube with 25 mg of fish tissue sample, that was grinded by disposable grinder, mixed by vortex briefly, incubated the sample tube at 65^0 C for ten minutes, added 150 µl of GS solution 2 into the tube, mixed by inverting five to ten times, incubated the prepared sample on ice for ten minutes and centrifuged at 10,000 rpm for ten minutes.

A new 1.5 ml microcentrifuge tube containing 500 µl of isopropanol was prepared, transferred 300 µl of supernatant was transferred into the isopropanol-prefilled 1.5 ml microcentrifuge tube and mixed by inverting five to ten times. After that, the tube was centrifuged at 10,000 rpm for ten minutes, the supernatant was slowly decanted, and washed the pellet by adding 800 µl of 75% ethanol into the tube, again centrifuged the tube at 10,000 rpm for five minutes, removed supernatant and let the pallet to be dried at room temperature. Finally, the dried pellet was resuspended with 100 µl of GS solution 3.

Proteinase K solution-based extraction method

A total of 180 µl buffer ATL was added into a 1.5 ml microcentrifuge tube with 25 mg of fish tissue sample and 20 µl proteinase K solution, and then mixed by vortex and incubated at 56^0 C until completely lysed. It was Vortexed occasionally during incubation and vortexed for 15 seconds directly before proceeding to step 2. Added 200 µl buffer AL and mixed thoroughly by vortex. 200 µl of ethanol was added to the tube and vortexed thoroughly.

The mixture was transferred the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube and centrifuged at ≥6000 x g (8000 rpm) for 1 min, and the mixture was discarded from the collection tube. The spin column was placed in a new 2 ml collection tube, and 500 µl added Buffer AW1 was added. It was centrifuged for 1 min at ≥6000 x g, and then the discarded collection tube with the mixture. The spin column was placed in a new 2 ml collection tube, and 500 µl Buffer AW2 was added and centrifuged again for 3 min at 20,000 x g (14,000 rpm). Discarded the flow-through and collection tube. The spin column was transferred to a new 1.5 ml

microcentrifuge tube. Finally, eluted the DNA by adding 200 µl Buffer AE to the center of the spin column membrane. It was incubated for 1 min at room temperature (15-25°C) and centrifuged for 1 min at ≥6000 x g.

Recording on time-consuming for each method per sample

In our analysis, we recorded the time taken for steps that were the lysis step, washing step, DNA rehydration, and elution step for each extraction method applied to individual samples according to the time-consuming steps during this conducting experiments. The average time taken (mean) and standard deviation were calculated for each method to assess the variation in processing times. These calculations allowed us to compare the efficiency and consistency of each method based on the time required for processing.

Evaluation of DNA Quality

In this study, based on spectrophotometric evaluation, DNA can be considered pure when the absorbance ratio of A260/280 is 1.8 to 2 and the secondary absorbance ratio of A260/230 is 1.8 to 2.2 for all methods according to (Green and Sambrook, 2012). The quality of DNA was measured using the Nanodrop spectrometer (One C spectrophotometer, USA). The average values were calculated for DNA concentration, purity ratio, and contamination ratio.

Results

In the present study, the total genomic DNA was extracted from three individuals of catfish *Clarias batrachus* species by using three different methods. The quantity and quality of total genomic DNA were measured using 4 µl of each sample on the Nanodrop spectrometer. The DNA concentrations and purity ratios were calculated to assess the quality and quantity of the extracted DNA.

In using Silica solution-based DNA extraction method, the DNA concentration of MAF 1Sili, MAF 2 MAF 3 Sili were 19.1 ng/µl, 20.8 ng/µl, and 25.9 ng/µl, purity ratio of DNA at A260/280 reading were 2.05, 2.06 and 1.99, and the contamination ratio at A260/A230 reading were 0.06, 0.05 and 0.06 respectively from 4 µl of sample (Table 1).

In using GS solution-based DNA extraction method, the DNA concentration of MAF 1GS, MAF 2 GS and MAF 3 GS were 167.2 ng/µl, 75.6 ng/µl, and 372.0 ng/µl, purity ratio of DNA at A260/280 reading were 1.92, 2.09 and 1.99, and the contamination ratio of DNA at A260/A230 reading were 0.80, 0.85 and 1.30 (Table .2).

In using proteinase K solution-based DNA extraction method, the DNA concentration of MAF 1 QG, MAF 2 QG and MAF 3 QG were 124.2 ng/µl, 164.0 ng/µl, and 85.6 ng/µl, purity ratio of DNA at A260/280 reading were 2.14, 2.09 and 2.14, and the contamination ratio of DNA at A260/A230 reading were 1.67, 1.73 and 1.35 (Table. 3).

Table 1. The result of the Silica solution-based extraction method for three samples on Nanodrop spectrometer

Sample	Silica-based extraction method		
	DNA concentration	Purity ratio (A260/280)	Contamination Ratio (A260/230)
MAF 1 Sili	19.1	2.05	0.06
MAF 2 Sili	20.8	2.06	0.05
MAF 3 Sili	25.9	1.99	0.06

Table 2. The result of the GS solution-based extraction method for three samples on Nanodrop spectrometer

Sample	GS solution-based extraction method		
	DNA concentration	Purity ratio (A260/280)	Contamination Ratio (A260/230)
MAF 1GS	167.2	1.92	0.8
MAF 2GS	75. 6	2.09	0.85
MAF 3GS	372	1.99	1.3

Table 3. The result of Proteinase K solution-based extraction method for three samples on Nanodrop spectrometer

Sample	Proteinase K-based extraction method		
	DNA concentration	Purity ratio (A260/280)	Contamination Ratio (A260/230)
MAF1QG	124.2	2.05	1.67
MAF 2 QG	164	2.06	1.73
MAF 3 QG	85.6	2.14	1.35

Table 4. The average time taken for each method per sample

Time taken steps	Silica solution-based	GS solution-based	Proteinase K solution-based
Lysis(min)	25	15	10
Washing(min)	30	45	10
DNA rehydration(min)	18	20	0
Elution(min)	20	2	2
Average	23.25	20.5	5.5
SD	±5.38	±18	±5.25
Total (min)	93	82	22

The average time taken for each method per sample

The average time taken per sample were record for each method, including time spent on lysis, washing, DNA rehydration and elution. The average and standard deviation were as follow 23.23±5.38 minutes in Silica-based extraction method, 20.5±18 minutes in GS solution-based extraction method, 5.5±5.25 minutes in Proteinase K solution-based conduction (Table 4). The total duration required per sample was 93 minutes, 82 minutes and 22 minutes. The longest method was Silica-based extraction method.

Comparison of average DNA Concentration, purity ratio and contamination ratio across of three methods

When comparing the average DNA concentration across three methods, GS solution-based method was the highest DNA concentration with average value (204.93 ng/µL), significantly more than the other two methods. Proteinase K-based method with a moderate concentration (124.6 ng/µL) and Silica solution-based method was the lowest concentration (21.93 ng/µL). (Fig. 2)

When comparing the average DNA purity ratio across three methods, GS solution-based method was the highest purity ratio with average value (2.00), Silica solution-based method was slightly lower purity (2.03) and Proteinase K-based method was the lowest purity (2.08). (fig 3)

When comparing the average DNA purity ratio across three methods, Proteinase K-based method was lower contamination with average value (1.58) and GS solution-based method had a moderate contamination level (0.98). Silica-based method showed the highest contamination (0.06) respectively. (fig 4)

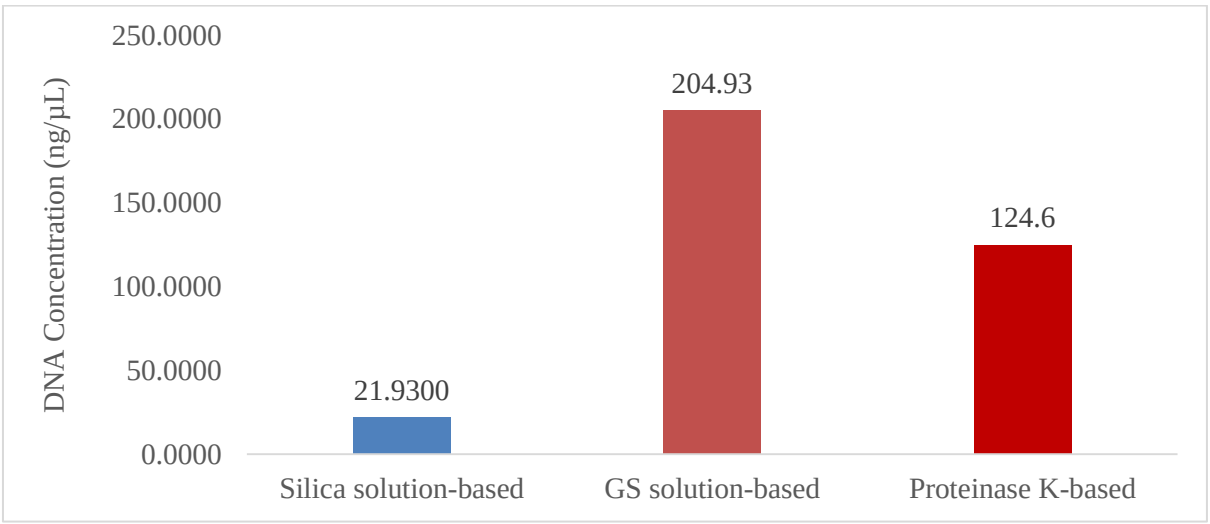

Figure. 2. Comparison on average three methods of DNA concentration

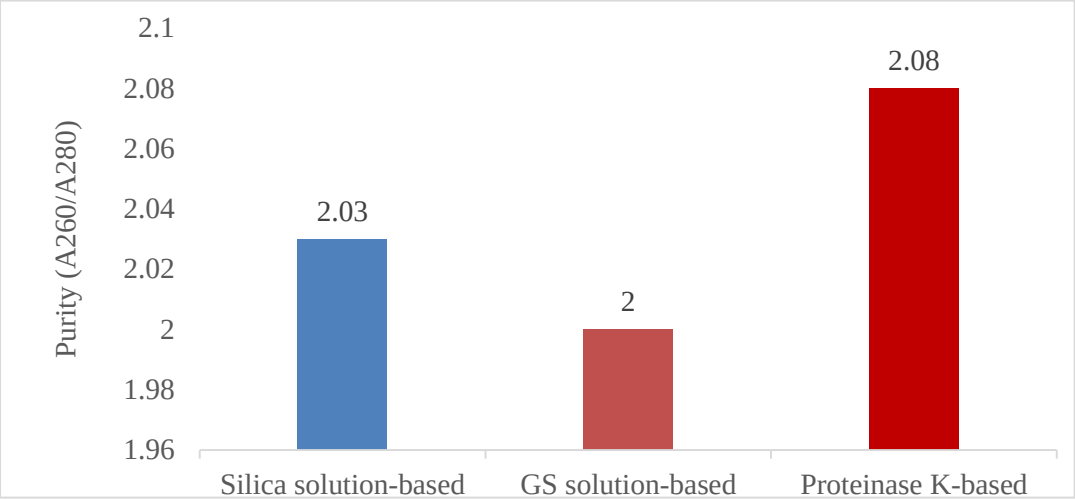

Figure. 3 Comparison on average three methods of DNA purity ratio

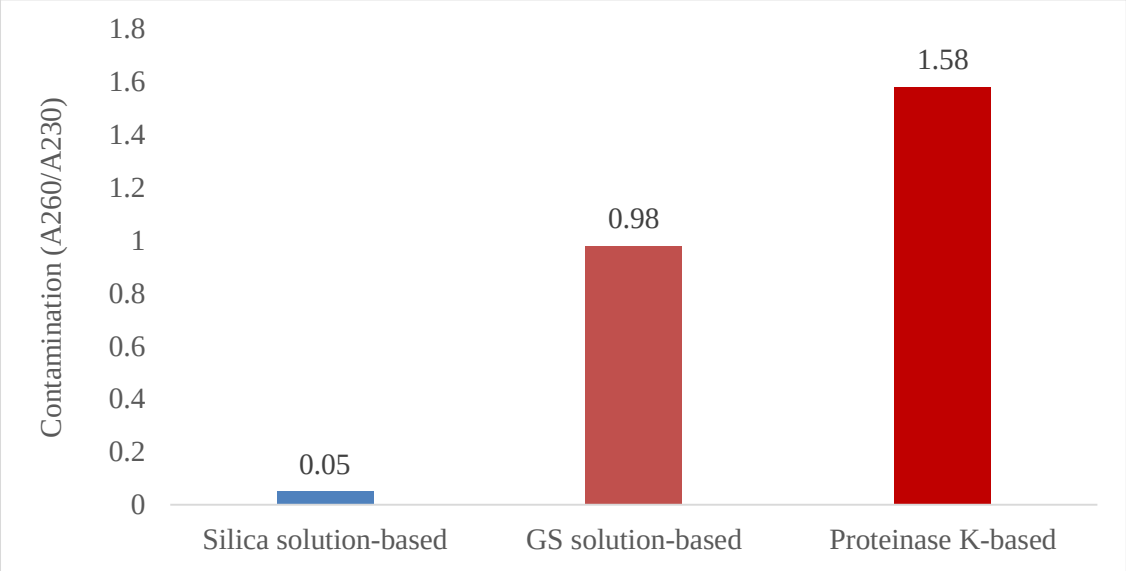

Figure. 4. Comparison on average three methods of contamination ratio

Discussion

The present study evaluated the efficiency of three different DNA extraction methods on catfish specimens: Silica solution-based, GS solution-based, and Proteinase K solution-based methods. DNA concentrations and purity ratios were assessed using a Nanodrop spectrometer, with results obtained from 4 µL of each sample.

The goal was to determine the most suitable method for downstream applications such as PCR and sequencing, where both the quantity and purity of DNA are crucial factors for successful PCR and sequencing. Contaminants such as proteins, salts, and organic compounds can inhibit enzymatic reactions. (Wilson. 1997)

Therefore, when we choose the ideal extraction method, we should consider not only sufficient DNA yield but also ensure high purity. In present study, the Silica solution-based method was the highest contamination level and also the lowest DNA yield.

The GS solution-based method was the highest DNA concentration but higher contamination level than Proteinase k-based, may be less desirable for some applications, but the best purity ratio (2.00). The Proteinase K-based method was the lowest contamination levels among three methods. This indicates that this method was the least number of contaminants like salts and organic compounds.

The time required for DNA extraction can vary based on the ingredients and components of the extraction kits used. Different kits have different protocols, reagents, and technologies that can impact the efficiency and duration of the extraction process. (Wilfinger *et al.,* 1997).

In present study, when comparing time taken for each method per sample for all specimens, including lysis, washing, DNA dehydration and elution.

Silica solution was the most time-consuming, with a total duration of 93 minutes because it might be depended on ingredients of method but offers a balance between speed and consistency.

GS solution took 82 minutes in total during per sample. The lysis time was relatively short, but the washing phase was longer. The brief elution time was a quick final step and had significant variability in time taken steps, making it less reliable. Proteinase K-based was the fastest method, took only 22 minutes.

The choice of extraction method often depends on the specific application and priorities. However, we should consider the overall balance of concentration, purity, and time consistency. Generally, methods with lower SDs are more reliable for time estimation and we can have more confidence in predicting the time for each step.

The Proteinase K and Silica-based methods compared to the GS solution-based method. and the fastest overall, both in terms of average time taken per step and total time. Therefore, the Proteinase K-based method is the best option if saving time is important and a slightly lower DNA yield is acceptable and it was the most efficient and consistent.

Generally, methods with lower SDs are more reliable for time estimation and we can have more confidence in predicting the time for each step. The Proteinase K and Silica-based methods compared to the GS solution-based method. and the fastest overall, both in terms of average time taken per step and total time. Therefore, the Proteinase K-based method is the best option if saving time is important and a slightly lower DNA yield is acceptable and it was the most efficient and consistent.

Conclusion

This study successfully compared three DNA extraction protocols on *Clarias batrachus*. The GS solution-based method produced the highest yield but moderate contamination. The proteinase K-based method offered high purity and fast processing. The silica solution-based method had lower yield and higher contamination. All methods showed variations in DNA quality, despite using the same species. Further research is needed to understand these variations and optimize extraction methods.

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GENETIC CHARACTERIZATION OF FISHING CAT, *PRIONAILURUS VIVERRINUS* (BENNETT, 1833) BASED ON MITOCHONDRIAL CYTOCHROME B GENE AT YANGON ZOOLOGICAL GARDEN, MYANMAR

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Abstract

The fishing cat (*Prionailurus viverrinus*), categorized as a vulnerable species by the International Union for Conservation of Nature (IUCN), is increasingly threatened across its range. The fishing cat (*Prionailurus viverrinus*) is currently listed as "Vulnerable" on the IUCN Red List, with a population decline of approximately 30% over the past three generations (2010–2015) due to habitat loss and human-wildlife conflict. This study aimed to evaluate the genetic diversity of *P. viverrinus* individuals in Myanmar, particularly those housed at Yangon Zoological Garden, and to assess their genetic status in comparison with reported populations from other countries. In December 2023, fecal samples from three captive fishing cats (N=3) were collected for genetic analysis. DNA was extracted and PCR (Polymerase Chain Reaction) was used to amplify the mitochondrial cytochrome b (cyt b) gene from fecal DNA samples of fishing cats. Of the three samples, three were successfully amplified and subsequently sequenced using Big Dye terminator sequencing. Results from the NCBI database indicated that the Myanmar samples showed a 99% genetic similarity to fishing cat populations from India. Phylogenetic analysis revealed that the two Myanmar cats comprised a distinct cluster with identical haplotypes, suggesting minimal genetic divergence within this population. Comparative analyses also showed the closest genetic distances to fishing cats from Thailand and Indochina and sundaland. This study underscores the genetic relatedness of fishing cat population from Yangon Zoological Garden with other regional populations, providing valuable insights into the species' distribution and aiding conservation efforts across their range.

Keywords: Fishing cat, *Prionailurus viverrinus*, Cytochrome b, Genetic characterization, Phylogenetic analysis.

Introduction

The Fishing cat (*Prionailurus viverrinus*) is a type of wild cat under the family Felidae. Among wild cats, the family Felidae is reported to include the genus *Prionailurus* consisting of the two subspecies of *Prionailurus viverrinus* (Bennett, 1833) and *Prionailurus rhizophoreus*. The species is primarily distributed across South and Southeast Asia, inhabiting coastal mangroves, swamps, marshlands, and riverine ecosystems in countries such as India, Nepal, Sri Lanka, Bangladesh, Myanmar, and Thailand, with possible populations in Cambodia, Malaysia, and Indonesia. In Myanmar, it is recorded in the Ayeyarwady Region Meinmahla Kyun Wildlife Sanctuary; and possibly distributed in Indawgyi Wetland Wildlife Sanctuary. It is known by the local name of kyaung ta-nga (Lin and platt,2019).

The fishing cat (*Prionailurus viverrinus*), a medium-sized wild cat adapted to wetland environments, is categorized as "Vulnerable" on the IUCN Red List due to substantial habitat loss and fragmentation (Mukherjee et al., 2016).The fishing cats faced with all threats, hunting for the illegal trade has the greatest potential to do the maximum harm in minimal time (Nowell

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and Jackson, 1996) although this is likely true for some species more than others and the situation in Myanmar needs further study. It is listed as endangered in the National Red Lists of Bangladesh and Nepal (WWF, 2020).

Most species of wild cats are threatened due to a combination of habitat loss, conflict with humans, poaching and illegal wildlife trade (MacDonald et al. 2010). Wild feline species are important predators with various body types, diverse food habits, and great adaptability to the surrounding environment. These species play key roles in the natural ecosystem. Most feline species are secretive and highly vigilant, which makes them a very difficult target for field research and conservation. With the rapid development of non-invasive sampling among wild animals, fecal samples are becoming informative objects in research on wild cats.

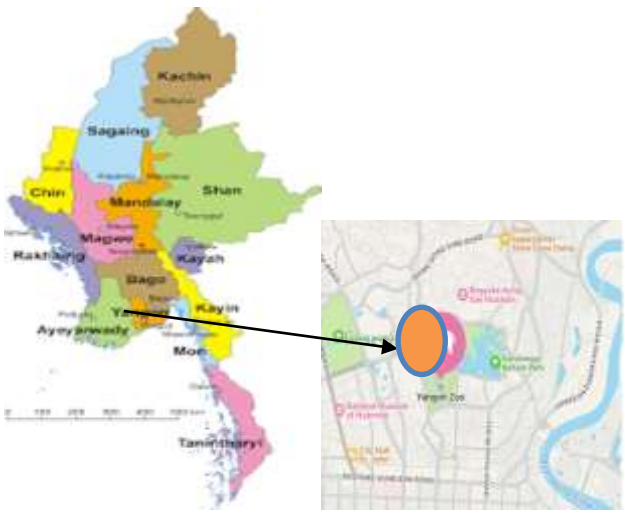
The cyt b gene is used to assess genetic diversity, species identification, evolutionary relationships, and phylogenetic studies in felid species, including fishing cats (Mukherjee et al.2016). It is frequently used in phylogenetic studies due to its stable mutation rate, making it useful for analyzing species relationships (Irwin et al.1991). It is located in mitochondrial DNA, which is maternally inherited and plays a crucial role in cellular respiration. These methods utilize various genetic markers to enhance accuracy in species identification. In this study, the suitability of mitochondrial DNA sequencing was assessed as a fundamental requirement to advance research on the fishing cat.

The study aimed to explore DNA sequencing identification and the phylogeny of the fishing cats from Yangon Zoological Garden, Myanmar. The objective was to examine molecular techniques for precise species identification, contributing to a deeper understanding of the fishing cat population in the specified region.

Materials and Methods

Sampling site and the study period

The three fishing cats sampled in this study were captive-born at the Yangon Zoological Garden, located in Mingalar Taung Nyunt Township, Myanmar. The study site is situated north of downtown Yangon, near Kandawgyi Lake, at coordinates 16° 47′ 30″ N, 96° 09′ 34″ E (Fig. 1). The research period spanned from December 2023 to September 2024.



Myanmar Yangon Zoological Garden

Figure 1 Map showing location of Yangon Zoological Garden, Myanmar(www.googlemap.com)

Sample collection

To adhere the research ethics by avoiding invasive tissue sampling, a more sustainable method of DNA extraction was used for *P. viverrinus*. Therefore, non-invasive fecal samples were collected instead of tissue or blood samples. A total of three fishing cats from the study site were assessed (Plate 1A). Fresh fecal samples were obtained from one male and two female fishing cats at the Yangon Zoological Garden with the assistance of cat keepers. Collection took place early in the morning, prior to the enclosure cleaning, ensuring fresh samples. Each individual and its fecal sample were assigned a code—'FC' for Yangon Zoological Garden—followed by a serial number (FC1, FC2, and FC3).

(a) FC1 (female) (b) FC2 (female) (c) FC3 (male)

Plate 1 Three fishing cats (*Prionailurus viverrinus*) from study site

DNA extraction of the fecal samples for molecular identification

A total of three fishing cats were utilized for DNA extraction and subsequent gene sequencing. The feces were preserved in the sterile plastic container containing 75% ethanol, as illustrated in Plate (2A) and kept in an icebox for transport to the Molecular Biology Laboratory at the Department of Zoology. Prior to DNA extraction, samples were stored at 4°C. The Silica Extraction Kit was employed for the extraction of DNA from the feces of fishing cat, as depicted in Plate (2B). The DNA extraction procedure followed the guidelines provided by the Silica Extraction Kit. Observations and analysis were conducted in the Molecular Laboratory at the University of Yangon.

(A) (B)

Plate 2 (A) Preservation of the fecal samples in the plastic container containing 75% ethanol
(B) Silica Extraction Kit

PCR amplification

To amplify the mitochondrial cytochrome gene, we designed universal primers targeting the cyt b region (Table 1). The PCR reaction consisted of 17.5 µL of extracted DNA template, 7.5 µL of each primer, 25 µL of Gold Taq DNA polymerase.

The PCR conditions involved an initial activation step at 95°C for 15 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 40 seconds, extension at 72°C for 40 seconds, and a final extension at 72°C for 15 minutes (Rana, 2021). Subsequently, PCR products (around 559bp) were separated and visualized via electrophoresis on a 1% agarose gel stained with Gel green stain.

For DNA sequencing, DNA was then extracted and purified from distinct bands using the FastGene® Gel/PCR Extraction Kit (Nippon Genetics Europe GmbH, Bunkyo, Tokyo). Briefly, to isolate DNA, first excise the fragment from an agarose gel using a clean scalpel. Transfer up to 300 mg of the gel slice into a microcentrifuge tube, add 500 µl of binding buffer GP1, vortex, and incubate at 55°C for 10-15 minutes, inverting the tube every 2-3 minutes. Next, apply up to 800 µl of the sample mixture from previous step into the FastGene® GP Column and centrifuge at 13,000 rpm for 30 seconds. Then, add 600 µl Wash Buffer GP2, and centrifuge at 13,000 rpm for 30 seconds. Discard the flow-through, return the column to the tube, and centrifuge again for 2 minutes to dry the column. Finally, add 20-50 µl elution buffer GP3 to the column, let it stand for 2 minutes, and centrifuge at 13,000 rpm for 2 minutes to elute the purified DNA.

Table 1. Primer details for the PCR detection of the fishing cat

Primers	Direction	Sequences	Tm
PrionCytb 2F	Forward	(5'-GGCTGAATCATCCGATACATAC-3')	52°C
PrionCytb 2R	Reverse	(5'-GAGGATTAGCGGGGATGTAGTTA-3')	52°C

Tm = melting temperature

Analysis of DNA Sequence and constructing of phylogenetic tree

Purified DNA products were quantified and directly used for DNA sequencing with an ABI gene sequencer employing the PCR primers. The sequences are then aligned, edited and deleted whether there are stop codons or not that by using MEGA 11 software (Tamura *et al*., 2013). The fishing cat's gene sequence was compared with that of other fishing cat species using the BLAST search available at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nih.gov).

Molecular identification was conducted using mitochondrial DNA sequences within the cytochrome b (cyt b) region. The obtained sequence was then compared to mitochondrial cyt b sequences from the GenBank database, specifically those associated with the Felidae family. The analysis confirmed the species from Yangon Zoologial Garden belonging to the genus *Prionailurus,* including *Prionailurus viverrinus*, *Prionailurus bengalensis.*

A phylogenetic analysis of the mitochondrial cytochrome b gene (mtDNA cyt b) was performed by bootstraps 1000 replicates using the Kimura-2-parameter model to create a

Neighbour-Joining tree (Kimura, 1980). The genetic result of the *P. viverrinus* species is compared with various species of *Prionailurus* with reference GenBank samples.

Results

Morphological description

The fishing cat has a broad, rounded head with relatively small, rounded ears set low on the head. The eyes are large, forward-facing, and typically greenish-yellow. Its coat is gray-brown, adorned with distinct black spots arranged in longitudinal rows along the body. It has short legs with strong muscular limbs. The toes are partially webbed, an adaptation that aids in swimming and hunting in water. Its tail is short and thick, less than half the body length. It has powerful jaws and sharp canines suited to grasp slippery prey like fish. Its carnassial teeth are well developed. According to morphological characters, the fishing cat was tentatively identified as belonging to the species *Prionailurus viverrinus.*

Concentration of DNA extraction from collected fecal samples

Total genomic DNA concentration ranged from 54.2 ng/µl of FC1,53.2 ng/µL of FC2 and 8.3 ng/µl of FC3 from Yangon Zoological Garden. The purity (260/280 nm) were from 0.03-0.09 nm (Table 2).

Table 2. Concentration and purity of extracted genomic DNA from fecal samples

Sr.No	Sample code	Nucleic acid conc-ng/µL	A260/280nm	A260/230nm
1.	FC1	54.2	1.9	0.09
2.	FC2	52.3	1.82	0.03
3.	FC3	8.3	1.9	0.05

DNA sequencing and constructing of phylogenetic tree

The PCR analysis performed on the fishing cat specimen successfully amplifies a mitochondrial DNA of the cyt b region about 559 base pairs (bp) (Figure 2). Figure 4 illustrates DNA sequences of a 1140 bp fragment were obtained from the green mussel. Blast results revealed that our sample is 99% similar to *Prionailurus viverrinus* with the accession numbers KF754958.1 and KF754956.1 (Table 3). These accession numbers with sequence submissions originated in India. The findings revealed that it is a *Prionailurus viverrinus* species that clustered together with members of the Felidae family. Moreover, 99.03 % identities with the species *Prionailurus viverrinus* (KF754958.1) from (India) and 99.03 % to *Prionailurus viverrinus* (KF754958.1) from (Indochina and sundaland).

Phylogenetic analysis was conducted using the Neighbor-Joining (NJ) method, based on the aligned sequences of 522 bp. Each cluster in the phylogenetic tree represented distinct fishing cat species derived from the analyzed fecal samples, aligned against reference sequences from GenBank. The phylogenetic tree was constructed with the 12 similar sequences obtained from the GenBank database; the accession numbers are shown in Fig (5). The graphic blast visualization is shown in Figure 5.

A notable clade in the NJ tree comprised a single cluster containing haplotypes from two fishing cats (FC1 PrionCytbF2 and FC2 PrionCytbF2) collected from the Yangon Zoological

Garden, which clustered closely with another haplotype (FC3 PrionCytbF2) from the same facility. The fishing cat populations from this study area, particularly samples FC1, FC2, and FC3, showed close genetic relationships with populations from India, Thailand, Southeast Asia, Indochina, and Sundaland. The phylogenetic tree illustrated strong support for these relationships, as indicated by high confidence values in the NJ analysis.

Notably, the haplotype from India (KF75496.1) clustered closely with the haplotype from Thailand (AB210239.1), and also related haplotypes from Indochina and Sundaland (KF754958.1). Furthermore, a haplotype from Indochina (OR126345.1) clustered with a haplotype from Southeast Asia (AB210238.1), reflecting connections between populations across these regions.

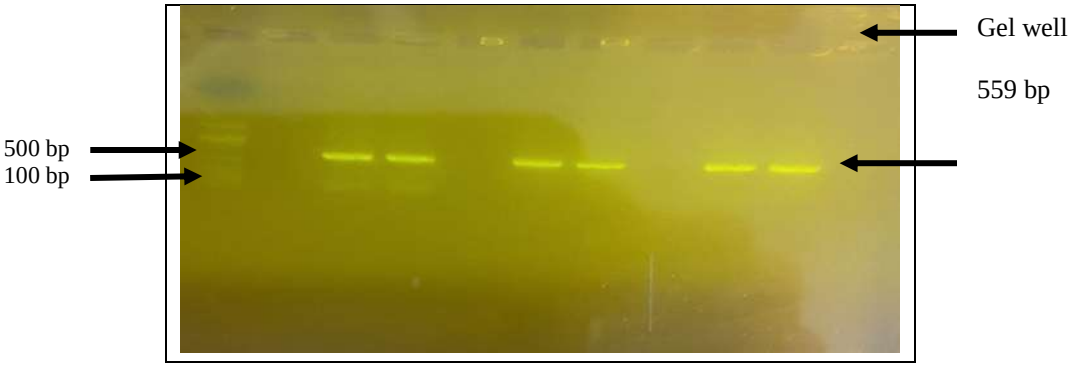


Figure 2 PCR amplification of mitochondrial DNA of the cyt b region of fishing cats after examination with agarose gel exhibits a fragment of 559 bp

GAGCGGGGACTCATATTCTTTATCTGTCTATACATGCACGTGGGCCGAGG……50
AATATATTAGGTTCCTACACCTTCTCAGAAACATGAAACATTGGAATCAT…… 100
AAAATGAAAGGTTATATAATGTTGTAGTAACAACTCATGCATTAGTAATA……150
ACTGCTATTCGCAACCATAGCCACAGCTTTCATAGGTTACGTACTACCATG……200
GGGCCAAATATCCTTCTGAGGGGCAACCGTAATCACCAACCTCCTATCAG… …… 250
CAATCCCATAATGGAACCAACTTAGTAGAATGGGTCTGAGGGGGTTTCTC…… 300
AGTAGACAAAGCCACCTAACACGATTCTTCGCTCTCCATTTCATTCTCCCA……350
TTCATTATTTCAGCCTTAGCAGCAGTACACCTCCTATTCCTCCATGAAACAG…… 400
GATCCAATAACCCCTCAGGAGTCACATCCGACTCGGACAAAATCCCATTCC…… 450
ACCCATATTATACAATCAAGGACATCCTGGCCTTCTAGTACTAATCTCAACAC…… 500
TTATACTACTTGTTCTATTCTCACCAGACCTGTTAGGAGACCCAGATAACTACA…… 550
TCCCGCTTAAATCCCCAAAAAAGCCTTCGATCCTTGCTTGGTTATTATTCCTGG…… 600
ATAAACTAGAGTCGAGCATTAAACTGCGCGTGCCACGACGTAGCATTATGGAC……650
TGACTGGATTGACCCCGCCATGTAATATGCACAAGTATTTCTCTCGAAAGAGTA…… … 700
ATGCGCCAGTCTAGTTATAACTTGCCCTCAATCAGTCTGTCACACTCAGCCGTC……750
AGAACTCTGCAGGCGATGGGGAATAACCATCCTGACAGTCAGTGTCCGGTAGC……800
TCATTAGTTAGGAGCAGATCGAAACGTCAGAATTGATTCCATCGGTAGCTCACAT……900
CACTCTTGTTCGTACGAGCACGGGATAGTGAGCCTGCATAGCTCGTCCACAAAG……950
TGGAGTCATTTCCGAGCTGGACTTATGCGGATTCGGAGCGGGACACGCTAAGA……1000
GGGAAACGTAGAGACTGAACGGGAACACACTTTTCGTACTGAGAACTGATACG……1050
TGACTTAACAAGCGCAGGGACGAAAATGGGGGCGCGTGGTACGCGATCTG……1100
ACACTCATGTCTGTAATAATGTCCGGAGATCAGGGTGATGTGTCATGCGT……1140

Figure 3 Partial sequences of mitochondrial cytochrome gene of the studied fishing cat

Table 3 Mitochondrial cytochrome gene partial sequence identities of the studied fishing cat to other species in the Gene Bank

Species	Locality	Percent Identity (%)	Assessor number in NCBI
Prionailurus viverrinus			
FC1-PrionCytb F2	Myanmar	99	This study
FC2_PrionCytb F2		98	
FC3_PrionCytb F2		98	
Prionailurus viverrinus	India	99.03	KF754956.1
Prionailurus viverrinus	Thailand	97.63	AB210239.1
Prionailurus viverrinus	Indochina & sundaland	99.03	KF754958.1
Prionailurus bengalensis	Indochina	94.94	OR126345.1
Prionailurus viverrinus	Southeast Asia	95.21	AB210238.1
Prionailurus bengalensis	Continental Far East	94.55	AB210226.1
Prionailurus viverrinus	Thailand	97.47	AB210240.1
Prionailurus bengalensis	Thailand	94.41	AB210234.1
Prionailurus bengalensis	Tsushima islands	94.55	AB194818.1
Panthera tigris	Bangladesh	100	JQ409474.1
Panthera tigris	Bangladesh	100	JQ409475.1

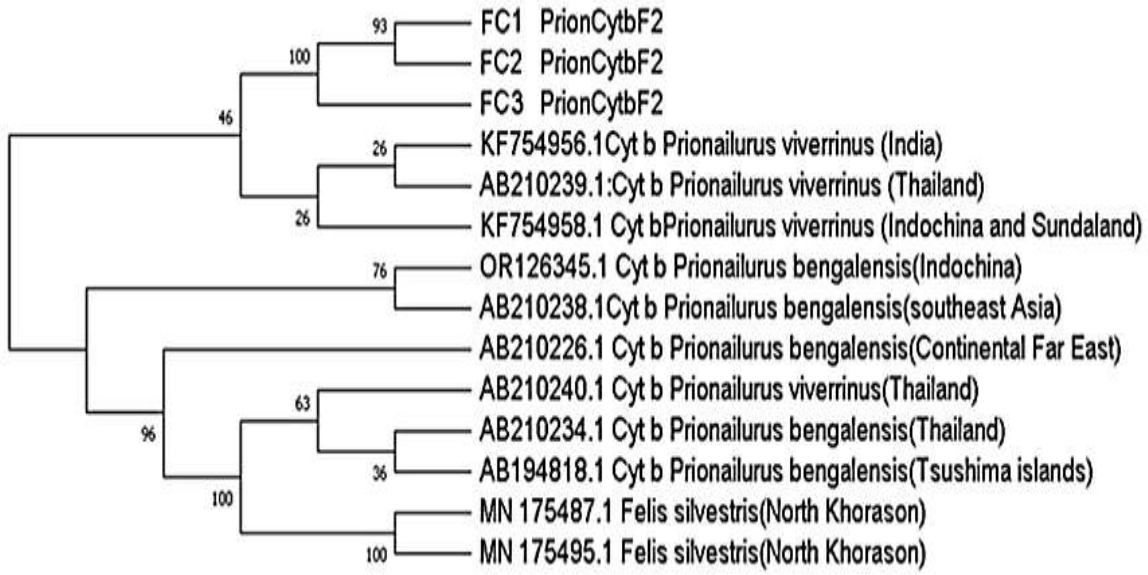


Figure 4 Phylogenetic trees are constructed using the Mitochondrial DNA of the cyt b region

Discussion

In the present study, morphological identification along with molecular identification using the mitochondrial cytochrome b gene sequencing for the identification of fishing cat was conducted. Based on morphological inspection, the specimen closely resembles the fishing cat (*Prionailurus viverrinus*) as described by Rajagopal *et al*. (2006).

The fishing cat (*Prionailurus viverrinus*) is a globally threatened small cat categorized as "Vulnerable" in the IUCN Red List (Mukherjee *et al*., 2016). However, very little is known about

the genetic status of different parts of the country. Therefore, the present research was conducted to assess the genetic status of fishing cats at Yangon Zoological Garden.

Partial cytochrome b (cyt b) gene of the mitochondrial DNA was used as a genetic marker sequence to analyze and compare the haplotypes from study sites. Genetic characterization is essential for understanding the genetic diversity of a species, which is crucial for conservation and management efforts. It can help identify species boundaries and distinguish between fishing cats and other felid species.

A significant finding was that the cyt b sequences from the study showed a high genetic similarity-ranging from 98% to 99%-to the fishing cat species, *Prionailurus viverrinus*. This high level of similarity indicates a close genetic relationship, although some evidence of admixture within the haplotypes of *P. viverrinus* was also observed.

Further examination of the phylogenetic relationships revealed that haplotypes from India (KF75496.1) clustered tightly with those from Thailand (AB210239.1), indicating a shared genetic background. Focusing on Clade I in the NJ tree, a distinct cluster composed of two specific haplotypes (FC1 and FC2) from the Yangon Zoological Garden were noted, which grouped with another haplotype (FC3) from the same location. Haplotypes FC1, FC2, and FC3 from Yangon Zoological Garden formed a distinct cluster (Clade I) in the Neighbor-Joining (NJ) tree. This cluster showed strong genetic similarity and bootstrap support, indicating that the fishing cat populations in Yangon Zoological Garden, Myanmar are closely related to those from India, Thailand, Southeast Asia, Indochina, and Sundaland. The high bootstrap support values associated with this cluster lend strong confidence to the evolutionary relationships proposed by the NJ analysis.

Overall, the results of the NJ phylogenetic analysis not only elucidate the genetic relationships among fishing cat populations but also provide insights into their potential evolutionary history and geographic distribution. These findings underscore the importance of conservation efforts for this species, particularly in managing gene flow and preserving the unique haplotype diversity present across its range.

High-quality samples as blood or tissue are ideal for generating genomic data. Non-invasive samples like hair, feces, and antlers though comparatively easy to obtain are generally poor in quality because of on-invasive samples like fecal samples often have an abundance of bacterial DNA apart from the DNA of the host and the prey species. Using the specific primers in PCR amplification allows for targeted amplification of particular DNA sequences, enhancing the specificity and sensitivity of the process.

In conclusion, the results of the Neighbor-Joining (NJ) phylogenetic analysis clarify the genetic relationships among fishing cat populations and offer valuable insights into their evolutionary history and geographic distribution. These findings emphasize the critical need for conservation efforts aimed at managing gene flow and preserving the unique haplotype diversity found across the species' range. Future research should consider expanding on these results by incorporating additional genetic markers or exploring the ecological factors that impact these populations.

Conclusion

The DNA extraction from fecal samples of fishing cats was successfully achieved, underscoring the effectiveness of non-invasive methods that minimize pain and stress for the animals involved. the genetic clustering analysis revealed a close genetic relationship among the samples from Yangon Zoological Garden, indicating a genetic connection to fishing cat populations in India, Thailand, Southeast Asia, Indochina, and Sundaland. These findings highlight the potential for genetic exchange among fishing cat populations and underscore the importance of conservation efforts to maintain their genetic diversity.

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SPECIES DIVERSITY OF ODONATES IN INYA LAKE AND KANDAWGYI LAKE ENVIRONS, YANGON REGION

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Abstract

The species composition, relative abundance, species diversity and evenness of the odonates from Inya Lake and Kandawgyi Lake environs of Yangon Region were assessed from February 2024 to July 2024. The species were collected weekly from three different habitat types; grasslands, wetlands and shrub lands. A total of 1107 individuals of 15 species belonging to 12 genera under 3 families and 2 suborders were recorded from Inya Lake and 1066 individuals of 12 species belonging to 12 genera under 3 families and 2 suborders were recorded from Kandawgyi Lake environs. The highest recorded species from Inya Lake environs was *Crocothemis erythraea* and Kandawgyi Lake environs was *Brachythemis contaminata*. *Ischnura aurora* from the family Coenagrionidae was the lowest recorded species of both Inya Lake and Kandawgyi Lake environs. According to the observation, the diversity and evenness of odonates from Inya Lake environs were greater than Kandawgyi Lake environs. The wing venation of *Brachythemis contaminata* and *Crocothemis erythraea* were analyzed and their unique structures under Scanning Electron Microscope (SEM) were also described in the present study.

Keywords: odonates, species diversity, environs, individuals, evenness, venation, SEM

Introduction

Insects, one of the largest group of arthropods are the most numerous and diverse organisms on the earth. They have many kinds of interactions with humans and other forms of life on earth. There are 29 orders of insects within the class Insecta and most of which can be found within forest ecosystems (Redak, 2023). They may be found in almost all environments, even though a small number of species occur in the oceans. They have invaded every niche, except the oceanic benthic zone (Grimaldi and Engel, 2005). Insect identification is an increasingly common hobby, with butterflies and dragonflies being the most popular (Gullan and Cranston, 2014).

Pterygota is a subclass of insects that includes the winged insects. The Odonata is a good candidate as the oldest living member of the Pterygota (Shagufta, 2017). Odonates are an extremely primitive and ancient group of insects around 300 million years ago (Grimaldi and Engel, 2005). Based on morphology, the order Odonata is divided into three suborders: Anisoptera, Zygoptera and Anisozygoptera (Subramanian and Babu, 2018). Odonata contained 5956 described species as of 2010 (39 families, 659 genera). 3012 species belong to the suborder Anisoptera (348 genera, 11 families), 2942 to the suborder Zygoptera (309 genera, 27 families) and 2 to the Anisozygoptera (Suhling *et al.*, 2015).

Odonates are notable for the beauty and brilliance of their coloration (Richards and Davies, 2013). They are typically large and active by day. They are observed near lakes, ponds, rivers, streams, canals of all sizes and all over the marshy places (Ameilia *et al.,* 2006). The Anisopteran are larger and more robust than Zygopteran.

Adult odonates have two pairs of elongate and transparent wings. Their wings are mainly composed of veins and membranes (Zhang *et al.,* 2018). Dragonflies spread their wings when

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they are resting but damselflies hold together dorsally over the thorax and abdomen when at rest (Berger, 2004). In Anisoptera, forewings and hindwings are dissimilar in form and venation. Their hindwings are distinctively broader at the base than the forewings. However, forewings and hindwings of Zygoptera are closely similar in form and venation. Most possess hyaline wings, but there are certain groups in which they are conspicuously coloured (Orr *et al.*, 2004). Dragonflies tend to be strong, fast fliers, and their flight appears purposeful, directed. Damselflies look weak and a little aimless in flight; they are more likely to flit and flutter (Richards and Davies, 2013). In the past, wing venation was used as the main guide for classifying the order Odonata. The details of venation are very important in identification and classification (Suhling *et al.*, 2015).

Odonates are hemimetabolous and amphibiotic insects which inhabit all kinds of freshwater habitats. The immature stages of the odonates are totally aquatic. Their mature stages have highly specific habitat preferences (Miguel *et al.,* 2017). Odonates have a wide distribution among habitats, but specific species occur in each habitat, thus making them useful as indicators of environmental quality (Junior et.al, 2015). They are of great importance in destroying noxious flies and mosquitoes, blackflies and other blood sucking flies, as well as the smaller moths which are regarded as pests and act as an important biocontrol agent of these harmful insects (Fraser, 1933). The sudden absence of some odonates may indicate rapid environmental degradation and we can monitor environmental changes by observing odonate populations (Kannagi *et al.,* 2016).

Odonates are reliable indicators of the ecological integrity for ecosystem structures, the ecological quality of the land-water-interface and the connectivity of aquatic ecosystems to other landscape units (Chovanec and Raab, 1997). Since larvae inhabits in aquatic, their diversity and abundance are positively correlated with overall aquatic macro-invertebrate diversity and abundance. Aquatic macro-invertebrates have frequently been used as biological indicators in lotic environments but much less commonly so in lentic habitats. Dragonflies and damselflies (Order: Odonata) satisfy most selection criteria for lentic bio-indicators of grazing impacts (Lee Foote and Rice Hornung, 2005). The main objectives for the present study were to identify and record the occurrence of odonates and their abundance, to determine the species composion, relative abundance, species diversity and evenness of odonates from Inya and Kandawgyi Lake environs and to analyse the wing venations of the two most dominant dragonflies from the recorded odonates under Scanning Electron Microscope (SEM).

Materials and Methods

The odonates (both dragonflies and damselflies) were collected from Inya Lake and Kandawgyi Lake environs which are located at Yangon Region, Myanmar. Inya Lake is situated at north latitude 16°50'25" and east longitude 96°08'27". Kandawgyi Lake is located at north latitude 16°47'40" and east longitude 96°09'54". For the observation, Inya Lake environs were divided into two study sites and also two from Kandawgyi Lake environs. The two study sites from Inya Lake environs were Inya Park and Myanmar Rowing and Canoeing Federation. Inya Park is in front of Yangon University of Economics and near University of Yangon. Myanmar Rowing and Canoeing Federation is in front of Yadanar Hall, University of Yangon. It is only 3 miles away from Inya Park. From Kandawgyi Lake environs, the odonates were recorded near the Western Park Royal Restaurant and in front of Café Garden Bristo at Kandawgyi Park during

the study period. Both of them are in the Kandawgyi Natural Garden situated on the Bahan road (Fig. 1).

A. Location maps of the study sites in Inya Lake environs

B. Location maps of the study sites in Kandawgyi Lake environs

Figure. 1. Location maps of the study sites in Inya Lake and Kandawgyi Lake environs (Sources: Google Earth, 2024)

Specimens collection

The specimens were collected weekly from February 2024 to July 2024. The three habitat types (grasslands, wetlands and shrub lands) were chosen for the specimen collection based on the odonates favorable habitats (Plate 1). Since odonates are diurnal, the specimens were collected between 09:00 A.M and 12:00 A.M on every sampling day. The specimens were collected from an area of approximately 50m× 25m at each site. Near the water edges, collections were made within an area of about 50m× 2m (1m along the edge and 1m extending into the water). Each site required one and a half hours for specimen collection. They were collected by using insect sweep net and put them in the plastic containers with slotted covers. Some of the specimens were sacrificed by gentle squeezing of their thorax with thumb and index finger, without giving them chances to struggle and break wings. To avoid mechanical injuries, the sacrificed specimens were kept in the triangular envelopes for later detailed studies. The habitat types of the observed specimens were recorded on the containers along with the date of collection.

Grasslands Wetlands Shrub lands Grasslands Wetlands Shrub lands

A. Various habitat types of Inya Lake environs

B. Various habitat types of Kandawgyi Lake environs

Plate 1 Various habitat types of Inya Lake and Kandawgyi Lake environs

Identification of the specimens

The collected specimens were examined under stereo microscope in the microscopy laboratory, University of Yangon and their general morphologies, coloration, abdomen length and wingspan were described with scaled photographs. The identification of the specimens was followed by Fraser (1933,1934 & 1936).

Preparation of wings for Electron Microscopy

The unique structures of the selected dragonflies's wings were observed in the Universities' Research Center (URC), located in the University of Yangon. The wings were needed to dry and clean before scanning for electron microscopy. Subsequently, three sections of each wing were cut to fit on the sample holder and put the carbon double tape on the sample holder together with the sections of wings. The samples' surface was coated with a thin film of proper material, gold (Au) to achieve high-resolution and clear images. Then the samples were put into SEM (Zeiss EVO 18) and observed pterostigma, node, vein, cells and spike on the vein at different magnification. The venations of wings were described according to Fraser (1933 and 1936).

Data analysis

Species compositions of the recorded odonates were calculated after Thursfield, 1995.

Species composition = Number of species in each order/ family / Total number of species recorded * 100%

The relative abundance (RA) of the recorded species was calculated by using formula (Kumar and Sivaperuman, 2005).

Abundance categories:

- Rare = (0.1-2.0)
- Uncommon = (2.1-4.0)
- Frequent = (4.1-6.0)
- Common = (6.1-8.0)
- Abundant= (8.1-above)

The species diversity for Inya Lake and Kandawgyi Lake environs was determined by using Shannon Diversity Index "H'" (Shannon and Weiner,1949).

$H' = -\sum (Pi \ln Pi)$

Where, $Pi = ni/N$

H' = Shannon diversity index

Pi = Proportion of individuals of i-th species in a whole community

ni = Total numbers of individuals belonging to i-th species

N = Total number of individuals belonging to the sampled population

ln = Natural logarithm

Σ = sum symbol

*The higher the (H'), the greater the diversity.

The evenness of the recorded species was calculated by using Pielou's evenness index(J).

$J = H' / H_{max}$

Where, J = Pielou's Evenness index

H' = Shannon-Wiener index

H_{max} = ln S

S = Total number of species

*The values are between 0-1. When the value is getting closer to 1, it means that the individuals are distributed equally.

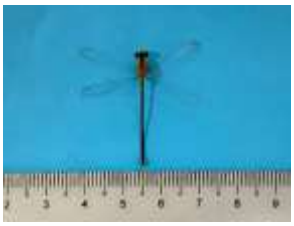
Results

A total of 2173 individuals of 18 species (12 dragonfly and 6 damselfly species), 15 genera, 3 families and two suborders of odonates were recorded from Inya lake and Kandawgyi lake environs from February 2024 – July 2024 (Table 1). Only 9 species were recorded in both Inya and Kandawgyi Lake environs (Plate 2).

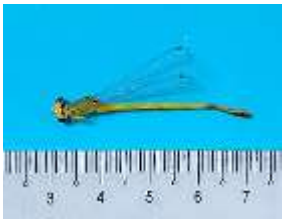
Table 4.1 Recorded odonate species found in Inya Lake and Kandawgyi Lake Environs

Sr. no.	Suborder	Family	Species	Common Name	Inya	Kandawgyi
1.	Anisoptera	Libellulidae	*Acisoma panorpoides*	Trumpet tail	-	+
2.			*Brachythemis contaminata*	Ditch jewel	+	+
3.			*Crocothemis erythraea*	Scarlet dragonfly	+	+
4.			*Diplacodes trivialis*	Ground skimmer	+	+
5.			*Libellula saturata*	Flame skimmer	+	+
6.			*Neurothemis tullia*	Pied paddy skimmer	+	-
7.			*Orthetrum pruinosum*	Crimson tail marsh hawk	+	+
8.			*Orthetrum sabina*	Slender skimmer	+	-
9.			*Pseudothemis jorina*	Banded skimmer	+	+
10.			*Rhyothemis phyllis*	Yellow-striped flutter	+	-
11.			*Tholymis tillarga*	Coral-tailed cloudwing	+	-
12.		Gompidae	*Progomphus obscurus*	Common sanddragon	+	+
13.	Zygoptera	Coenagrionidae	*Agriocnemis pygmaea*	Pygmy dartlet	+	+
14.			*Ceriagrion coromandelianum*	Coromandal marsh dart	-	+
15.			*Enallagma antennatum*	Rainbow bluet	-	+
16.			*Ischnura aurora*	Golden dartlet	+	+
17.			*Ischnura elegans*	Common bluetail	+	-
18.			*Ischnura senegalensis*	Marsh bluetail	+	-
Total Number of species					15	12

+ (present) ; - (absent)



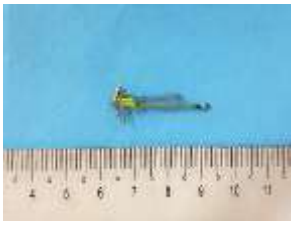
A. *Agriocnemis pygmaea*



B. *Ceriagrion coromandelianum*

C. *Enallagma antennatum*

D. *Ischnura aurora*



E. *Ischnura elegans*

F. *Ischnura senegalensis*

G. *Progomphus obscurus*

H. *Acisoma panorpoides*

I. *Crocothemis erythraea*

J. *Diplacodes trivialis*

K. *Brachythemis contaminata*

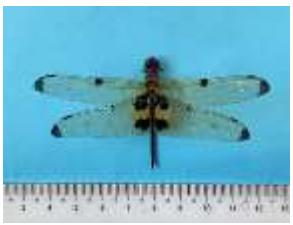
L. *Libellula saturata*

M. *Neurothemis tullia*

N. *Orthetrum pruinosum*

O. *Orthetrum sabina*

P. *Pseudothemis jorina*



Q. *Rhyothemis phyllis*

R. *Pseudothemis jorina*

Plate 2 Recorded damselfly and dragonfly species

Species composition of odonates found in Inya Lake and Kandawgyi environs

A total of 1107 individuals of 15 species, 12 genera, 3 families and two suborders of odonates (dragonflies and damselflies) were recorded from Inya Lake environs and 1066 individuals of 12 species belonging to 12 genera under three families and two suborders of dragonflies and damselflies were recorded from Kandawgyi Lake environs during study period. 85 individuals of damselflies belonging to family Coenagrionidae and 1022 individuals of dragonflies belonging to family Gomphidae and Libellulidae were recorded from Inya Lake environs. 67 % of the recorded species were from the family Libellulidae, 6 % from Gomphidae and 27 % from Coenagrionidae (Fig. 2). A total of 63 individuals of damselflies under family Coenagrionidae and 1003 individuals of dragonflies under family Gomphidae and Libellulidae were recorded from Kandawgyi Lake environs. 59% of the recorded odonates were from the family Libellulidae, 8% from Gomphidae and 33% from Coenagrionidae in Kandawgyi Lake environs. Coenagrionidae (Fig. 3). Family Libellulidae from suborder Anisoptera was the major component of odonates community in both Inya Lake and Kandawgyi Lake environs.

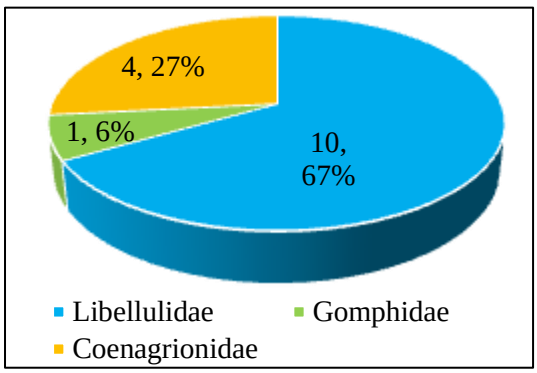


Figure. 2. Percentage of recorded odonate species with respective families in Inya Lake environs

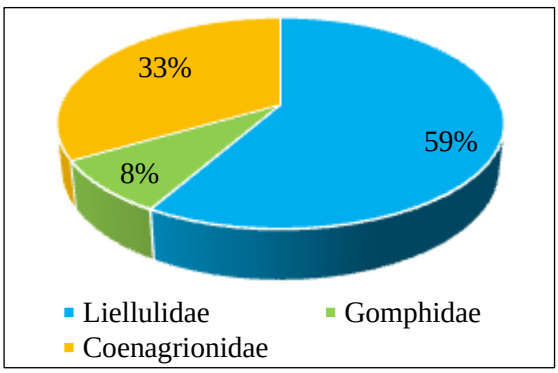


Figure. 3. Percentage of recorded odonate species with respective families in Kandawgyi Lake environs

Habitat types of the odonates found in Inya Lake and Kandawgyi Lake environs

Out of 1107 individuals, 398 individuals of 15 species were recorded in grasslands. 388 of 12 species were collected in wetlands and 321 of 7 species were collected from shrub lands from Inya Lake environs. *Crocothemis erythraea* was the most collected species found in grasslands and shrub lands. *Brachythemis contaminata* was the most recorded species found in wetlands compared with other species. The least collected species from grasslands, wetlands and shrub lands are *Pseudothemis jorina, Orthetrum sabina* and *Tholymis tillarga* respectively. *Neothemis tullia* from suborder Anisoptera and *Ischnura aurora* from the suborder Zygoptera were only found in grasslands (Table 2). In Kandawgyi Lake environs, among 1066 individuals, 386, 380, 300 individuals were recorded in grasslands, wetlands and shrub lands respectively. Only 8 species were found in shrub areas of Kandawgyi Lake environs. The most recorded species found in grasslands and shrub lands was *Crocothemis erythraea* from the family Libellulidae. *Brachythemis contaminata* also from the family Libellulidae was the most dominant species found in wetlands. *Ischnura aurora* from the suborder Zygoptera was the least dominant

species found in grasslands and shrub areas. Meanwhile *Ceriagrion coromandelianum* from the family Coenagrionidae was the least dominant species recorded in wetlands. *Pseudothemis jorina* from suborder Anisoptera was observed exclusively in wetlands (Table 3).

Table 2 Habitat types of recorded odonates species from Inya Lake environs

Sr. no.	Suborder	Family	Species	Habitat types		
				Grasslands	Wetlands	Shrub lands
1.	Anisoptera	Libellulidae	*Brachythemis contaminata*	58	82	68
2.			*Crocothemis erythraea*	89	45	98
3.			*Diplacodes trivialis*	47	14	56
4.			*Libellula saturata*	46	69	33
5.			*Neurothemis tullia*	12	-	-
6.			*Orthetrum pruinosum*	63	89	50
7.			*Orthetrum sabina*	3	6	8
8.			*Pseudothemis jorina*	1	14	-
9.			*Rhyothemis phyllis*	27	16	-
10.			*Tholymis tillarga*	5	-	8
11.		Gomphidae	*Progomphus obscurus*	5	10	-
12.	Zygoptera	Coenagrionidae	*Agriocnemis pygmaea*	9	22	-
13.			*Ischnura aurora*	3	-	-
14.			*Ischnura elegans*	20	11	-
15.			*Ischnura senegalensis*	10	10	-
Total Number of Individuals				**398**	**388**	**321**

Table 3 Habitat types of recorded odonates species from Kandawgyi Lake environs

Sr. no.	Suborder	Family	Species	Habitat types		
				Grasslands	Wetlands	Shrub lands
1.	Anisoptera	Libellulidae	*Acisoma panorpoides*	9	8	-
2.			*Brachythemis contaminata*	74	89	72
3.			*Crocothemis erythraea*	104	43	80
4.			*Diplacodes trivialis*	18	10	34
5.			*Libellula saturata*	65	83	52
6.			*Orthetrum pruinosum*	85	77	52
7.			*Pseudothemis jorina*	-	29	-
8.		Gomphidae	*Progomphus obscurus*	6	10	3
9.	Zygoptera	Coenagrionidae	*Agriocnemis pygmaea*	5	8	5
10.			*Ceriagrion coromandelianum*	6	1	-
11.			*Enallagma antennatum*	11	22	-
12.			*Ischnura aurora*	3	-	2
Total Number of Individuals				**386**	**380**	**300**

Relative abundance and abundance categories of the recorded odonates found in Inya Lake and Kandawgyi Lake environs

During the survey period, a total of 15 species were recorded in Inya Lake environs. Among them, 5 species were abundant, 7 species were rare and 3 species were uncommon. From another point of view, 33 % of the species was abundant, 47 % was rare and 20 % was uncommon in Inya Lake environs (Table 4). Among the recorded species of Kandawgyi Lake environs, 4 species were abundant, 3 were rare, 4 were uncommon and 1 was frequent. *Diplacodes trivialis* was the only species as frequent. In total, 34 % of the species was abundant, 25% was rare, 33 % was uncommon and 8 % was frequent in Kandawgyi Lake environs (Table 5).

Table 4 Relative abundance and abundance categories of recorded odonates species from Inya Lake environs

Sr. no.	Suborder	Family	Species	Individuals	RA	Abundance categories
1.	Anisoptera	Libellulidae	*Brachythemis contaminata*	208	18.79	Abundant
2.			*Crocothemis erythraea*	232	20.96	Abundant
3.			*Diplacodes trivialis*	117	10.57	Abundant
4.			*Libellula saturata*	148	13.37	Abundantm
5.			*Neurothemis tullia*	12	1.08	Rare
6.			*Orthetrum pruinosum*	202	18.25	Abundant
7.			*Orthetrum sabina*	17	1.54	Rare
8.			*Pseudothemis jorina*	15	1.36	Rare
9.			*Rhyothemis phyllis*	43	3.88	Uncommon
10.			*Tholymis tillarga*	13	1.17	Rare
11.		Gomphidae	*Progomphus obscurus*	15	1.36	Rare
12.	Zygoptera	Coenagrionidae	*Agriocnemis pygmaea*	31	2.80	Uncommon
13.			*Ischnura aurora*	3	0.27	Rare
14.			*Ischnura elegans*	31	3.34	Uncommon
15.			*Ischnura senegalensis*	20	1.81	Rare
Total Number of Individuals				**1107**		

Table 5 Relative abundance and abundance categories of recorded odonates species from Kandawgyi Lake environs

Sr. no.	Suborder	Family	Species	Individuals	RA	Relative Abundance
1.	Anisoptera	Libellulidae	*Acisoma panorpoides*	17	1.59	Rare
2.			*Brachythemis contaminata*	235	22.05	Abundant
3.			*Crocothemis erythraea*	227	21.29	Abundant
4.			*Diplacodes trivialis*	62	5.82	Frequent

Sr. no.	Suborder	Family	Species	Individuals	RA	Relative Abundance
5.			*Libellula saturata*	200	18.76	Abundant
6.			*Orthetrum pruinosum*	214	20.08	Abundant
7.			*Pseudothemis jorina*	29	2.72	Uncommon
8.		Gomphidae	*Progomphus obscurus*	19	1.78	Uncommon
9.	Zygoptera	Coenagrionidae	*Agriocnemis pygmaea*	18	1.69	Uncommon
10.			*Ceriagrion coromandelianum*	7	0.66	Rare
11.			*Enallagma antennatum*	33	3.10	Uncommon
12.			*Ischnura aurora*	5	0.47	Rare
Total Number of Individuals				**1066**		

Diversity and evenness of the recorded odonates found in Inya Lake and Kandawgyi Lake environs

The value of Shannon Diversity Index in Inya Lake environs was 2.15548 and Pielou's evenness was 0.795953 and in Kandawgyi Lake environs, 1.93492 and 0.778669 respectively. Both the values of diversity and evenness was greater in Inya Lake environs than Kandawgyi Lake environs (Table 6).

Table 6 Species evenness of Inya Lake and Kandawgyi Lake environs

Study Area	Total number of individuals (N)	Total Number of species (S)	Shannon diversity index (H')	Pielou's Evenness index (J)
Inya Lake environs	1107	15	2.15548	0.795953
Kandawgyi Lake environs	1066	12	1.93492	0.778669

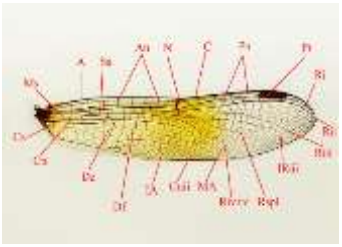
Wing venation of the dominant dragonflies; *Brachythemis contaminata* and *Crocothemis erythraea* from Inya Lake and Kandawgyi Lake environs

Family - Libellulidae
Genus - *Brachythemis* Brauer, 1867
Species - *Brachythemis contaminata* (Fabricius, 1793)

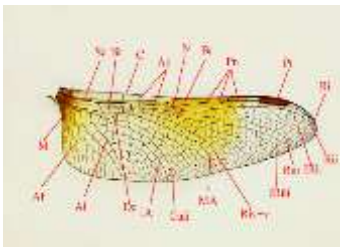
Wings - Hyaline and short, rather rounded at apices; reddish with a broad bright orange fascia extending from base to within 2 to 3 cells of pterostigma in both forewing and hindwing of male. This structure is absent in female. This is variable in extent and depth of color is according to the age.
Reticulation - Closed.
Pterostigma - Moderately large; rust- red in male with posterior border brown and bright ochreous between dark nervures in females.

Discoidal cell - Only the costal side is narrow in forewing and about half the length of basal or distal are traversed; hindwing is entire with base at arc.
Discoidal field - Beginning with 3 rows of cells, its border parallel to wing border.
Arc - Situated between the first and second antenodal nervures.
Sectors of arc - Very shortly fused at origin.
Cubital nervures - Only one in both wings.
Second cubitus - Rising from or separated from the posterior angle of discoidal cell in hindwing.
Anal loop - Rather broadly dilated distally and with distal side strongly angulated.
Anal Field - Developed and many cell rows.
Nodal index - $\frac{8-7\frac{1}{2}}{7-5}\left|\frac{7\frac{1}{2}-8}{5-7}\right.$
Sex - Male
Size - Forewing : 23 mm
Hindwing : 22 mm

The venation is similar in both sexes (Fig. 4). The SEM images of unique structure of the wings; pterostigma, node, vein, cells and the spike on the vein (Plate 3).

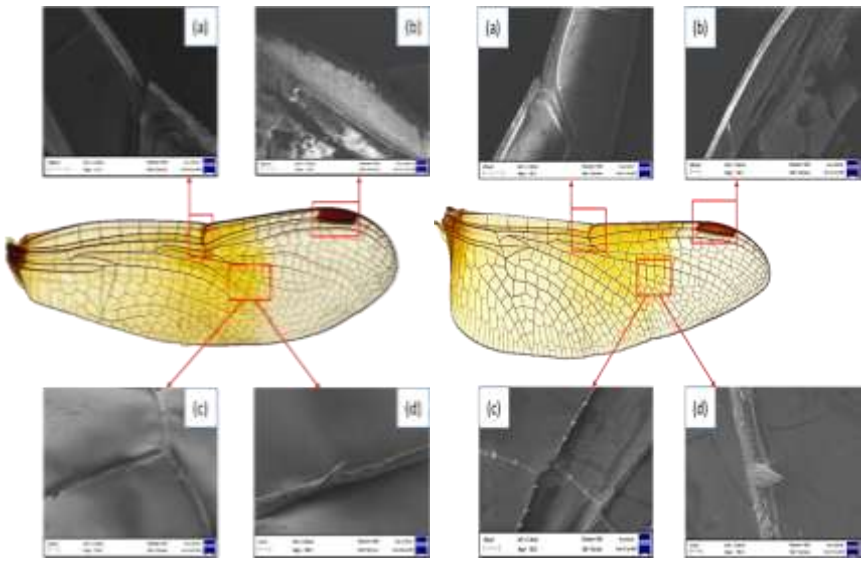


(A)



(B)

Figure.4. The wing venation of *Brachythemis contaminata*'s (A) Forewing (B) Hindwing



(A) (B)

Plate 3 SEM images of unique structure of wing (a) node, (b) pterostigma, (c) vein and cells, (d) spike on the vein of *Brachythemis contaminata's* (A) Forewing (B) Hindwing

Family - Libellulidae
Genus - *Crocothemis* Brauer, 1867
Species - *Crocothemis erythraea* (Brulle, 1832)

Wing - Long and narrow, pointed at apices; hyaline or partly colored at extremely base, the basal amber-tinted marking slightly more extensive. This basal marking is bright orange in male and bright yellow in female.

Reticulation - Closed.

Pterostigma - Large; dark ochreous between blackish nervures.

Discoidal cell - The costal side is straight and narrow in forewing and only half the length of distal and basal sides are traversed; hindwing is usually entirely at the base level of arc.

Discoidal field - Beginning with 3 rows of cells, widely divergent at wing border.

Arc - Situated between the first and second antenodal nerves.

Sectors of arc - Very shortly fused at origin.

Cubital nervures - Only one in both wings;

Second cubitus - Usually arising from the posterior angle of hindwing's discoidal cell.

Anal loop - Widely dilated at distal end and the distal side right-angled.

Anal field - Developed and numerous cell rows.

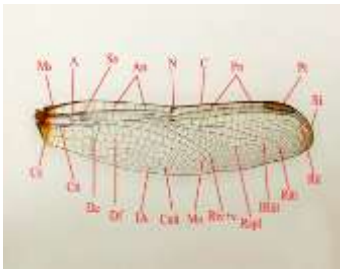
Nodal index - $\frac{10-10\frac{1}{2}}{10-9} \mid \frac{10\frac{1}{2}-10}{10-9}$

Sex - Female

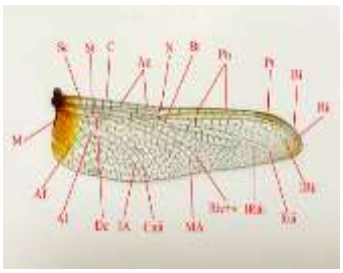
Size - Forewing : 30 mm

Hindwing : 27 mm

The venation is similar in both sexes (Fig. 5). The unique structure of the wings; pterostigma, node, vein, cells and spike on the vein under SEM were described (Plate 4).

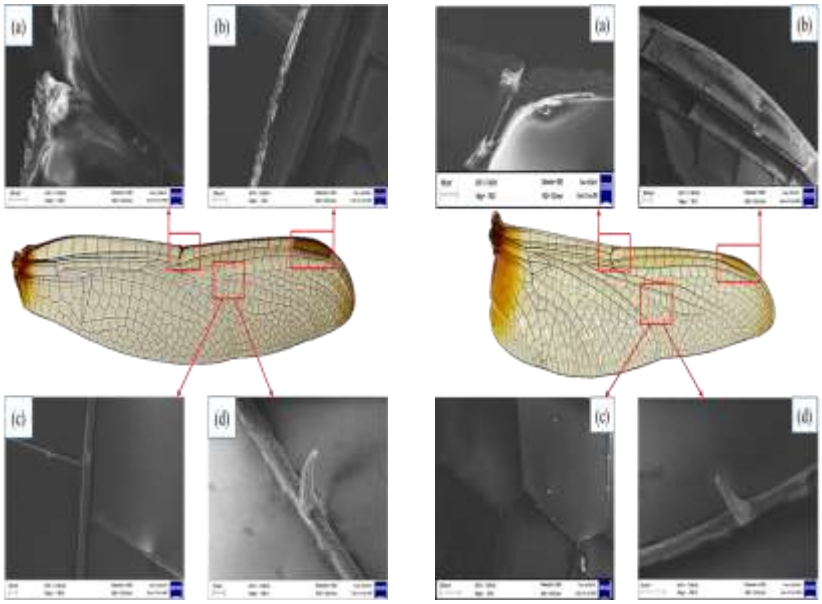


(A)



(B)

Figure. 5. The wing venation of *Crocothemis erythraea*'s (A) Forewing (B) Hindwing



(A) (B)

Plate 4 SEM images of unique structure of wing (a) node, (b) pterostigma, (c) vein and cells, (d) spike on the vein of *Crocothemis erythraea's* (A) Forewing (B) Hindwing

Discussion and Conclusion

A total of 1107 individuals of 15 species belonging to 12 genera under 3 families and 2 suborders of odonates (1022 dragonflies and 85 damselflies) were recorded in Inya Lake environs and 1066 individuals of 12 species belonging to 12 genera under 3 families and 2 suborders including 1003 dragonflies and 63 damselflies) were recorded in Kandawgyi Lake environs during the study period. It is presumed that the environmental impact of Inya and Kandawgyi Lake provide for a large variety of dragonflies but not for most damselflies. Inya Lake environs had more genera and species than Kandawgyi Lake environs. This may be due to the fact that Inya Lake environs have more suitable food sources, habitats and fewer predators for odonates.

From the total recorded species, most of the species were in the family Libellulidae as a dominant family with 61% in both study sites. From Inya Lake environs, the major component of odonates were in the family Libellulidae with 67% and from Kandawgyi Lake environs with 59%. This finding is agreed with Khin Hnin Soe (2011) who found the species community in Thanlyin and Kyauktan environs, Kathy Khine (2013) who studied the species occurrence in Inya and Kandawgyi Lake environs and May Myat Thu (2013) who recorded dragonfly species in Kandawgyi Lake environs. That's why the family Libellulidae may be assumed to be the dominant family in many places of Yangon Region. Only 9 species (7 anisopterans and 2 zygopterans species) were found in both Inya and Kandawgyi Lake environs throughout the study period. It is assumed that these species may be adapted to a wide range of environmental conditions.

Among the recorded species, *Crocothemis erythraea* under the family Libellulidae and *Brachythemis contaminata* also under the family Libellulidae were the most abundant species in Inya Lake and Kandawgyi Lake environs respectively. *Ischnura aurora* under family Coenagrionidae was the least abundant species in both Inya and Kandawgyi Lake environs in the present study. According to Kathy Khine's finding, the most abundant species is *Brachythemis contaminata* in Inya and Kandawgyi Lake environs.

The highest number of individuals were recorded from grasslands in both Inya and Kandawgyi Lake environs. The maximum number of species and individuals were recorded also in grasslands in Inya Lake environs. From Kandawgyi Lake environs, the maximum number of species were observed in grasslands and wetlands. Khin Hnin Soe (2011) and Kathy Khine (2013) pointed out that a greater number of species occupied in wetlands than other habitats. This finding of Khin Hnin Soe (2011) and Kathy Khine (2013) showed slight differences which suggests that habitat conditions may directly influence the presence of odonate species. May Myat Thu (2013) observed that the highest number of individuals were recorded in grasslands. This observation aligns with the present study. This may be assumed that odonates were found in wetlands and grasslands because larvae can only survive in water and it is also their birth place. Grassland may serve as feeding ground for the adult odonates.

Among 5 categories of relative abundance, only 3 categories (47% of the species were rare, 20% were uncommon and 33% were abundant) in Inya Lake environs. In Kandawgyi Lake environs, there are 4 relative abundance categories (25% were rare, 34% were abundant, 33% were uncommon and 8% were frequent). In the present study, Shannon-Wiener index (H') was

2.15548 for the Inya Lake environs and 1.93492 for the Kandawgyi Lake environs. Since the higher the 'H', the value indicates greater diversity, this suggests that the species diversity in the Inya Lake environs is greater than that of Kandawgyi Lake environs. The value of Pielou's evenness index (J) is between 0 and 1. When the value is getting closer to 1, it means that the individuals were distributed equally. The evenness index (J) in Inya and Kandawgyi Lake environs was 0.79593 and 0.778669 respectively. These values indicate a fairly high degree of species evenness in both areas, with some species potentially dominating. However, the overall distribution of individuals among species remains relatively balanced. If a more comprehensive study covering the seasons could be conducted, more diverse odonate species could be observed. The study expends the understanding of odonate biodiversity in Inya and Kandawgyi Lake regions, offering valuable information that can aid in future ecological and conservation efforts.

Both *Brachythemis contaminata* and *Crocothemis erythraea* belong to the family Libellulidae. Species within this family Libellulidae can be distinguished based on the coloration, wings shape, wings markings and the number of antenodal and postnodal nervures present on their wings. The pterostigma also serves as one distinctive feature when identifying the species. The color of the pterostigma in *Brachythemis contaminata* is rust-red with posterior border brown or bright ocherous between dark nervures but it is dark ochreous between blackish nervures in *Crocothemis erythraea*. The cells or membrane of the wings are hyaline in some species and some are partly colored. On the wings of *Brachythemis contaminata*, there is a bright orange fascia extending from base to within 2 to 3 cells of pterostigma in both fore and hindwing but in *Crocothemis erythraea*, the basal amber-tinted marking is present only at the extremely base.

Khaing Sabai (2000) studied 16 species of wing venation but these two species were not included. The position of the node or nodus varies between taxa. According to Needham (1903), the pterostigma, the pigmented spot is developed upon the costa of the wing at the point of greatest impact against the air (Sun and Bhushan, 2012). The unique structure like pterostigma, node, vein, cells and spike on the vein were also studied under Scanning Electron Microscope (SEM) with different magnifications in the present study. Knowing the wing venation patterns can also help in conservation efforts by enabling accurate identification of rare or endangered species. This analysis revealed various types of venation patterns and structural features that are critical for species classification and identification. These findings not only contribute to the field of odontology but also provide essential data for biodiversity conservation and species identification.

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ISOLATION, SCREENING AND IDENTIFICATION OF CELLULOLYTIC BACTERIA FROM WOOD INDUSTRY AND BAMBOO TREES SOIL SAMPLES

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Abstract

Cellulases are key enzymes that hydrolyze cellulose into simpler sugars, making them vital for applications such as bioethanol production and use in the textile and paper industries. Bacteria obtained from different soil samples in South Dagon Township, Yangon Region were assessed for cellulose degradation. Cellulose degrading bacteria were isolated from wood industry soil and bamboo tree soil samples using serial dilution and pour plate method. This study was directed towards the isolation and screening of cellulase producing bacteria from wood industry soil and bamboo tree soil samples on CMC (carboxymethyl cellulose) agar plates, by Congo Red staining. A total 18 bacteria were isolated from wood industry soil and bamboo tree soil. Out of 18 isolated bacteria, six isolates were showed clear zones of hydrolysis on CMC agar. Among cellulolytic bacteria, two cellulolytic bacteria were isolated from wood industry soil and four cellulolytic bacteria were isolated from bamboo tree soil. Among the six strains, *Bacillus* spp.2 was showed the highest cellulose hydrolysis activity from bamboo tree soil. Bacteria identification was carried out according to colony morphology, gram stain appearance and their biochemical reaction based on Bergey's Manual of Determinative Bacteriology. These findings highlight the potential of local soil environments as a source for highly active, industrial-relevant cellulolytic bacterial strains.

Keyword: wood industry soil and bamboo tree soil, isolation, screening and identification, cellulolytic bacterial strains

Introduction

Bacteria are the major cellulose-producing microbes involved in the degradation process. Cellulolytic bacteria have been isolated from a wide diversity of environments such as soils, composts and decaying plant litter, which play a specific role in the biodegradation of organic materials during decomposition (Medie *et al*., 2012). Soil is the richest ecosystem which is the most imperative source for the presence of hydrolytic enzyme-producing microbes such as bacteria, fungi, actinomycetes etc. The microorganisms in the soil produce different hydrolytic enzymes such as amylases, proteases, cellulases, lipases etc. There are several reports available on the production of cellulases from microbes (Bhagat *and Kokitkar*., 2021).

Moreover, they play a pivotal role in plant residue decomposition since microorganisms are the main producer of enzymes that participate in the degradation processes of plant cell wall polymers, including cellulose, hemicellulose and lignin in soils (Lopez-Mondejar *et al.*, 2016). Cellulose is an abundant organic compound present on earth. Each cellulose molecule contains 10,000 glucose units (Daba and Krishna, 2019). Cellulose is the most common organic polymer. Cellulose composes a liner polymer of glucose. Cellulose need biodegradation before using an energy source of glucose. Biodegradation of cellulose using cellulolytic bacteria can be an effective way of serving low price of high-quality feed. Cellulolytic bacteria synthesize a set of enzymes capable of breaking down cellulose by hydrolyzing the glycosides β-1.4 polymer

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cellulose bond (Whistler and Smart, 1953). Fermentation with cellulolytic bacteria can increase the sugar and crude protein levels, degrade cellulose and crude fiber content (Ardiansyah and Aspet, 2019).

Wood industry soil is a rich source for obtaining cellulose degrading bacteria because of abundant diverse group of cellulolytic microorganisms owing to the presence of cellulose in wood works and timber. Further, its wide availability, ease of collection and cost effectiveness also plays important role for its selection (Tolulop *et al.*, 2019).

Cellulose, the largest component of plant residues- terrestrial ecosystem and huge source of energy for microorganism, indirectly responsible for soil organic matter decomposition. Degradation processes of plant cell wall polymers, including cellulose, hemicellulose and lignin in soils (Arman *et al*., 2022). There is huge demand for cellulase enzyme in many industries like textile and paper production factories. (Daba and Krishna, 2019). To improve the quality of nutrients and digestibility of feed ingredients at a cheaper price than the use of commercial cellulase enzymes (Wenny *et al*., 2017). The objective of this study are as follow,

- To investigate the cellulose degrading activity of bacteria from wood industry and bamboo tree soil
- To determine the cellulolytic activity from isolated bacteria
- To identify the isolated cellulolytic bacteria

Materials and Methods

Study area period

Soil samples were taken from wood industry soil and bamboo trees soil in South Dagon Township, Yangon Region. The samples were carried out the Microbiology Laboratory, Department of Zoology, Dagon University. The study period lasted from November, 2023 to May, 2024.

Materials

The apparatus and equipment used for the laboratory work were water distiller, analytical balance, biosafety cabinet, vortex mixture, stirrer hotplate, autoclave, refrigerator, hot air oven, incubator, colony counter, various kinds of glassware, pipettes, microscope glass slides, inoculation nichrome wire loop, aluminium foil, masks, glove, long coat and sterilized cotton.

The culture media and biochemical test media used in bacteriology study consisted Plate Count Agar, Nutrient agar, Carboxy Methyl Cellulose agar, Gram staining kits, Triple Sugar Iron (TSI) agar, Simmon's Citrate agar, Methyl-Red Voges-Prokauer (MR VP) broth, Urea agar, Sulphide Indole Motility (SIM) medium, Geltinase Enzyme, Oxidase reagent, Starch Hydrolysis test, Salt tolerance test and Sugar fermentation test etc. Methyl Red reagent, α-naphthol reagent, 40% KOH solution containing 0.3% creatine, 3% H_2O_2 and Kovac's reagent.

Methods

Sample collection

Soil samples were collected from the wood industry and bamboo tree soil in South Dagon Township, Yangon Region. Each soil sample (100g) was randomly collected from the superficial

soil (depth 1 to 10 cm) aseptically and stored in sterile plastic bags. After labeled and transported to the Microbiology Laboratory of Zoology Department, Dagon University.

Preparation of samples

Wood industry soil was designed to WS and bamboo soil was designed to BS. One gram of each soil samples were serially diluted with 9mL of distilled water by using a vortex mixer.

Serial dilution

One gram of collected soil sample was serially diluted in 10 mL of sterile distilled water up 10^{-1} to 10^{-10}. 1 g of each dilution (10^{-3} to 10^{-10}) was inoculated on plate count agar using pour plate method and incubated at 37^0 C for 24 hours. Subsequently, the number of growing colonies on each plate were counted for estimation of the number of total viable bacteria. The colony numbers only between 30 to 300 in each plate were used to calculate the colony forming unit (CFU/g-1). Then the number of colony were multiplied with the dilution factor and the bacteria counts in CFU/ g-1 were calculated (Dubey and Maheshawri, 2002).

Morphological characterization of the bacterial isolates

The bacteria isolates were isolated and recorded the colony morphology such as, color, size, shape, surface, elevation, margin and texture, were recorded. (Bisen and Verma, 1998).

Determination of carboxy methyl cellulase (CMC) activity of bacteria isolates

Pure cultures of bacterial isolates were individually transferred in Carboxy Methyl Cellulose (CMC) agar plates. After incubation for 48 hrs, CMC agar plates was flooded with 1 % Congo red and allowed to stand for 15 mins at room temperature. One molar NaCl was thoroughly added and used for counterstaining of the plates. Clear zones appeared around growing bacterial colonies indicating cellulose hydrolysis. The bacterial colonies having the largest clear zone were selected for identification and cellulase production in submerged system (Andro *et al*., 2004).

Morphological identification - Gram staining procedure

A pure colony from culture plate was picked up and smears on clean glass slide, air-dried, heat-fixed and stained with crystal violet for one minute. The slide was washed in a gentle and indirect stream of tap water. The slide was flooded with the mordant: Gram's iodine for one minute and washed again. The slide was decolorized with acetone for 30 seconds or added drop by drop to slide until decolorizing agent running from the slide runs clear and washed out. Lastly, the slide was flooded with counterstain, safranin for 30 seconds to one minute and washed out. The gram stained smear was examined under oil immersion field of Brightfield microscope. Gram-negative bacteria will be stained pink or red and gram-positive bacteria will be stained blue or purple.

Biochemical characterization of the cellulolytic bacteria

All isolates were subjected to the following standard biochemical tests described using dehydrated media. These tests included (i) Simon’s citrate utilization test, (ii) urea test, (iii) gelatinase test, (iv) sulfur indole motility (SIM) test, (v) triple sugar iron (TSI), (vi) Oxidase test, (vii) catalase test, (viii) starch hydrolysis test, (ix) sodium chloride test and (x) glucose fermentation test. The characteristic features of isolated bacteria like colony and cell morphology, gram staining nature and biochemical properties obtained in the present work were

compared to the standard described in Bergey's Manual for Determinative Bacteriology (Breed *et al*., 1994); Cowan and Steel's Manual for Identification of Medicine Bacteria (Cowan, 1975).

Results

Bacteria counts of the wood industry and bamboo tree soil samples

The total viable count of bacteria on plate count agar of soil sample from wood industry soil was 3.92 x 10^8 CFU/ g-1 and soil sample from bamboo tree soil was (2.27 x 10^7 CFU/ g-1) as shown in (Table 1).

Cellulolytic activity and cell morphology of the isolated bacteria

In the present study, a total of 18 bacteria were isolated from wood industry and bamboo tree soil samples. All cellulolytic bacteria were designated into SI.B and SI.F from wood industry soil and SII.D, SII.E, SII.F and SII.J from bamboo tree soil. Out of these 18 isolates, 6 isolates showed a distinct clear zone around colonies in CMC agar plate after congo red staining as shown in (Plate.1). Among all cellulolytic bacteria, SII.D exhibited the highest cellulose-degrading activity range of 38 mm, while SI.F was the lowest cellulose-degrading activity range of 10 mm as shown in (Table 2).

After gram staining one isolate from SI.B was gram positive, rod shape arrange in chain and five isolates (SI.F, SII.D, SII.E, SII.F, SII.J) were gram positive, rod shape bacteria as shown in (Plate.2). The biochemical characteristics of cellulolytic bacteria were designated into two genera; *Streptobacillus* sp. and *Bacillus* spp. was identified according to Bergey's Manual for Determinative Bacteriology (Breed *et al*., 1994); Cowan and Steel's Manual for Identification of Medicine Bacteria (Cowan, 1975) as shown in (Table 4). In wood industry soil, both *Streptobacillus* sp. and *Bacillus* sp. were found and *Bacillus* spp.2, *Bacillus* spp.3, *Bacillus* spp.4 and *Bacillus* spp.5 were isolated only in bamboo tree soil.

Table (1) Colony forming unit of cellulolytic bacteria from wood Industry soil and bamboo tree soil samples

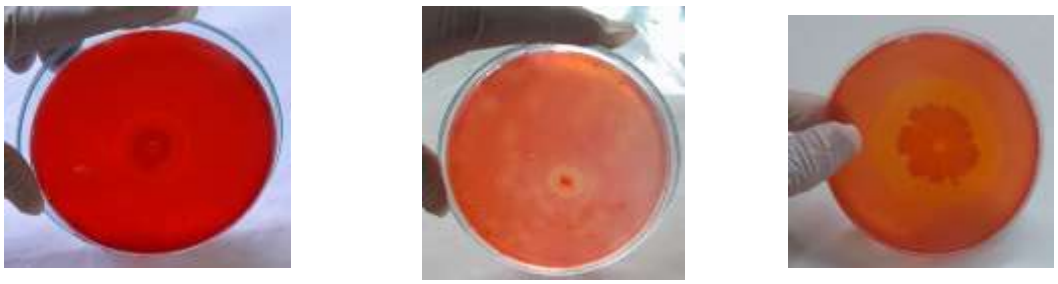
Sr. no.	Sample code	First Count CFU/g	Duplicate Count CFU/g	Total CFU/g	Average Count CFU/g
1	WS	7.11 x 10^8	7.28 x 10^8	7.84 x 10^8	3.92 x 10^8
2	BS	2.30 x 10^7	2.24 x 10^7	4.54 x 10^7	2.27 x 10^7

WS = wood industry soil, BS = bamboo tree soil

Table (2) Evaluation of highly potential cellulolytic bacterial isolate in CMC agar medium

Sr.no.	Bacteria isolates code	Zone diameter (mm)
1.	SI. B	28 mm
2.	SI. F	10 mm
3.	SII. D	38 mm
4.	SII. E	27 mm
5.	SII. F	12 mm
6.	SII. J	36 mm

A. SI. B B. SI. F C. SII. D



D. SII.E E. SII. F F. SII. J

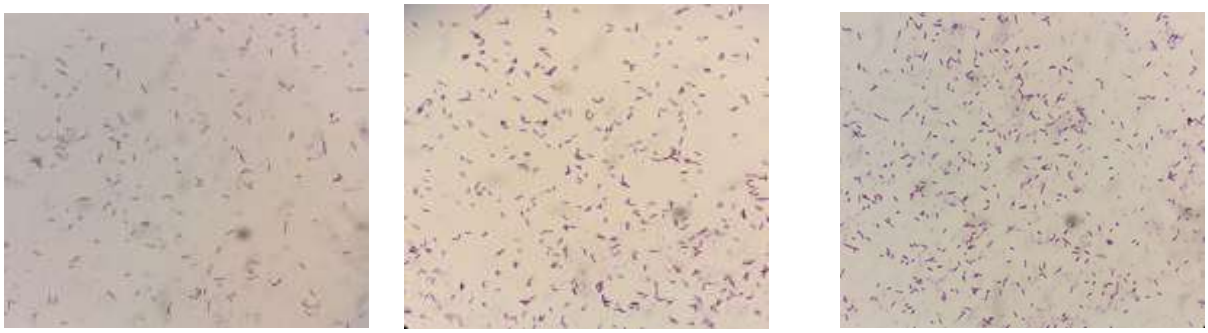
Plate 1. Cellulolytic activity of isolated bacteria from wood industry and bamboo tree soil samples on CMC agar for two days old culture

Table (3) Cell morphology and gram staining reaction of cellulolytic bacteria six isolates

No	Isolates strains	Shape	Margin	Elevation	Size	Texture	Appearance	Colony color	Shape	Gram-staining	Motility
1.	SI.B	Circular	Entire	Flat	Large	rough	glistening	White	rod	+	Motile
2.	SI.F	Circular	Entire	Convex	Large	smooth	dull	Cream	rod	+	Motile
3.	SII.D	Circular	Entire	Convex	Large	rough	dull	Cream	rod	+	Motile
4.	SII.E	Circular	Entire	Convex	Large	rough	dull	Cream	rod	+	Motile
5.	SII.F	Circular	Entire	Convex	Large	rough	glistening	Cream	rod	+	Motile
6.	SII.J	Circular	Entire	Convex	Large	rough	dull	Cream	rod	+	Motile

+ = positive, - = negative

A.SI. B B.SI. F C.SII.D



D. SII.E E. SII. F F. SII. J

Plate 2. Gram staining appearance of cellulolytic bacteria from wood industry soil and bamboo tree soil

Table (4) Biochemical characteristics of cellulolytic bacteria isolates from wood industry soil and bamboo trees soil

Sr. no	Isolate code	SC	Urea	TSI			VP	Gel	SIM			Oxi	Cata	Star	Salt tolerance	Glu	Tentative genera
				S	B	Gas			S	I	M						
1.	SI. B	+	+	K	A	-	+	-	-	-	+	-	+	+	+	+	*Streptobacillus* sp.
2.	SI. F	-	+	K	K	-	-	+	-	-	+	+	+	-	+	+	*Bacillus* spp.1
3.	SII. D	+	+	K	A	-	+	-	-	-	+	+	+	-	+	+	*Bacillus* spp.2
4.	SII. E	+	+	K	A	-	-	+	-	-	+	+	+	-	+	+	*Bacillus* spp.3
5.	SII. F	-	+	K	A	-	-	-	-	-	+	+	+	-	+	+	*Bacillus* spp.4
6.	SII. J	-	+	K	A	-	-	-	-	-	+	+	+	+	+	+	*Bacillus* spp.5

(+) = positive reaction, (-) = negative reaction, No gas
S = slant, B = butt, G= gas, SC=Simmon Citrate Utilization, MR = Methyl Red, VP = Voges Proskauer,
Gel = Gelatinase, Urea = urea test, SIM = Sulphide Indole Motility, Ca = catalase, A= acid, K= alkaline,
ST= Salt Tolerance test, Glu = Glucose concentration test, Cata = Catalase test, Star = Starch Hydrolysis test.

Table (5) Summary of isolated bacteria from wood industry soil and bamboo trees soil samples

No.	Tentative genera of species	WS	BS
1	*Streptobacillus* sp.	1	-
2	*Bacillus* spp.	1	4
3	Unidentified specie	5	7
4	Total isolates	7	11

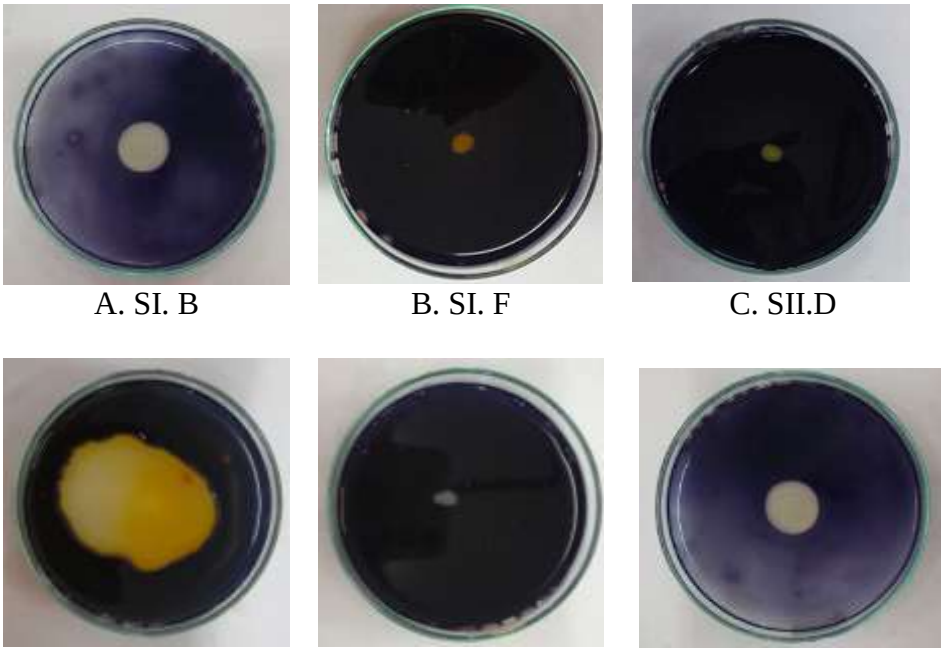
WS = Wood industry soil, BS=Bamboo tree soi

A. S.I B B. S.I F C. S.I B and S.I F for salt tolerance test D. S.I B and S.I F for glucose test

Plate 3. Biochemical tests from wood industry soil

A. S.II D B. S.II E C. S.II F D. S.II J

SC=Simmon Citrate Utilization, Urea = urea test, MR = Methyl Red, VP = Voges Proskauer, Gel = Gelatinase, SIM = Sulphide Indole Motility,
Plate 4. Biochemical tests from bamboo tree soils



A. SI. B B. SI. F C. SII.D

D. SII. E E. SII. F E. SII. J

Plate 5. Starch hydrolysis test from wood industry soil and bamboo tree soil

Plate 6. Salt tolerance test for S.II D, S. II E, S. II F and S. II J

Plate 7. Sugar fermentation test (glucose) for S.II D, S. II E, S. II F and S. II J

Plate 8. Catalase test from wood industry soil and bamboo tree soil

A. SI. B B. SI. F

Plate 9. Oxidase test form wood industry tree soil

A. SII. D B. SII. E C. SII. F D. SII. J

Plate 10. Oxidase test form bamboo tree soil

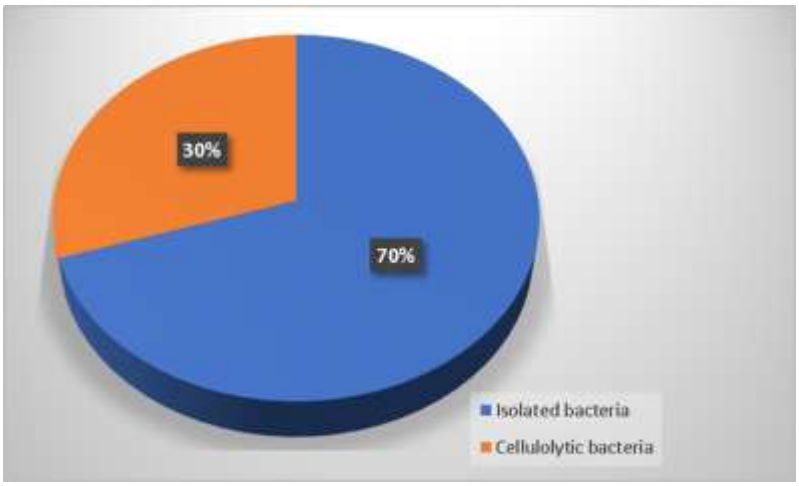
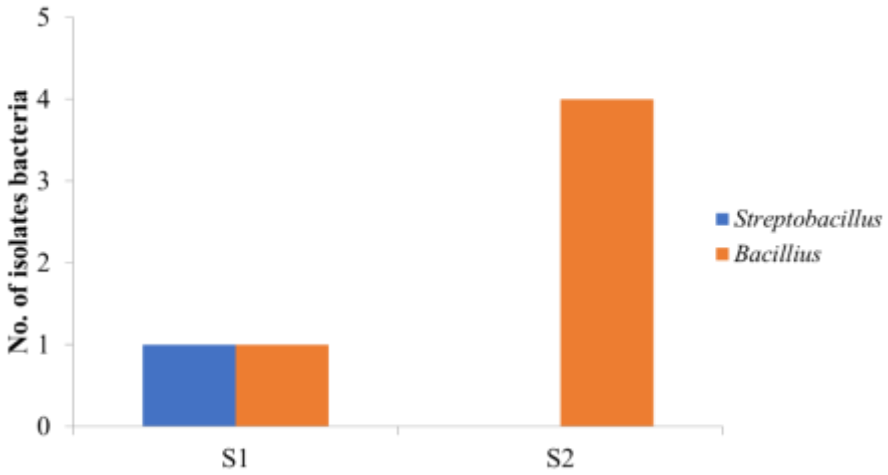


Figure. 1 Percentage of cellulolytic bacteria isolated from the wood industry soil and bamboo tree soil**.**



SI = wood industry soil, S II = bamboo tree soil

Figure.2 Number of cellulolytic bacteria isolated from the wood industry soil and bamboo tree soil samples

Discussion

A total of 18 isolates were obtained from wood industry soil and bamboo tree soil samples. Out of 18 isolates, 6 isolates were produced cellulose enzymes and showed cellulolytic activity on CMC agar. The isolated cellulolytic bacteria were identified as *Streptobacillus* spp. and *Bacillus* spp. according to their colony morphologies, gram staining and biochemical characteristics. In the present study, the total viable count (CFU/ g-1) of cellulolytic bacteria in the analyzed soil samples were ranged from (2.27 x 10^{-7} CFU/ g-1) to (3.92 x 10^{-8} CFU/ g-1) which indicated a significant population of isolated bacteria. Yi Lwin Kyaw Zaw, (2013) in Yangon, cultivated soils from Garbage and Garbage dump showed higher bacterial population than present study which were ranged from (1.3 x 10^{-9} CFU/g) and (6.0 x 10^{-8} CFU/g). It was possible due to soils sample taken from different areas with different microbial community composition. Khotimah *et al*., (2020) from Indonesia also found the total bacteria count of peat forests soil were (2.1×10^{3} CFU/ g-1) to (5.9×10^{4} CFU/ g-1) which was lower than the present study. The different in total viable count of bacteria compared with above findings might be due to different locations, climate and temperature of soils.

In the current study, out of 18 bacterial isolates, 6 (30%) isolates showed cellulose degrading activity. Misbah *et al*., (2020) in Pakistan reported among 24 isolates from different soils, 6 strains (40%) showed cellulase production. Riad *et al*., (2020) in Bangladesh isolated 42 bacterial isolates from various soil samples (sawdust). Among then, 24 (57%) isolates showed cellulolytic activity with a distinct clear zone. The different percentage of cellulolytic activity of bacterial isolates in compared with above studies may be due to different soils that may contain substrates with higher proportion of cellulose and the existence of microenvironments where different growth conditions for cellulose degrading bacteria are present.

Among six cellulolytic bacteria, the maximum clear zone diameter of cellulolytic bacteria was about 38 mm (SII.D), while the minimum was 10 mm (SI.F). These results showed higher than the findings observed by Hatami *et al*., (2008) in Iran who reported the hydrolysis zone diameter of cellulose-degrading aerobic bacterial isolates from farming and forest soil were 0.15 cm to 1.37 cm. However, Mohammed *et al*., (2017) in Egypt who observed 42 mm to 23 mm of the clear zone diameter from garden soil. The zone diameter in present study was lower than of their findings.

In this study, among six isolated cellulolytic bacteria, 5 isolates were identified as *Bacillus* spp. and one isolate was identified as *Streptobacillus* spp. These results are in agreement with those reported in previous studies where the cellulose degrading bacteria have been identified and isolated from different environments like soil. Rastogi *et al*. (2009) also observed *Bacillus* spp. in soil. This finding was consistent with Abdel-Aziz *et al*., (2021) in Egypt who also identified *Bacillus cereus, Bacillus licheniformis* from farm fields soils. Another study reported by Misbah *et al*., 2020 in Pakistan also identified predominance of *Bacillus spp.* such as *Bacillus megaterium, Bacillus aerius, Bacillus paralichniformis* and *Bacillus flexus* from old paper, and old wood soil.

Moreover, Yi Lwin Kyaw Zaw (2013) in Yangon, also isolated *Bacillus subtilis and Bacillus cereus* from cultivated soil and Garbage dump soils. Vimal, (2016) in Kerala, were mentioned *Bacillus subtilis* and *Bacillus cereus* from industrial and agricultural soil. Shweta and Seema, (2021) in India, were identified *Bacillus subtilis, Bacillus flexus, Bacillus licheniformis* and *Bacillus paralicheniformis* from garden soil and forest soil. The different study sites and the current study sites of cellulolytic bacterial isolates in compared with above studies may be due to differences in sampling sites that may contain substrates with higher proportions of cellulose. Additionally, the present of unit microenvironments may offer distinct growth condition that favor specific cellulose-degrading bacteria.

Conclusion

Six highly potent cellulolytic bacterial strains were successfully isolated and screened from wood industry and bamboo tree soil samples in South Dagon Township. These strains were tentatively identified as *Bacillus* spp. and *Streptobacillus* sp., with *Bacillus* being the dominant and most active genus. The isolate *Bacillus* spp. 2 (SII.D) from bamboo tree soil demonstrated the highest cellulolytic activity (38 mm clear zone), warranting further research into its enzyme characteristics for biotechnological utilization.

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GONADAL DEVELOPMENTAL STAGES OF *JOHNIUS COITOR* (HAMILTON, 1822) IN GYAING RIVER SEGMENT BETWEEN GADOE AND KAWBEIN AREA

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Abstract

Amphidromous croaker *Johnius coitor* (Hamilton, 1822) is one of the commercially important demersal fish of the Gyaing River segment between Gadoe and Kawbein Area. The irregular pattern of GSI and HSI of studied species were now an evidence of potential indicator which directly reflect the hazard conditions of aquatic medium. Macroscopic and microscopic studies were made on the gonadal development of this species, *J.coitor*. Microscopic study for male spermatogenesis were identified into four stages; spermatogonia phase, spermatocyte phase, spermatid stage and spermatozoa stage. For oogenesis, oocytes were identified as chromatin nucleolus phase, prenucleolus phase, primary yolk phase, secondary yolk phase, tertiary yolk phase, germinal vesicle migration phase, germinal vesicle breakdown phase and spent phase. The female gonadal developmental stages will be compared with that of "oocytes ranged from 13.10µm to 520.40µm in *Johnius coitor*. Thus, this study suggested to take necessary steps are needed to monitor the aquatic medium to protect the fish reproductive physiology, thus fish population as a whole. These findings are very important to fishery management to fix closed season and closed areas for conservation of fish. Suggestions for future works outlined.

Keywords: GSI, HSI, Condition factor, macroscopic and microscopic structure, J. *coitor*,

Introduction

Johnius coitor, commonly known as coitor croaker, is an amphidromous fish species belonging to family Sciaenidae under order Perciformes, popularly known as Jew fish, croakers or drums. Besides India, coitor croaker is widely distributed in Indo-West Pacific region with reports of occurrence from Australia, Bangladesh, Brunei, Indonesia, Malaysia, Myanmar, Nepal and Singapore (Froses and Pauly 2017).The family Sciaenidae consists of approximately 300 species from genera throughout Atlantic, Indian and Pacific Oceans, in coastal marine, brackish and freshwater; many associate with muddy or sanday bottons (Chao *et al.*, 2015; Lin *et al.*, 2019). Some sciaenids form large aggregations for spawning, feeding and over-wintering, which makes them vulnerable to overfishing (Liu and Sadovy de Mitcheson 2008). In recent years, small- and medium-sized croakers enter fisheries and become predominate in Chinese and adjacent water, e.g., the genera *Collichthys, Johnius* and *Pennahia* (Yamaguchi *et al.*, 2006; Wang *et al*., 2011; Xie *et al.*, 2012).*Johnius coitor* is an amphidromous fish species and popularly known as Jew fish, croakers or drums are commercial fish species of the family Sciaenidae. This species can be caught by Gyaing River almost year-round and is important local food fish.

Gonadosomatic index (GSI) is a suitable indicator of the gonad development that can be used for determining the reproductive period (Le Cren, 1951). Hepatosomatic Index (HSI) provides an indication on status of energy reserve in an animal. Condition factor (K) is widely

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used in fisheries and fish biology studies. A piece of complete knowledge on the reproductive system and biology of fishes is critical to understand the reproductive strategy and annual reproductive cycle of many fish species. Information on reproductive biology is very much essential for the development of the aquaculture industry. Therefore the present study was carried out the reproductive strategy of *Johnious coitor*.

Therefore, the objectives of this paper are

- To determine GSI, HSI, and K values of the studied fish species
- To investigate the macroscopic and microscopic gonad developmental stages of *Johnious coitor*

Materials and Methods

Study area and period

The present work was carried out in Gyaing River segment between Gadoe and Kawbein Area. It is located at North latitude 16º34´29.98"N and East longitude 97º39´20.07"E (Fig .1). The study period lasted from January, 2023 to December, 2023

Figure. 1 Topographic map of the study area (Source: Office of Land Record, Hpa-an Township, Kayin State, 2022)

Specimen collection

A total of 220 specimens of *Johnius coitor* were collected and recorded from the local fisherman at the study site during the study period (January 2023 to December 2023). In this research work, 20 fresh fish samples of the species were randomly collected monthly from the local fishermen in the Gyaing River segment between Gadoe and Kawbein Area (Plate 1).

Identification and classification

The collected samples were identified referring to Talwar and Jhingran 1991 and Talwar 1995.

Morphometric Measurement

The monthly fish specimen was caught with the help of fishermen and total length (tip of the snout to the end of the caudal pentacle), Standard length (tip of the snout to the end of the caudal fork) was measured to the nearest millimeters (mm) by a measuring scale.

Total body weight was measured (g) by a digital balance. Fish specimens were dissected out ventrally to remove gonads carefully and then surface moisture of gonads was removed using

blotting paper. They were photographed and weighted before fixation in 10% natural buffer formalin for histology.

Gonado-somatic index (GSI)

Gonadosomatic Index (GSI) for each fish was calculated using the formula, (Nikolsky, 1963)

GSI= GW/BWx100

Where GW= gonad weight of fish in grams (g) and BW= Body weight of fish in grams.

Hepatosomatic Index (HSI)

Hepatosomatic Index (HSI) for each fish was calculated using the formula, (Biswas, 1993).

HIS= LW/BW x 100

Where LW= liver weight of fish in grams (g) and BW= Body weight of fish in grams.

Condition Factor 'K'

The condition factor 'K' for each fish was calculated using formula (Jones, 1970)

$K= W/L^3 \times 100$

K = condition factor, W= Body weight of fish in grams, L= Body length of fish in cm.

Macroscopic and Microscopic observation of Gonad Maturation Stages

Macroscopic Examination

The gonads appearance, color, size, space occupied in the body cavity, size of the gonads were noted down. Macroscopic gonads maturity stages were classified according to Jacob (2005), Bucholtz (2008), and Selman (2003).

Microscopic Examination

For the histological study, gonads (testes and ovary) were collected by dissecting out the fish. The tissues were trimmed into 5 to 6 mm size for better penetration of fixatives into it. The tissues were put into 10% neutral buffer formalin a few day as per the size of tissues.

Results

Correlation between GSI, HSI and K

In male *Johnius coitor*, the GSI values fluctuated throughout the study period and then increased in December as the peak GSI value (6.8%). The lowest GSI value was found in January (0.03%). But for females, the peak GSI values were observed in December (11.29%). The lowest GSI values were found in January (1.45%) A gradual increase in GSI value was found during the spawning period and gradually decreased during the post-spawning and resting periods(Fig. 2&3) In male *J.coitor*, the peak HSI values were found to be 2.71% in May, and the lowest values were found to be 0.44% in August and 0.71% in December. But *J.coitor* for female the highest HSI values were observed in May (2.76%), and the lowest HSI values were found in December (0.44%) (Fig. 2&3). The monthly values of condition factor (K) for males and females were determined from the number of fish studied per month. The K values ranged from 0.41 to 1.0 in

males and 0.40 to 1.05 in females. The K value is close to 1, therefore males and females were in good condition in the study area (Fig.2&3).

Structure of Reproductive Organs

The ovary was elongated sacs, paired organs. Right, and left ovaries were fused forming a single, medium ovary. The right ovary was slightly longer than the left and observed various sizes according to their maturation stages. Testes were elongated paired organs, Y-shaped and consisting of numerous finger-like projections. Length and sizes were varied according to their maturation stages. Testes were located in the posterior half of the body cavity, just ventral to the trunk kidney (Plate 1 A, B).

Macroscopic Developmental Stages of Female Reproductive organ

Immature ovaries

The immature ovary was a small, elongated, transparent, and narrow tube-like structure. The right ovary was slightly longer than the left. Oocytes were not visible with the naked eye. Immature ovaries were found from January to December (Plate 3 A)

Developing Ovaries

Developing ovary was slightly larger than the above stages. The ovary was observed transparent in color and ova was visible through the thin ovarian wall. This stages was found from January, May, August to December (Plate 3,B)

Mature Ovaries

Ovaries size was became larger than the previous stages and filled with third- fourth of the body-cavity. The ovary was contained distinctly visible oocytes with obvious blood vessels. Ova were translucent and yellowish. This stage was found in January –April and July – August (Plate 3, C)

Ripe Ovaries

The ovary was yellow and filled the entire length of the body cavity. The ovarian wall thin, Ova were translucent and yellowish. This stage was found in April – May and July – November (Plate 3, D)

Spent Ovaries

The ovary was much shrunken, blood-shoot, and contained numerous small ova. This stages was found in August and October (Plate 3, E)

Macroscopic Developmental Stages of the Male Reproductive Organ of *Johnius coitor*

Immature Testes

Testes were tiny, thin, and transparent in color. This stage was found from January to November (Plate 4, A)

Developing Testes

Developing testes were found from May and August to November. In developing stage testes lobes were increased in size and length, slightly thicken whitish and occupied one half of the body cavity length (Plate 4, B)

Mature Testes

Mature testes were observed in January – April and July to December. Testes were reached the largest, more than one half of the body cavity, and released milt on applying pressure on the abdomen (Plate 4, C)

Ripe Testes

In the ripe stage, testes were occupied two –thirds or more of the body cavity. The weight and volume were more increased than the mature stage. Testes were becoming opaque and milky white. Ripe testes were observed in April and July - November (Plate 4, D)

Spent Testes

Spent testes were found in August and October. Testis shape was found like fats in the body cavity.

Microscopic Developmental Stages of Oogenesis of *J.coitor*

Oogonia

Germ cells or oogonia were embedded in the ovigerous tissue. Oogonia cells were observed in cluster and originated from the germinal epithelium, 13.10±0.87µm (Plate 5, A)

Chromatin nucleous stage

These cells were youngest cells, strongly basophilic cytoplasm with large nucleus and one to two darkly stain nucleoi were found. The shape of the cells were irregular and sizes were small, 90.50±5.17µm (Plate 5, B)

Pre-nucleolus stage

Oocytes were rounded or oval or polygonal shape. In this stage, cytoplasm was less basophilic and different size of nucleoi was situated in the periphery of the nucleus. This stage was found throughout the year. The sizes of the cells were 141.03±12.09µm (Plate 5, C)

Primary yolk stage

Oil droplets (Od) and yolk granules (Yg) were initially appeared in the cytoplasm. Cytoplasm was weak basophilic and nucleus was still presented in central position. The size of the cells were 234.63±27.74µm (Plate 5, D)

Second yolk stage

Cells were rapid growth and yolk granules (Yg) and oil droplets (Od) were increased in number and coalescence. Nucleus was irregular shaped. The size of the cells were 244.63±10.10µm (Plate 5, E)

Tertiary yolk stage

Size and number of yolk granules (Yg) and oil droplets were increased. The size of the nucleus was small and it seen nearly disappeared. The sizes of the cells were 590.50± 48.64µm (Plate 5, F)

Germinal vesicle migration stage

In this stage, nucleus (N) was moved toward the pole and oil droplets (Od) were accumulated in the cyctoplasm (Cy). The nucleolus (Nu) were scattered in the nucleus. The size of the cells were 433.17±46.14µm (Plate 5, G)

Germinal vesicle breakdown stage

In this stage, nucleus substance were released into the cytoplasm (Cy) and nucleoli (Nu) were not clearly seen in the nucleus. Yolk globules (Yg) were fused to each other in the cytoplasm. The size of the cells were 520.40±35.62µm (Plate 5, H)

Postovulatory follicle stage

In this stage, various types of postovulatory follicle (Pof) was found, Hydrolyse yolk granules, Primary growth oocyte and oogonia were observed found in this stage (Plate 5, I)

Microscopic developmental stages of spermatogenesis of *Johnius coitor*

Spermatogonia stage

The largest size of spermatogonia cells were observed and localized at the inner layer of seminiferous tubule wall. This cell was contained basophilic nucleus and unstained cytoplasm (Plate 6, A)

Spermatocytes stage

Spermatocytes cell became smaller in size than the spermatogonia. Spermatocytes were found the most abundant stage during the process of spermatogenesis (Plate 6,B)

Spermatids stage

Spermatids was strongly basophilic spherical cell and observed dense as clusters. They were found in the inner layer of seminiferous tubules (Plate 6, C)

Spermatozoa stage

Mature spermatozoa were contained spherical nucleus at the head region and a faint long tail. They were found in cluster in the central position of the lumen of the lobules (Plate 6, D)

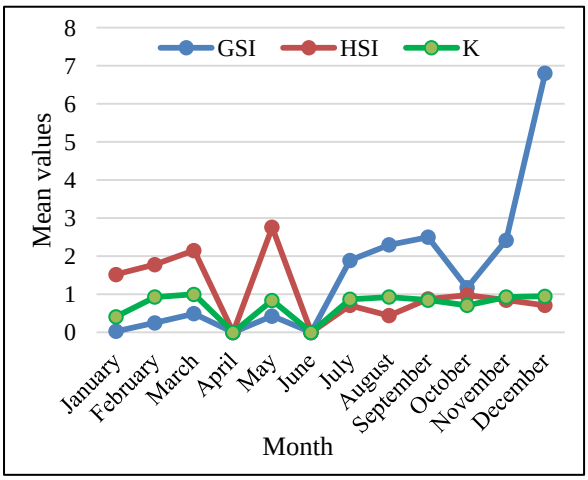


Figure.2 Monthly values of GSI, HSI, and K of male *Johnius coitor*

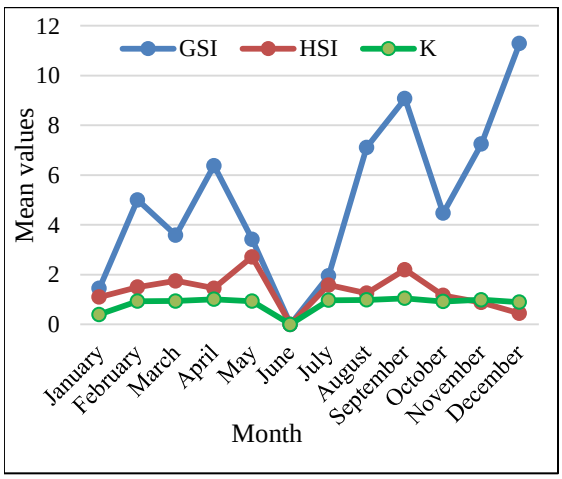


Figure.3 Monthly values of GSI, HSI, and K of female *Johnius coitor*

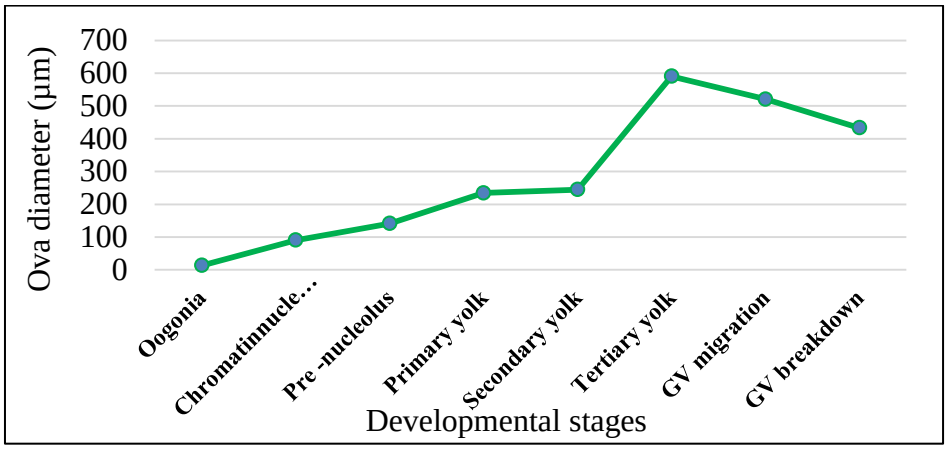
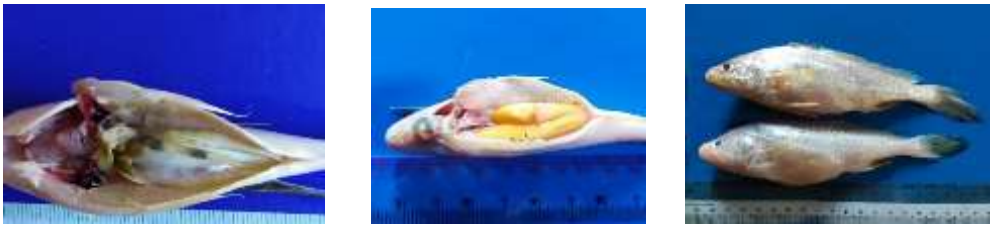


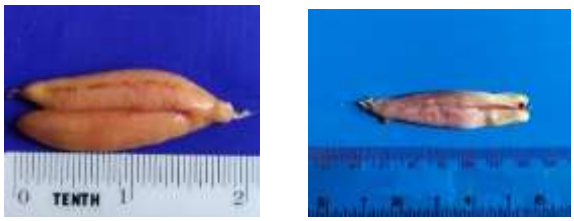
Figure. 4 Measurement oocyte diameter for *Johnious coitor*



(A) Dissected male (B) Dissected female C. male& female of

Plate 1 Reproductive organ of *Johnius coitor*

A. Immature stage B. Developing stage C. Mature stage

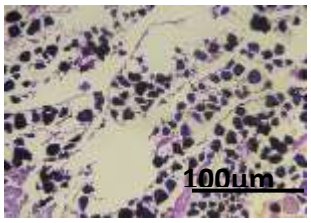


D. Ripe stage E. Spent stage

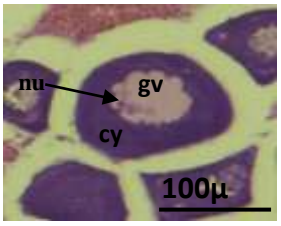
Plate 3. Macroscopic developmental stages of female reproductive organ of *J.coitor*

A.Immature stage B. Developing stage C. Mature stage D. Ripe stage E. Spent stage

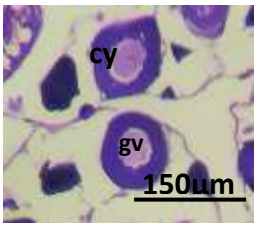
Plate 4. Macroscopic developmental stages of male reproductive organ of *J.coitor*



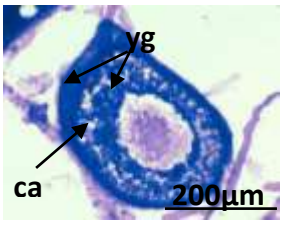
A. Oogonia



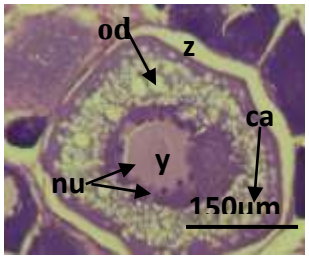
B. Chromatin nucleolus stage



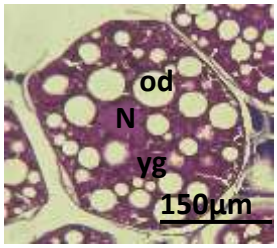
C. Prenucleolus stage



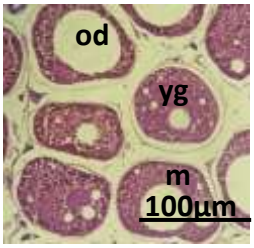
D. Primary yolk stage



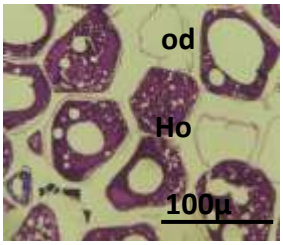
E.Second yolk stage



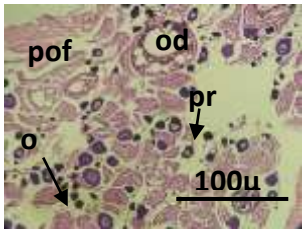
F.Tertiary yolk stage



G. Germinal vesicle

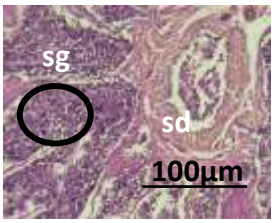


H. Germinal vesicle Breakdown stage

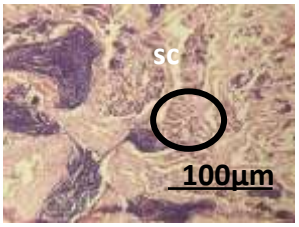


I.Spent stage

Plate 5. Continued Microscopic developmental stages of oogenesis in *J.coitor* H& E stain, transverse section, Oogonia (Og),Cytoplasm (Cy), Nucleus (N), Primary growth oocytes (Pr),Nucleolus (Nu), Yolk granule (Yg), Oil droplet (Od), Zona radiate (Zr), Migration nucleus (Mn), Hydrated oocyte (Ho), and Post ovulatory follicle (Pof)



A.Spermatogonia(Sg)



B. Spermatocytes (Sc)

C. Spermatid (St)

D. Spermatozoa (Sz)

Plate 6. Microscopic developmental stages of spermatogenesis in J.coitor, H &E stain, transverse section, Spermatogonia (Sg), Spermatocytes (Sc), Spermatid (St) and Spermatozoa (Sz).

Discussion

Developmental stages of gonads of *Johnius coitor* were examined from Gyaing River segment between Gadoe and Kawbein Area.Among teleost fishes, the testes are generally a paired organ that develops longitudinally, elongating horizontally concerning the body axis, even though testes morphology varies among species, and they are located either dorsally or ventrally

in the abdominal cavity (Helfman *et al.,* 1997). In the present result, testes were examined on the dorsal side of the body cavity in *Johnious coitor*.

In most teleosts, the ovary is a hollow paired organs, however, in some species paired structures become fused into one solid, single organ during their early development (Priyadharsini *et al.,* 2013). The ovaries were observed elongated sacs, paired organs, and right and left ovaries fuse forming a single, medial ovary and two lobes were not the same size according to this result.

Lagler (1962) staged that the several fish species spawn more than once a year and more or less continually. The GSI values are higher in spawning period and declining in the post spawning period (Le Cren, 1951). According to the monthly GSI, *Johnius coitor,* peak GSI values were found in December for both males and females. The lowest GSI value was found in January for both males and females. So that peak GSI value indicates the peak spawning season of *J.coitor* is during post-monsoon periods. Similar results are found in Kumar *et al.,* (2013), who described that during peak breeding season (maximum July–September > January–May >October–December minimum) throughout the year for *J.coitor.* Quang Minh Dinh *et at.,* (2022) also concluded that the fish (*J.coitor*) was a multi-spawner releasing eggs all year-round with a peak in the wet season.

In the present finding, the highest HSI value of *J.coitor* was found in May for both males and females. The lowest HSI value occurred in August for males and in November to December for females. The GSI and HSI were negative correlated as the oocyte development in fish is intimately associated with hepatic synthesis of egg-yolk precursor protein vitellogenin which is secreted into the blood and ultimately transported to developing oocyte and deposited as yolk (Sudarshan and Kulkarni, 2013).Wootton (1990) also stated that the liver weight (HSI) decreased as the ovary weight increased during vitellogenesis. This showed that HSI has reversed action on GSI.

Sarkar *et al.,* (2018) recorded that condition factor (K) of *J.coito*r was lower in December and higher in January. In the present study, condition factor (K) values of male was higher in March and December and lower in January and then of female was higher in April and September and lower in January. The present finding was disagreement with the previous author. It may be suggested that different in food resources, environmental temperature or evolutionary adaptation of different population to the specific ecological properties of specific ecosystem.

Different authors have divided the gonad developmental stages according to their prominent features of changes during the process of gametogenesis (Jacob, 2005; Thida Aung 2006; Thinzar Aung 2018;).Based on gross morpho-histological changes occurred in the ovary and testes, the occurrence of developing oocytes was divided into eight stages, and observation of developing spermatogonia was divided into four stages during the study period.

Oogonia give rise to immature oocytes with multiple peripheral nucleoli, in which the number of nucleoli was not more than 20 in the present study. Yolk vesicles were the first type of inclusions appearing in the cytoplasm of second-growth phase oocytes. These vesicles were likely to contain lipid, as the appeared as empty non-staining vacuoles on paraffin sections, suggesting that their contents were dissolved during routine histological procedures (Jacob, 2005). In the present study, during oocyte maturation, the lipid vesicles were found in the

peripheral of the cytoplasm, which might help the eggs to keep buoyant but the cytoplasm was filled with yolk granules instead of lipid droplets. Zona radiate was also distinctly appeared in the mature stage of oocytes in this study. The distinction between germinalvesicle migration and germinalvesicle breakdown was histologically differentiated as the migration of the nucleus to the animal pole in the former stage and absent of the nucleus in the later stage.

As a result of histological stages of testes, accumulation of unstained spermatogonia and dot-like structure of stained spermatocytes were dominant over immature stages, all stages, spermatogonia, spermatocytes, spermatids, and spermatozoa were observed in developing. The abundance of deep stain, basophilic, and oval-shaped head with distinct tail spermatozoa were observed in maturation stages during this study period. During oogenesis, the size of the oocytes increased considerably due to a progressive accumulation of lipid and protein-yolk within the cytoplasm by vitellogenesis (Montchowui *et al.*, 2012). The present study agreed with this statement as the size of oocytes were larger nearer to the germinalvesicle breakdown stage, ripe stage (13.10µm to 520.40µm).

The monthly GSI analysis and histological finding of the present study showed that ovarium types were group synchronous and this species spawn once in a breeding season. According to the finding results, the highest mean value of GSI corresponds to where the gonads were at ripe and while the lowest value indicates spent stage or starting developing stage.There are eight developmental phase or stages; Oogonia, chromatin nucleolus phase, prenucleolus phase, primary yolk phase, secondary yolk phase, tertiary yolk phase, germinal vesicle migration phase, and germinal vesicle breakdown phase were found in the month where the values of GSI was highest. However, the number of germinalvesicle migration and germinal vesicle breakdown phases was dominant over the other stages. In the findings of this study, the highest number of germinalvesical migration and germinal vesical breakdown occurred in October to December.

Indeed, the present finding of the different developmental stages of gonads of the studied fish species from natural brackish water, Gyaing River will provide valuable information of fishery resources concerning with their breeding season and also evidence outcomes for the closing season to conservation and management on threatened fishes from natural water fishery resources.

Conclusion

The irregular pattern of GSI and HSI of the studied species, *J.coitor* were now evidence of potential indicators that directly reflect the hazardous condition of aquatic medium. The study on maturity stages of oocytes in the ovaries of these species was firmly supported by ovarian developmental stages of histological slides with photographs. Indeed, the present finding of the different developmental stages of gonads of the studied fish species from natural brackish water,The data provides valuable baseline information for the fishery management of this species, specifically for implementing closed fishing seasons or areas during the peak spawning months (October–December) to ensure the sustainability of the population.

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DISTRIBUTION AND ABUNDANCE OF YELLOWTAIL CATFISH *PANGASIUS MYANMAR*, ROBERTS AND VIDTHAYANON 1991, IN THE ESTUARY ENVIRONMENT OF YANGON RIVER*

Thin Thin Soe[1], Ko Myint[2]

Abstract

This study investigates the abundance and distribution of the endemic yellowtail catfish, *Pangasius myanmar*, within the Yangon River estuary. Fish data, including length and weight measurements, were collected through field observations and interviews. Surface water samples were collected using standard sampling procedures for laboratory quality analysis. Pearson's correlation coefficient was used to evaluate the relationship between fish length and weight. Field observations and interviews revealed that the species is widely distributed across the coastal, river mouth, and intertidal zones, extending up to 23 miles (38.34 km) upstream from the river mouth, which itself stretches approximately 40 miles (63.37 km). Seasonal variations in catch were noted, with increased captures upstream during the dry season and active fishing in downstream during the wet season, particularly in the brackish water zone where freshwater and saltwater converge. Remarkably, fishing activity yielded the highest catches near mangrove areas during high tides, with fishermen using lift nets to exploit the catfish's foraging behavior. Over the study period from November 2023 to October 2024, a total of 2,839 fish were recorded, with monthly catches highest in November (475 fish) and reducing in May (75 fish). The total weight of the harvest reached 16,512.7 kg, with the highest monthly weight recorded in October (2,671.2 kg). The findings indicated the ecological significance of the Yangon River as a breeding and feeding habitat area for *Pangasius Myanmar* which is endemic species to Myanmar, Yangon Region. The correlation between fish length and weight is both strong and statistically significant, meaning that fish grow proportionally in both dimensions. This suggests healthy growth status in terms of both body length and weight. Fluctuations in water quality, particularly during the reproductive periods such as egg maturation in April directly influence the species' health and population viability. Therefore, the protection and appropriate fishery management of this estuarine ecosystem, which is rich in mangroves and salt marshes, is vital for the sustainability of the local fishery and the continued presence of this endemic species in the Yangon River.

Key words: Distribution, abundance, estuary habitats, Yangon River, *Pangasius myanmar*

Introduction

The fish species *Pangasius myanmar*, commonly known as the yellowtail catfish for its distinctive yellow tail, was first identified as a new species by the California Academy of Sciences in 1937. It was later scientifically documented by Roberts and Vidthayanon in 1991 and is locally known as "Myit Nga Tan" in Myanmar because it inhabits the rivers. It is native to Myanmar, particularly in the Yangon region and the Yangon River. This yellowtail catfish is a benthopelagic tropical species can grow up to 120 cm in length (FishBase, 2015). This species is one of the most commercially important and nutritious riverine fish (Monalisa et al., 2012). It is a carnivorous and voracious fish that also eats mangrove fruits, with juveniles found in high estuaries, sub-adults migrating to brackish waters, and adults reaching river mouths and inshore areas.

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Estuarine river waters where freshwater from rivers mixes with seawater, which serve as critical habitats for a range of aquatic species (NGS, 2023). These habitats characterized by mangrove ecosystems, salt marsh grasses and sediment-rich mudflats, provide food and shelter for various aquatic species including catfishes (NOAA Fishery, 2022). Distribution is narrow range, native only to the river systems of Myanmar, particularly the Yangon region. The fish plays a vital role both ecologically and commercially. This benthopelagic species feeds voraciously on smaller aquatic organisms and mangrove fruits, its feeding habits showing the connection between the estuary system, mangrove ecosystems, the survival of fish populations and its specific geographic location. Mangroves, with their dense roots, act as nurseries, offering shelter to juvenile fish while providing abundant nutrients through detritus and organic matter. As the fish grow, they move to brackish waters and eventually reach the open river mouth. Therefore, understanding the distribution of species, population and habitat preferences in the estuarine environment of Yangon River is essential for informing sustainable fisheries management and conservation efforts. The aim of this study is to examine the abundance and distribution of *Pangasius myanmar* within the Yangon River estuary.

The objectives of the study are to examine the abundance and distribution of *Pangasius myanmar* (Yellowtail catfish) within the Yangon River estuary, to understand the role of estuarine habitats, particularly mangrove ecosystems and brackish waters/water quality, in supporting the life cycle and feeding habits of *Pangasius myanmar* and to provide insights for sustainable fisheries management and conservation efforts by studying the species' population, habitat preferences, and geographic distribution in the Yangon region.

Materials and Methods

Study area

The Yangon River was selected as the study area because it provides habitats for the species Pangasius in Myanmar. This study site is located along the Yangon River, near Chaung Wa Village in Kyauktan Township, Yangon Region (Figure 1). The study area, Yangon River estuary, formed by the confluence of the Pegu and Myitmaka rivers, is an estuarine system. It flows from Yangon to the Gulf of Martaban which is part of the Andaman Sea and experiences significant tidal influences. The river has strong water currents and turbid conditions particularly during the rainy season and on days of spring tides. Mangrove and salt marsh grass habitats are found in the segment of the Yangon River mouth, an estuary environment featured by the influence of the intertidal zone (Figure 2). A total of six mangrove species commonly occur in the mangrove area along the river within the study segment. They are *Avicennia officinalis* (Lame); *Sonneratia apetala* (Kambala); *Sonneratia caseolareis* (Lamu) *Nipa fruticans* (Dani); *Clerodendrum inerme* (Pinle-kyauk-pan); *Cyperus malaccensis* (Nil). In addition, there is a large area of salt marsh grass in the muddy area along the river bank downstream of the river. These mangrove and salt marsh habitats provide shelters, foods and nursery grounds for aquatic organisms, including study fish (NOAA, 2022). The Yangon River is highly productive and plays a vital role in supporting the local fishery. Many residents living along the river relies on fishing, as well as the selling and buying of fish, for their livelihoods. They use various types of fishing gear, including drift gill nets, stow nets, trammel nets, beach seine nets, cast nets, lift nets, and push nets. Of these, drift gill nets are the most commonly used, particularly for catching

commercially valuable fish such as pangas catfish, hilsa, and seabass. Approximately 350 fishing boats fish in the study area.

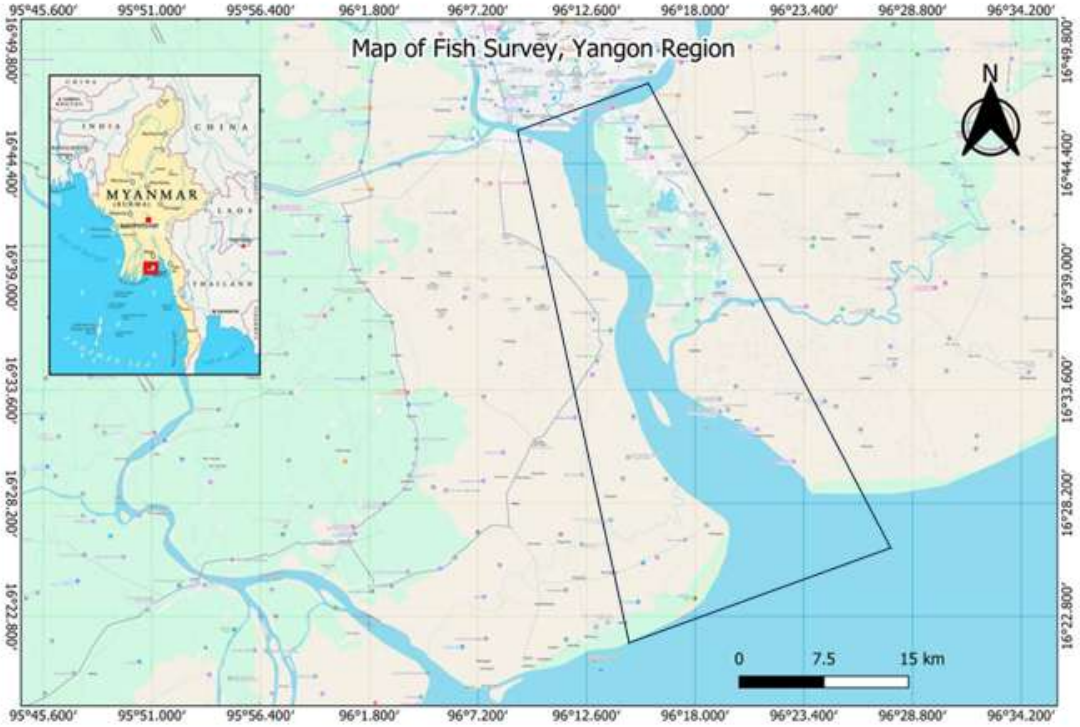

Figure 1. Study area of Yangon River estuary habitat influenced by the intertidal zone showing highlight by rectangle

(a) (b)

Figure 2. Mangrove and salt marsh grass habitats (a) and fishing catfish using lift net encircle the mangrove during high tide (b) in the segment of the Yangon River mouth, the estuary habitat influenced by the intertidal zone

Data Collection and Sampling

Field observations were conducted during both the dry and wet seasons. Observations were conducted in both the dry (April 2024) and wet (October 2024) seasons, focusing on fish caught using drift gill nets and lift nets. Sampling was based on fieldwork and interviews. Fish data were collected from fishermen catching the fish in the surrounding area of the Yangon River in the selected study area, as well as from fish purchase centers and through interviews. The distribution of mangrove and salt marsh grass habitats have been recorded which provide food and shelter for aquatic organisms, including studying catfish.

Water parameters relevant to the effects on health, growth, population and distribution of the study fish were measured both in the field and in the laboratory. Surface water sample was

collected from designated study sites. The water sample from 2 feet depth was collected for determining river water quality. The clear sampling plastic bottles of 1 L capacity was rinsed three times with deionized water and river water, then, filled and screwed tightly with cap. Some physicochemical parameters such as pH, temperature, electrical conductivity, total dissolved solids and dissolved oxygen of collected water samples were onsite tested and other parameters were determined within 24 hrs at the laboratory.

Those properties of water quality parameters such as temperature, pH and salinity were determined by Exact® pH+ meter master kit (S/N: PHP 1947513), dissolved oxygen (DO) was determined by Dissolved Oxygen Meter(HI 9146-04, Hanna, USA), turbidity, total hardness, ammonia and nitrate were determined by Exact micro 20 + photometer with Bluetooth SMART, 525 nm +638 nm wavelength (S/N: M 20 BTA 00009 HACH test kit), biochemical oxygen demand (BOD) was determined by APHA Standard Test Method. Some heavy metals such as mercury, lead and cadmium were determined by GBC-932 plus Atomic Absorption Spectrometer (GBC Scientific Equipment Ltd., Australia) at Fershwater Aquaculture Laboratory, Department of Fisheries, Ministry of Agriculture, Livestock and Irrigation. The results of water sample parameters and their effects on the health, growth, survival of fish distribution and abundance depending on the the river ecosystem are shown in Table (2).

Statistical analysis

Pearson's correlation coefficient was used to assess the relationship between fish length and weight. This measure assesses the linear correlation between two variables, with values ranging from -1 (perfect negative correlation) to 1 (perfect positive correlation). A correlation coefficient close to 1 suggests a strong positive relationship, meaning that as the fish's length increases, the weight also increases proportionally. A p-value is also computed to assess the statistical significance of this correlation.

Results and Discussion

Description of the study species

The *Pangasius myanmar* is a species of catfish that belongs to the family Pangasiidae, commonly known as the yellowtail catfish for its distinctive yellow tail (Figure 3(a)). Observation data indicated that *Pangasius myanmar* closely resembles *P. pangasius*, but differs from it by having a narrower and more transverse mouth; shorter barbels; palatal dentition with short oval palatine patches and a median oval vomerine patch; fewer gill rakers with several distinct morphological characteristics. It has streamlined body with a moderately elongated shape. The head is small, and the mouth is terminal with shorter barbels not reach to operculum. Its dorsal fin is relatively short but features a sharp spine while the pectoral fins are wide and strong also possessing spines. The caudal fin is forked. The body color is silver-gray with a lighter underside (Roberts and Vidthayanon , 1991).

Distribution

Based on field observations and interviews, yellowtail catfish were found throughout the coastal, river mouth, and intertidal areas of the Yangon River estuary, extending up to 23 miles (38.34 km) from the river’s mouth, which spans about 40 miles (63.37 km). During the dry season, more catfish are caught upstream, while in the wet season, the catch is higher downstream and near the river mouth, where saltwater and freshwater mix to form estuarine

waters. Additionally, the fish catch is greater in the waters near the mangrove area. The fisherman catches fish in the mangroves during high tide using a lift net, as the fish search for food among the mangrove. The yellowtail catfish is a carnivorous and voracious fish that eat fish, crustaceans, mollusks and also eats mangrove fruits with juveniles found in high estuaries, sub-adults migrating to brackish waters, and adults reaching river mouths and inshore area.

Its distribution in the estuary is closely tied to seasonal variations in water flow and salinity. During the early monsoon season, when rainfall increases freshwater input into the river, and during the late monsoon, when the last surge of rainwater mixes with marine water from the sea, *Pangasius myanmar* is particularly abundant in the estuarine zone. However, despite its economic importance and relatively high abundance in the region, little scientific attention has been given to the species and comprehensive data on its distribution and population trends is rare (Fishbase, 2015 ; Roberts and Vidthayanon , 1991).

During the field observation, the fish were primarily found near the river mouth, within estuary and mangrove habitats. This finding is revealed with the distribution of this species described by Fishbase, 2015. The data shows a similar count of fish during both seasons (7 in the dry season and 8 in the wet season), with an average total length of 96.68 cm (n=15). All observed fish were adults. In April, the presence of mature eggs in their abdomens (Figure 4B) indicated that reproductive activity began in April and happen during pre-monsoon (May to June), and breeding might occur in the study area, Yangon River. This suggests that the fish *Pangasius myanmar* not only inhabit but also breed in Yangon River, potentially influenced by habitat and environmental conditions.

(a) (b)

Figure 3. Recorded (a) *Pangasius myanmar* and (b) Examination of eggs in adult fish

Abundance

A total of 2,839 fish were recorded over the year with the highest number in November (475) and the lowest in May (75). The total weight harvested for the year was 16,512.72 kg with the highest monthly weight recorded in October (2,671.2 kg) and the lowest in May (490.6 kg). It summarizes the monthly recorded quantities *of Pangasius myanmar* (Yellowtail catfish) harvested from the Yangon River, collected from two purchase centers in Chaungwa village, Kyauktan Region, between November 2023 and October 2024 (Table 1 and Figure 4). The data indicates the highest abundance in November and the lowest in May, reflecting seasonal fluctuations in the fish population and availability in the Yangon River.It was suggested that Yangon River is home for endemic fish of *Pangasius myanmar.* This species plays a crucial role in the local fisheries, contributing to both livelihood and commercial markets in Yangon and surrounding areas. *Pangasius myanmar* is an estuarine and freshwater species native to the Yangon Region, recorded seasonally in Yangon River with a known distribution limited to this

area. IUCN (2015) described that its conservation status is Data Deficient (DD) that there is insufficient information to make a direct or indirect assessment of a species' risk of extinction. According to Gupta et al. (2016), the decline in fish populations is attributed to overfishing, habitat degradation, pollution, and migration barriers such as dams and embankments.

Table 1. Monthly recorded *Pangasius myanmar* (yellowtail catfish) from Yangon River, data collected from two purchase centers at Chaungwa village, Kyauktan Region.

ZMT+ UHW	Nov	Dec	Jan	Feb	M	A	May	Jun	Jul	Aug	Sep	Oct	Total
No. of fish	475	330	159	182	75	100	180	220	268	200	235	415	2839
Weight (kg)	2402.4	2217.6	860.2	1008	490.6	630	924	1108.8	1344	1344	1512	2671.2	16512.7

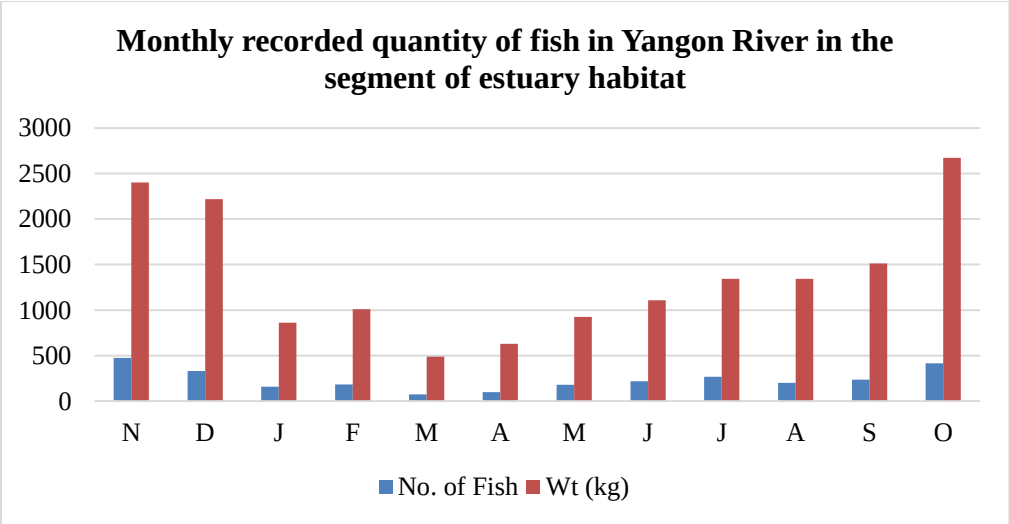

Figure 4. Monthly recorded *Pangasius myanmar* from Yangon River, data collected from two purchase centers at Chaungwa village, Kyauktan Region

Relationship of length and body weight of Catfish

The statistical analysis yielded a Pearson's correlation coefficient (r): 0.936. This value indicates a very strong positive correlation between the length and weight of fish. P-value: 3.08 x 10 -14, which is extremely small, suggesting that this correlation is statistically significant (Figure 5). The scatter plot above also visualizes this relationship, with a best-fit line showing that as the fish's length increases, its weight increases in a near-linear fashion. The correlation between fish length and weight is both strong and statistically significant (Figure 6), meaning that fish grow proportionally in both dimensions. This finding suggests a healthy growth status where body dimensions increase proportionally.

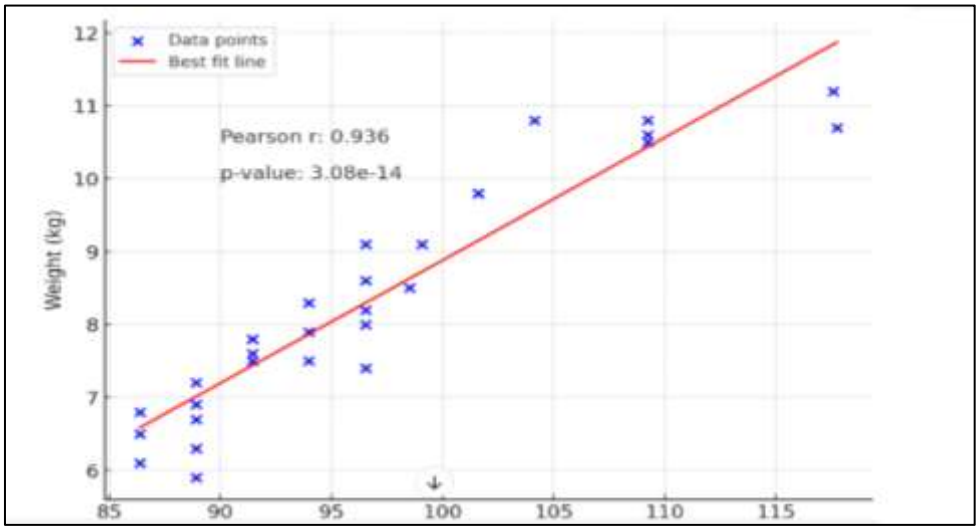

Figure 5. Correlation between length and weight of *Pangasius Myanmar*

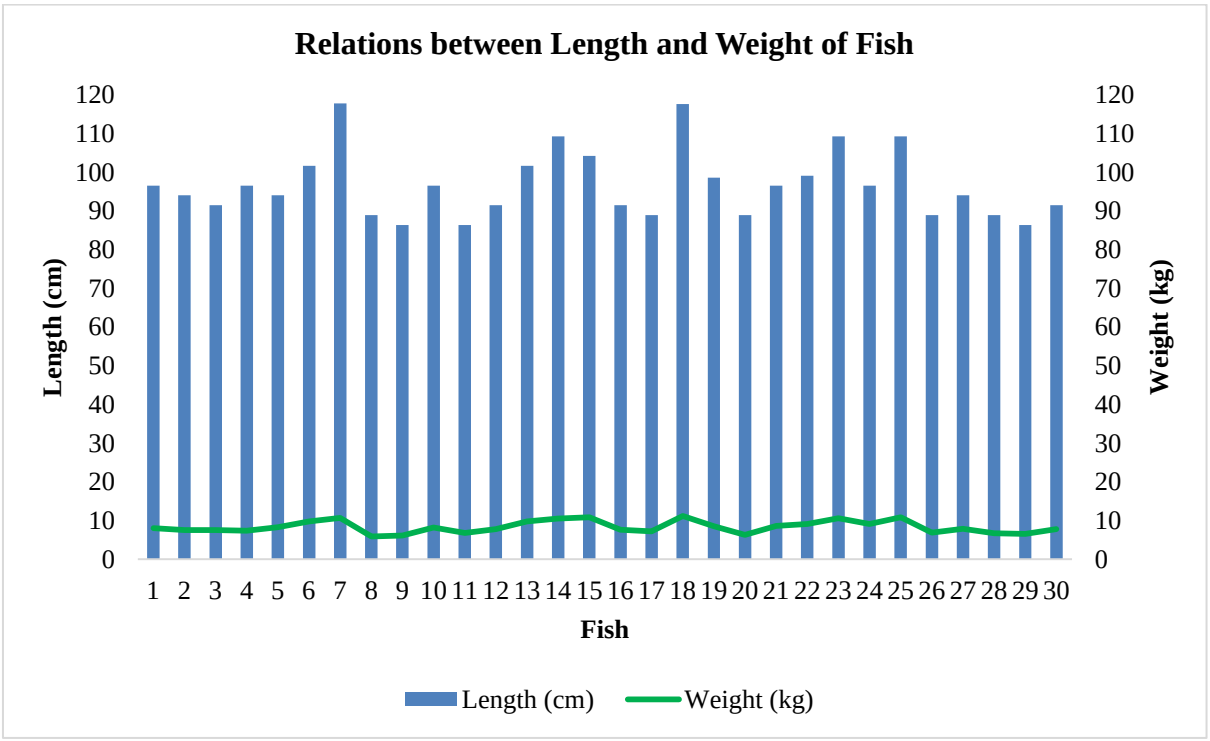

Figure 6. Relationship between length and weight of-*Pangasius myanmar*

Relationship between water quality, health and survival of fish

The water quality in the study area of Yangon River as shown in Table 2, plays a critical role in the ecology of *Pangasius myanmar*, an endemic species adapted to salinity fluctuations. Key parameters such as temperature, pH, dissolved oxygen (DO), salinity, and turbidity significantly influence the reproduction, population dynamics, health, and survival of this species. Temperature fluctuations between 26-29°C align with the optimal range (25-30°C) for metabolic processes, behavior, and reproduction. Similarly, pH levels (6.5-7.5) support metabolic functions and reproductive health within the tolerable range for the species. DO concentrations (6.29-12 mg/L) are conducive for respiration and survival, while BOD levels are low, indicating

favorable conditions for aquatic health. However, elevated salinity (0.02-15 ppt) suggests the estuarine environment's fluctuations, which *Pangasius myanmar* can adapt to as they thrive in variable salinity. High turbidity (15-50 NTU) may inhabit feeding and reproduction, especially if it crosses the threshold of 20 NTU, affecting visibility and oxygen exchange. Low levels of harmful substances like ammonia (0.033 mg/L) and nitrates (5.0 mg/L) suggest a relatively safe environment for growth, though heavy metals like cadmium (0-0.19 mg/L) and lead (0-0.03 mg/L) could present long-term health risks. In this estuarine system with mangroves, the water quality fluctuations directly impact *Pangasius myanmar's* reproductive cycles (notably egg maturation in April), population viability, and overall health, especially as they adapt to the freshwater and brackish environments (Table 2).

Table 2. Summary of surface water quality parameters and their effects on the health, growth, survival of fish,-and the river ecosystem

Sr. No	Parameter	Effects on health and survival fish	Observed value	Standard value for fish (Clesceri *et a.l,* 1999)
1	Temperature (°C)	Effects on metabolism, behavior, reproduction, and overall health	26-29	25-30
2	pH	Effects on metabolism, growth, and reproductive health	6.5 -7.5	6.5 - 9
3	DO (mg/l)	Effect on respiration	6.29 -12	5-14
4	Salinity (ppt)	Effect on metabolism, growth and reproduction	0.02 -15	10-25
5	Turbidity (NTU)	Effects on feeding, breathing, and reproduction	15- 50	<20
6	BOD (mg/l)	Effect on the health of aquatic environments and the survival of fish	2.5	<3
7	Ammonia(mg/l)	Effect on growth and health	0.033	<0.25
8	Total hardiness (mg/l)	Effect on growth and health	100-150	>200
9	Nitrate (mg/l)	Effect on growth and health	5.0	0.5 -10
10	Hg (mg/l)	Poses significant risks to fish health and toxic to the ecosystems they inhabit	0	~ 0
11	Pb (mg/l)	Poses significant risks to fish health and toxic to the ecosystems they inhabit	0 – 0.03	~ 0
12	Cd (mg/l)	Poses significant risks to fish health and toxic to the ecosystems they inhabit	0 - 0.19	~ 0

Conclusion

The Yangon River is home of *Pangasius myanmar* which has been observed the whole year. The Yangon River, particularly in its estuarine segment downstream, serves not only as a

breeding ground but also as a favorable habitat. This species is not only ecologically significant but also economically important to local fisheries. Known locally as "Myit Nga Tan", its high protein and fat content make it a prized catch, contributing to the livelihoods of fishing communities in this region. In this estuarine system with mangroves, the water quality fluctuations directly impact *Pangasius myanmar's* reproductive cycles (notably egg maturation in April), population viability, and overall health, especially as they adapt to the freshwater and brackish environments. Protecting the delicate balance of the estuarine habitats, especially the mangrove and salt marsh areas, is the key to ensuring sustainable local fisheries and the preservation of *Pangasius myanmar* in the Yangon River.

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REVIEW ON ESTUARINE AND MARINE MOLLUSCS OF MYANMAR: PART I*

Naung Naung Oo[1]

Abstract

A total of 40 orders, 164 families, 375 genera, and 1720 species of estuarine and marine molluscs were collected from intertidal, subtidal and deep-water areas from Nantha Island in northern Rakhine Coastal Region to Dawei Point in Tanintharyi Coastal Region from 2004 to 2024 in Myanmar coastal water. The collected specimens were recognized as 1 species of Aplacophora, 20 species of Polyplacophora, 2 species of Monoplacophora, 1106 species of Gastropoda, 529 species of Pelecypoda (= Bivalvia), 10 species of Scaphopoda, and 52 species of Cephalopoda. In the present study, 954 species of estuarine and marine molluscs were recorded from the Rakhine Coastal Region, followed by 598 species from the Tanintharyi Coastal Region, 134 species from the Ayeyawady Delta and Gulf of Martaban Coastal Region, and 34 species from unspecified localities, respectively.

Keywords: Estuarine, marine, molluscs, Myanmar water.

Introduction

Myanmar has diverse finfish and shellfish resources, productive inshore and offshore waters, with a 2,831 km coastline facing the Bay of Bengal and the Andaman Sea in the Indian Ocean. Estuarine and marine molluscs were classified into seven classes, such as Aplacophora von Jhering, 1876; Polyplacophora de Blainville, 1816; Monoplacophora Wenz, 1940; Gastropoda Cuvier, 1795; Pelecypoda (= Bivalvia) Linnaeus, 1758; Scaphopoda Bronn, 1862; and Cephalopoda Cuvier, 1795 by Seed (1983).

The Sclerite-bearing deep-sea shells of aplacophorans; Chiton shells of polyplacophorans; Bearing one plate or cup-like shells of monoplacophorans; Angaria shells, Keyhole limpets or slit limpets, Abalones, Lottiid limpets, Nacellid true limpets, Nerites, Patellid limpets, Top shells, Turban shells, Sundial shells, Frog shells, Hat or cup shells, Helmet and bonnet shells, Ceriths, Cerithiopsis, Triton's trumpet, Cowries, Staircase or ladder shells, Violet snails, Parasitic sea snails, Fig shells, Hoof shells, Iravadiid sea snails, Periwinkles, Moon snails, Egg cowries, Distorsios, Planaxids and cluster-winks, Swamp-ceriths or horn shells, Triton shells, Rissoinid sea snails, Conchs, Tun shells, Triphorid sea snails, Bean cowries, Turret shells, Worm shells, Carrier shells, Babyloniid whelks, Whelks, Clathurellid sea snails, Dwarf tritons, Dove shells, Cone shells, Costate miters, Horse conchs or spindle shells, Harp shells, Coral snails, Mangeliid sea snails, Marginellids, Melongenas, Miter shells, Purpuras, murex and rock shells, Nassa mud snails, Olive shells, Pisaniid sea snails, Raphitomid sea snails, Auger shells, Vase shells, Turrids, Volutes, Fragile bubble shells, Sea hares, Bubble shells, Haminoeid bubble shells, Lozenge-shaped slugs, Barrel-bubble snails, Canoe-bubble shells, Floating sea snails, Chromodorid nudibranchs, Dendrodorid nudibranchs, Discodorid nudibranchs, Sea lemon, Hexabranchid nudibranchs, Phyllidiid nudibranchs, Polycerid nudibranchs, Aitengid slugs, Side-gilled slugs, Sea butterflies, Pyramid shells, Bubble sea slugs, Sacoglossan sea slugs, Mangrove slugs, Cassidula and pythia snails, and False limpets of gastropods; Knife and razor clams, Ark shells, Cucullaeid ark shells, Bittersweet clams, Limopsis ark shells, Noetiid ark shells, Cockles, Donax clams, Sunset clams or sanguins, Semeles, Short razor clams, Tellins, Giant clams, Carditas, Lantern clams, Penicillid clams, Tiny galeommatid clams, Kelly clams, Flask cockles, File shells, Lucinas, Little basket clams or box clams, Zebra mussel, Angel wings or piddocks,

*First Prize(2024)

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Shipworms, Sea mussels, Swan neck shells, Yoldias, Nut clams, Honeycomb oysters, Tree oysters, Hammer oysters, Pearl oysters, Edible oysters, Pen shells, Winged oysters, Jingle shells or saddle oysters, Scallops, Windowpane shells, Kitten's paws, Thorny oysters, Awning clams, Jewel box shells, Marsh clams, Glauconomyas, Trough shells, Trapezid clams, and Venus clams of pelecypods; Tusk shells of scaphopods; Octopus, Squid, Cuttlefish, and Nautilus of cephalopods were predominantly found in Myanmar water.

The aims of this study are 1) to record the real existence of estuarine and marine molluscs in Myanmar water, and 2) to investigate the seashell resources and their utilization in the daily life of the local population.

Materials and Methods

Shells were collected from Nantha Island, northern Rakhine Coastal Region, down to Dawei Point, northern Tanintharyi Coastal Region, from 2004 to 2024 (Fig. 1) in the estuarine habitats of mangrove swamps, mudflats, salt marshes, sandflats, rocky fringes, and marine habitats of offshore Islands and deep-water zones along Myanmar coastal regions. **Rakhine Coastal Region:** Sittway Point (20˚ 06′ N, 92˚ 53′ E), Kyauk Phyu (19˚ 25′ N, 93˚ 31′ E), Kyauk Layaine Gyaing (19˚ 50′ N, 93˚ 25′ E), Sing aung (18˚ 32′ N, 94˚ 14′ E), Mazin (18˚ 27′ N, 94˚ 17′ E), Ngapali (18˚ 26′ N, 94˚ 18′ E), Shwewar Gyaing (18˚ 24′ N, 94˚ 19′ E), Lonetha Gyaing (18˚ 21′ N, 94˚ 20′ E), Kyauk Phone Gyi Maw (18˚ 18′ N, 94˚ 20′ E), Maung Shwe Lay Gyaing (18˚ 18′ N, 94˚ 19′ E), Kywe Thauk Gyaing (18˚ 17′ N, 94˚ 22′ E), Kwinwaing Gyaing (18˚ 17′ N, 94˚ 20′ E), Hmaw Chay Gyaing (18˚ 13′ N, 94˚ 25′ E), Gyaing Kauk Gyaing (17˚ 47′ N, 94˚ 28′ E), Hlyaw Gaung Taung Gyaing (17˚ 45′ N, 94˚ 30′ E), Yay Myet Taung Gyaing (17˚ 42′ N, 94˚ 31′ E), Maw Shwe Gyaing (17˚ 41′ N, 94˚ 32′ E), Chan Pyin Gyaing (17˚ 38′ N, 94˚ 33′ E), Yahaing kutoe (Gwa Aw) (17˚ 38′ N, 94˚ 34′ E), Makyeengu Gyaing (Gwa Aw) (17˚ 35′ N, 94˚ 33′ E), Shweya Gyaing (17˚ 35′ N, 94˚ 33′ E), Baw Di Gyaing (17˚ 29′ N, 94˚ 33′ E), Jade Lett Gyaing (17˚ 17′ N, 94˚ 30′ E), Tapin Maw (17˚ 16′ N, 94˚ 29′ E), Phoe Htaung Gyaing (17˚ 10′ N, 94˚ 29′ E), Wet Thay Gyaing (17˚ 08′ N, 94˚ 27′ E), Kyauk Nagar (17˚ 04′ N, 94˚ 27′ E), Shwe Thaung Yan (Ma Gyi) (17˚ 04′ N, 94˚ 27′ E), Boung Kyun I. (17˚ 04′ N, 94˚ 26′ E), Inn Din Gyi (17˚ 03′ N, 94˚ 26′ E), Thae Phyu Kyun (17˚ 01′ N, 94˚ 18′ E), Chaung Tha (16˚ 57′ N, 94˚ 25′ E), Ngwe Saung (16˚ 52′ N, 94˚ 22′ E), Thathanar Dauk (16˚ 36′ N, 94˚ 19′ E), Ngayoke Kaung Aw (16˚ 32′ N, 94˚ 17′ E), Ohn Kyun I. (16˚ 23′ N, 94˚ 13′ E), Cape Negrais (16˚ 02′ N, 94˚ 11′ E), Ngwe Taung Pagoda (16˚ 01′ N, 94˚ 12′ E), Zea Gyaing (16˚ 01′ N, 94˚ 12′ E), Mawtin Point (15˚ 57′ N, 94˚ 14′ E); **Ayeyawady Delta and Gulf of Martaban Coastal Region:** Letkokkon (16˚ 19′ N, 96˚ 08′ E), Kyauk Chaung (15˚ 59′ N, 94˚ 16′ E), Kha Mauk Hmaw (15˚ 59′ N, 94˚ 16′ E), Kyar Kan (15˚ 59′ N, 94˚ 13′ E), Haing Gyi I. (15˚ 58′ N, 94˚18′ E); **Tanintharyi Coastal Region:** Ahlyat (16˚ 37′ N, 97˚ 27′ E), Kyaikkhami (16˚ 05′ N, 97˚ 34′ E), Setse (15˚ 57′ N, 97˚ 36′ E), Kalegauk I. (15˚ 32′ N, 97˚ 39′ E), Sitaw (15° 11′ N, 97˚ 48′ E), Ka Byar Wa (15˚ 04′ N, 97˚ 48′ E), Maungmagan (14˚ 07′ N, 98˚ 05′ E), Thabawseik (Mwe Taung) (14˚ 06′ N, 98˚ 05′ E), Kanpani (14˚ 03′ N, 98˚ 04′ E), San Hlann (13˚ 54′ N, 98˚ 04′ E), South Moscos I. (13˚ 51′ N, 97˚ 55′ E), Nyaw Pyin Aw (13˚ 38′ N, 98˚ 08′ E), Wa Maw (13˚ 37′ N, 98˚ 08′ E), Myin Kwar Aw (13˚ 33′ N, 98˚ 08′ E).

All live shells were preserved in 10% formalin in seawater. The collected specimens were taxonomically identified up to the lowest possible taxonomic units and housed in the Museum of Mawlamyine (MLM.MS), Sittway (MSS), and Pathein universities (PMS). Available shell books such as Indo-Pacific Mollusca, Monographs of Marine Mollusca, Molluscan Research and The Veliger were also examined. An extensive literature and internet search was conducted, including the World Register of Marine Species (WoRMS; WoRMS Editorial Board [2023]), to determine the current valid name and family placement.

Figure 1. Collection areas of molluscs in some coastal areas of Myanmar

Results and Discussion

There were 1833 species of estuarine and marine molluscs in some coastal areas of Myanmar in the present study, but 113 species were rejected or considered uncertain: 66 on geographic grounds because the species does not occur in the Southeast Asian marine biogeographic region; 34 on a taxonomic basis; 8 for being fossils; and 5 for being freshwater species.

Table 1. Estuarine and marine species in each class of mollusc recorded from Myanmar

Class	RCR	ACR	N.TCR	NSL	Total
Aplacophora von Jhering, 1876	0	1	0	0	1
Polyplacophora de Blainville, 1816	8	2	9	1	20
Monoplacophora Wenz, 1940	2	0	0	0	2
Gastropoda Cuvier, 1795	673	84	336	13	1106
Pelecypoda (= Bivalvia) Linnaeus, 1758	245	35	234	15	529
Scaphopoda Bronn, 1862	5	0	3	2	10
Cephalopoda Cuvier, 1795	21	12	16	3	52
Total	954	134	598	34	1720

Symbols: RCR = Rakhine Coastal Region; ACR = Ayeyawady Delta and Gulf of Martaban Coastal Region; N. TCR = Northern Tanintharyi Coastal Region; NSL = No Specific Locality.

The remaining 1720 species are classified under 7 classes, 40 orders, 164 families and 375 genera and are considered valid by the WoRMS Editorial Board (2023) and other sources. The species diversity was the highest in-class Gastropoda (1106 species), followed by Bivalvia (529 species) and Cephalopoda (52 species) (Table 1). Based on taxonomic criteria, seven classes of estuarine and marine molluscs were recorded from Myanmar coastal water as shown in Table 2.

Table 2. Taxonomic status of estuarine and marine molluscs recorded from Myanmar

Class	**Orders**	**Families**	**Genera**	**Species**
Aplacophora von Jhering, 1876	1	1	1	1
Polyplacophora de Blainville, 1816	2	5	13	20
Monoplacophora Wenz, 1940	1	1	1	2
Gastropoda Cuvier, 1795	13	88	204	1106
Pelecypoda (= Bivalvia) Linnaeus, 1758	16	50	126	529
Scaphopoda Bronn, 1862	2	3	4	10
Cephalopoda Cuvier, 1795	5	16	26	52
Total	40	164	375	1720

The aplacophoran shells are tiny, relatively barrel, worm-like structures, without outer shells and the length is 1 mm to 30 cm but usually less than 5 cm long (Fig 2A). The epidermal layer secretes calcareous spicules or scales embedded in the dorsal mantle. They live in the sediment of the deep-sea floor. The single species of *Falcidens crossotus* Salvini-Plawen, 1968 was recorded during Dr Fridtjof Nansen Marine Ecosystem and Oceanographic Survey (2013) in Myanmar waters.

The chitons are composed of eight overlapping rough shell plates, three layers in young individuals, small tubes that photoreceptors pierce plates and various sizes of marine molluscs groups that are found in the class of Polyplacophora which was formerly known as Amphineura. These shells were grazed on rocky intertidal zones and hard substratum (Fig 2B). In Rakhine and Tanintharyi coastal regions, chiton shells were collected by local people for their daily consumption and the sale of dry meat at local markets.

The monoplacophorans are worm-like molluscs, without shells, crystalline style, and feet; they live in vertical burrows on the deep-sea floor. Its shell resembles a cap. Shells are found in soft and hard substrates on the continental shelf and in the deep sea. Only two species of

Neopilina bruuni Menzies, 1968 and *N. galatheae* Lemche, 1957 were recorded in deep water depths of 100-500m in the Rakhine Coastal Region (Fig 2C).

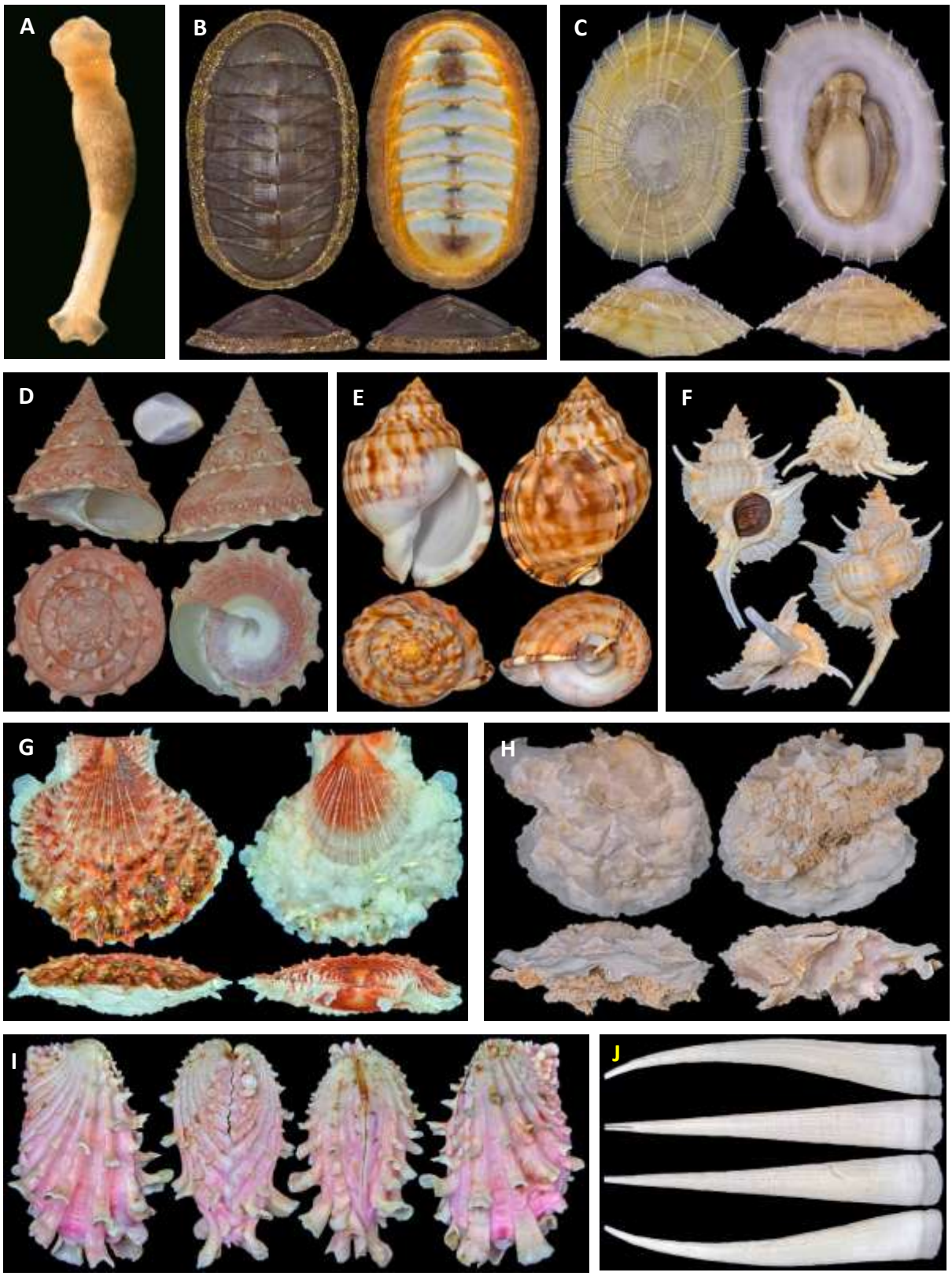

Figure 2. (A-J): Typical species of estuarine and marine molluscs in Myanmar. A) Aplacopod; B) Polyplacopod; C) Monoplacopod; D-F) Gastropods; G-I) Bivalves; J) Scaphopod.

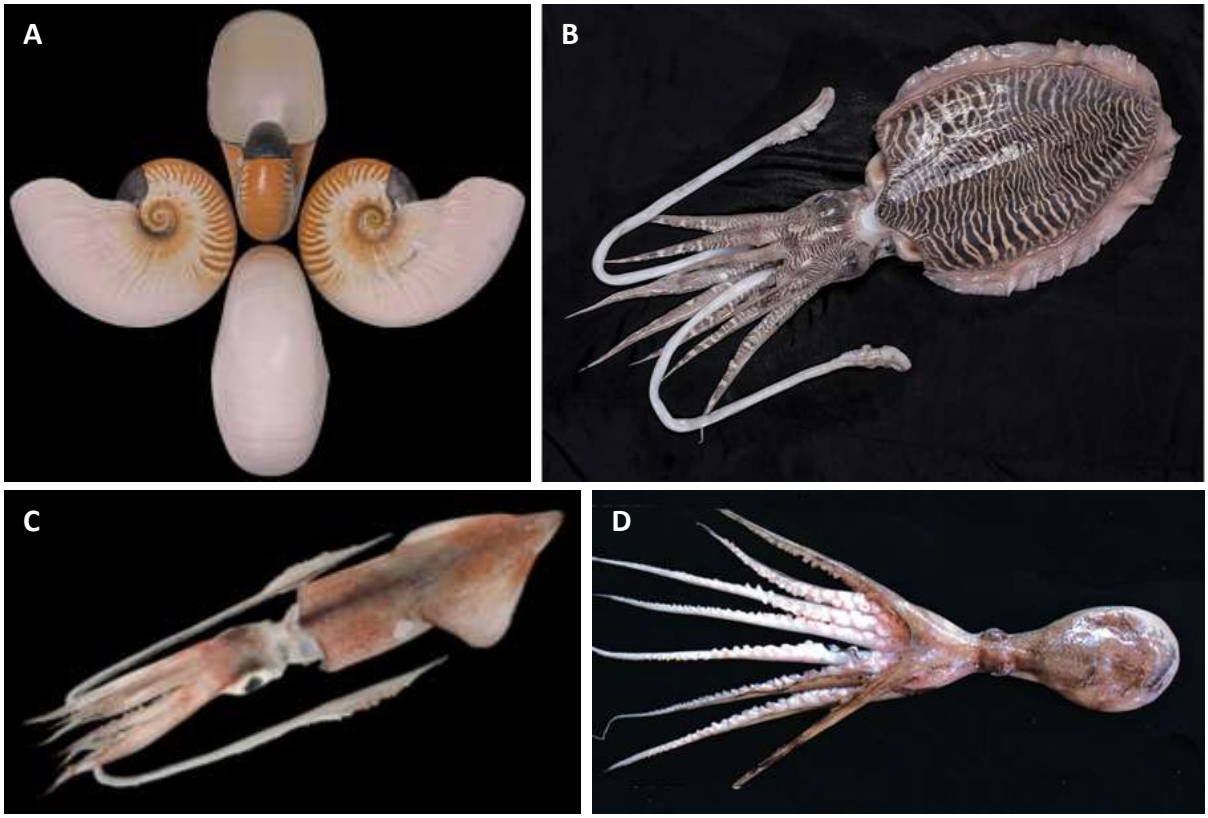

Figure 3. (A-D): Typical Cephalopod species in Myanmar. A) Nautilus species; B) Cuttlefish species; C) Squid species; D) Octopus species.

Gastropods are spirally coiled and asymmetrical shells. Their soft body is divided into 4 main regions: the head, the foot, the visceral mass, and the mantle (Fig 2D-F). Gastropods are usually divided into 4 main subclasses: Prosobranchia, Opisthobranchia, Pulmonata, and Gymnomorpha. Among the marine-shelled gastropods, the prosobranchs contained the most commercial interest to fisheries. In this study, 1106 known species were recorded along Myanmar water. The highest species was recorded at 673 species in the Rakhine Coastal Region followed by 336 species in the northern Tanintharyi Coastal Region, 84 species in the Ayeyawady Delta and Gulf of Martaban Coastal Region, and the last 13 species had no specific locality.

The pelecypods or bivalves are composed of 2 calcified valves which are equally convex (equivalve shell), but they may differ in size and shape (inequivalve shell) as a result of an alteration of bilateral symmetry. The valves are characterized by the hinge, the ligament, 1 or 2 (sometimes 3) adductor muscles, well-defined imprints, and adductor muscle scars. The soft and unsegmented body of bivalves is laterally compressed, fused and covered by the mantle, pallial lobes, pallial line, foot and elastic filaments (byssus) (Fig 2G-I). These shells were the second largest species outnumbered in the present study and most of the shells were consumed and traded by coastal populations.

Tusk shells or scaphopods are curved, tooth-like shells open at both ends, bilaterally symmetrical marine forms, lack eyes and burrow with shovel feet in marine mud and sediments (Fig 2J). Scaphopod shells are truly marine organisms that can be found at an incredible depth of up to 4570 meters. In this study, 10 species of scaphopods were recorded in deep water areas of Rakhine and northern Tanintharyi coastal regions.

Table 3. Reviews of estuarine and marine molluscs in Myanmar

Sr. No.	Author	Year	Field of study and area
1	Mason	1850 1852 1882	Taxonomy (Northern Rakhine Coastal Region and Tanintharyi Coastal Region)
2	Mann	1888	
3	Soe Thu	1970	Taxonomy (Ngapali, Rakhine Coastal Region)
		1971	Taxonomy (Maungmagan, Tanintharyi Coastal Region)
		1972	Taxonomy (Myanmar Coastal Water)
		1980	Taxonomy (Myanmar Coastal Water)
4	Kyaw Myint	1971	Taxonomy (Moscos Islands, Tanintharyi Coastal Region)
5	Khin May Gyi	1973	Morphology and Taxonomy
6	Maung Win	1975	Morphology and Taxonomy (Myanmar Coastal Water)
7	Kyi Bin	1979	Anatomy of *Octopus* sp.
8	Aye Thida Thein	1982	Morphology and Taxonomy (Myanmar Coastal Water)
9	Mar Lar Myo Sein	1982	Morphology and Taxonomy (Myanmar Coastal Water)
10	Wai Wai Kan Oo	1982	Anatomy of *Sepia* sp.
11	Tint Tun	1984	Anatomy and Histology of Pearl Oyster
12	Lwin Lwin	1985	Aquaculture (Ye River Mouth, Mon State)
13	Tin Nu	1985	Aquaculture (Ye River Mouth, Mon State)
14	Htay Aung	1987	Aquaculture (Setse Coastal Water, Mon State)
15	Phyu Phyu Khin Win	1990	Taxonomy (Bottom Trawled, Myanmar Coastal Water)
16	Su Su Khin	1992	Anatomy of *Sepia* sp.
17	Mya Mya Htwe	1995	Anatomy and Histology of Spider Conch
18	Myint Myint Khaing	1995	Aquaculture (Setse Coastal Water, Mon State)
19	Hla Hla Aye	1996	Anatomy and Histology of Top Shell
20	Htwe-Nyunt	1996	Aquaculture (Setse Coastal Water, Mon State)
21	Nan Nwe Nwe Aye	1998	Ecology (Chaung Tha Coastal Water, Ayeyawady Region)
22	Taat Tun Thu	1998	Taxonomy (Tanintharyi Coastal Region)
23	Aye Thant Zin	2007	Taxonomy (Sin Ma Coastal Water, Ayeyawady Region)
24	Kay Thwe Tun	2007	Aquaculture of Pearl Oyster
25	San San Aye	2007	Aquaculture of Pearl Oyster
26	Sandar Win	2009	Ecology (Chaung Tha Coastal Water, Ayeyawady Region)
27	Htay Aung	2011	Aquaculture of Oyster spp.
28	Jar San	2011	Taxonomy (Mon State, Tanintharyi Coastal Region)
29	Naung Naung Oo	2012	Taxonomy (Mon State, Tanintharyi Coastal Region)
30	Phoo Thet Su Win	2012	Taxonomy (Setse Coastal Water, Tanintharyi Coastal Region)
31	Phyu Phyu Than	2012	Ecology (Myeik Coastal Water, Tanintharyi Region)
32	Sint Sint Hlaing	2012	Taxonomy (Palaw Coastal Water, Tanintharyi Coastal Region)
33	Thaw Zin Naing Tun	2012	Taxonomy (Kampani Coastal Water, Tanintharyi Coastal Region)
34	Phoo Thet Su Win	2013	Taxonomy (Mon State, Tanintharyi Coastal Region)
35	Khin Myo Thin	2015	Taxonomy (Ye River Mouth, Mon State)
36	Su Pyae Tun	2016	Taxonomy (Myanmar Coastal Water)
37	Aung Ko Latt	2017	Taxonomy (Kawdut Coastal Water, Tanintharyi Coastal Region)
38	Khin Yu Nwe	2017	Fishery Biology (Myeik, Tanintharyi Coastal Region)
39	Aung Pyae Phyo	2019	Taxonomy (Mon State, Tanintharyi Coastal Region)
40	Nu Sein	2019	Ecology (Bo Ron Ga I., Northern Rakhine Coastal Region)
41	Win Win Nwe	2019	Aquaculture (Ye River Mouth, Mon State)
42	Hsu Thawdar Hnin	2021	Population Dynamics (Ye River Mouth, Mon State)

A total of 52 species of cephalopods, including nautiluses, cuttlefish, squids, and octopuses were included in the present study. They may not be as diverse as other mollusc groups or as the bony fishes in terms of the number of species (about 600 cephalopod species described worldwide), but these species are very common and some have large body sizes. Hence, they are of considerable ecological and commercial fisheries importance globally and in Myanmar (Fig 3A-D).

The works of literature concerned with Myanmar seashells have been very few in the past. The earliest study on Myanmar seashells is that of Mason (1850, 1852, 1882) and Mann (1888) collected from the northern Rakhine Coastal Region and Tanintharyi Coastal Region during the colonial period. In this way, there was an entire lack of modern literature on Myanmar molluscs, and information gained from this literature was very scarce and inadequate. The seashells study of Myanmar coastal water was revised and compiled by Naung Naung Oo (2022) (Table 3 and Fig 4).

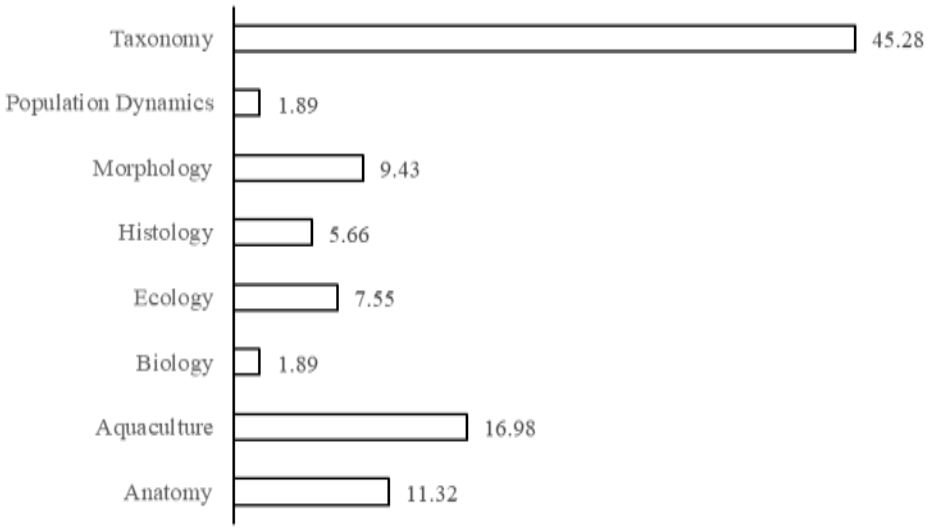

Figure 4. Percentage field of study of estuarine and marine molluscs in Myanmar

Conclusion

The three coastal regions of Myanmar are huge habitats with rich biodiversity. The shellfish biodiversity holds significant social, economic and cultural values for the local population. The intertidal zones of Myanmar are all aware of the role of coastal ecosystems, particularly mangrove swamps, coral reefs, seagrass meadows, dunes, mudflats, and rocky fringes in sustaining the recruitment of shellfish and in supporting fisheries too. The coastal water from Nantha Island to Dawei Point has 1833 species of estuarine and marine molluscs, 1 species of aplacophora, 20 species of polyplacophora, 2 species of monoplacophora, 1106 species of gastropoda, 529 species of pelecypoda, 10 species of scaphopoda, 52 species of cephalopoda, and 113 species are considered uncertain as of the studies done so far. However, much information on deep-sea and offshore species of molluscan fauna in Myanmar water remained unknown.

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MORPHOMETRICS, LENGTH WEIGHT RELATIONSHIP AND CONDITION FACTOR OF *COILIA DUSSUMIERI* VALENCIANNES, 1848 FROM YE ESTUARY, MON COASTAL AREA

Zarni Ko Ko[1]

Abstract

The morphometrics analysis, length weight relationship and condition factor of *Coilia dussumieri* were estimated in Ye Estuary between January and October 2022. During this study, the interrelationship of total length (TL) with various morphometric characters, namely SL, HL, ED, BD, PDL, PAL, PPL, and PVL, was found to be statistically insignificant ($p > 0.05$). A high correlation coefficient ($r = 0.95$) showed a highly significant relationship between total length and standard length, while the remaining characters showed moderate or low correlations ($0.5 < r < 0.7$). Moreover, the growth pattern between total length and other morphometric characters was negative because the value of 'b' was lower than unity ($b < 1$). The length-weight relationship of the fish indicated negative allometric growth ($b < 3$), and the condition factor showed a poor condition in fish ($K < 1$).

Keywords: *Coilia dussumieri*, morphometry, length weight relationship, condition factor, Ye Estuary, Mon Coastal Area.

Introduction

Coilia dussumieri is known as gold spotted grenadier anchovy (Nga-kyan-ywat) in Mon Coastal Areas. *C. dussumieri* is a commercial fish marketed in dried form and has become one of the major food commodities in different regions. If the intensity of fishing continues to rise, it is feared that anchovy populations will decrease.

C. dussumieri is widely distributed in the Indian Ocean, from Gujarat to West Bengal, Bangladesh, Myanmar, Thailand, and Malaysia. It is an important pelagic resource along the West Bengal coast, landed by trawlers and 'Doll' netters (Uddin and Ghosh, 2021). Furthermore, it is a major fish resource along the Southern Mon Coastal Areas, landed mainly by stationary bagnets.

When discussing fish conservation, the aims are to: (1) protect endangered fish species, (2) maintain the diversity of fish species, (3) maintain the balance and stability of the ecosystem, and (4) utilize fish resources in a sustainable manner. Therefore, the first step is to study the taxonomic status of anchovy (*Coilia dussumieri*) using morphological characteristics, which includes the early stages of identifying fish (Nugroho et al., 2015).

Morphometric characters in fish are widely used for taxonomic studies, as they contribute to the identification of populations and species inhabiting different bodies of water in different geographical regions. Statistical relationships between the measurements of various body parameters have been recorded in systematic studies worldwide (Jalbani et al., 2014).

In general, the measurement of morphometrics can be defined as a method for describing body shape. It is a technique widely used in ichthyological or taxonomic systematic studies through measurable components (measuring the length or distance between physical features or landmarks) of fish anatomies, such as the size of body parts, fins, and body length ratio (Kumaladewi et al., 2022).

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The study of the length-weight relationship of fish provides a mathematical relationship between length and weight, serving as a means of inter-conversion for estimating weight from a known length and vice versa. Knowledge of a fish's length-weight relationship is essential, as various important biological aspects, such as the general well-being of the fish, the appearance of first maturity, and the onset of spawning, can be assessed with the help of the condition factor (Le Cren, 1951). The length-weight relationship and condition factors are useful parameters for assessing the well-being of individuals and for determining possible differences among different stocks of the same species. The length-weight relationship is also important in fisheries science, particularly for scaling length frequency samples to total catch or for estimating biomass from underwater length observations (Pal et al., 2014).

Zarni Ko Ko (2021) studied the length-weight relationship, condition factor, and stock assessments for *Coilia dussumieri* from Setse and Sinpone. However, there has been no published information to date on the bagnet fishery of *C. dussumieri* from the Ye Estuary. As no previous study had been conducted on all selected morphometric characters of *C. dussumieri* along the Ye Estuary, the present study aims to examine the growth patterns of various body parts of this species in relation to total body length (TL) and also to observe the relationship between length and weight, and the condition factor for the species from the landing area of Ye Estuary.

Materials and Methods

Sampling site and Sample Collection

The samples of *Coilia dussumieri* were collected from Ye Estuary, Asin Landing Area, Mon Coast, with coordinates 15° 13' N and 097°47' E (Fig. 1) in the months from January 2022 to October 2022.

Analysis of Morphometric Characters

Nine morphometric characters were analyzed in the present study using standard procedures previously followed by Mahapatra et al. (2015). All these morphological parameters and their acronyms are listed in Table 1.

Table 1. Quantitative morphometric characters used for differentiation analysis of *Coilia dussumieri* collected from Ye Estuary.

No.	Morphometric characters	Acronyms
1	Total body length	TL
2	Standard length	SL
3	Head length	HL
4	Eye diameter	ED
5	Body depth	BD
6	Pre dorsal fin length	PDL
7	Pre pectoral fin length	PPL
8	Pre anal fin length	PAL
9	Pre pelvic fin length	PVL

Morphometric characters were measured to the nearest centimeter (nearest ± 0.1cm) with the help of a measuring scale. To obtain the length - length relationship among various

parameters with dependent variables, Total length (TL) and independent variables, various lengths was established with the formulae.

$$Y = a + b X$$

Where, 'Y' is the dependent variable, 'X' is the independent variable, 'a' is constant (intercept) and 'b' is the regression coefficient (slope).

Linear regression relationship and coefficient of correlation (r) were calculated to determine the relationship between total body length (TL) and all these morphometric characters. The significance of regression was assessed by t-test analysis at $p < 0.05$. These values of the t-test were obtained during linear regression analysis, which provides the method for observing the statistically significance of correlations between total length and all selected morphometric characters at 5% level ($p<0.05$). All statistical analysis of data was calculated using Microsoft Excel 2010 version.

Analysis of Length Weight Relationship and Condition Factor (K)

A total of 430 fish samples were collected from Setse and Sinpone fish landing sites from January 2022 to October 2022. The total length (TL) of each individual fish was measured in centimeters from the snout to the end of the caudal fin using the measuring board and the weight was measured in grams (g) by using the digital balance. The monthly length frequency data were pooled into groups of 1.7 cm length intervals by using Microsoft Excel.

The length weight relationship was determined by the methods of least square using the formula, which was followed after Le Cren (1951) as; $W = a L^b$

Where, W = weight of fish (g), L = total length of fish in (cm), a = constant (intercept), b = length exponent (slope).

The mean weight and length of the experimental fish were used to estimate condition factor using the equation below: $K = W/L^3 \times 10^2$ (Fulton, 1904)

Where, W= Weight in gram (g), L = Total length of fish in centimeter (cm)

Figure 1. Map showing the present study site of Ye Estuary

Results

Morphological characteristics of *Coilia dussumieri* during the present study.

In the present study, the external morphology of the fish body was observed as strongly compressed, with the ventral and dorsal profiles being in convex shapes. The minimum, maximum and mean with standard deviation values of morphometric characters are depicted in Table 2. Although morphometric measurements of the current study disclosed that total length (TL) was found in a range from 8.6 to 16.1 cm (in male) and from 8.3 to 16.5 cm (in female), standard length (SL) ranged from 6.8 to 14.5 cm (in male) and 5.8 to 14.6 cm (in female), head length (HL) varied from 1.5 to 2.6 cm (in male) and from 1.1 to 2.4 cm (in female), eye diameter (ED) was between 0.2 and 0.5 cm (in male and female), body depth ranged from 1.6 to 2.7 cm (in male) and from 1.3 to 2.8 cm (in female), pre dorsal length (PDL) ranging from 2.2 to 5.2 cm (in male) and 2.1 to 4.1 cm (in female), pre pectoral length (PPL) was between 2.1 and 4.2 cm (in male and female), per anal length (PAL) ranged from 3.2 to 5.6 cm (in male) and from2.8 to 5.8 cm (in female), and pre pelvic length (PVL) varied from 0.9 to 2.7 cm (in male) and from 0.9 to 2.4 cm (in female), as shown in Table 2.

The percentage of morphometric characteristics with respect to total length (TL) is shown in Table 3. The head length of *C. dussumieri* was 15.14 % (in males) and 17.01 % (in females) of total body length. The body depth was 16.25 % (in males) and 18.82 % (in females) of the total body length. The percentages of pre dorsal length, pre pectoral length, pre anal length and pre pelvic length for male were 23.85 %, 23.25 %, 34.09 % and 14.90 % while the percentages of pre dorsal length, pre pectoral length, pre anal length and pre pelvic length for female were 28.02 %, 27.41 %, 39.43 % and 17.27 %, respectively.

Table 2. Range, mean, and standard deviation values of morphometric characters of male and female for *Coilia dussumieri* during the present study.

Sir No.	Morphometric characters	Male (N=115)			Female (N= 315)		
		Min (cm)	Max (cm)	Mean±SD	Min (cm)	Max (cm)	Mean±SD
1	TL	8.6	16.1	13.29±1.27	8.3	16.5	11.64±1.98
2	SL	6.8	14.5	11.84±1.22	5.8	14.6	9.49±2.29
3	HL	1.5	2.6	1.98±0.21	1.1	2.4	1.98±0.24
4	ED	0.2	0.5	0.37±0.07	0.2	0.5	0.34±0.08
5	BD	1.6	2.7	2.16±0.28	1.3	2.8	2.19±0.29
6	PDL	2.2	5.2	3.17±0.39	2.1	4.1	3.26±0.41
7	PAL	3.2	5.6	4.53±0.52	2.8	5.8	4.59±0.64
8	PPL	2.1	4.2	3.09±0.41	2.1	4.2	3.19±0.42
9	PVL	0.9	2.7	1.98±0.28	0.9	2.4	2.01±0.28

Symbol: N=sample size, S.D = standard deviation

Table 3. Percentage of morphometric characteristics with respect to total length (TL) of male and female for *Coilia dussumieri* during the present study.

Morphometric Characteristics Percentage of Total length (TL)	Average (%)	
	Male	Female
HL (% TL)	14.90	17.01
ED (% TL)	2.78	2.92
BD (% TL)	16.25	18.81
PDL (% TL)	23.85	28.01
PAL (% TL)	34.09	39.43
PPL (% TL)	23.25	27.41
PVL (% TL)	14.90	17.27

Linear regression relationships between total body length (TL) and morphological characters of *Coilia dussumieri* during the present study.

In order to determine the linear regression relationships between total length (TL) and various morphometric characteristics, the total length (TL) was taken as X (Independent variable) and various morphometric measurements were taken as Y (dependent variables). The regression coefficient 'b' and the correlation coefficient 'r' of different variable characters (Y), including standard length, head length, dorsal fin length, pectoral fin length, anal fin length etc., on the total length (X) were described in Table 4.

The morphometric study of *Coilia dussumieri* for males indicated that a strong correlation ($r > 0.70$) exists between total length (TL) against standard length (SL), body depth (BD), and pre anal length (PAL). The moderate correlation ($r = 0.51$-0.69) was observed between total length (TL) against head length (HL), pre dorsal length (PDL), and pre pectoral length (PPL). And then, the weak correlation ($r < 0.50$) was found between total length (TL) and eye diameter (ED), and pre pelvic length (PVL), while the correlation between head length (HL) and eye diameter (ED) was also weak correlation ($r < 0.50$). The regression coefficient 'b' of total length against other morphometric characters ranged from 0.69 to 1.06. The relationship showed negative allometric growth except standard length and body depth. Moreover, the present findings of linear regression relationships between total length (TL) and all morphometric characters of males reveal insignificant correlations ($p > 0.05$) (Table 4).

The study of the morphometrics in females showed a strong correlation between total length (TL) and standard length (SL) ($r > 0.70$). And then the moderate correlation ($r = 0.51$-0.69) showed between total length (TL) and head length (HL), pre pectoral length (PPL), and pre pelvic length (PVL) while the correlation between head length (HL) and eye diameter (ED) was also moderate ($r = 0.51$-0.69). Furthermore, the weak correlation observed between total length (TL) against body depth (BD), eye diameter (ED), pre dorsal length (PDL), and pre anal length (PAL) ($r < 0.50$). The regression coefficient 'b' of total length against other morphometric characters ranged from 0.33 to 1.33. The relationship showed negative allometric growth except for standard length. Moreover, the present findings of linear regression relationships between total length (TL) and all morphometric characters of females reveal insignificant correlations ($p > 0.05$) (Table 4).

Table 4. Linear relationship between total body length (TL) and various morphometric characters of male and female for *Coilia dussumieri* during the present study.

Variables		Male					Female				
X	**Y**	**a**	**b**	**r**	**CT**	**p-value**	**a**	**b**	**r**	**CT**	**p-value.**
TL	SL	-0.12	1.06	0.95	***	0.49^{NS}	-0.33	1.33	0.95	***	0.49^{NS}
TL	HL	-0.51	0.72	0.66	**	0.49^{NS}	-0.20	0.47	0.58	**	0.45^{NS}
TL	ED	-0.36	0.82	0.45	*	0.44^{NS}	-1.01	0.54	0.42	*	0.16^{NS}
TL	BD	-0.80	1.01	0.74	***	0.49^{NS}	-0.01	0.33	0.39	*	0.44^{NS}
TL	PDL	-0.42	0.82	0.67	**	0.48^{NS}	0.13	0.36	0.46	*	0.43^{NS}
TL	PAL	-0.30	0.85	0.71	***	0.48^{NS}	0.20	0.43	0.49	*	0.46^{NS}
TL	PPL	-0.41	0.80	0.58	**	0.49^{NS}	0.07	0.44	0.51	**	0.44^{NS}
TL	PVL	-0.48	0.69	0.43	*	0.48^{NS}	-0.14	0.42	0.55	**	0.44^{NS}
HL	ED	-0.66	0.75	0.45	*	0.46^{NS}	-0.72	0.98	0.60	**	0.45^{NS}

Note: CT = correlation type; *** shows the strong correlation (r > 0.70); ** shows moderate correlation (r = 0.51-0.69); * represent weak correlation (r < 0.50); – shows negative correlation; ^{NS} shows insignificant correlation when p > 0.05; ^{a} shows significant correlation when p < 0.05 (Massod *et al*., 2024).

Length frequency distribution, length weight relationship and condition factor of *Coilia dussumieri* during the present study.

During the present study, a total of 430 *Coilia dussumieri* (315 males and 115 females) were examined. The total length ranged from 8.6 to 16.1 cm and weight from 1.89 to 8.9 g for males, and for females, the length ranged from 8.3 to 16.5 cm and weight from 1.74 to 7.5 g. Therefore, total length varied between 8.3 cm and 16.5 cm and body weight varied between 1.79 g and 8.9 g during the present period.

The variation of length frequency in the number of *C. dussumieri* recorded in different size groups is presented in figure 2 after grouping them at intervals of 1.7 cm. The maximum number (150) of fishes were found in 11.5 to 13.2 cm size group, followed by the 13.2 to 14.9 cm (148) sized group. The remaining fish are distributed from 8.1 to 9.8 cm (26), 14.9 to 16.6 cm (43), and 9.8 to 11.5 cm (63).

From the results, it is observed that weight of *C. dussumieri* bears a linear relationship with the length of a logarithmic transformation (Figure 3). The regression equation for length weight relation in males, females and combined was Log W = 2.2463 Log TL- 1.8184 (males), Log W = 1.3613 Log TL- 0.7649 (r =0.44652) (females), and Log W= 1.4917 Log TL- 0.9536 (combined). The value of the exponent ('b') for males was 2.25, for females it was 1.36, and for combined it was 1.49 (Table 4), and the exponent value was tested against '3' and was found to be negative allometric growth in fish. The length and weight measurements of the fish are related to each other with a high degree of correlation coefficient for males, r = 0.79, and females and combined showed a moderate degree of correlation coefficient, r = 0.69 (Table 4**).** Moreover, the condition factor of *C. dussumieri* was observed; 0.221 for males, 0.690 for females and 0.252 for combined was presented in Table 5.

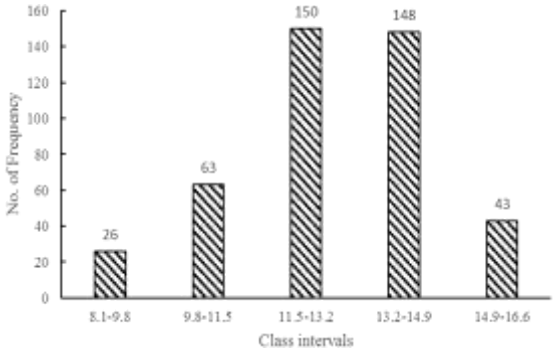

Figure 2. Length frequency distribution of *C. dussumieri* during the present study (Combined).

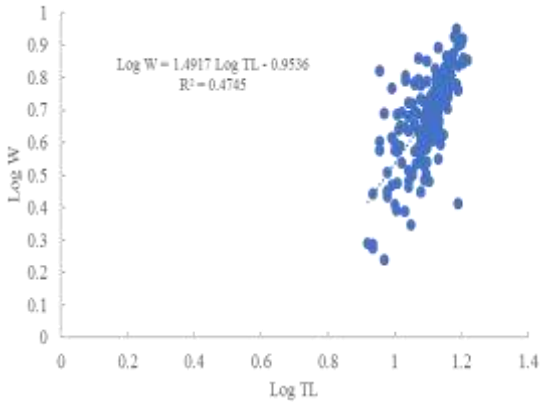

Figure3. Length weight relationship of *C. dussumieri* during the present study (Combined).

Table 5: Length weight relationship equation and "K" value of *Coilia dussumieri* during the present study.

Sex	Regression Equation	"b" value	K value	r
Male	Log W= 2.2463 Log TL- 1.8184	2.25	0.221	0.79
Female	Log W = 1.3613 Log TL- 0.7649	1.36	0.690	0.69
Combined	Log W = 1.4917 Log TL- 0.9536	1.49	0.252	0.69

Disccusion

The morphometric measurement of a species is important in fisheries management activities. In the present study, the maximum values of morphometric characters of males were lower than those of females. The value of 'b' = 1.0 shows an isometric pattern of growth between length to length of various parts of fish's body, b > 1 shows allometric growth (positive), while b < 1 shows allometric growth (negative) (Khalid and Naeem, 2017). In the present study, the regression coefficient 'b' ranged from 0.69 to 1.06 (males) and 0.33 to 1.33 (females).

During the present study, HL, ED, PDL, PAL, PPL and PVL (for males) showed 'b' value less than unity, with TL representing negative allometric growth, while isometric growth was indicated in SL and BD. For females, the negative allometric growth was observed in HL, ED, BD, PDL, PAL, PPL and PVL with TL. SL indicated 'b' value more than unity, with TL, representing positive allometric growth, while ED with HL was found to have isometric growth because the value of b (0.98) was very close to 1.0. Therefore, the overall present findings of morphometric characters showed a negative growth pattern, revealing the indirect relationship between total body length and growth of various body parts of this species.

Moreover, the overall findings of linear regression relationships between total body length (TL) and all morphometric characters of males and females of *Coilia dussumieri* revealed weak or moderate correlations (r < 0.70) except the standard length (SL) that was accessible, a very strong type of correlation. According to the present results, there were slight variations and no significant differences (t-test, p > 0.05) in all morphometric characters between males and females of *C. dussumieri*. However, Dhanya *et al*., (2004) reported that the linear regression

relationships between total body length (TL) and morphometric characters showed positive correlation with high values of correlation coefficient (r).

The study of morphological characters of fishes is reasonably necessary for detecting

the taxonomic classification of a genus or species and finding the differences between geographically variant populations (Masood *et al*., 2024). Therefore, the present findings of morphological characters of *C. dussumieri* supports the taxonomic classification of species in the Mon Coastal Areas.

During the present study, the head length of *C. dussumieri* was 15.14 % (in males) and 17.01 % (in females) of total body length. In the findings of Mahapatra *et al*. (2015), the head length of *C. dussumieri* was 15.14% of total body length, which was similar to the present finding.

The regression coefficient between total length and weight helps to understand the type of growth. Fish showing ideal growth, having the value of 'b' lies between 2 and 4, mostly close to 3. The b = 3 value of b above or below 3 shows positive and negative allometric growth. In the case of positive allometric growth (b greater than 3), a fish becomes heavier for its length as the fish increases in size, while negative allometric growth (b less than 3) indicates that the fish becomes lighter for its length as it grows (Khalid and Naeem, 2017).

In the present study, the value of "b" was less than 3, showing a negative allometric growth pattern. The calculated value 'b' (1.36-2.25) in length weight relationship of the present study closely matched the reported value of 'b' from other findings on *C. dussumieri* by Su Su Hlaing (2016) and Zarni Ko Ko (2021) from the landing centers of Mon Coast. Moreover, the negative allometric growth of this fish was also observed in previous findings, such as Fernandez and Devaraj (1988), Dhanya, *et al*., (2004), Shingadia (2014), Mahapatra *et al*., (2015), and Uddin and Ghosh (2021). Nevertheless, Bal and Joshi (1956) reported the positive allometric growth of *C. dussumieri* from the Sassoon Dock and Danda landing centers, Bombay.

When the condition factor of a fish is equal to or greater than 1, it means that the fish is healthy, thus its health is in a good state, but if the value of K is less than 1, it implies a poor health state and the fish is unhealthy (Jin *et al*., 2015). In the present study, the condition factor of *C. dussumieri* was calculated as 0.69 (for males) and 0.79 (for females and combined), indicating the poor condition of fish. The present findings are similar to the previous results; Dhanya, *et al*., (2004), Shingadia (2014), Mahapatra *et al*., (2015), and Uddin and Ghosh (2021). However, Zarni Ko Ko (2021) reported the K value of fish was greater than 1, so that their health is in good condition. Condition factor (K) is a short-term indicator of fish health status, and the variation in K values depends on many factors such as changes in food availability, gonadal development, and other environmental parameters (Pope and Willis, 1996). However, no such changes were observed in the present investigation. Therefore, the low values in condition factor reported in the current study may be attributed to various factors such as time of the year, stages of maturity and stomach contents.

Conclusion

This is the first report on the morphometric and length weight analysis of *Coilia dussumieri* from Ye Estuary. The present study indicates negative allometric and poor growth of the fish. The results of this study would be helpful for ichthyologists and taxonomists to compare

morphometric relationships of *C. dussumieri*. The result of the current study will be useful to researchers and policy planners. Moreover, further studies on the biology and stock assessment of *C. dussumieri* should be conducted in Ye Estuary.

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VERTICAL DISTRIBUTION OF HEAVY METALS IN MUDFLAT AND MANGROVE SEDIMENTS OF BILU ISLAND, MYANMAR

Ei Thinzar Phyo[1], Nyo Nyo Tun[2], Kyaw Thura[3]

Abstract

Mangroves are examples of coastal blue carbon ecosystems that play significant roles in the environment. This study measured the concentrations of heavy metals (Cd, Cr, Pb, and Cu) using profile samples from two different habitats: mangrove and mudflat. The study was conducted on Bilu Island in Mon State, Myanmar. Findings from this study demonstrated that the top 20 cm of the mangrove sediment profile had higher concentrations of heavy metals compared to the mudflat sites. This research suggests that coastal blue carbon ecosystems are effective at retaining trace elements, which can help to mitigate the negative effects of heavy metals on marine environments.

Introduction

Mangrove forests are tropical or subtropical habitats defined by the presence of plant species that have adapted to high temperatures, high levels of organic matter, and high salinity. These ecosystems also protect coastlines from the effects of tides and waves, and serve as nurseries for many species of birds, fish, and shellfish. They also support a complex community of saltwater benthic organisms and provide a significant portion of the fish, shellfish, and crustaceans that coastal communities consume or sell (Lee et al., 2014).

Globally, mangrove sediments have been shown to have elevated concentrations of heavy metals, which often indicates long-term pollution from human activity (Lacerda et al., 1992; Perdomo et al., 1998). Due to their toxicity, persistence, and bioaccumulation potential, heavy metals are among the most dangerous pollutants in the natural environment (MacFarlane and Burchett, 2000). The exceptional ability of mangrove muds to retain contaminants released into the near-shore marine environment is a result of their natural physical and chemical characteristics (Harbison, 1986). Mangrove sediments are rich in organic matter and sulfides, and they are also reduced and anaerobic, which is conducive to the retention of heavy metals in the sediment (Silva et al., 1990; Tam and Wong, 2000). The concentrations of heavy metals in sediments are typically three to five orders of magnitude higher than those in the surrounding water (Zachetoglou et al., 2002). Because of these high concentrations, the bioavailability of even a small portion of the total metal content in the sediment is crucial for the bioaccumulation of metals in mangrove-dwelling animal and plant species. Heavy metals are not biodegradable, so they are taken up and concentrated in plant tissues from the soil, causing long-term harm to plants (Defew et al., 2005).

Cadmium is a hazardous heavy metal that builds up in living organisms. Workers in the electroplating, battery manufacturing, and pigment industries are most at risk for exposure, and elevated blood and urine cadmium levels have been reported in these populations. Due to its long half-life, cadmium bioaccumulates in aquatic invertebrates, vertebrates, and plants. In addition to being found naturally—volcanoes and rock weathering are two processes that mobilize cadmium from the earth's crust—anthropogenic activities can also release cadmium into the environment. These activities include the disposal of food and agricultural wastes, urban refuse, municipal sewage sludge, various organic wastes, and atmospheric fallout (WHO, 2003; Akguc et al., 2008; Sabiha-Javied et al., 2009; Osma et al., 2012; Ozyigit et al., 2016).

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Because of its widespread industrial use, chromium is a common metal contaminating groundwater, soil, and sediments, which presents a severe environmental risk. When Cr builds up in plants, it can cause structural alterations and is highly toxic, leading to reduced growth and biomass accumulation. Certain plant species can accumulate large amounts of Cr without experiencing harm. According to ATSDR (1998), Cr is a naturally occurring element found in rocks, soil, vegetation, animals, and volcanic dust and gases. Concentrations in most finfish species ranged from 0.1 to 0.6 mg Cr/kg fresh weight (Hall et al., 1978).

This research aims to determine the concentrations of specific heavy metals [cadmium (Cd), chromium (Cr), copper (Cu), and lead (Pb)] and to compare their concentrations in mangrove and mudflat sediments across different sediment depths.

Materials and Methods

Study Area

The present research was conducted at Sepala and Nathmaw in the Mon Coastal Region (Figure 1). The sampling sites are located in the estuary where freshwater runs off from the Thanlwin River and encounters the semi-diurnal tide.

Sample Collection

The soil samples were collected from the mangrove and mudflat areas of Nathmaw and Sepala villages (Bilu Island) in July 2022. The sampling methods will be adopted from the methods by Foruquean *et al.* (2014). The PVC (poly vinyl chloride) pipes with a length of 50 cm were used for sampling and labeled with the types of Core ID and locations systematically. Triplicate samplings were carried out for each habitat (mudflat or mangrove). After that, the collected cores were wrapped with aluminum foil to reduce gaseous exchanges and then carried to Marine Science Department at Mawlamyine University. The cores were kept in the ice box before sample preparation.

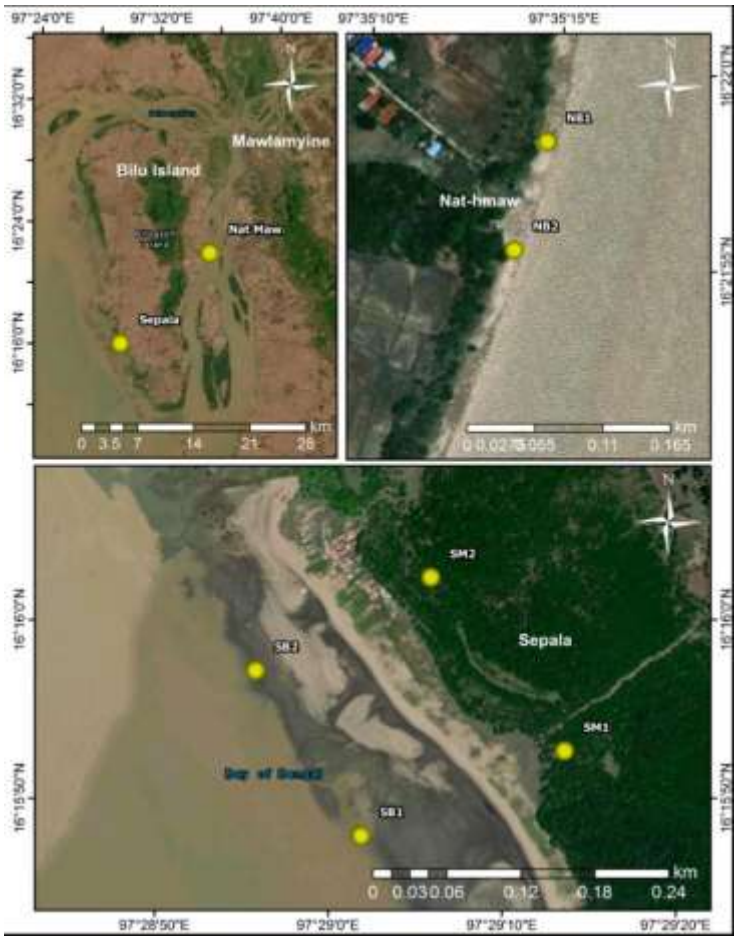

Figure 1. Map showing the location of the study area in Bilu Island and sampling sites. SM: Sepala Mangrove Soils, SB: Sepala Mudflat Soils and NB: Sepala Mudflat Soils

Sample preparation

The collected PVC cores will be halved lengthwise using an electric saw. The soil samples were sectioned at 4 cm intervals with a blade and labelled with specific depth and Core ID. The wet samples were weighed using a precise electronic balance. The weighed samples will be dried in the oven at 60ºC. This cycle will be repeated until successive weight differences are less than 4% (always use the same balance). Typically, this process requires at least 48–72 hours. The weights were recorded every 24 hours.

Soil properties and heavy metal analysis

The physical properties of the soil samples and soil texture, were determined using the hydrometer method (Bouyoucos, 1962). For sediment chemical properties: soil pH and salinity at each depth will be determined by using the electrode pH meter (Eutech Instruments pH 700, Massachusetts USA) by using the method of Tan (2005) and a refractometer. Five grams of oven-dried soil were mixed with 25 mL of distilled water (1:5).

The heavy metal concentrations were determined using the Atomic Absorption Spectrophotometer (AAS) method. To extract the heavy metals from the samples before being analyzed in AAS, inorganic acids: hydrochloric acid (HCl) and nitric acid (HNO_3) were used. The AAS method was used because there are no spectral and ionization interferences.

The heavy metals analysis was carried out immediately on the consequence digests using atomic absorption spectrophotometer (AAS), with the use of prepared standards to determine the sample concentrations. To ensure the accuracy of AAS results, three replicates of each sample were run to ensure that measured absorbencies were consistent. These metal concentrations were calculated from each replicate absorbance value, which was then used to calculate an average

sample metal concentration. For mangrove and mudflat samples, four metals were analysed by using AAS: Cd, Cr, Cu and Pb. Heavy metal concentrations were expressed by ppm (mg/L) for each sample and an average was calculated from the three replicates.

Results

Physical and Chemical Properties in Soils

In this study, four cores were taken at Sepala: two from mangrove soils (SM1 and SM2) and the other two from mudflat soils (SB1 and SB2). At the other study area, Nathmaw, two cores were collected from mudflat soils (NB1 and NB2). The pH values from the study area are shown in Figure 2. A comparison of pH values in mangrove and mudflat soils at both sites showed that the highest value was 8.8 at a depth of 0-4 cm in mudflat soil at Sepala, while the lowest value was 8.09 at a depth of 0-4 cm at Nathmaw. Thus, the pH value at Sepala was consistently higher than that at Nathmaw (Figure 2).

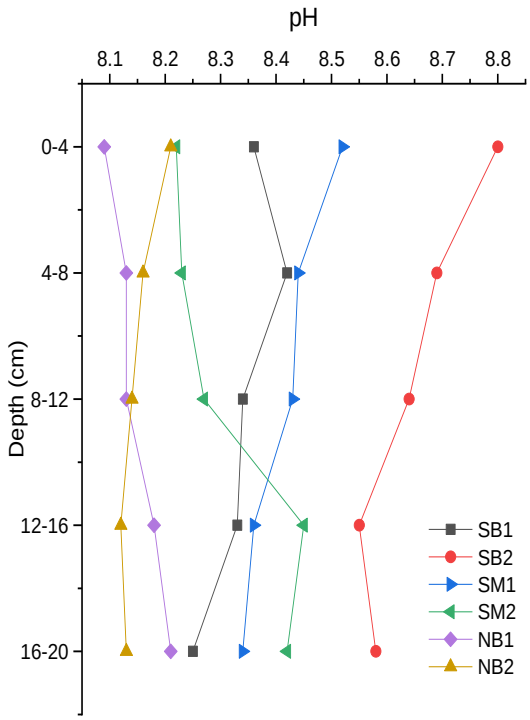

Figure 2. The comparison of pH at different depths of Sepala and Nathmaw in the soils

Heavy Metals Accumulation in Mangrove and Mudflat Soils

In this study, six cores were taken from Sepala and Nathmaw which are 2 cores from mangrove soils (SM1 and SM2) and the other 4 cores from mudflat soils (SB1, SB2, NB1 and NB2). Some heavy metals (Cd, Cr, Cu and Pb) concentrations were shown in Figure 4.2a-d. When Cd was taken into consideration at Sepala, the maximum value was found (0.025 mg/L) at 8-12 cm depth of mangrove soil and the minimum content was found (0.012 mg/L) at 4-8 cm depth in mudflat soil. The concentrations of Cd at 0-4 cm and 12-20 cm were nearly the same. The concentration of Cd is decreased more at the surface than near the deeper part of the surface. For the surface layer, Cd content in each point of the mangrove soils was found to be higher than that of the mudflat soils. When Cd was considered at Nathmaw, the maximum content was found at the depth (0.041 mg/L) in the surface layer of 0-4 cm at mudflat soil and the minimum value is (0.025 mg/L) in 4-8 cm depth at mudflat soil. The second highest value of Cd is 0.029 mg/L at 16-20 cm depth in the mangrove soil. The concentration of Cd is increased at the surface (0-4

cm) of the core sample when compared with that from 16-20 cm depth. The concentration of Cd at 0-4 cm and 8-16 cm were almost the same.

In the cases of mangrove and mudflat soils at Sepapla (Figure 3b), Cr content was found to have the highest value (0.344 mg/L) in 4-8 cm depth at mangrove soil and the lowest value (-0.074 mg/L) in 0-4 cm depth of mangrove soil. At Nathmaw, the maximum value of Cr was found to be (0.164 mg/L) in 12-16 cm depth and the minimum value is (-0.171 mg/L) in 0-4 cm depth of mudflat soils. In some areas, the presence of chromium contents is almost non-existent, as it showed negative signs (e.g. -0.034, -0.036 mg/L) and so on.

In the case of Sepala, the maximum content of Pb was found to be (1.646 mg/L) in the surface layer of 0-4 cm depth at mangrove soil and the minimum level is (0.013 mg/L) in 0-4 cm depth of mudflat soil. In the deeper layer of the core, values for Pb content ranged from 0.012 to 0.014 mg/L and 0.020 to 0.023 mg/L in 0 – 12 cm depth of mangrove and mudflat soils respectively (Figure 3). While it is compared with the mangrove soil, every layer of Pd concentration in the mangrove soils is found higher than that of the mudflat areas.

In the study area, the concentration of Cu is higher than that of Pb, Cr and Cd. According to Figure 3d, it is noticed that in the case of mangrove soil, the maximum value of Cu was found to be (1.747 mg/L) in the depth of 4-8 cm and the minimum value is (0.006 mg/L) in 12-16 cm depth of mudflat soil at Sepala. Copper concentration is higher in a depth of 16-20 cm (3.471 mg/L) and the lowest value is 0.120 mg/L in 12-16 cm depth in mudflat soils. The values for Cu content ranged from 0.006 to 0.020 mg/L in 0-12 cm depth, from 0.041 to 0.096 mg/L and in 12-20 cm depth, the range from 0.180 to 0.476 mg/L 0-12 cm depth and 12-20 cm depth are found 0.120 to 0.171 mg/L, respectively (Figure 3).

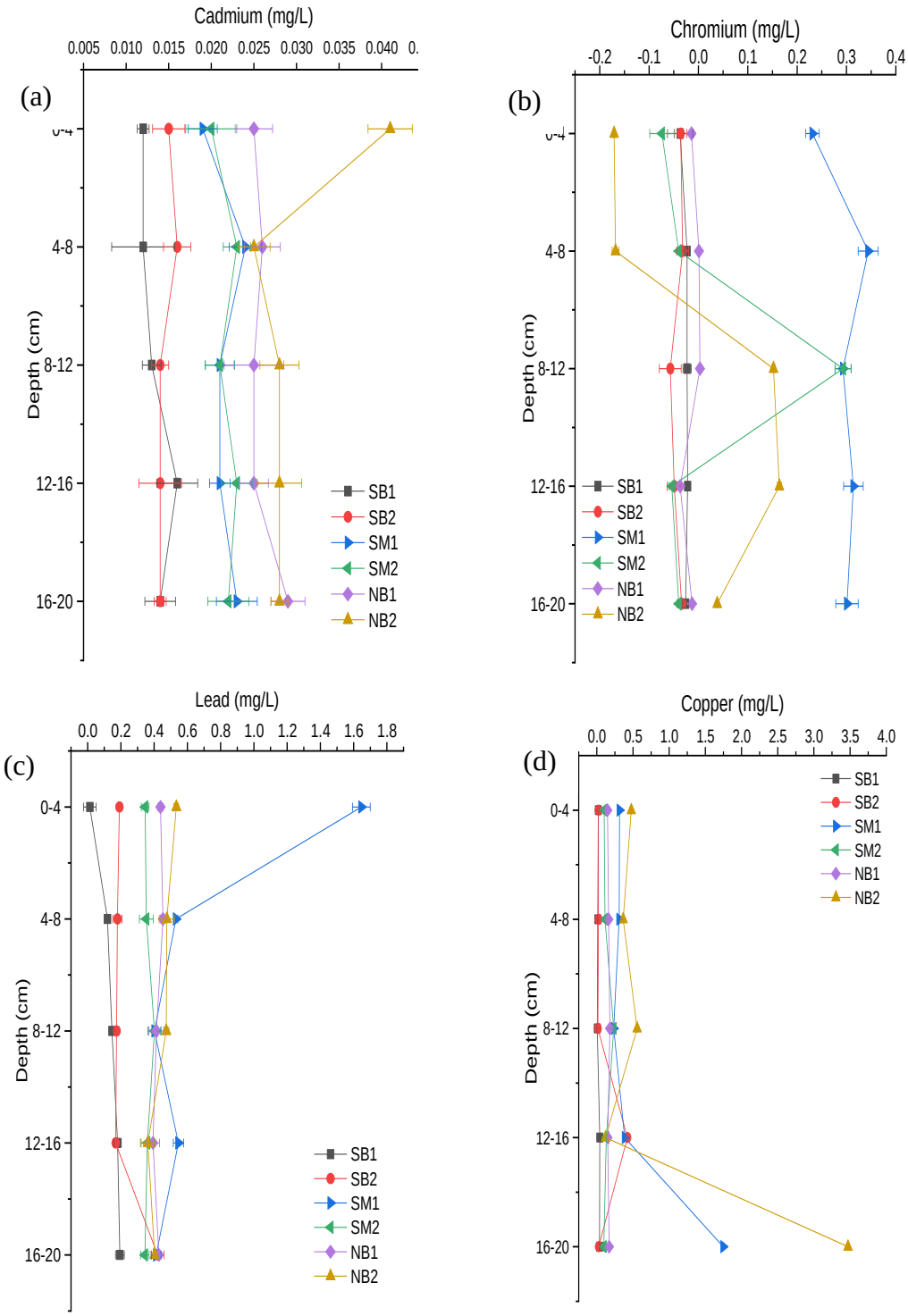

Figure 3. Comparison of heavy metals: a) Cadmium, b) Chromium, c) Lead and d) Copper in different depth intervals of mangrove and mudflat soils

Discussion

Generally, soil pH is one of the main parameters for determining the extent of pollution, as it has been identified as a principal factor affecting the mobility and availability of metals in soil (Inobeme et al., 2014). When comparing the pH range at Sepala and Nathmaw, Sepala had a higher pH value (8.8 > 8.09) than Nathmaw, indicating neutral to slightly alkaline soils.

The Cd concentration in every layer of mangrove soil was found to be higher than that of the mudflat soil. The highest Cd concentration was 0.041 mg/L, observed at a 12-16 cm depth in mudflat soil, while the lowest was 0.015 mg/L at a 12-16 cm depth of mudflat soil. In all samples from both mangrove and mudflat soils, Cd content exceeded the maximum permissible limit (0.0008 mg/L) recommended by WHO (1996).

The Cr concentration was higher in every layer of mangrove soil compared to mudflat soil, with the exception of the 4-8 cm depth, which had a value (0.344 mg/L) higher than the corresponding value in mudflat soil. The Cr content in all samples from both mangrove and mudflat soils did not exceed the maximum permissible limit (0.1 mg/L) recommended by WHO (1996).

When comparing mangrove and mudflat soils, Pb concentrations in mangrove soil were higher in every layer than in mudflat soil, with the exception of the 0-4 cm depth, which had a value (1.646 mg/L) higher than the corresponding mudflat soil value (0.013 mg/L). Contrary to the permissible limit, the Pb concentration in the 0-4 cm mangrove soil layer (1.646 mg/L) exceeded the WHO (1996) limit of 0.085 mg/L.

When comparing mangrove and mudflat soils, Cu concentration in the mudflat soil was higher in every layer than in the mangrove soil, except for the 16-20 cm depth, where the mudflat soil value (3.471 mg/L) was higher than the mangrove soil value (1.747 mg/L). In this study, Cu concentrations in both mangrove and mudflat soils exceeded the WHO (1996) permissible limit of 0.036 mg/L in all layers.

In comparison to unvegetated mudflats, the present findings showed that mangrove sediments accumulated comparatively higher concentrations of most of the heavy metals. The higher accumulation of heavy metals by blue carbon ecosystems is due to their increased carbon sequestration rate, which is essential for heavy metal retention. Due to their capacity to disperse waves and capture particles from the water column, mangroves and saltmarshes have been shown to increase the organic matter content in sediments and maintain greater carbon stocks than terrestrial ecosystems (Duarte et al. 2013). As a result, they are particularly effective at sequestering heavy metals in sediments (Zhou et al., 2011).

Conclusion

This study provides a baseline record of heavy metal concentrations in the sediment profiles of the region's blue carbon ecosystems. The present findings showed that the presence of mangroves in the coastal area is associated with significantly increased concentrations of most of the heavy metal components. The accumulation of Cd, Cr, Pb, and Cu toward the surface of both mangrove and mudflat sediments was likely accelerated by anthropogenic activity. While some heavy metals like Cu, Cd, and Pb exceeded the WHO permissible limits in certain layers, the findings from our quantitative contamination assessments suggest that the soils were not heavily contaminated throughout the entire profile.

Acknowledgements

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MANGROVE STATUS OF SETSE, MON STATE

Nway Thiri Htun1[1] ,Htway Thiri Htun[2], Kyaw Thura[3]

Abstract

This study recorded a total of 18 mangrove species, comprising 12 true mangrove species and 6 mangrove associates. In terms of relative dominance, *Avicennia alba* and *A. officinalis* were the most dominant species across all transect lines. *Avicennia alba* had the highest importance value index (IVI) at 145.22, while *Sonneratia apetala* had the lowest IVI value at 1.57. The application of Google Earth Pro was used to assess the loss and recovery status of the mangrove area from 2011 to 2023. According to historical images from Google Earth Pro, 3.51 acres (2011-2014), 13.37 acres (2014-2017), 0.65 acres (2017-2019), and 7.74 acres (2019-2023) were recovered, while 3.73 acres (2017-2019) and 2.54 acres (2019-2023) were lost.

Keywords: relative dominance, loss and recovered status

Introduction

Mangroves are a group of trees and shrubs belonging to several families of flowering plants (Santisuk, 1983). Mangroves host a large array of associated floral and faunal communities by providing food, habitats, and shelter for their survival (Spalding *et al*., 2010).

Myanmar has a high mangrove diversity, with 44 species of true mangrove plants. It holds the 8th largest mangrove forest in the world (4% of the world's mangroves) and stands 3rd largest in Southeast Asia (8.8% of Southeast Asia's mangroves) (Spalding *et al*., 2010). Myanmar's mangrove forests have been depleted and degraded over the last few decades due to urbanization, charcoal production, agricultural expansion, and conversion to fishponds, shrimp ponds, and rice paddies (Zockler and Cherry Aung, 2019).

Globally, several studies on mangrove identification and diversity have been conducted. However, there have been few studies in Myanmar. Although mangroves are distributed in Mon State, their ecological roles are unclear. Setse is located in the southern part of Mon State and is the location of the Marine Science Aquaculture Research Centre (MSARC). There were no prior studies on the status of mangrove forests in Setse. To address this gap, the present study was carried out with the following objectives: (1) to evaluate the relative dominance of the forest and (2) to compare the status of mangrove forests in 2011, 2014, 2017, 2019, and 2023.

Materials and Methods

The study area

The study was conducted in the mangrove areas of Setse (Lat. 15° 56′ N and Long. 97° 35′ E), Thanbyuzayat Township, Mon State (Figure 1). It is located in the southern part of Mon State, in the southern region of Myanmar. The total mangrove area is approximately 60 hectares (148.26 acres). It is adjacent to Damin-Seik Village. This mangrove area experiences semi-diurnal tides (two high tides and two low tides), with a tidal range of 4 m from the bottom of the tidal creek.

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Figure 1. Map showing the study area

Methodology

The present study area was divided into three areas (Area 1, Area 2, and Area 3) (Figure 3.1). Within the present study site, 13 transect lines (TLs) were established perpendicular to the tidal creek (6 transect lines in Area 1, 7 transect lines in Area 2, and no transect line in Area 3). There were no mangroves and reservoirs were present for grow-out farms in Area 3. Hence, sampling cannot be done in Area 3. Using a transect tape, 100-meter-long transect lines were established, with 100 meters between transect lines during the fieldwork. Five quadrats were plotted on each transect line, each plot having an area of 100 m^2 (10 x 10), with 5 m intervals among them (totally of 65 quadrats in the whole study area). The length of the transect lines was recorded using the transect tape, and the coordinates of each transect line were taken by the Global Positioning System (GPS).

Data Collection and species identification in the study area

The mangrove species and associate species within each quadrat were recorded. All specimens were photographed digitally in color and recorded. For species composition purposes, the plants were identified to species level if possible, during the fieldwork following Giesen *et al*. (2007), Reddy (2008) and Primavera (2009).

Determination of relative dominance

To understand the situations in Setse mangrove forest and how many areas of the tree were occupied in each area during the current study, the basal area, total basal area, and relative dominance were approached using the following equations (Aheto *et al*., 2011).

$$\text{Basal Area } (m^2) = \frac{\pi}{4} (DBH)^2$$

$$\text{Total basal area of all species } (m^2\,ha^{-1}) = \frac{\text{sum of all species basal area}}{\text{area of plot } (m^2)} \text{ x } 10000 m^2$$

**Acanthus ilicifolius* was not considered for basal area because of shrub type.

Where DBH is the diameter at breast height. The breast height of the tree will be measured at 1.3 m of stem height from the bottom without buttress and at 0.3 m with buttress. To calculate the DBH of a tree, girth breast height (GBH) is divided by π (pi). Then, to calculate the relative dominance, the following equation will be used:

$$\text{Relative dominance (\%)} = \frac{\text{total basal area of a species}}{\text{basal area of all species}} \text{ x } 100$$

Importance value index (IVI) in the study area

The importance value index (IVI) indicates the ecological importance of a species in a specified ecosystem, which can be used for species conservation and management, through which species with a low IVI value require soar protection preference (Kacholi, 2013). The importance value index (IVI) for the species was determined as the sum of the relative frequency, relative density, and relative dominance using the formulas presented by English *et al*. (1997).

Density (stems ha^{-1}) = (no. of living stems in a plot× 10000) / area of the plot

RD_1 = (density of a species / total density of all species) × 100

RF = (frequency of a species / total frequency of all species) × 100

RD_2 = (total basal area of a species / total basal area of all species) × 100

$$IVI = RD_1 + RF + RD_2$$

Where RD_1 is relative density, RF is relative frequency and RD_2 is relative dominance

Mapping the degradation and recovered status of mangrove areas in the study area

The degradation and recovered status of the mangrove areas in 2011, 2014, 2017, 2019, and 2023 using Google Earth Pro. The historical images within the time zone were used to analyze their status.

Data analysis

The necessary figures were made in Microsoft Excel. For the variations in mangrove status of Setse in 2011, 2014, 2017, 2019, and 2023, Google ArcMap and Earth Pro were used.

Results

Species Composition

A total of **18 mangrove species** were identified within the transect lines, consisting of 12 true mangrove species and 6 mangrove associates, belonging to 11 different families (Table 1).

Table 1. Taxonomic classification list of the identified true mangrove and mangrove associates observed in the study area

Family	Genus	No.	Species	Common name	Local name
True Mangroves					
Acanthaceae	*Acanthus*	1	*Aanthus ilicifolius*	Sea holly	Kayar
Avicenniaceae	*Avicennia*	2	*Avicennia alba*	Gray mangrove	Thame-ywet-chon
		3	*A. officinalis*	Gray mangrove	Thame-ywet-wine
		4	*A. marina*	Gray mangrove	Thame-ywet-leit
Plumbaginaceae	*Aegialitis*	5	*Aegialitis rotundifolia*	Mangrove swamp	Sar-byat
Primulaceae	*Aegiceras*	6	*Aegiceras corniculatum*	River mangrove	Yae-kayar
Rhizophoraceae	*Bruguiera*	7	*Bruguiera cylindrica*	White Burma mangrove	Byu-kyet-tet
	Ceriops	8	*Ceriops decandra*	Flat-leaved spurred mangrove	Ma-da-ma
	Rhizophora	9	*Rhizophora apiculata*	Tall-silt mangrove	Byu-chay-dauk-ywet-chon

Family	Genus	No.	Species	Common name	Local name
Euphorbiaceae	*Excoecaria*	10	*Excoecaria agallocha*	Blinding tree	Thayaw
Sonneratiaceae	*Sonneratia*	11	*Sonneratia apetala*	Mangrove apple	Kant-ba-lar
		12	*S. alba*	Mangrove apple	La-mu
Mangrove Associates					
Verbanaceae	*Clerodendrum*	1	*Clerodendrum inerme*	The glory bower	Pinle-kyaung-pan
Fabaceae	*Derris*	2	*Derris trifoliata*	Common derris	Home-nwe
	Dalbergia	3	*Dalbergia spinosa*	Thorny liana	Byeik-su
	Pongamia	4	*Pongamia pinnata*	Indian beech	Thinn-win
Asteraceae	*Pluchea*	5	*Pluchea indica*	Indian pluchea	Kha-yu
Malvaceae	*Hibiscus*	6	*Hibiscus tiliaceus*	Sea hibiscus	Shaw-pin

Relative dominance of mangroves in the study area

Based on the basal area, the highest relative dominance values of *Avicennia officinalis* (70.57% in TL2, 47.49% in TL3, 44.22% in TL4, 70.10% in TL5, 77.11% in TL6) were estimated followed by *Avicennia alba*, *Excoecaria agallocha* and *Sonneratia alba* in Area 1 (Figure 2).

According to the result of relative dominance, the most common species was *Avicennia alba* (64.25% in TL 7, 83.13% in TL 8, 69.09% in TL 9, 52.47% in TL 10, 79.65% in TL 11, 80.38% in TL 12, and 83.66% in TL 13), followed by *Avicennia officinalis* and *A. marina* in Area 2 (Figure 3).

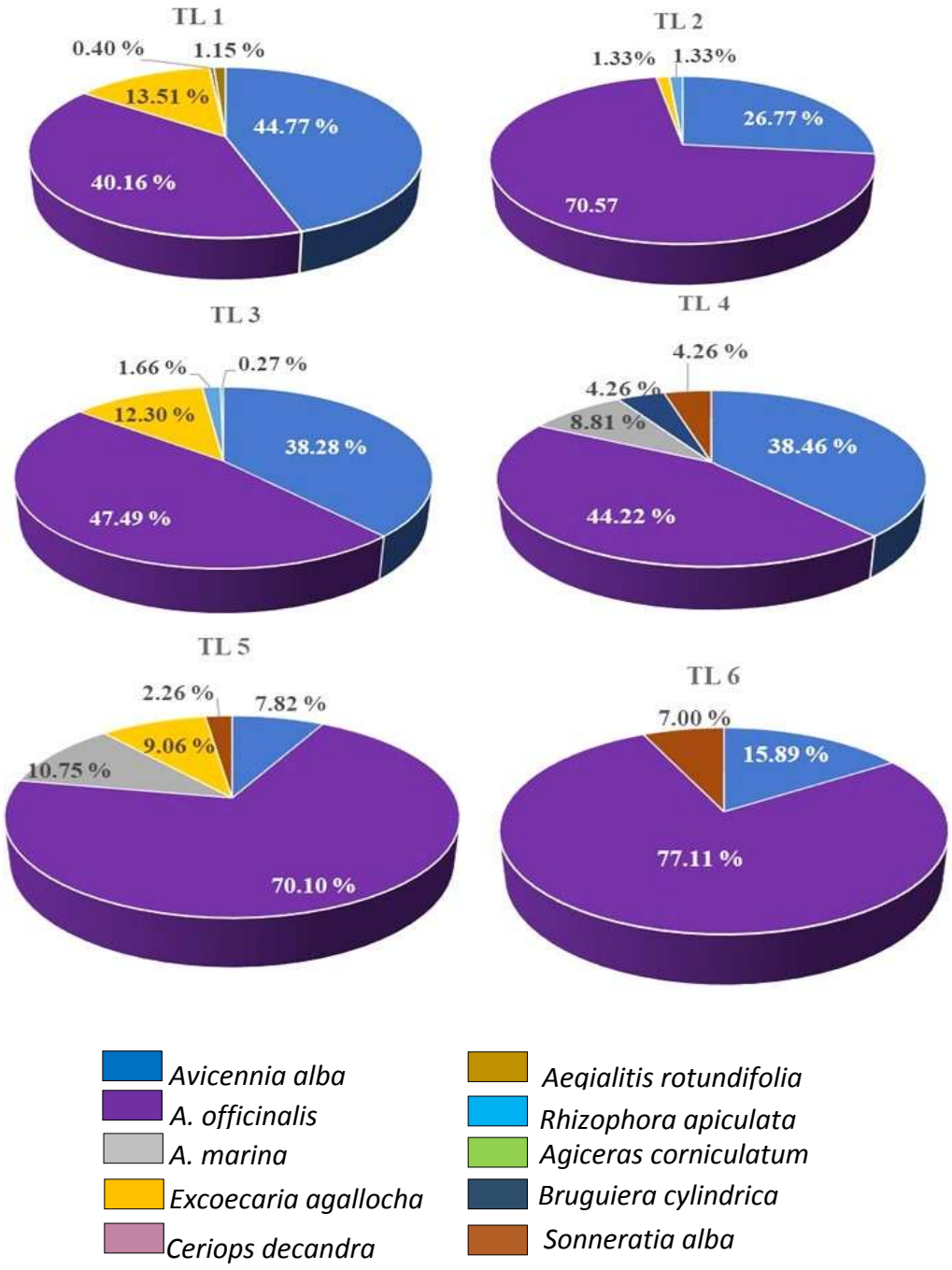

Figure 2. Relative dominance of each species found in different transect lines within Area 1

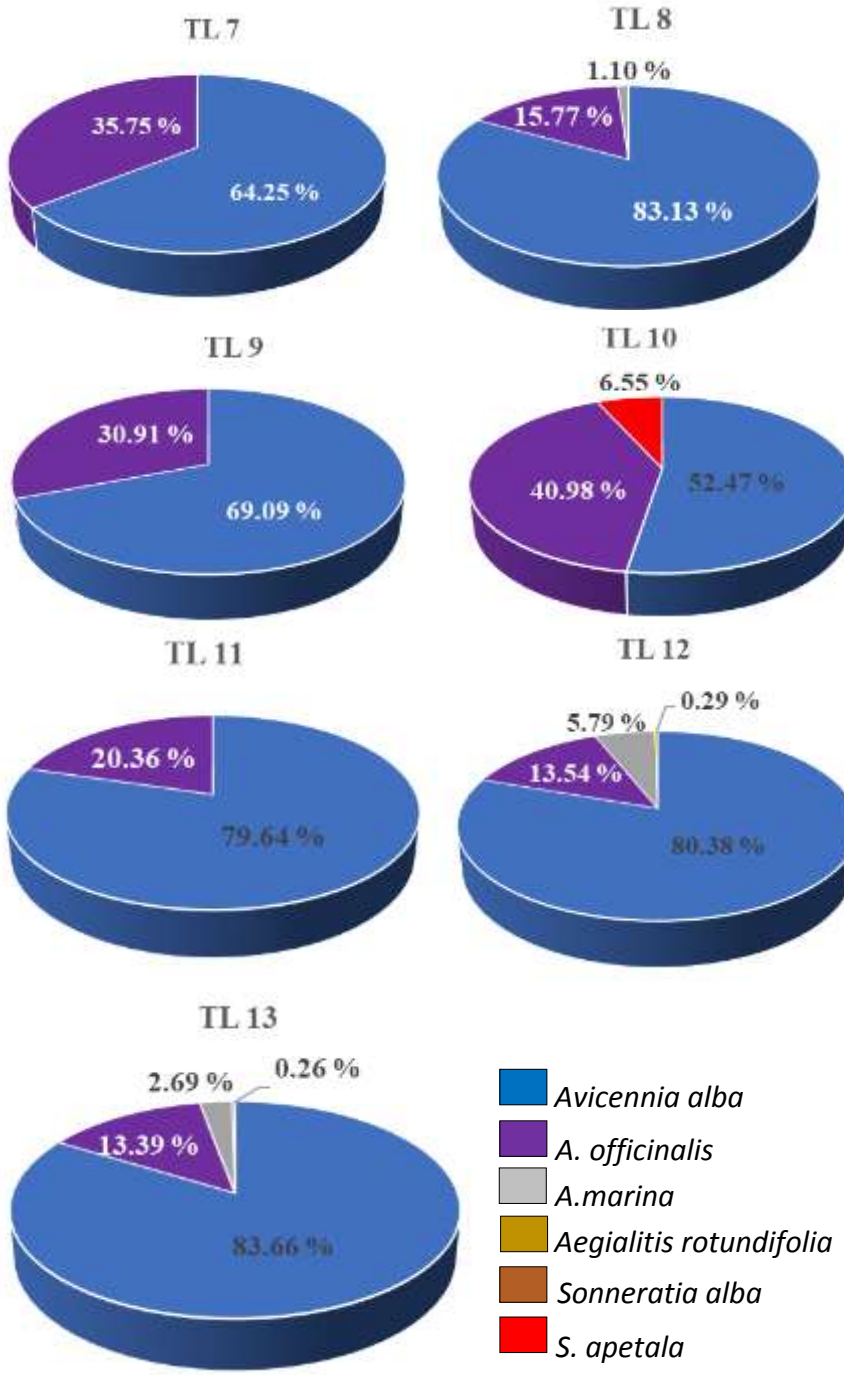

Figure 3. Relative dominance of each species found in different transect lines within Area2

Importance value index (IVI) in the study area

The density values showed that *Avicennia alba* has the highest stem density (532.31 stems ha^{-1}) among 12 true mangrove species, followed by *A. officinalis* (329.23 stems ha^{-1}), while the lowest stem density was recorded for *Aegiceras corniculatum* (1.54 stems ha^{-1}). The IVI results revealed that *A. alba* has the highest IVI value (145.22), followed by *A. officinalis* (111.62), while the lowest value was for *Sonneratia apetala* (1.57), followed by *Bruguiera cylindrica* (1.91). Table 2 shows the results of the density, relative density, relative frequency, relative dominance, and importance value index (IVI) of mangrove species for each category.

Table 2. Mangrove density (stems ha^{-1}) and importance value index (IVI) of trees in the study area

No.	Species name	Density (stems ha^{-1})	Relative density	Relative frequency	Relative dominance	IVI (%)
1	*Avicennia alba*	532.31	55.72	37.65	51.85	145.22
2	*A.officinalis*	329.23	34.46	37.65	39.51	111.62
3	*A.marina*	30.77	3.22	6.79	2.23	12.24
4	*Excoecaria agallocha*	29.23	3.06	1.23	4.09	8.38
5	*Rhizophora apiculata*	3.08	0.32	4.32	0.21	4.85
6	*Aegiceras corniculatum*	1.54	0.16	2.47	0.02	2.65
7	*Bruguiera cylindrica*	3.08	0.32	1.23	0.35	1.91
8	*Sonneratia apetala*	4.62	0.48	0.62	0.47	1.57
9	*S. alba*	9.23	0.97	1.23	0.95	3.15
10	*Ceriop decandra*	3.08	0.32	3.70	0.08	4.10
11	*Aegialitis rotundifolia*	9.23	0.97	3.09	0.24	4.29

Loss and recovered status of mangrove areas from 2011 to 2023 in the study area

An analysis of changes in mangrove areas was carried out from 2011 to 2023 using historical images from Google Earth Pro. From 2011 to 2014 and from 2014 to 2017, 3.51 and 13.37 acres were recovered. The trend of the recovered area continued from 2017 to 2019 and from 2019 to 2023, with an area of 0.65 and 7.74 acres, respectively. Meanwhile, loss of mangrove areas occurred in 2017–2019 and 2019-2023, with an area of 3.73 and 2.54 acres, respectively (Figure 4) (Table 3).

Table 3. Loss and recovered status of mangrove areas from 2011 to 2023

Year	Recovered (acres)	Loss (acres)
2011-2014	3.51	-
2014-2017	13.37	-
2017-2019	0.65	3.73
2019-2023	7.74	2.54

means Loss of mangroves and means Recovered mangrove

Figure 4. Changing of mangrove areas in: (a) 2011-2014, (b) 2014-2017 (c) 2017-2019 and (d) 2019-2023 using Google Earth Pro

Discussion

In the present study, the mangrove forest in the study area was found to be composed of **18 species from 11 different families**, with 12 true mangrove species and 6 associated species. The number of true mangrove species recorded was lower than the findings of San Mon Thu (2020) in the Myawyit coastal area, who recorded 13 species. The true mangrove species composition in the Myawyit coastal area differed from the present study area. In Myawyit, the family Rhizophoraceae was more dominant than in the current study area. However, in our study, the family Avicenniaceae was more dominant than others, which is likely due to the estuarine area receiving freshwater from the Thanlwin River, creating a suitable brackish water environment. Hsu Pyae Sone (2022) recorded 10 species of true mangroves in the Sepala mangrove forest, Bilu Island, a number nearly identical to our findings.

In terms of **relative dominance**, *Avicennia alba* and *A. officinalis* cover a large basal area in the study area. Relative dominance and basal area are primarily influenced by the girth and density of mangroves (Samson and Tubias, 2015). Therefore, the species *Avicennia alba* and *A. officinalis* occupied a larger area than other species. This is consistent with the previous studies by Hsu Pyae Sone (2022).

The **importance value index (IVI)** provides an overview of the influence or role of a mangrove species in the community; the higher the IVI value of a certain species, the more important its role in the community (Matatula et al., 2021). In the study area, the highest IVI value was recorded for *A. alba* (**145.22**), making it the most dominant species due to its high relative density, relative frequency, and relative dominance. It was followed by *A. officinalis* (**111.62**) and *A. marina* (**12.24**). Therefore, the family Avicenniaceae was the dominant family based on the species importance value index. Vijayan et al. (2015) also obtained the highest IVI values for Avicennia species in mangrove forests on the southwest coast of Kerala, India, with *A. officinalis* (**64.19**) followed by *A. marina* (**59.78**). This is likely because both study areas are located along the banks of estuarine water bodies, and the family Avicenniaceae is well adapted to the estuarine environment.

Cherry Aung (1999) studied the mangrove community of the Setse coastal area from 1997 to 1998, revealing a community with 17 true mangrove species and 15 species of mangrove associates. When compared to this previous study, the floral diversity in the present study was lower. Five true mangrove species—*Nypa fruticans*, *Xylocarpus granatum*, *Bruguiera gymnorhiza*, *Sonneratia griffithii*, and *Heritiera fomes*—were not found during the current study.

The absence of these species indicates the **degradation of the mangrove forest** in the Setse area.Although there are no charcoal kilns in this area, a few local people use the mangrove forest for firewood. Additionally, over the last twenty years (1999-2024), some areas of the mangrove forest have been degraded by **conversion to aquaculture** (shrimp ponds). In these instances, operators used machinery to clear the mangrove forest and build dikes with sluice gates to retain water. This alteration of the Damin Creek's hydrology led to the loss of some mangrove plants and species. Thus, the greatest threat to mangrove species loss was the rapid expansion of shrimp aquaculture.

From 2000 to 2002, 148.26 acres of mangrove forest were converted into shrimp ponds. However, according to historical images from Google Earth Pro, 25.27 acres of mangrove areas were recovered from 2011 to 2023. Prior to 2011, Google Earth Pro images were unclear and cloudy, so the situation could not be properly assessed. The mangroves recovered after the conversion of mangroves into marine aquaculture ponds because the sluice gates of the shrimp pond and seawater reservoir broke. Water thoroughly entered these areas, and the natural flow of water facilitated mangrove seed dispersion. Therefore, hydrology is a crucial factor in the distribution of mangrove species. However, from 2017 to 2019, the mangrove forest lost 3.73 acres. This was because shrimp pond operators once again implemented seawater reservoirs for their ponds. As a result, people cut down mangroves and created small channels that connected the shrimp ponds with irrigation systems. The mangrove forest also lost 2.54 acres from 2019 to 2023. During that time, shrimp pond operators killed the mangroves with continuous flooding. This is because aerial roots are especially sensitive to long periods of flooding; these specialized roots were covered for extended periods by water, and the mangroves died due to a lack of oxygen in the plant tissues.

Conclusion

The mangrove forest of Setse is composed of 18 species from 11 different families. Based on field observations, *Avicennia alba* and *A. officinalis* had the highest relative dominance values and were found in all transect lines. According to historical images from Google Earth Pro, 25.27 acres of mangroves were recovered and 6.27 acres were lost from 2011 to 2023. Therefore, this study provides basic information on the current situation of mangrove forests in Setse and establishes baseline data for rehabilitation, replantation, and conservation and management strategies.

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SOIL CARBON SEQUESTRATION OF SALT MARSHES IN BILU ISLAND, MYANMAR

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Abstract

In this study, vertical soil cores were collected from two habitats (mudflat and salt marshes) in Zeekone, Tawpun, Kalwi, and Karaikdo. The aim was to examine the organic carbon content and physicochemical properties of the soil's vertical profile (within 0-60 cm). Eight salt marsh species were identified based on their vegetation cover, including *Sesuvium portulacastrum*, *Phragmites australis*, *Distichlis spicata*, *Puccinellia* species, *Paspalum* species, *Spartina maritima*, *Typha angustifolia, and Schoenoplectus americanus*.The results showed that soil organic carbon (SOC) content decreased with depth in the Zeekone, Tawpun, and Karaikdo stations. In contrast, an increasing trend was observed in the Kalwi station. For soil physical properties, dry bulk density (gcm^{-3}) increased with depth at Zeekone, Tawpun, and Karaikdo, but not at Kalwi. The mudflat in the Kalwi station had the highest soil salinity value (5.98 ± 0.67), while the *Phragmites australis* salt marsh at the Zeekone station had the lowest (0.06 ± 0.01). Soil pH values across all stations ranged from weakly acidic to weakly alkaline.The highest SOC content was found in the *Sesuvium portulacastrum* salt marsh at Zeekone and the mudflat at Tawpun, with mean values of 1.00 ± 0.57% and 1.16 ± 0.71%, respectively. The sediment types in the Zeekone and Tawpun stations were sandy loam and sand. Kalwi station consisted of loamy sand and sand, and Karaikdo station had loam and sandy loam sediment types.

Keywords: Salt marsh, organic carbon, bulk density, soil pH, soil salinity

Introduction

Salt marshes are intertidal, vegetated wetland ecosystems that dominate protected shorelines and estuaries in a wide range of climates (Mitsh et al., 1994; Butler and Weis, 2009). These intertidal habitats are vital for healthy fisheries, coastlines, and communities (NOAA, 2024). They typically occur in the upper intertidal zones, between mangrove fringes and terrestrial vegetation, and are usually flooded by spring tides rather than daily tides (Johns, 2006).

Coastal wetlands, including salt marshes, are significant global carbon sinks, storing more carbon per unit area than terrestrial forests (Duarte et al., 2005; McLeod et al., 2011; Temmink et al., 2022). The carbon sinks in salt marshes consist of aboveground biomass, belowground biomass, and soils. Soil plays a crucial role, often storing more carbon than the vegetation itself (Dalal and Allen, 2008). In tidal salt marshes, prolonged flooding can create anoxic conditions, which reduces the efficiency of organic matter decomposition and promotes soil carbon sequestration (Megonigal et al., 2004).

The drivers of blue carbon stock in salt marshes include several factors. Environmental characteristics such as elevation and local hydrology influence the supply of sediment and allochthonous carbon (Wollenberg et al., 2018; Rogers et al., 2019; Mossman et al., 2022).

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Sedimentary characteristics like particle size affect the preservation of carbon stocks (Kelleway et al., 2016; Ford et al., 2019). Additionally, vegetation community composition and diversity affect both the supply of autochthonous carbon from primary production and the trapping of allochthonous carbon (Ouyang and Lee, 2014).

While numerous studies on blue carbon stock in salt marsh sediments exist globally, research on this topic is limited in Myanmar. Previous studies have focused on the soil organic carbon of mangrove areas in the Ayeyarwady Region (Ya Min Thant et al., 2012) and the Mon Coastal Area (Htwe Yee Lin, 2022; Min Thu Aung, 2023). However, there has been no specific study on soil carbon sequestration in salt marshes and mudflats. Therefore, this study was conducted to contribute to the understanding of the organic carbon status of salt marshes in Bilu Island. The research also covered soil organic carbon variations with depth and among different species. The specific objectives were to evaluate soil organic carbon (SOC) at different depth intervals, compare SOC variations among salt marsh species, and assess the relationship between SOC and physicochemical properties.

Materials and Methods

Study Area

The sampling sites selected for this study were Tawpun (Lat. 16°22' 4.92"N, Long. 97° 28' 15.16"E), Zeekone (Lat. 16°18' 32.89"N, Long 97° 27' 27.44"E), Karaikdo (Lat. 16°17' 0.79"N, Long. 97° 31' 2.12"E), and Kalwi (Lat. 16° 30' 4.69"N, Long. 97° 31' 34.10" E) on Bilu Island in the Thanlwin River. The Tawpun, Zeekone, and Kalwi sites are located in coastal regions, while Karaikdo is situated in a tidal creek. These areas contain various habitats, including mudflats, salt marshes, brackish marshes, and mangroves. All stations experience a semidiurnal tide. The Bilu Deltaic Island is significantly influenced by the influx of the Thanlwin River. The region has a monsoon-influenced tropical climate with five distinct seasons and an annual precipitation of 148.72 mm (Lwin, 2002).

Sampling Procedure

Soil samples were collected to a depth of 60 cm using a custom-made steel soil auger (5 cm in diameter, 1.5 m in length). All samples were sealed in polyethylene bags and transported to the laboratory for analysis of SOC, dry bulk density, soil pH, and soil texture.

Depth-aggregated sampling techniques were employed at depth intervals of 0-10, 10-20, 20-30, 30-40, 40-50, and 50-60 cm, as soil carbon content typically changes slowly with depth (Kauffman et al., 2011; Donato et al., 2011). To ensure proper identification and avoid confusion, each core was labeled with a unique ID.

Figure 1. Map showing the study areas

Analysis of Soil Organic Carbon Content (%SOC)

The dry bulk density (DBD) of the soil was determined by quantifying the mass of the dry soil and its original volume. Samples were placed in polyethylene bags and dried in an oven at 60°C until a constant weight was reached, which took between 48 and 72 hours, and sometimes longer. The dry bulk density was calculated using the equation:

DBD = (mass of dry soil) / (original sample volume)

Before analysis, the oven-dried soil was manually pulverized with a mortar to a consistent particle size and then passed through a 2 mm sieve. To determine the %SOC, 5 g of the homogenized soil was weighed into a crucible and heated in a muffle furnace at 450°C for four hours to completely ignite the organic matter. The temperature was gradually decreased, and the samples were cooled in a desiccator before being weighed again. The weight before and after burning was compared to calculate the loss on ignition (%LOI).

The %LOI was calculated as follows:

%LOI = [(Initial dry weight - Final weight after ignition) / Initial dry weight] × 100

The %Corg (organic carbon) for tidal salt marshes was then calculated using the following equation (Craft et al., 1991):

$\%Corg = 0.40 * \%LOI + 0.0025 * (\%LOI)^2$

Furthermore, SOC storage (Mg C ha^{-1}) within the 60 cm soil depth of mudflats and salt marshes was calculated using the equation:

SOC storage (Mg C ha^{-1}) = SOC (%) × DBD (gcm^{-3}) × SD (cm)

Where DBD is the dry bulk density and SD is the soil depth.

Determination of Physicochemical Parameters

Soil salinity and soil pH were determined by mixing soil samples with distilled water at a 1:2.5 (soil:water) ratio, following the method of Gao et al. (2019). After shaking the mixture thoroughly and allowing it to settle for 30 minutes, the soil pH was measured using a pH meter (Fisher brand, Accumet AB 150). Soil salinity was then measured with an electrical conductivity meter. Soil particle texture was determined using the soil hydrometer method (Allen et al., 1974).

Data Analysis

The mean and standard deviations for replicates were analyzed using Excel and SPSS. Vertical trends for each core were graphed using OriginPro.

Results

Eight salt marsh species with different vegetation covers were recorded. Summary statistics for SOC, DBD, soil pH, soil salinity, and soil texture (sand, silt, clay) are shown in Tables 1 and 2. The average soil salinity of the vertical profile ranged from 0.06 to 5.98‰. In most vertical soil cores, salinity declined from 0-20 cm and increased thereafter (Figures 2b, 3b, 4b, and 5b). Soil pH values across all stations ranged from weakly acidic to weakly alkaline (Figures 2a, 3a, 4a, and 5a). Soil DBD gradually increased with depth at Zeekone, Tawpun, and Karaikdo stations (Figures 2c, 3c, and 4c), but this trend was not observed at Kalwi station (Figure 5c).

Four soil texture types were found. Sandy loam sediment was found in the salt marshes of *Sesuvium portulacastrum*, *Distichlis spicata*, *Puccinellia* species, and *Schoenoplecus americanus*. Loamy sand sediment was found in the *Spartina maritima* salt marsh. Loam sediment was found in the *Typha angustifolia* salt marsh, and sand sediment was found in the *Phragmites australis* and *Paspalum* species salt marshes (Figure 6).

Soil Organic Carbon

The variations of soil organic carbon (SOC) with depth are shown in Figures 2d, 3d, 4d, and 5d. Carbon content in the surface soil layer (0-20 cm) was relatively higher than in the deeper layers at Zeekone, Tawpun, and Karaikdo (Figures 2d, 3d, and 4d). A decreasing trend of SOC with depth was observed in these three stations. In contrast, the SOC content in the bottom soil layer (30-60 cm) was higher than in the upper layers at the Kalwi station (Figure 5d).

Table 1. The mean values of physical and chemical properties and organic carbon contents (mean ± standard deviation, n = 12) within 60 cm soils in mudflats and salt marshes

Parameters	Zeekone				Tawpun		
	Mudflat	*Sesuvium portulacastrum*	*Phragmites australis*	*Distichlis spicata*	Mudflat	*Puccinellia* sp.	*Paspalum* sp.
Soil salinity	4.22 ± 1.01	4.33 ± 0.90	0.06 ± 0.01	3.38 ± 1.12	3.89 ± 0.93	4.32 ± 1.36	3.19 ± 0.62
Soil pH	7.87 ± 0.20	7.59 ± 0.22	7.89 ± 0.07	8.19 ± 0.14	7.96 ± 0.23	7.91 ± 0.23	7.61 ± 0.14
Sand (%)	71.3 ± 4.60	74.07 ± 13.79	98.5 ± 0.59	59.72 ± 5.18	70.72 ± 4.97	70.63 ± 6.30	95.78 ± 0.53
Silt (%)	20.5 ± 3.36	19 ± 11.42	0.60 ± 0.52	28.53 ± 2.98	21.95 ± 6.71	22.47 ± 6.19	1.78 ± 0.60
Clay (%)	8.2 ± 1.56	6.93 ± 2.59	0.90 ± 0.49	11.75 ± 2.5	7.33 ± 2.06	6.9 ± 1.20	2.43 ± 0.27
Soil type	Sandy Loam	Sandy Loam	Sand	Sandy Loam	Sandy Loam	Sandy Loam	Sand
DBD (gcm^{-3})	1.24 ± 0.11	1.25 ± 0.12	1.31 ± 0.06	1.26 ± 0.06	1.01 ± 0.31	1.10 ± 0.15	1.51 ± 0.07
SOC (%)	0.69 ± 0.23	1.00 ± 0.57	0.08 ± 0.01	0.60 ± 0.13	1.16 ± 0.71	0.83 ± 0.17	0.19 ± 0.03
$SOC_{storage}$ ($Mg\ C\ ha^{-1}$)	50.57	71.41	6.27	47.6	61.02	54.25	16.85

Table 2. The mean values of physical and chemical properties and organic carbon contents (mean ± standard deviation, n = 12) within 60 cm soils in mudflat and salt marshes

Parameters	Karaikdo		Kalwi	
	Typha angustifolia	*Schoenoplectus americanus*	Mudflat	*Spartina maritima*
Soil salinity	1.14 ± 0.21	2.96 ± 0.54	5.98 ± 0.67	4.34 ± 0.59
Soil pH	6.41 ± 1.37	7.52 ± 0.04	7.55 ± 0.10	7.44 ± 0.09
Sand (%)	46.57 ± 3.61	64.90 ± 4.63	87.90 ± 5.52	80.55 ± 8.79
Silt (%)	30.20 ± 1.26	26.23 ± 2.98	7.47 ± 4.34	12.62 ± 6.14
Clay (%)	23.23 ± 2.87	8.87 ± 1.83	4.63 ± 1.42	6.83 ± 2.7
Soil type	Loam	Sandy Loam	Sand	Loamy Sand
DBD (gcm^{-3})	0.68 ± 0.07	0.79 ± 0.13	1.11 ± 0.11	1.17 ± 0.11
SOC (%)	0.86 ± 0.10	0.87 ± 0.11	0.36 ± 0.07	0.44 ± 0.16
$SOC_{storage}$ ($Mg\ C\ ha^{-1}$)	34.67	41.64	24.05	30.35

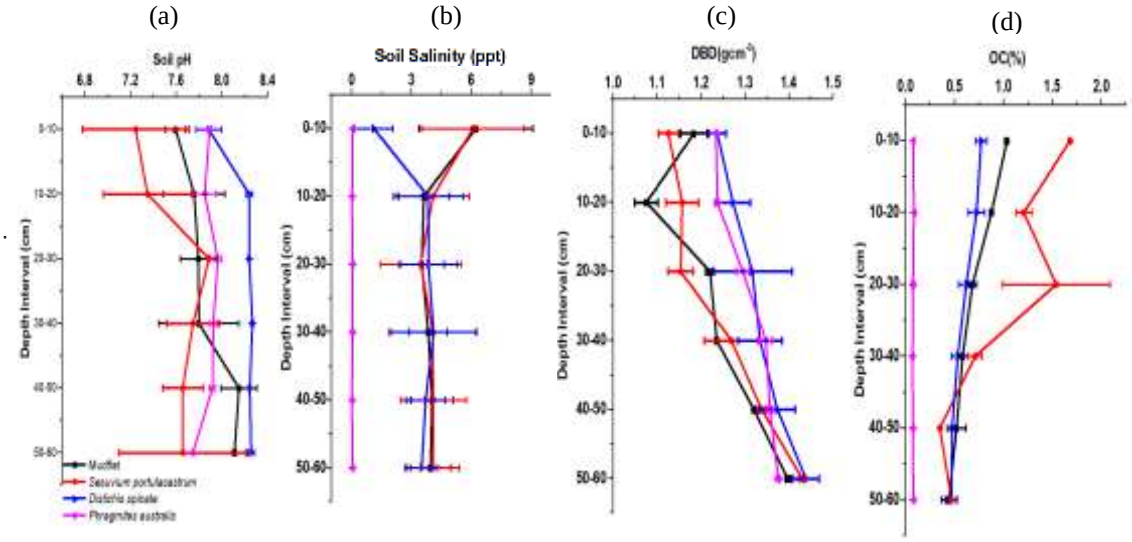

Figure 2. Physicochemical parameters and soil organic carbon in Zeekone: a) soil pH, b) soil salinity, c) DBD (dry bulk density) and d) %OC

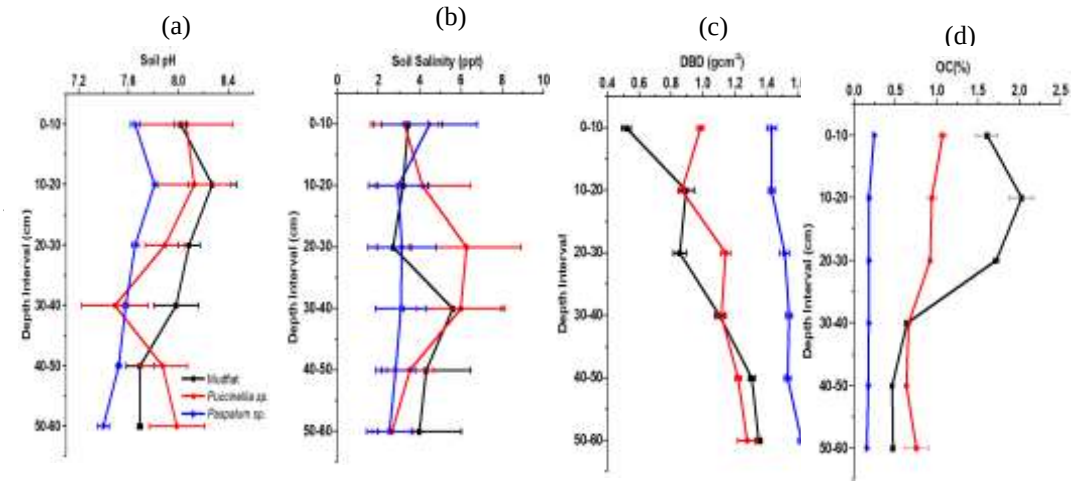

Figure 3. Physicochemical parameters and soil organic carbon in Tawpun: a) soil pH, b) soil salinity, c) DBD (dry bulk density) and d) %OC

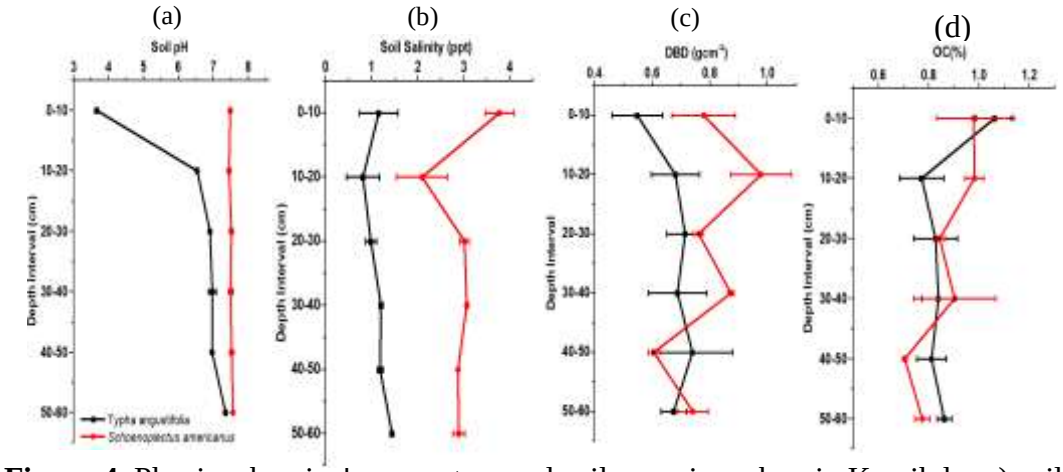

Figure 4. Physicochemical parameters and soil organic carbon in Karaikdo: a) soil pH, b) soil salinity, c) DBD (dry bulk density) and d) %OC

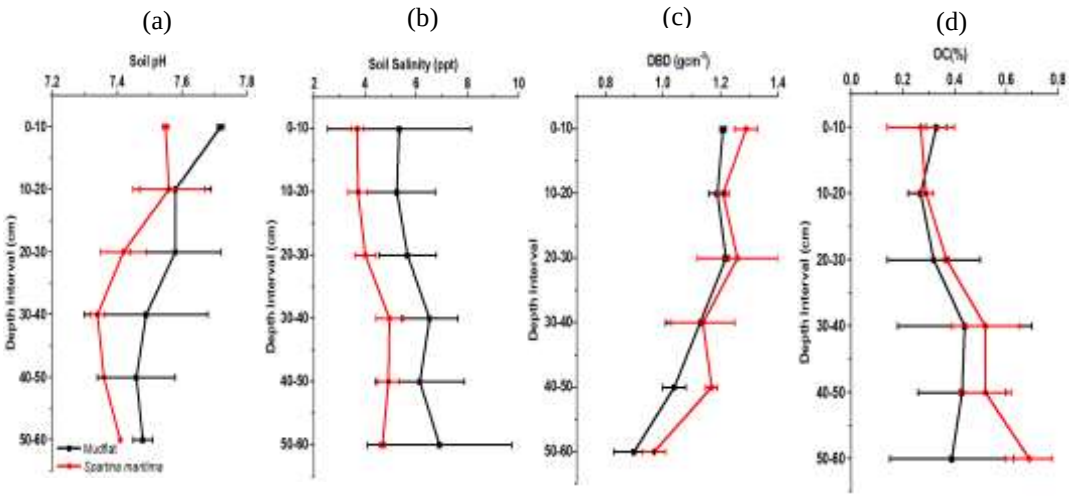

Figure 5. Physicochemical parameters and soil organic carbon in Kalwi: a) soil pH, b) soil salinity, c) DBD (dry bulk density) and d) %OC

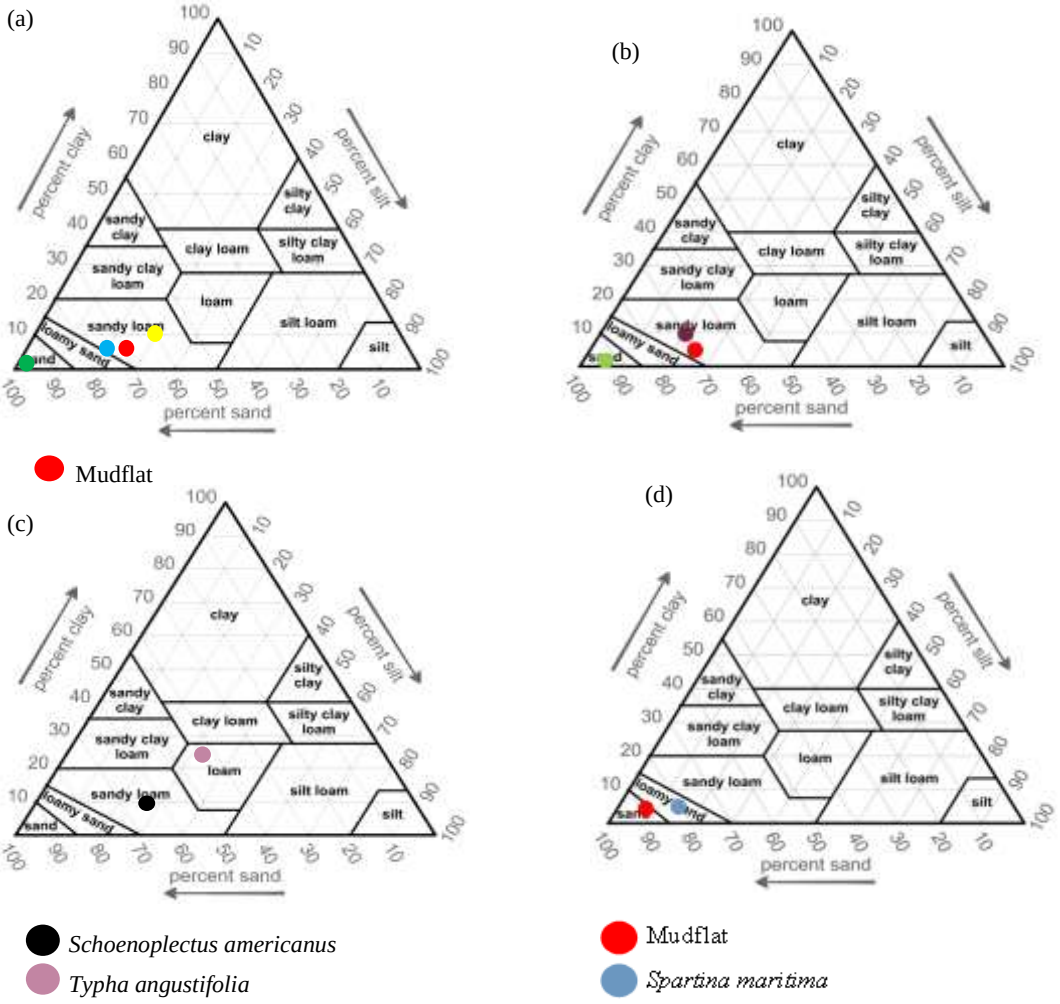

Figure 6. Soil texture types of mudflat and different salt marshes in: a)Zeekone, b)Tawpun, c)Karaikdo and d)Kalwi

Discussion

Variation in salinity across a marsh can alter the vegetation community (Deleeuw et al., 1993). Elevation is a key factor driving these salinity changes. In this study, the highest salinity values were observed at lower elevation sites in some habitats at Zeekone, Tawpun, and Kalwi stations, which experienced frequent tidal inundation. The lowest salinity values were found at the Karaikdo station, which is located on the landward side and has a higher freshwater influence.

According to previous research, salt marsh soils can be either acidic or weakly alkaline. Kadiri and Spencer (2011) found mean pH values of 6.8–7.7 in natural salt marshes and 7.0–7.5 in mudflats in Southeast England. Similarly, Bai et al. (2016) reported a mean soil pH of 8.05–8.30 in salt marshes of China's Yellow River Delta. The soil pH values in this study were consistent with these findings, ranging from weakly acidic to weakly alkaline. The average soil pH in the *Typha angustifolia* salt marsh was relatively low (6.41 ± 1.37), possibly due to the input of allochthonous organic matter (dried paddy straws) from adjacent paddy fields via water runoff.

Dry bulk density (DBD) is an indicator of soil compaction and water permeability (Han et al., 2010; Drewry et al., 2008). The observed increase in soil DBD with increasing depth at Zeekone, Tawpun, and Karaikdo stations is consistent with findings from salt marshes in the Yellow River Delta (Bai et al., 2016) and Tasmania (Ellison and Beasy, 2018). This trend can be attributed to autocompaction, where recent sediments become more compacted over time (Allen, 2000; Bird et al., 2004). However, this trend was not observed at the Kalwi station, where autocompaction may not be apparent at depths up to 60 cm, a finding similar to Valerie (2015). Dry bulk density is also strongly related to particle texture. In this study, sites with high sand concentrations (60-98%) showed higher bulk density than soils with high silt (1-30%) and clay (1-23%) concentrations. The study area is influenced by the Thanlwin River and the Gulf of Martaban, which transport an estimated 108 to 237 million tonnes of sediment annually (Baronas et al., 2020).

Within each vertical soil profile, the percent soil organic carbon (%SOC) showed a similar distribution pattern among different vegetation communities and mudflats, with the exception of the Kalwi station. SOC profiles decreased with depth at Zeekone, Tawpun, and Karaikdo stations, consistent with previous studies on salt marshes in China (Bai et al., 2016), Australia (Ellison and Beasy, 2018), and the southern Red Sea coast (Eid et al., 2022). These studies also reported that most soil organic carbon is concentrated in the top 20 cm, a finding that aligns with the results from most of the sampling sites in this study. In contrast, at the Kalwi station, %SOC content increased with depth, which could be due to the downward migration of soil organic carbon through leaching and microbial activities in the surface soil (Wang et al., 2010; Dosskey and Bertsch, 1997). The decreasing dry bulk density with depth at Kalwi also contributed to the higher SOC in deeper soil layers.

At Zeekone, the SOC content in the *Sesuvium portulacastrum* salt marsh was relatively high, which can be attributed to primary production by the vegetation (autochthonous carbon) and the input of allochthonous carbon from the adjacent mangrove area via tidal creeks. A different situation was observed at the Tawpun station, where the SOC content in the mudflat was significantly higher than in other mudflats and salt marsh species. This could be due to particulate organic matter and allochthonous carbon input.

At the Karaikdo station, the *Schoenoplectus americanus* salt marsh was located on a narrow tidal creek bank, and the *Typha angustifolia* salt marsh was adjacent to paddy fields in a high-tide zone. These sites had a lower chance of receiving organic matter from the tide. However, the SOC contents were higher than those in some of the other studied areas. This may be explained by their proximity to paddy fields and other terrestrial forests.

At the Kalwi station, the mudflat and the *Spartina maritima* salt marsh stored lower amounts of SOC compared to other areas. Within these two habitats, the *Spartina maritima* salt marsh had a higher %SOC than the mudflat, as it receives both autochthonous and allochthonous organic matter from neighboring mangrove habitats.

The %SOC values in this study (0.08–1.16%) were higher than those reported by Yu et al. (2014) and Bai et al. (2016) in the Yellow River (0.04–1% and 0.16–0.4%, respectively), but significantly lower than those from Dutch salt marshes (2.4–4.2%) (Van Deelen, 2018). The %SOC in the salt marsh species studied was: 1.00 ± 0.57% in *Sesuvium portulacastrum*; 0.60 ± 0.13% in *Distichlis spicata*; 0.08 ± 0.01% in *Phragmites australis*; 0.83 ± 0.17% in *Puccinellia* species; 0.19 ± 0.03% in *Paspalum* species; 0.86 ± 0.10% in *Typha angustifolia*; 0.87 ± 0.11% in *Schoenoplectus americanus*; and 0.44 ± 0.16% in *Spartina maritima*. These differences in %SOC may be influenced by the daily tidal range and vegetation cover types, which affect the depth distribution pattern of SOC (Bai et al., 2016).

Among the different salt marsh species, the highest SOC storage was found in *Sesuvium portulacastrum* (71.41 Mg C ha^{-1}), and the lowest was in *Phragmites australis* (6.27 Mg C ha^{-1}). For mudflats, the highest SOC storage was at the Tawpun station (61.02 Mg C ha^{-1}), while the lowest was at Kalwi (24.05 Mg C ha^{-1}). These differences are likely related to variations in elevation, accretion and erosion rates, vegetation cover, and soil texture (Van Deelen, 2018).

Conclusion

This study highlights the depth distribution patterns of organic carbon content in the mudflats and salt marsh species of Zeekone, Tawpun, Kalwi, and Karaikdo on Bilu Island, Myanmar. These findings are valuable for future monitoring and assessment of salt marshes and their biodiversity. The *Phragmites australis* salt marsh at Zeekone had the lowest carbon content (0.08%) among all habitats. The highest SOC content was found in the mudflat at Tawpun (1.16%), which can be attributed to allochthonous organic matter from adjacent mangrove areas during tidal inundation and tidal feedback. Regular monitoring and assessment of these salt marshes can provide further insights into their protection and restoration, which is crucial for enhancing carbon sequestration and mitigating climate change on Bilu Island.

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CULTURE POTENTIAL OF THE PACIFIC CLAM, *ASAPHIS VIOLASCENS* (FORSSKAL, 1775), IN THE MYEIK ARCHIPELAGO

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Abstract

The Pacific clam, *Asaphis violascens* (Forsskal, 1775), locally known as "Gin-Phyu," is a benthic bivalve species native to the Indo-Pacific region. This study demonstrated the potential for aquaculture by successfully breeding the Pacific clam from its embryonic and larval stages to the juvenile stage in a laboratory. While the clams are currently a low-priced seafood, which may limit commercial-scale culture, their production from natural habitats is mainly for local consumption. By studying the clam's life cycle through laboratory culture, we gained insights into its population dispersion and optimum environmental parameters. This culture practice can be used in the future to enhance its abundance in natural beds. The primary objective of this research was to establish suitable rearing conditions for induced spawning, larviculture, and grow-out culture. In this study, adult, mature clams were collected from the sandy beaches of Doris Island. Induced spawning was attempted using emersion and thermal shock methods. Larvae rearing and grow-out culture were investigated in a hatchery setting. Eight species of microalgae (*Chaetoceros simplex* I & II, *Chaetoceros calcitrans*, *Chaetoceros ceratosporum*, *Chaetoceros gracilis* I & II, *Nannochloropsis oculata*, and *Tetraselmis suecica*) were used as food. A sand substrate was found to be essential for the grow-out culture of post-larval clams to reach the adult stage.

Keywords: *Asaphis violascens*, bivalve, clam hatchery, emersion, induced spawning, larviculture, Pacific clam, thermal shock.Keywords: *Asaphis violascens*, bivalve, clam hatchery, emersion, induce spawning, larviculture, Pacific clam, thermal shock.

Introduction

The Pacific clam, *Asaphis violascens* (Forsskal, 1775), is a benthic bivalve in the phylum Mollusca and family Psammobiidae. They are found throughout the Indo-Pacific region, from East Africa to eastern Polynesia and from Japan to central Queensland and New Caledonia. They inhabit intertidal sandy beaches, dwelling in sandy or coarse gravel substrates at depths of 0 to 20 meters (Poutiers, 1998). This species naturally occurs in estuaries and can thrive under a broad range of pH (8.4-7.2) and salinity (10-35‰) conditions. As a dominant species, it can sometimes be found in pure strands within a habitat. One of its notable characteristics is its ability to cope with desiccation and high temperatures during low tide (Subagjo, 1989).

Clams are generally suspension-feeding bivalves that primarily depend on phytoplankton and detritus for nutrition. Microalgae constitute the main food source for most cultured Pacific clams. The average edible weight of the clam is approximately 9 g. They are dioecious (having separate sexes) and reach sexual maturity in about 6 months, at a length of 18-20 mm (Rimmer et al., 2001; Bell, Lober, & Trevor, 2001). Spawning can occur in spring, summer, or fall, with mass spawning typically happening in late summer. The larval stage lasts about 21 days.

While a variety of clam species in Myanmar have been documented, the Pacific clam has been minimally studied despite its wide distribution along the Myanmar coast, especially in the Myeik Archipelago. This study represents the first attempt at induced spawning of the Pacific clam in Myanmar. Samples were collected from Doris Island, Myeik Archipelago, and the induced spawning, larvae rearing, and grow-out culture of the clams were investigated in a

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laboratory. The objective of this research was to determine the optimal conditions for completing the clam's life cycle in a controlled environment.

Materials and Methods

The adult clams were collected from Doris Island (Lat. 11.240930˚N and Long. 98.272172˚E), Myeik Archipelago, Myanmar, in April 2023. As the clams live buried in the sand, they were collected by digging to a depth of 30 cm during low tide. Their location was identified by searching for siphonal openings on the beach. Their habit of living in accessible groups made their collection relatively easy. Approximately 700 individuals, with a size range of 40-80 mm, were collected for induced spawning. The collected clams were cleaned and stocked in a tank containing 30‰ seawater with strong aeration and food. Feeding was observed when the siphons were protruding from their shells.

After a one-day conditioning period, the clams were subjected to various induced spawning methods, including thermal shock, salinity shock, emersion, hormone induction, sperm activation, and stripping. Seawater filtration systems, hatchery facilities, spawning and larvae rearing tanks, aeration systems, and food culture were prepared before spawning. The following phytoplankton species were used: *Chaetoceros simplex* I & II, *Chaetoceros calcitrans*, *Chaetoceros ceratosporum*, *Chaetoceros gracilis* I & II, *Nannochloropsis oculata*, and *Tetraselmis suecica*. A sand substrate was also required for the grow-out culture of the clams in the laboratory.

Figure 1. A) Pacific Clam, *Asaphis violascens*: B) Siphon opening

Results

Induced Spawning

Broodstock with a shell length of 60-80 mm were acclimated in 500 L tanks containing UV-filtered seawater at a temperature of 29°C and a salinity of 31‰. Spawning induction was attempted using thermal shock, salinity shock, emersion, hormone induction, sperm activation, and stripping. After testing several methods, it was observed that the emersion method was the most effective for spawning.

Approximately 30 individuals were used for each treatment. For the emersion method, clams were removed from the water and exposed to air for 2 hours (at an air temperature of 33°C). Afterward, they were placed in 200 L spawning tanks with UV-filtered seawater at 29°C. A large amount of food was provided with gentle aeration during this period. Additional stimuli, such as stripped eggs or sperm from a opened clam, were also used. The process was repeated once the clams started to spawn.

Pacific clams have two short siphons through which they filter water. The inhalant siphon, which takes in water, is located on the ventral side, while the exhalant siphon, which

expels water, is on the dorsal side. During spawning, gametes are ejected through the exhalant siphon. Males began to expel sperm like smoke, and females released eggs next to the males. Based on observations of different spawning designs, most males were moved to a separate tray during spawning to prevent an excess number of sperm for fertilization. When the release of gametes stopped, all broodstock were removed, and the remaining liquid was left undisturbed for approximately 1 hour to allow fertilization to occur.

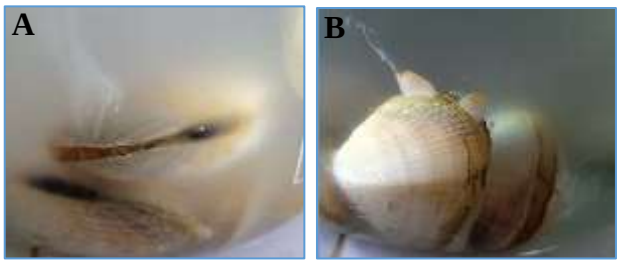

Figure 2: Pacific Clams: A) Spawning female; B) Spawning male

Fertilization and early larval stage

After spawning, the fertilized eggs were sieved through a 20-µm polyethylene screen and transferred to 200 L tanks filled with filtered seawater. Gentle aeration was provided to enhance survival. Through repeated cell division, the fertilized eggs transformed into D-hinged veliger larvae within 20 hours. After 20 hours, the larvae were transferred to 500 L larvae rearing tanks using a 40-µm screen, and the water was renewed.

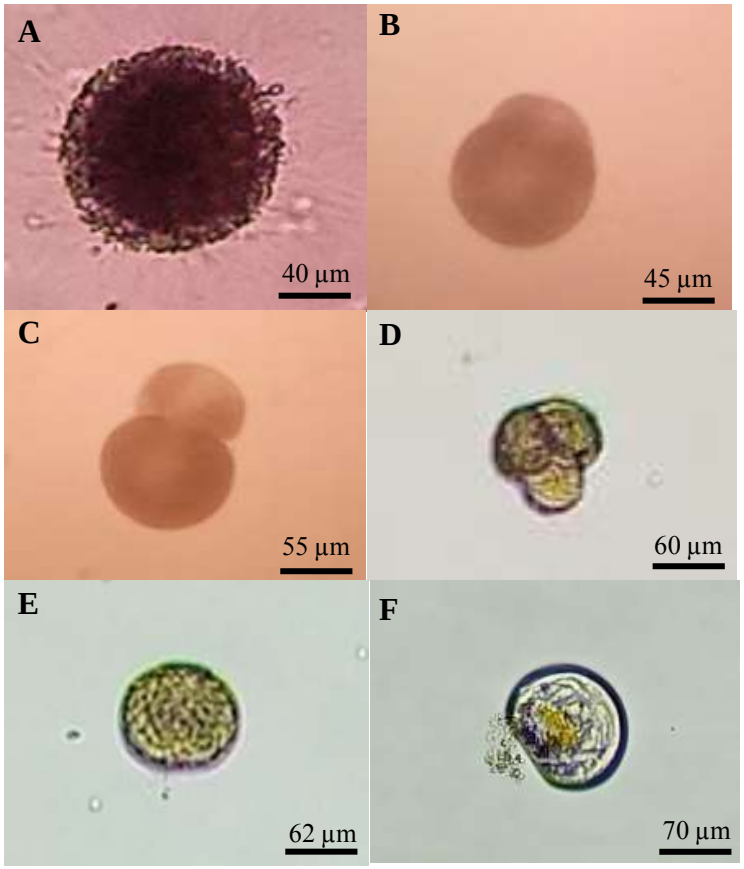

Figure 3. Early development of fertilized egg: A) sperm around egg; B) first polar body; C) second polar body; D) four cell stage; E) trochophore larva; F) D-hinged veliger larva

Larval Rearing

The 'D' larva was actively swimming. Larvae were not fed until 48 hours. after fertilization and then they were fed with phytoplankton twice daily. Seawater was renewed every alternate day. On the third day, the larva was slightly oval and started to reach early umbo stage. On the seventh day, the umbo becomes distinct. The late umbo stage was reached on 12-13 days. Eye spots formation stage was reached on 14-16 days. At this stage, the larvae were transferred into a transparent glass tank containing cleaned sand for grow up culture. During larval rearing stage, Antibiotics viz., mixed penicillin and streptomycin, were used to avoid bacterial infection and increase survival rates. Eight species of microalgae, *Chaetoceros simplex* I and II, *Chaetoceros calcitrans*, *Chaetoceros ceratosporum*, *Chaetoceros gracilis* I and II, *Nannochloropsis oculata* and *Tetraselmis suecica*, were used as food.

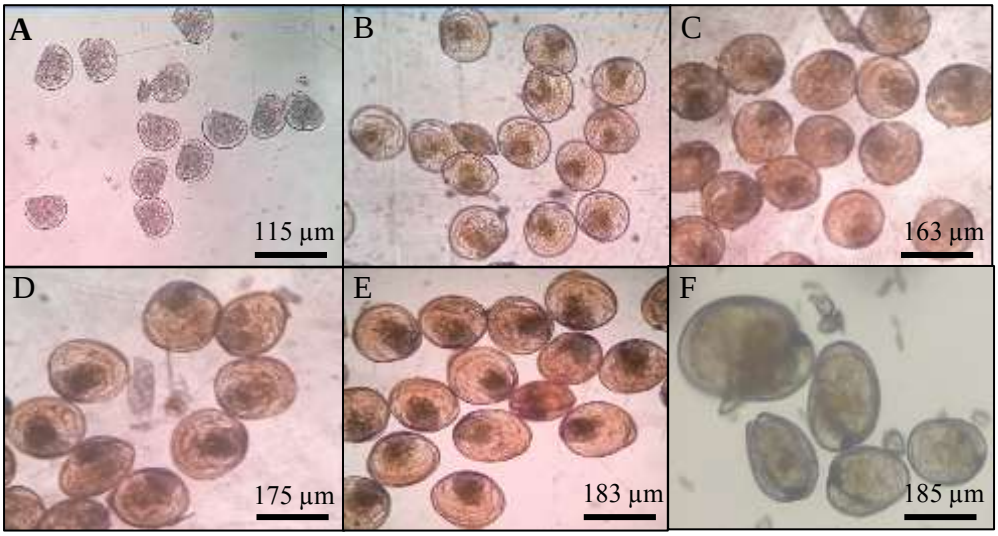

Figure 4. Larval development: A) 3 days-old; B) 7 days-old; C) 10 days-old; D) 12 days-old; E) 14 days-old; F) 16 days-old

Grow up culture

After 16 days, the larvae developed ciliated velum, which is used for locomotion and feeding. After 21 days, the veliger larvae develop a foot and become pediveliger stage. Soon after, the clam larvae developed into juveniles in which their swimming organs will lost and develop siphons, which are used for respiration and feeding. At this stage, the juveniles were already settling to the bottom and buried in the sand substrate of the tanks. The grow up culture has been conducted in the sand tanks up to 162 days-old in which the clam reached 30.76 mm in trial 1 and 27.88 mm in trial 2. In this stage, the water can be changed once a week and no need to add the antibiotics. Moreover, the phytoplankton which were directly collected from natural water can be fed to young clams for food. The growth of larvae and juveniles of Pacific clam was shown in Table 1.

Table 1. Average length (mm) of growth of Pacific clam, *Asaphis violascens*

Age (days)	Trial 1		Trail 2	
	No. of measured individuals	Average larval length	No. of measured individuals	Average larval length
5	44	119.4 µm	28	119 µm
6	25	127 µm	32	127 µm
7	22	142 µm	104	134 µm
8	32	150 µm	128	147 µm
9	60	150.8 µm	99	151 µm
10	80	163 µm	59	162.6 µm
11	64	171.4 µm	56	167.8 µm
12	81	175.2 µm	70	174.6 µm
14	36	183.8 µm	94	180.4 µm
16	65	185.2 µm	136	182.4 µm
44	16	3.12 mm	18	2.52 mm
58	15	4.02 mm	16	2.92 mm
65	20	4.94 mm	17	3.12 mm
72	24	5.88 mm	15	3.46 mm
80	20	8.42 mm	15	7.12 mm
94	22	12.5 mm	12	9.26 mm
112	20	15.2 mm	6	13.4 mm
162	8	30.76 mm	5	27.88 mm

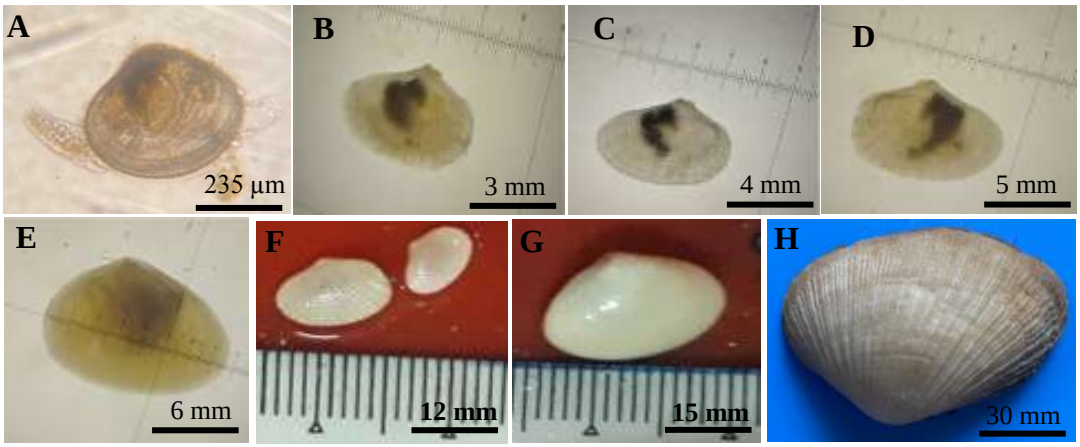

Figure 5. Juveniles' development: A) 20 days-old; B) 44 days-old; C) 58 days-old; D) 65 days-old; E) 72 days-old; F) 94 days-old; G) 112 days-old; H) 162 days-old

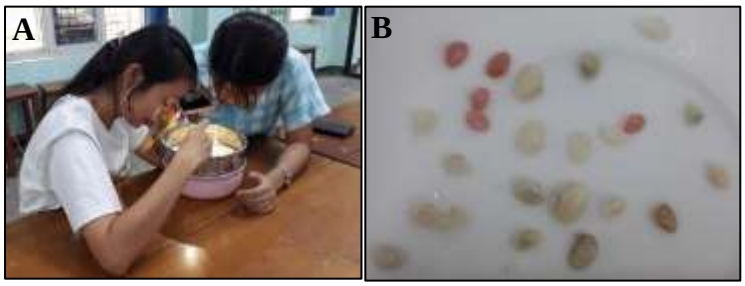

Figure 6. Numerical assessment of juvenile clam: A) select the clam from the sand; B) counting and measuring the size in the laboratory

Discussion

Induced spawning and grow up culture of Pacific clam, *Asaphis violascens*, have been investigated in the laboratory. The natural habitats of the clams are intertidal sandy beaches and they are dwelling buried in the upper layer of the sand. For the grow up culture of juveniles, the sand substrate was prepared in the rearing tanks. In the present study, the emersion method was reached successful spawning. Some other induced methods were also conducted; however; no spawning occurred in the study period.

Within larva rearing period, the antibiotic Chloramphenicol was used for trail 1 and mixed Streptomycin and Penicillin was used for trial 2 and observed that Chloramphenicol was better than the rest for the growth of larva. Hidu & Tubiash (1963) reported that the larval culture of clam *Mercenaria mercenaria*, usually resulted in a significant increase in growth rate of larvae by adding "Combi-strep" (dihydrostreptomycin-streptomycin sulfates) antibiotic.

Fertilized eggs of most mollusks are transformed into D-shaped larvae within 18 hours. after fertilization. However, Duisan *et al*. (2022) observed that fertilized eggs of hard clam, *Meretrix meretrix* changed into the D-shaped larvae were first observed after 24 hours of fertilization. From the results, the fertilized eggs of *A. violascens* reached the D-shaped lava 20 hr. after fertilization.

Owing to its low price, culture of Pacific clam in Myanmar is very rare. However, mass production of it was first developed in Palau, a Pacific island nation, by Mr. Gerald Heslinga in 1970s, mainly to produce its adductor muscle for overseas markets, particularly in Asian countries (Okada 1997). Pacific clams can have optimal reproduction at any salinity within the range 20-30‰. Peak gonad development is between April and May in Myanmar.

For the larva development, types of diets have a strong influence on their growth and mortality. Among these species, *Chaetoceros* sp and *Tetraselmis* sp are the most popular feeds for Pacific clam culture (Chícharo & Chícharo, 2001). In the present study, *Chaetoceros simplex* I and II, *C. calcitrans*, *C. ceratosporum, C. gracilis* I and II were fed to larval stage whereas a mixture of *Nannochloropsis oculata*, *Tetraselmis suecica* and *Chaetoceros* sp were fed to juveniles.

It was observed that sand substrate is ideal for the growth and survival of the Pacific clam, *A. violascens.* Several trials were tested for the grow up culture of juveniles in the tanks without sand, observing that they can survive only for a few days. High survival was observed in the tanks with sand substrate in which the post larvae was stocked when they needed to settle on the substrate.

Larval development stages of *A. violascens* are similar to other tropical bivalves; however, planktonic period was observed 16 days in the present study. Bolasina (2022) reported that *A. violascens* has a 10-day planktonic period. The present study observed the larval stage from induced spawning to adulthood, achieving a length of 2.5-3 cm over a period of about 5 months in the laboratory.

The implementation of small-scale rural aquaculture of Pacific clams may be an interesting option for improving household food security and supplementing family income of the rural poor (Bolasina, 2019). Besides this, bivalve aquaculture can bring ecosystem services, improve water quality, and provide wildlife habitat, bringing positive results on coastal habitat conservation and restoration (Theuerkauf *et al*., 2021). In the majority of cases, bivalve farming needs the collection of seeds from the sea (Chávez *et al*., 2002). Being the basis of their diet, the phytoplankton species occurring (Bricelj & MacQuarrie, 2007) and their abundance (Chícharo & Chícharo, 2001) have a strong influence on their growth and mortality, thus limiting the supply of seeds from the wild.

Conclusion

According to the literature, Pacific clam, *Asaphis violascens*, can be successfully induced to spawn using various methods. Emersion method is the best method for the induced spawning in the present study. Broodstock was collected in April when the gonad is fully developed. Peak spawning season in natural habitat is in late summer. Induced spawning and an attempt to culture the life cycle of clams over a period of five months have been conducted for the first time in Myanmar. Among the several experiments, two trials (1000 indv. /10L each) using different antibiotics and sandy substrate were successfully cultured. The sizes reached about 30.76 mm and 27.88 mm in two experiments. It was observed that sandy substrate is the main factor for the survive in the laboratory. Food availability, water quality maintenance and aeration are the important factors that influence the growth and survival of the clams.

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