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EFFECT OF DIFFERENT LIVE FEEDS ON THE GROWTH PERFORMANCE, SURVIVAL RATE AND NUTRITIONAL PROFILES FOR EARLY LIFE STAGES OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) (LINNAEUS, 1758)*

Cho Cho Thin[1], May Thu Rein Oo[2], Aye Aye Khaing[3], Kay Lwin Tun[4]

Abstract

The success of larval rearing depends mainly on the availability of optimal diets during their growth stages. Live feed is considered to be critically important for larviculture of aquatic animals. Different live feed will affect the growth and survival rate of fish larvae. The objectives of present study are to assess the growth and survival rate of Tilapia fingerlings feeding on the different live feed and to analyze the nutrient composition (moisture, protein, lipid, and ash) of Tilapia treated with three kinds of live feed. The research was carried out in the Wet Lab and Live food Laboratories, Fisheries and Aquaculture, Department of Zoology, University of Yangon. Three kinds of live feed, freshwater rotifer, water fleas (*Moina*), and *Haematococcus* sp. were used in the present study. Firstly, three kinds of live feeds were inoculated to obtain the sufficient amount of feed for the experiment. Rotifer species were fed on *Chlorella* sp. during the culturing period. Similarly, *Moina* species was cultured by using shrimp powder and rice bran (1:1) as feed in this species. *Haematococcus* sp. by using nutrient media. Tilapia fingerlings were introduced with different livefood after completing the mass production of live foods. Three experimental designs were set up for Tilapia culture. Experiment I was fed with Rotifer, experiment II with *Moina* while Experiment III was treated with *Haematococcus* sp. Each experiment was set up in triplicate. A total of 25 fingerlings were introduced in each tank for rearing period 35 days. Sampling was carried out at seven days interval. The highest length-specific growth rate was observed in the group fed with Moina (1.546%), followed by the *Haematococcus*-fed group (1.343%), and, in turn, the Rotifer-fed group (0.864%). The maximum weight-specific growth rate was observed in the Moina-fed group (5.254%), followed by the Rotifer-fed group (4.769%), and then the *Haematococcus*-fed group (4.508%). There was no significant difference on both specific growth rate (length and weight) and the survival rate of Tilapia fingerlings among the various treatments. The nutritional composition (moisture, protein, lipids, and ash) of Tilapia fingerlings varied with different live feed. According to the result, *Moina* is considered as the most appropriate live food when they were tested.

Keywords: Tilapia fingerling, Live feeds, Mass production, Survival rate, Growth rate, Nutritional value

Introduction

Over the past two decades, aquaculture, or fish farming, has experienced rapid growth in Myanmar, assuming an increasingly significant role in the nation's fish supply (Belton, *et al*., 2015). The Ayeyarwady Region comprises 50 percent of the total aquaculture fishpond area, followed by the Yangon Region (27 percent) and the Bago Region (13 percent). In aquaculture fishponds, Burmese farmers cultivate over 20 species of freshwater fish, including Indian major carps, Grass carp, Mrigal carp, Silver carp, Chinese carps, Tilapia, Pangasius, Striped catfish, Catla, Rohu, Common carp, Walking catfish, and Pacu (USDA, 2022). Tilapia has been reported as the most important aquaculture species of the 21st century (Shelton, 2002). Tilapia is an excellent species for aquaculture due to its high tolerance to adverse environmental conditions, relatively fast growth, resistance to disease, excellent quality of its firm-textured flesh, short generation time, and appealing taste to consumers (Corpei, 2001). In the late 1990s, improved

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[1] Department of Zoology, Fisheries and Aquaculture, University of Yangon
[2] Department of Zoology, Fisheries and Aquaculture, University of Yangon
[3] Department of Zoology, Fisheries and Aquaculture, University of Yangon
[4] Department of Zoology and Fisheries and Aquaculture, University of Yangon

strains of the larger Nile tilapia (*Oreochromis niloticus*) were introduced and observed to exhibit faster growth. Soon after, tilapia barbecue become as one of the most popular fish dishes in restaurants, pubs, and roadside stalls. The current annual tilapia harvest stands at approximately 45,000 metric tons, with the vast majority being sold in the domestic market. Myanmar boasts one of the highest annual per capita consumption rates in the world, with individuals consuming 56 kilograms per person (Holmyard, 2017). In 2016, WorldFish Myanmar introduced GIFT into the Hlawgar Hatchery in Yangon Region and Daedeye Hatchery in the Ayeyarwady Delta in collaboration with the DOF to enhanced tilapia aquaculture in the country (Lwin, *et al.,* 2022). Tilapia is extensively cultured by both smallholder farmers and commercial intensive operators in Myanmar. In recent times, tilapia species are extensively cultured in intensive farms due to high demand from local consumers and an increasing demand from restaurants and barbecue shops (FAO, 2002). Therefore, a substantial number of tilapia seeds are required for production in culture system.

When Tilapia larvae or fry are maintained in a larva rearing tank, their growth and survival rates are crucial to obtain a high yield at harvest time. The larvae need to be fed as soon as the yolk sac disappears. If not fed, the larvae may die. The success of larval rearing depends mainly on the availability of optimal diets that are easily consumed, efficiently digested, and provide the required nutrients to support good growth, survival, and health (Giri *et al*., 2002). Live food organisms are able to swim in water column and are constantly available to larvae of aquatic animals. Live feeds play an important role in the aquaculture production of various fish larvae (Das, *et al.,* 2012).

The success of aquaculture relies on maintaining a healthy cultured stock. To achieve a disease-free, healthy stock, it is essential to provide live feed along with supplemented artificial feed. In terms of acceptance, nutritional content, and other factors, artificial larval feeds cannot match live feed. Live food organisms, often referred to as “Living capsules of Nutrition”, contain essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids, and fatty acids.

In this study, various live feeds were produced in the laboratory to determine the optimal live feed species crucial for survival and growth performance of fish during early life stages. The fish were then subjected to three different types of live feed, and their growth and survival rates were measured. Additionally, the nutritional composition of the experimental groups was estimate to elucidate the impact of different live foods on fish. Therefore, the present research aimed to assess the suitable live feed for the early life stages of Tilapia, with the following objectives:

- To produce the mass production of different live feed (Rotifer, *Moina* sp., and *Haematococcus* sp.,) for Tilapia fingerlings
- To examine the growth and survival rate of Tilapia fingerlings with various live feeds
- To analyze the nutrient composition (moisture, protein, lipid, and ash) of Tilapia fingerlings treated with different live feeds

Materials and Methods

The research was conducted in the Wet Laboratory and livefood Laboratory, Fisheries and Aquaculture, Department of Zoology, University of Yangon (Fig. 1).

Figure. 1. Map of the study area, Fisheries and Aquaculture, Department of Zoology (Source: Google)

The study period lasted from April 2022 to May 2023.

The tilapia fingerling (2-3 cm) was obtained from Department of Fisheries, Hlaw-Ga Fisheries Experimental station, Yangon.

Preparation of Live Feeds

Three kinds of life feed, freshwater rotifer, water fleas (*Moina*), and *Haematococcus* sp. were used in the present study.

Culture of Fresh water Rotifer

Fresh water Rotifer with a size range of approx. 90-100 μm in length was cultured in the Livefood Laboratory, Fisheries and Aquaculture, Department of Zoology, University of Yangon. *Chlorella* sp. were used as feed for Rotifer species during the cultured period. The density of Rotifer was determined daily with Sedgwick Rafter counter and Microscope (100x magnification). Partial harvests of Rotifer were conducted regularly, serving as live feed for Tilapia fingerling being cultured in the experimental tanks.

Culture of Water fleas (*Moina*)

Water fleas (*Moina*) were cultured in the same laboratory. A mixture of shrimp powder and rice bran (1:1) served as the feed for *Moina* sp. throughout the cultured period. Once the *Moina* population in the cultured tanks reached approx. 30-50 individual per mL, they were harvested to be used as food for tilapia fingerlings.

Culture of Microalgae Haematococcus sp.

Haematococcus sp. was cultured using fertilizer in the Live food laboratory. The growth of *Haematococcus* sp. population was monitored using a Hemocytometer and a Microscope with 100x magnification. On the sixth day, when the population density reached its peak at approximately 15,000 cell mL^{-1}, they were partly harvested as live feed for Tilapia fingerlings.

Water quality parameters were recorded daily during live food (Rotifer, *Moina*, and *Haematococcus* sp.) cultured period.

Experimental Design

Three experimental designs were set up for Tilapia culture. Experiment I was fed with Rotifer, experiment II with *Moina* while Experiment III was treated with *Haematococcus* sp. Each experiment was set up in triplicate. A total of 9 aquaria (120cm×60cm×45cm) was used for the experiment. All the aquaria were filled up with water and labeled according to the

experimental design. The aerator was used continuously for 24 hours throughout the experimental period. A total of 25 fingerlings was added to each aquarium. On the first day, no feed was given. Starting from the second day of stocking, the fish were provided with feed at a rate of 5% of their body weight. Feed was consistently supplied based on the body weight of the fish. The experiment was conducted in triplicate for each live food variant. Tilapia fingerlings were cultured for a duration of 35 days. Sampling was carried out on the 7^{th}, 14^{th}, 21^{nd}, and 28^{th}, and 35^{th} days of the experimental period, with subsequent measurement of individual fish's length and weight for further analysis.

The survival rate of fingerlings was recorded throughout the study period. Water quality parameters (pH, temperature, DO, and ammonia) were also analyzed daily. At the end of the experiment, fish samples were analyzed for the nutritional values (ash, lipid, protein, and moisture) by Association of Official Analytical Chemists (AOAC, 1995) at Fish Nutrition Laboratory, Department of Fisheries (DoF), Yangon.

Data Analysis

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

Length-specific growth rate = [(In final length – In initial length)] / days of rearing x 100

Weight-specific growth rate = [(In final weight – In initial weight)] / days of rearing x 100

Survival rate = (final number of fish / initial number of fish) x 100

Significant differences in growth and survival rate were tested using a one-way analysis of variance (ANOVA).

Results

The specific growth rate and survival rate of Tilapia fingerlings, which were fed with different live feeds (freshwater rotifer, water fleas (*Moina*), and *Haematococcus* sp.), were calculated at the end of the experiment. Moreover, the nutritional compositions of these study species were analyzed.

Firstly, three kinds of live feeds were inoculated in the Livefood Laboratory to obtain the sufficient amount of feed for the experiment. The highest population densities of rotifer (100 ind/mL) were found in 6^{th} day, those of Moina (30 ind/mL) in 5^{th} day, and those of *Haematococcus* sp. (9.00×10^6 cells/mL) in 6^{th} day of culture period (Fig. 2, Fig. 3, and Fig. 4). Tilapia fingerlings were introduced with different livefood after completing the mass production of livefood.

The highest length-specific growth rate was observed in the group fed with Moina (1.546%), followed by the *Haematococcus*-fed group (1.343%), and, in turn, the Rotifer-fed group (0.864%) (Fig. 5). The maximum weight-specific growth rate was observed in the Moina-fed group (5.254%), followed by the Rotifer-fed group (4.769%), and then the *Haematococcus*-fed group (4.508%) (Fig. 6). The highest survival rate was observed in Rotifer-fed group (84%), followed by *Moina* (80%) and *Haematococcus* (72%) (Fig. 7). There was no significant difference on both specific growth rate (length and weight) and the survival rate of Tilapia fingerlings among the various treatments.

The nutritional composition (moisture, protein, lipids, and ash) of Tilapia fingerlings was varied with different live feed (Fig. 8). The protein content of Tilapia fingerlings was found to be

highest in the Moina-fed group, while the moisture, lipid, and ash contents were highest in the Rotifer-fed group.

It was observed that there was no significant difference in water quality parameters among different treatments (Table 1). The range of temperature from (26.6 ± 0.95°C) to (26.8 ± 0.94°C), dissolved oxygen (DO) from (5.9 ± 0.28) mg/L to (5.97 ± 0.32) mg/L, and pH from (7.9 ± 0.03) to (7.913 ± 0.01) were recorded among different treatments throughout the study period. The value of ammonia was not detected during the study period.

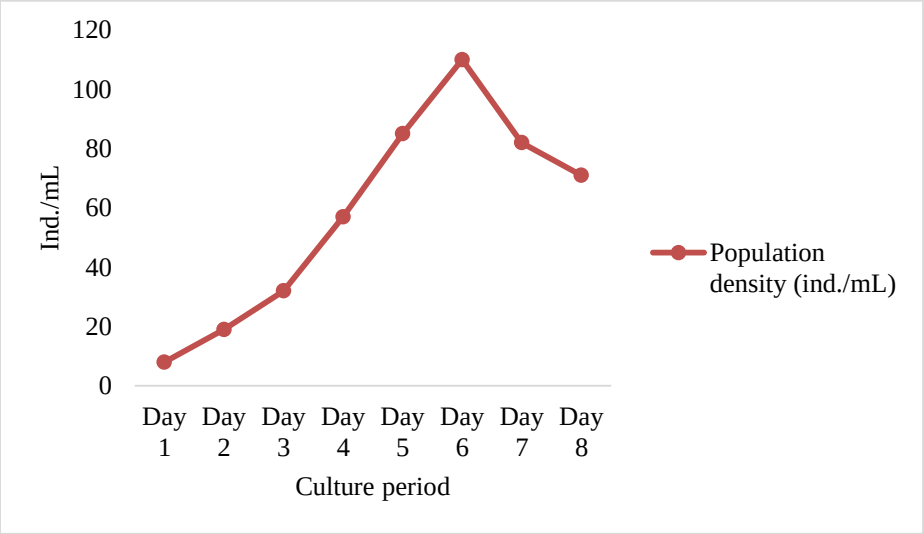

Figure. 2. The curve of population density of Rotifer *Brachionus* sp.

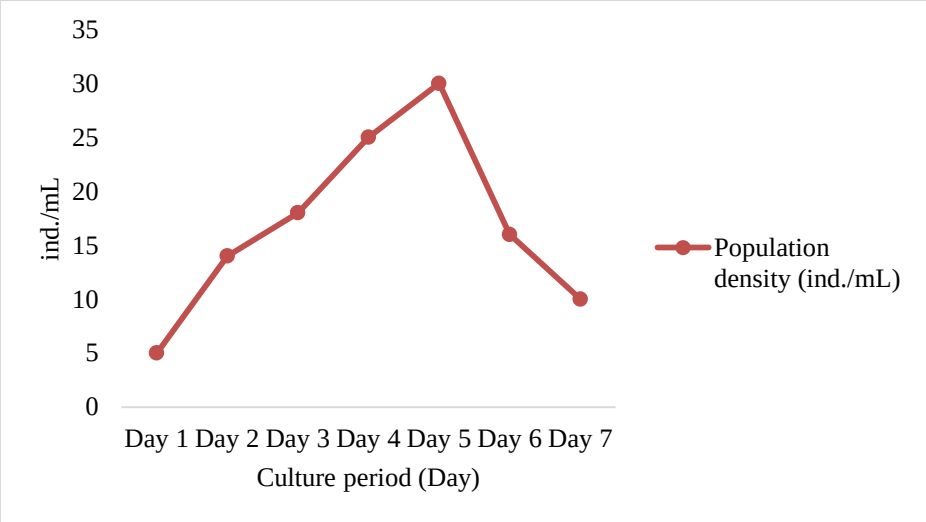

Figure. 3. The curve of population density of Moina

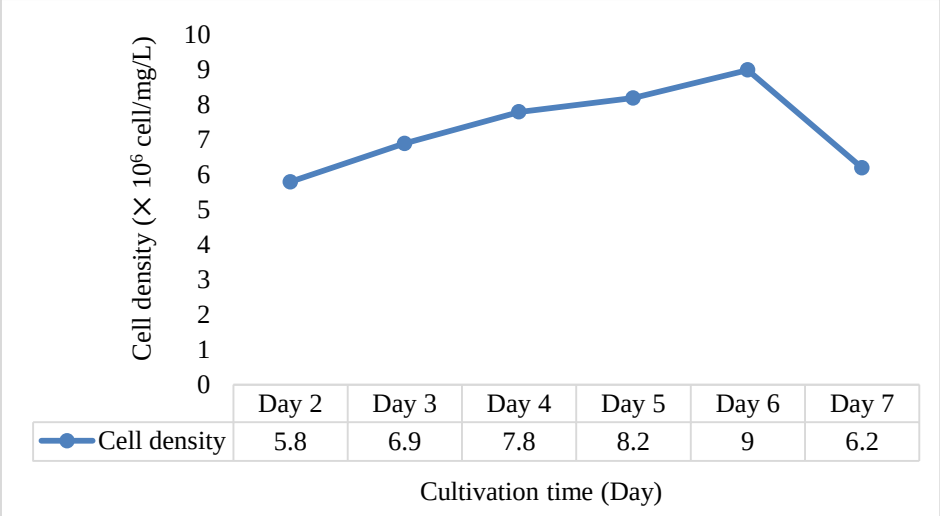

Figure. 4. The curve of population density of *Haematococcus* sp.

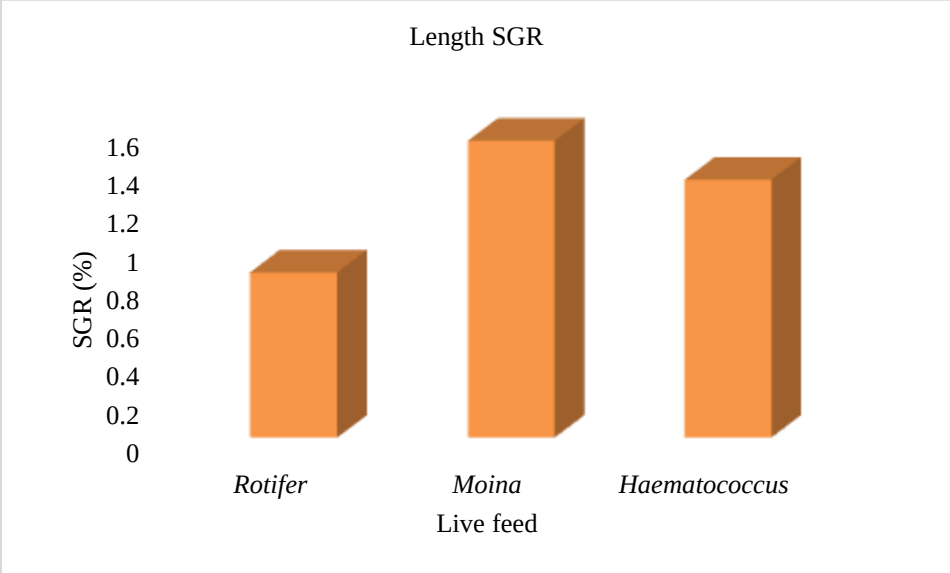

Figure. 5. Length Specific Growth Rate of Tilapia fingerlings treated with different live feed

Figure. 6. Weight Specific Growth Rate of Tilapia fingerlings treated with different live feed

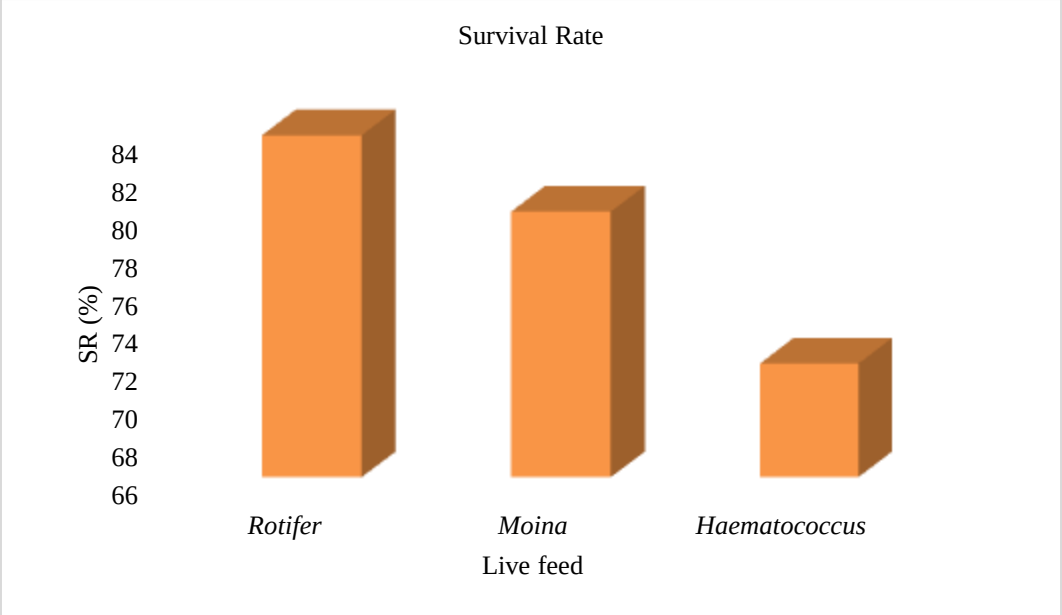

Figure. 7. Survival Rate of Tilapia fingerlings treated with different live feed

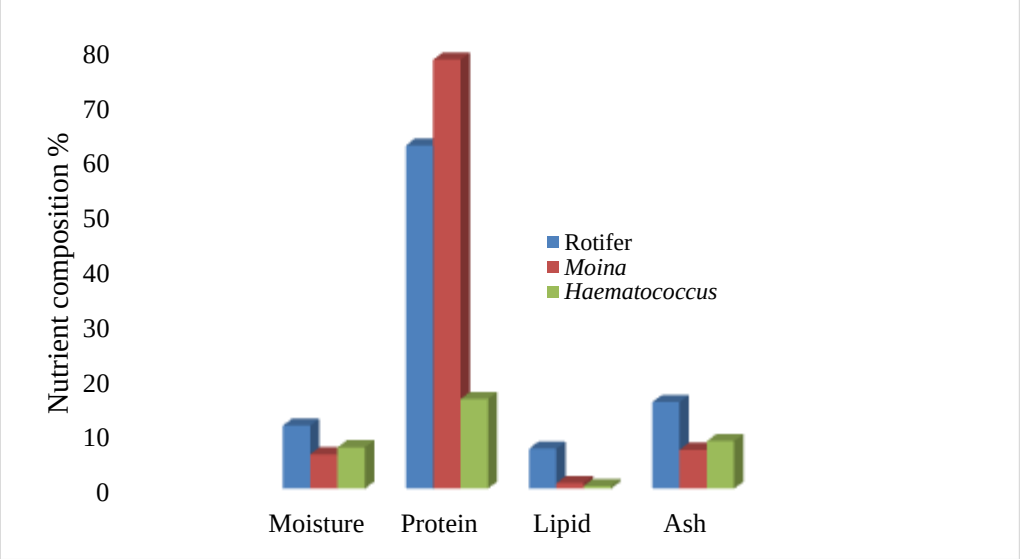

Figure. 8. Nutrient composition of Tilapia fingerlings treated with different live feed

Table 1 Water quality parameters in Nile tilapia, *Oreochromis niloticus* cultured tank under different treatment during the study period

Live feed	T (°C)			DO (mg/L)			pH			Ammonia (mg/L)		
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mea	±	SD
Rotifer	26.809	±	0.9420	5.915	±	0.353	7.913	±	0.01	0	±	0
Moina	26.738	±	0.945	5.972	±	0.315	7.906	±	0.01	0	±	0
Haematococcus	26.603	±	0.948	5.904	±	0.281	7.9	±	0.03	0	±	0

Discussion

Larval rearing is one of the most difficult points in aquaculture due to its high mortality. The risk of mortality can be overcome by feeding suitable live feed. In the present study, three kinds of live feed (Rotifer, *Moina,* and *Hametococcus*) were cultured as food for Tilapia fish larvae. Production of nutritious Rotifers depends on the production of microalgae used to feed them. Many researchers have recently chosen *Moina macrocopa* as a potential live feed to be studied as food sources for tilapia fry rearing (Ramesh *et al*., 2014). *Moina* species are an important source of essential nutrients such as amino acids, protein, lipids, fatty acids, enzymes and vitamins (El-Naggar *et al.,* 2019). Rice bran and shrimp powder are potential feed for *Moina* since they contain various nutrients such as protein (12–13%), lipid (16–20%), linoleic acid, acids α linolenate, vitamin B (Faria *et al*., 2012; Murtaza *et al*., 2011). So, rice bran and shrimp powder are used as the food source for *Moina* species in the present study.

Haematococcus sp. is a unicellular freshwater microalga that is a promising source of bioactive substances, such as carotenoids, proteins, and fatty acids (FAs), particularly astaxanthin, a powerful antioxidant (Mehariya, *et al.,* 2020). *Haematococcus* sp. was inoculated with nutritive media as feed for Tilapia fingerlings in the current experiment. The highest population density was reached in the 6^{th} day of culture period (9.00×10^6 cells /mL) in the present study.

The specific growth rate and survival rate of Tilapia fingerlings were calculated at the end of the experiment to assess their growth performance. The highest length-specific growth rate was observed in the group fed with *Moina* (1.546%), followed by the *Haematococcus*-fed group (1.343%), and, in turn, the Rotifer-fed group (0.864%). The maximum weight-specific growth rate was observed in the Moina-fed group (5.254%), followed by the Rotifer-fed group (4.769%), and then the *Haematococcus*-fed group (4.508%). Statistical analysis of the average Specific Growth Rate (SGR) among treatments reveals that there is no significant difference ($p > 0.05$) in both body length and body weight. Hussain *et al*., (1987) recorded that the survival rate of Tilapia ranged from 82% to 90% by feeding on live feed. In the present study, the highest survival rate was observed in tilapia fingerlings fed with Rotifers (84%), *Moina* (80%) and *Haematococcus* (72%). There was no significant difference ($p>0.05$) in the survival rate of Tilapia fingerlings among the various treatments. According to Rocha *et al.,* (2017), the

utilization of zooplankton as an initial feed for fish larvae, such as in the rearing of codfish larvae, can enhance fish growth and survival. Compared to artificial feed, live feed contains relatively higher nutrients and is easily obtained at relatively low costs. It also has a size that matches the mouth opening of the fish, particularly the size of the fingerlings (Putra, *et al.*, 2019). So, live feed was observed as ideal feed for survival and growth of Tilapia fingerlings in this study.

The nutrient compositions of Tilapia fingerlings after 35 days rearing period were analyzed. The highest moisture content was recorded in Tilapia fingerlings that fed on Rotifer (11.51%) followed by *Haematococcus* (7.56%) and *Moina* (6.28%). A feed that contains a high protein content is favorable because it serves as the essential nutrient provider that supports fish development and survival (Rocha *et al*., 2017; Karlsen *et al.,* 2015). Furthermore, the lipid content in Tilapia fry varied with the different nutrient used for live feed. Both protein and lipids are essential nutrients that play a significant role in the rearing of fish fry (Radhakrishnan *et al*., 2020). In the present study, tilapia fingerlings that were fed Miona enriched with rice bran exhibited the highest protein content (78.12%), while the highest lipid content was found in Tilapia fingerlings that feed on Rotifer (7.3%). The percentage of ash in Tilapia fingerlings that consumed various live feeds was 7.1% for Moina, 8.7% for *Haematococcus*, and 15.8% for Rotifer, respectively. Ash comprises various minerals that play essential roles in the structural integrity of organisms, such as calcium, magnesium, phosphorus, iron, zinc, and so on (Pilot, 2014).

Water quality is very importance in aquaculture farming. Maintaining balanced levels of water quality parameters is essential for both the health and growth of farmed aquatic species.

Water temperature can affect the metabolism, feeding rates, and the degree of ammonia toxicity in fish and shrimp. The temperature range was 26.81±0.94°C to 26.60± 0.95°C during the experimental period. De Verdal *et al.,* (2018) observed that the water temperature plays a key role in regulating the metabolic processes of fish. The optimum temperature for Tilapia culture is 26°C to 32°C (Khan *et al.*, 2008). The water temperature in the experimental tanks was found to be suitable for the culture unit. In this experiment, no value of ammonia was detected because live feed can reduce the water quality deterioration. Dissolved oxygen concentration is an important water quality parameter that affects the growth and survival of fish. A reduction in dissolved oxygen content has negative effects on the growth, reproduction, and other biological activities of fish, and extremely low dissolved oxygen levels can be lethal to fish. Tilapia can tolerate dissolved oxygen concentrations as low as 0.1 mg/L (Dan and Little, 2000). In the present study, the average value of DO was around 5.97±0.32 mg/L in each treatment during the study period. A higher level of dissolved oxygen concentration was recorded in the experimental tanks as a result of aeration throughout the experiment. The level of pH was found to be optimal range 7.9±0.03 to 7.913±0.01during the experiments. In the current study, water quality was found to be improved as the Tilapia fingerlings fed on live feed. The ideal pH range for fish life is 6.5 to 9.0, while pH values below 4 and above 11 leads to fish mortality (Craig, and Helfrich, 2009).

Conclusion

This research was provided what kind of live food is the best for the growth and survival rate of Tilapia fingerling. The information was valuable knowledge for Tilapia aquaculture in Myanmar. In addition, the composition of the nutritional value of experimental fish was evaluated intend to produce high protein fish for human consumption. We contribute the information to the farmers as well as to the researchers on how to produce live feed for Tilapia to obtain a high yield in the aquaculture farm. The findings of this study indicated that for

aquaculture purposes, tilapia fingerlings can be successfully cultivated in aquatic environments with different live feeds, leading to increased fish production and improved economic returns.

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EFFECT OF FEEDING FREQUENCY ON GROWTH PERFORMANCE OF GIFT TILAPIA (*OREOCHROMIS NILOTICUS* LINNAEUS , 1758) IN DEEP WATER CULTURE AQUAPONICS SYSTEM*

San Htet San[1], Yu Yu Aye[2], Kay Lwin Tun[3]

Abstract

Aquaponics integrates aquaculture and hydroponics into a common closed-loop eco-culture where a symbiotic relationship is created in which water and nutrients are recirculated and reused, concomitantly fully utilized and conserved. In this study, GIFT Tilapia (*Oerochromis niloticus*) (2.8 ± 0.5g) collected from Hlawga Hatchery Station; Yangon which was cultured with different feeding frequency in DWC aquaponics systems to investigate the appropriate feeding frequency for optimal growth of GIFT tilapia. These experiments were divided into three treatments on the basis of feeding frequency (2 times (2T), 3 times (3T) and 4 times (4T)) per day respectively, having three replications. Then 24 seedlings of lettuce were introduced aquaponic system. Fish were fed floating pellet for 5 % of body weight. Fish were cultured from July to November, 2022. The highest total mean weight was found in 4T (356 g), followed by 3T (326 g) and 2T (281.6 g) respectively. The highest final weight gains were recorded in 4T (219.6 g) while the lowest weight gain in 3T (186 g) followed by 2T (161 g) in the end of experiment. In November, the highest FCR was found in 2T (1.1) while the lowest FCR was recorded in 4T (0.9) followed by 3T (1). High specific growth rate (11) was found in 4T treatment in two production cycles. For plant quality index, the highest number of grade A plants were observed in 4T. However, 12 and 13, Grade B plants were found in 2T, 3T and 4T in first and second harvest times. According to this study, it is understood that feeding frequency has an important effect on growth rate: 4T system shows the most favorable outcome for tilapia and lettuce than other frequencies.

Keywords: Tilapia, feeding frequency, growth performance, lettuce

Introduction

Aquaculture is the culture of aquatic organisms in a designated water body. The water needs to be treated whenever toxicants in it have built up beyond animal's safe level. Toxicants such as ammonia and nitrite are derived from decomposition of unconsumed feed and metabolites or waste of the animals. Hydroponics is the culture of aquatic plants in soilless water where nutrients for plant's growth come entirely from a formulated fertilizer (Liang and Chien, 2013). Aquaponic systems are integrated recirculating aquaculture with hydroponics as fast emerging food production technology (Rakocy *et al*., 2004). In aquaponic systems, the wastewater from aquaculture system that is rich in nutrients is circulated to vegetable grow beds (Rakocy *et al*., 2006).

As the microbes break down fish waste metabolites into soluble nutrients. Thus, plants can uptake nutrients directly from water. Already treated, cleansed and safe water for the fish flows back to aquaculture system for reuse (Somerville *et al*., 2014). Aquaponics productions are known to be natural, organic, eco-friendly and free of pesticides and herbicides (Blidariu and Grozea, 2011). Other advantages are: less usage of water through reuse, the recycling of nutrients and management of waste, and minimize adverse environmental impacts such as pollution (Al-Hafedh *et al*., 2003; Rakocy *et al*., 2004b). In addition to the ecological benefits, aquaponics systems are capable of offering several economic benefits such as: savings in the costs of the treatment of water in the aquaculture system (Adler *et al*., 2000; Liang and Chien, 2013).

Tilapia is the most commonly used fish in aquaponics systems (Rakocy *et al*., 2006) for their high availability, fast growing, stress and diseases resistant and easy adaptation to indoor environment (Hussain, 2004). The mostly grown plants in aquaponics include lettuce, water spinach, tomato, cucumber, pepper and herbs (Rakocy and Hargreaves, 1993; Adler *et al*., 2000;

*Second Prize (2023)

[1] Fisheries and Aquaculture (Department of Zoology), University of Yangon
[2] Fisheries and Aquaculture (Department of Zoology), University of Yangon
[3] (Department of Zoology), University of Yangon

Savidov *et al*., 2005). Among those, Lettuce (*Lactuca sativa*) is commonly used because it is well adapted to aquaponic systems. It can be harvested within 3 to 4 weeks, with relatively fewer pest problems and low to medium nutritional requirements (Diver, 2006; Rakocy *et al.*, 2006).

Feeding frequency is important to ensure a maximal food conversion ratio and weight of cultured organisms (De Silva and Anderson, 1995). Higher feeding frequencies decrease aggressive behavior may resulting the faster growth and uniformity in size (Zhou *et al*., 2003). Moreover, feeding frequency can affect growth performance, survival, body composition and water quality (Zakes *et al*., 2006). Furthermore, the feed cost is one of the largest operational costs in the aquaculture industry (De Silva and Anderson, 1995).

An important approach for reducing feed costs in commercial aquaculture is to develop proper feed management, husbandry strategies (Lovell, 1998). Hence, the act of feeding may be pointed as one of the most vital elements in the culture practice (Pouomogne and Ombredane, 2001). Several authors had already studied the influence of feeding frequency on growth performance for various species (Kasiri *et al*., 2011). However, the effect of feeding frequency on growth performance of GIFT tilapia reared in aquaponics system is yet quite limited.

Therefore, the present research work was carried out with an aim to find the more effective and a suitable feeding frequency for optimal growth and survival during rearing.

Materials and methods

The present study was conducted at the Laboratory of Aquatic Bioscience, Department of Zoology, University of Yangon.

The experiment was carried out from July to November 2022.

Experimental design

In this experiment, aquaponic systems included fish tanks, hydroponic tanks and biofilter. A fiber tank (0.85m x 0.61m x 0.43m) was used for the aquaponic system while fiber tank (1.2m ×1.2m×0.43m) was applied for hydroponic tanks. In the hydroponic tank, Styrofoam block(1.1m×1.1m) entirely covered the surface area of hydroponic tank. A water pump (75 Watt) was used in all systems. The running water system was set up between the hydroponic tank and the fish tank with the aid of a pump through a biofilter (Fig. 1 and Plate 1). The biofilter tank is one of the most important components in an aquaponics system as it reduces the toxicity of the nitrogenous waste for fish. In the present study, shells from bivalve were used as substrate in the biofilter to growth the nitrifying bacteria in large surface area. Large plastic bucket (0.39 m in diameter ×0.43 m in height) was filled with shell of bivalves until two-thirds of the bucket. This experiment system was set up for triplicate. The DWC unit, also called the float or raft system, set up a fish tank, filters, canals, and floating rafts (Plate 2).

Sample collection

A total of 400 fingerlings GIFT Tilapia (*Oreochromis niloticus* Linnaeus, 1758) was collected with oxygen filled plastic bags from Hlawga Hatchery Station to laboratory of Aquatic Bioscience, Department of Zoology, Yangon. Genetically Improved Farmed Tilapia (GIFT), a strain of tilapia, is one of the most-farmed aquaculture fish. Each fish tank is contained, 40 fingerlings GIFT tilapia (2.8 ± 0.5g) were put in each tank (0.85 x 0.61 x 0.43 m3).

Feeding regime

Three feeding frequencies were tested: two meals per day (9:00 and 16:00 hours; 2T), three meals per day (9:00, 12:00 and 16:00 hours; 3T), and four meals per day (8:00, 11:00, 14:00 and 17:00 hours; 4T). Three replications were allocated for each feeding frequency.

Preparation of vegetables

Seeds were put in seedling trays with coconut coir and soil. Seeds were germinating within four days.Seethings of lettuce (Lactuce sativa) were used as vegetable in this study.

Firstls, seeds were put in seelling trays with cocount coir soil . After two weeks, seedling of lettuce were transplanted in pot. Seedlings of lettuce were cultivated in the whole of styrofoam in DWC. The density of plant was 24 plants m^2. Plants were put to the pot together with substrate (coconut coir) which help the plants to stand vertical (Plate 3).

Growth parameters analysis of fish

Fish growth performance such as weight gain, specific growth rate and feed conversion ratio were evaluated in accordance with Cerozi and Fitzsimmons (2017).

$$\text{Weight gain (WG, g)} = \frac{Wf - Wi}{\text{number of fish per replicate}}$$

$$\text{Specific growth rate (SGR, \%)} = 100 \times \frac{\ln Wf - \ln Wi}{\text{days of feeding period}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{feed intake (g)}}{\text{weight gain (g)}}$$

wf = final weigth

wi= initial weigth

Growth parameters analysis of plants

Final plant parameters were: height of leaves (cm), weight of leaves and roots (g), number of leaves per plant, and specific growth rate (SGR = [(ln final leaves wet weight − ln initial leaves wet weight) × $time^{-1}$] × 100) (% day^{-1}).

Additionally, a plant quality index (PQI) was evaluated by grades attributed to visual aspect of the leaves. Visual parameters included abnormalities in the leaf surface such as yellowish color and/or imperfections (wrinkles and burns). The grades were from A to D as follows: (Pinho *et al*., 2017).

A = Excellent, up to 5% of the leaves surface with imperfections

B = Good, 33% imperfections

C = Average, 66% imperfections

D = Poor, 100% imperfections

Plants grades assessed using a “blind” approach where three valuators did not know which treatment the plants were grown.

Water quality analysis

The water quality parameters; pH, dissolved oxygen and temperature were monitored by probes (ID-1100, USA and ID-150, Iijima Electronics Corporation) every day in all tanks. Nitrate and ammonia equipment calibrated from the experimental tanks were measured twice a week using colorimetric test kits.

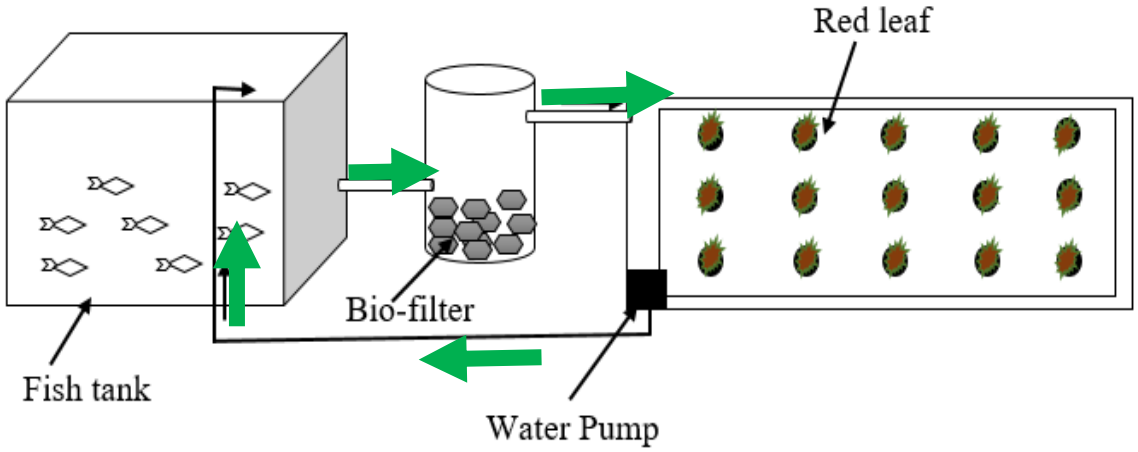


Figure. 1 Schematic diagram of Deep Water Culture (DWC) aquaponic system

Plate 1 Deep Water Culture (DWC) aquaponic system in experiment

Plate 2 Preparation the three different units in aquaponic system

Plate 3 Germination of Lettuce before introducing to aquaponic systems

Results

Growth of GIFT Tilapia

Growth of fish was studied during the period of five month culture in DWC aquaponic systems. In the beginning of experiment, mean weight of tilapia was 2.8g in all systems. Fish weight gradually increased during study period. At the end of experiment, the highest total mean weight was found in 4T (356 g), followed by 3T (326 g) and 2T (281.6 g) respectively (Fig. 2).

The highest final weight gains were recorded in 4T (219.6 g) while the lowest weight gain in 3T (186 g) followed by 2T (161 g) in the end of experiment (Fig. 3). Final specific growth rate of fish was (3.5) followed by 3T (3.2 g) and 2T (2.7 g) respectively (Fig. 4).

Food conversion ratio (FCR) in October, were 0.9, 1 and 1.1 in 2T, 3T and 4T respectively. In November, the highest FCR was found in 2T (1.1) while the lowest FCR was recorded in 4T (0.9) followed by 3T (1). Although the highest FCR was found in 4T, in the end of experiment in November, the lowest FCR was recorded (Fig. 5). The mortality of GIFT tilapia was found 98% in the beginning of experiment.

Lettuce production

During experimental period, the condition of plants was measured in all aquaponic systems. Plants were cultivated and harvested for two production cycles of lettuce during the study period. High specific growth rate (11) plants were found in 4T treatment in two production cycles (Fig. 6).

Plant quality index was assessed as A, B, C and D according to the quality of plant. For plant quality index, the highest number of grade A plants were observed in 4T. However, 12 and 13, Grade B plants were found in 2T, 3T and 4T in first and second harvest times. None of grade D lettuce was found in 4T while 1 grade D lettuce was found in 2T and 3T in September 15 - October 30, 2022 (Fig. 7).

Water quality

The water quality was recorded from July to November, 2022. In all experiments, at the end of experiment, ammonia and nitrite levels were 0.2 mg/L in all tanks. pH level of ranged between 6.8 to 7.5 in all tanks. Temperature ranged between 24 to 28 ºC in all systems. Dissolved oxygen ranged between 6 to 6.8 mg/L in all tanks (Table 1).

Table 1 Water Parameters during experimental period

Parameters	July			August			September			October			November		
	2T	3T	4T	2T	3T	4T	2T	3T	4T	2T	3T	4T	2T	3T	4T
Dissolved Oxygen (mg/L)	6	6	6	6.8	6.6	6.5	6.8	6.8	6.6	6.5	6.8	6.5	6.7	6.6	6.4
Temperature(°C)	24	24	24	25	25	25	27	27	27	27	27	27	28	28	28
pH	7.3	7.3	7.3	7.5	7.4	7.2	7.4	7.4	6.9	7.5	7.4	6.8	7.4	7.2	6.8
Ammonia (mg/L)	0	0	0	0	0	0	0	0	0	0.1	0.1	0.1	0.1	0.1	0.2
Nitrate (mg/L)	0	0	0	0	0	0	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2

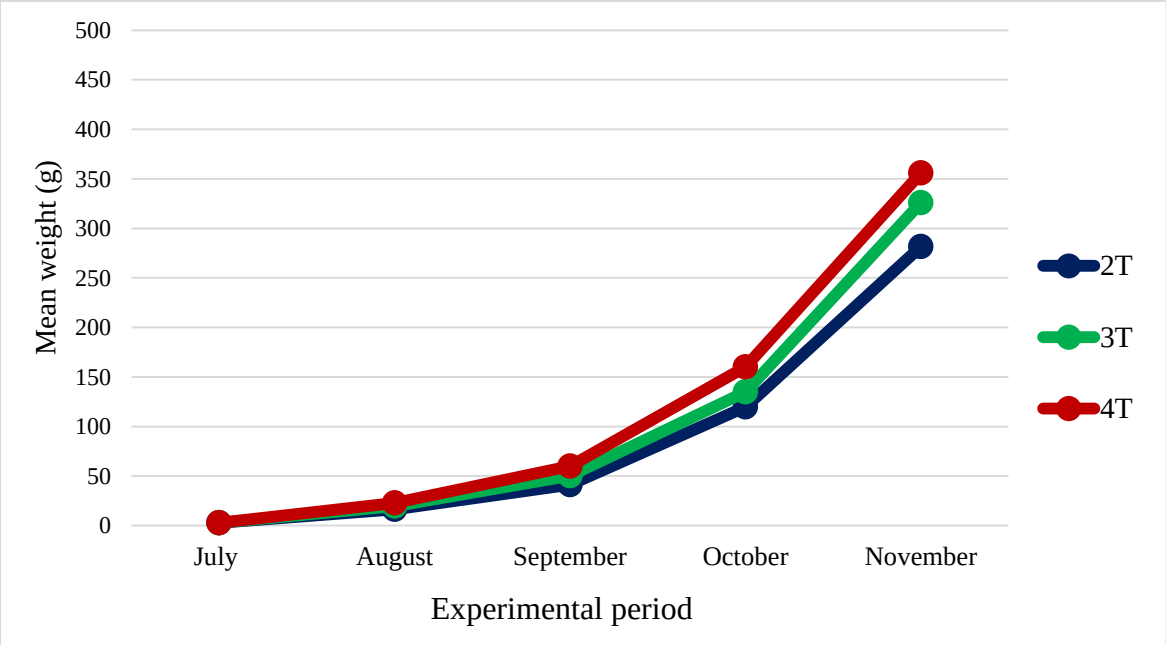


Figure. 2 Total Mean weight of GIFT Tilapia in all experiments

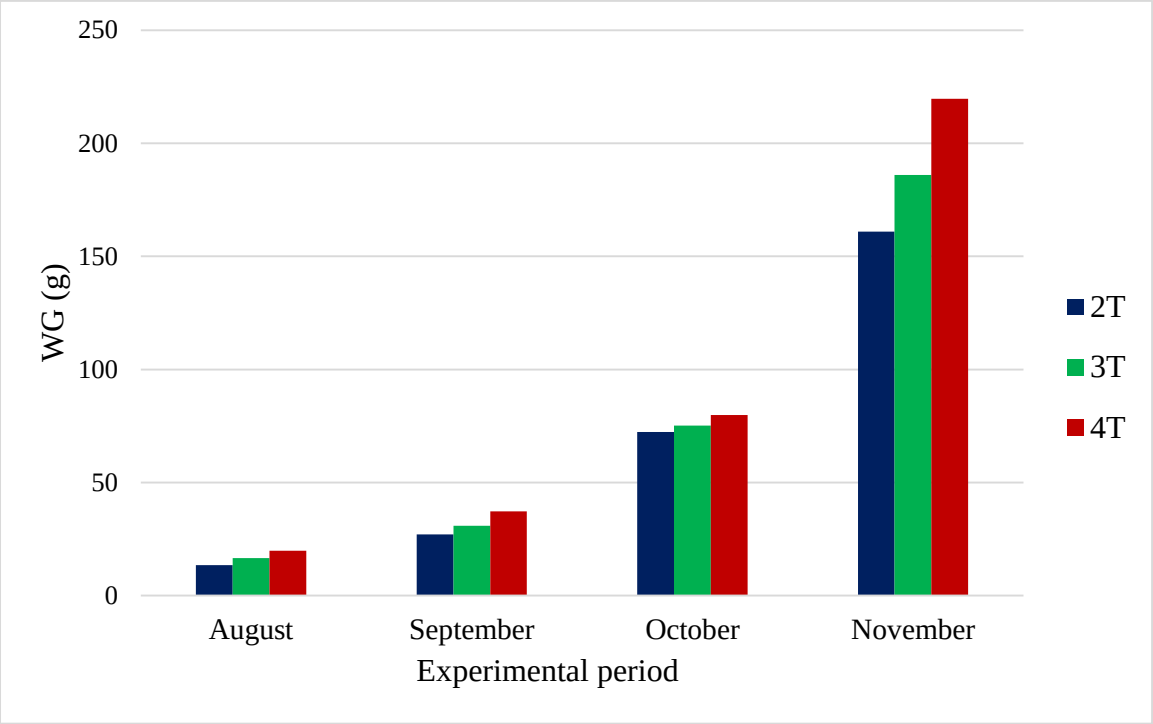


Figure. 3 Weight gain of GIFT Tilapia in all experiments

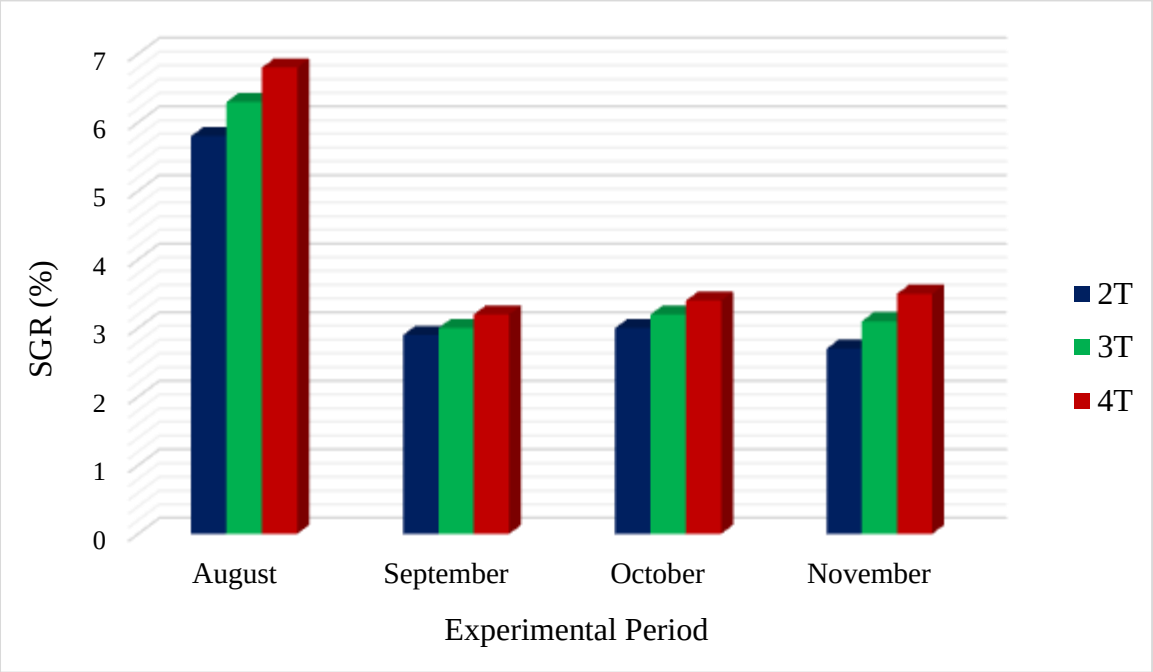


Figure. 4 Specific growth rate of GIFT Tilapia in all systems

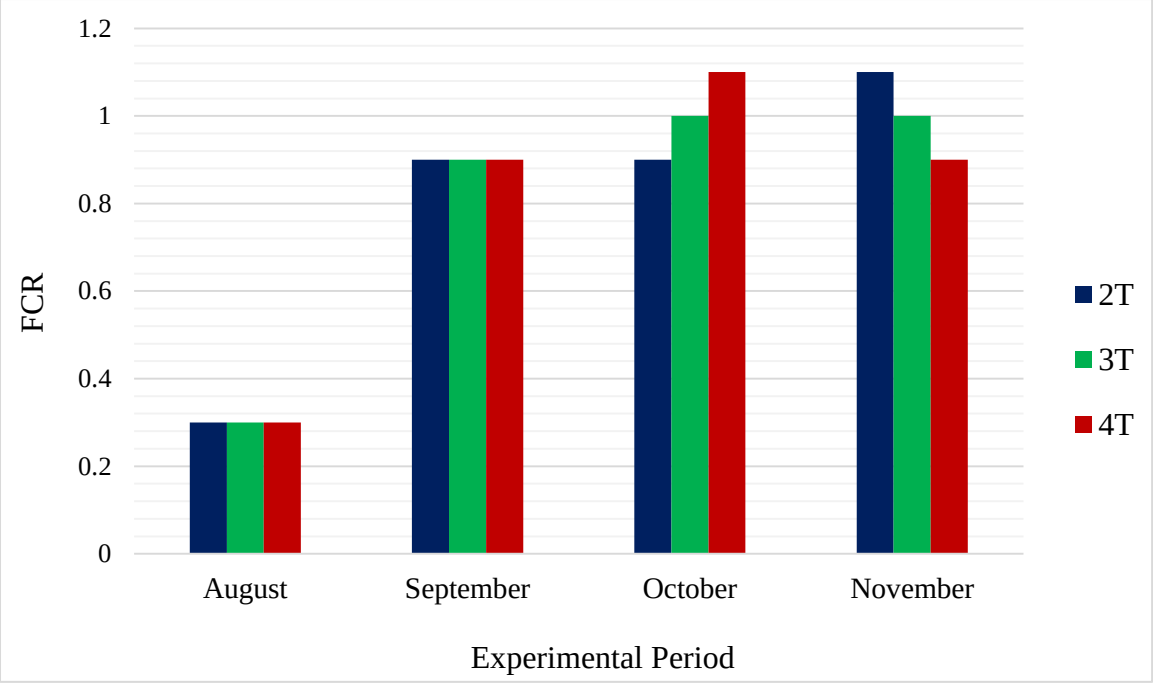


Figure. 5 Food conversion ratio in experimental tanks

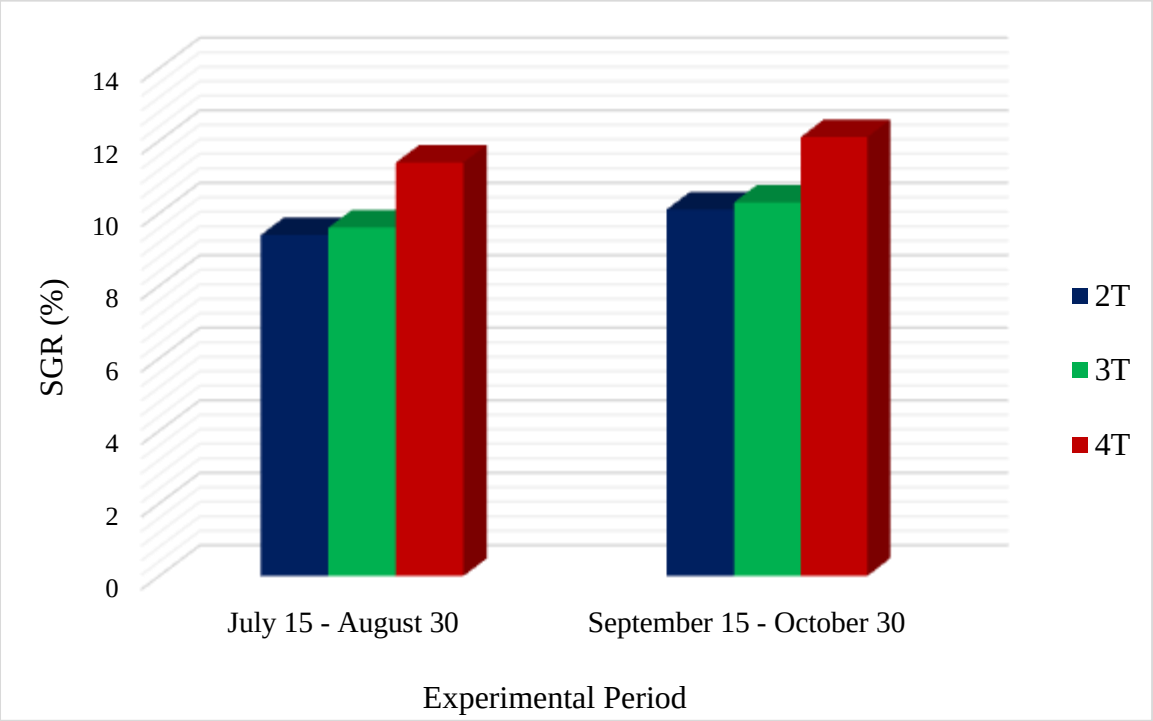
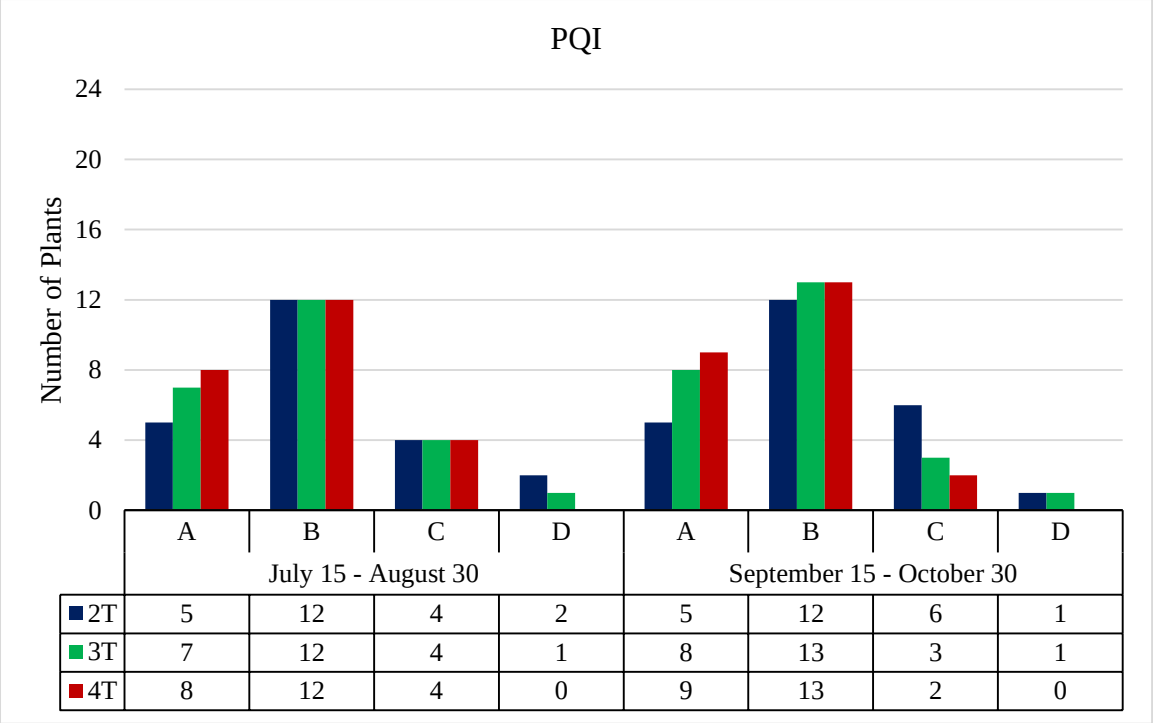


Figure. 6 Specific growth rate of lettuce in aquaponic systems



	A	B	C	D	A	B	C	D
	July 15 - August 30				September 15 - October 30			
2T	5	12	4	2	5	12	6	1
3T	7	12	4	1	8	13	3	1
4T	8	12	4	0	9	13	2	0

Figure. 7 Plant Quality Index of lettuce in all aquaponic systems

Discussion

Feeding frequency is one of the most important considerations in aquaculture practice that can affect overall growth, survival as well as habitat of fish. Again, the optimization of feeding frequency is considered as a significant factor as profit is the main motivating reason in fish culture. In the present study, Tilapia *Oreochromis* sp. growth and feed conversion values as related to feeding frequencies, initial weights and survival rate was similar in all treatments.

However, the final weight, weight gain, specific growth rate, feed intake and feed conversion ratio (FCR) significantly increased in all experimental tanks. Where fish were fed four times a day, followed by those tanks with fish fed thrice and twice a day. During the experimental period, three types of feeding frequency were conducted to observe the growth performance of tilapia. The mean final weight of tilapia was 356 g in 4T followed by 326 g in 3T and 281.6 g in 2T. Ali *et al*. (2017) studied that three-feeding frequency with four (T1), three (T2) and two times (T3) for four months. Their results were similar to the present findings that the highest mean final weight of tilapia was found in the tank treated with four times (4T).

The highest weight gain and SGR (%) were found in 4T which might be due to the effect of having four times feeding frequencies in a day. The lowest weight was found in 2T having feeding frequency two times a day. Yousif (2004) that had emphasized the effects of feeding frequency on growth performance of Nile tilapia juveniles. He reported that 3 meals/ day of feeding frequency was significantly better growth performance of tilapia. However, lower growth performance was observed in all groups of fish fed twice/day.

With regards to FCR, the lowest values (0.9) were recorded in *Oreochromis* sp. fed with four times (4T) per day followed by (1) three times (3T) and (1.1) two times (2T). According to Somerville *et al*., (2014), FCR for tilapia cultured in earthen pond is 1.4 to 1.8 which is higher than present study. Feeding frequency not only improved the growth indices, but also had a great impact on survival of tilapia. Liang & Chien (2013) reported that higher feeding frequency is well-effected tilapia survival and weight gain. The best plant quality index was recorded in 4T during the experiments. Plants indirectly used fish feed from fish waste (Rana *et al*., 2009).

In this study, the better production of lettuce (*Lactuca sativa*) was found in four times feeding frequency/day and also has better yield of tilapia production. The results indicated that the four times/day feeding frequency is more suitable for recirculation aquaponics of tilapia and lettuce production. The present findings are similar to the findings of Liang and Chien (2013) and Rakocy *et al*., (1997) who stated that higher feeding frequency would have an effect on plant growth, SGR and yield performance of tilapia and water spinach in raft aquaponics system.

Rakocy *et al*., (2006) indicated that water dissolved oxygen and pH plays a vital role in aquaponics system, tilapia culture and Lettuce *L. sativa* cultivation. In this study, the higher feed frequency experiment showed the slightly low level of Dissolved Oxygen (DO) and pH level; but it was within the acceptable level of tilapia growth. Lowest ammonia levels ranged 0 to 0.3 mg/L in all systems. Biofilter in aquaponic system convert fish waste ammonia into plant food nitrate (Rakocy, 2004a). Although, fish were feed with four times/day, water quality especially for ammonia in all tanks were acceptable levels.

Conclusions

The production of tilapia and lettuce using the DWC aquaponic system was studied in this experiment. The highest production of Tilapia and lettuce was observed in 4T (feed four times per day) during the study period. The water quality parameters are the favorable condition for the growth and survive for fish and lettuce in the study period. It was concluded that the maximum feeding frequency of four times per day (4T) is recommended to achieve maximum profitability. It is also recommended to use either of them with any selection of the other one, based on the business requirements of the system.

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THERMAL INDUCED SPAWNING FOR LARVAL AND SPAT PRODUCTION *PINCTADA MAXIMA* (JAMESON, 1901)*

Su Mon Theint[1], San San HmwE[2], Moe Thida Htway[3]

Abstract

Species *Pinctada maxima* (Jameson,1901) is one of four major pearl oyster species utilized by the cultured pearl industry for pearl production. They are rich in the banks of Mergui Archipelago in the Andaman Sea. Thermal stimulation is a successful spawning induction in all major commercial pearl oyster species in hatchery. In the present study, the induce spawning method on hatchery of gold-lip oyster and silver-lip oyster *Pinctada maxima* was used for larval and spat oyster culture conduction in Myanmar Pearl Enterprise, Pearl Island, Myeik Archipelago. Different developmental stages of larval oyster were found after the fertilization in the hatchery such as cleavage stage, trochophore stage, D-shape stage, umbo stage, eye-spot stage and spat stage, etc. the time was taken from the selection for mother oysters until the spat collection stage was for 22days. The measurements of larvae were 80-90 µm with the mean size 87.6 µm in gold-lip oyster and 90-110 µm with the mean size 105.2 µm in silver-lip oyster on six days after fertilization. After 20 days, the size of measurement was increased to 170-260 µm with the mean size 234.4 µm whereas 180-280 µm with the mean size 243.0 µm for gold-lip and silver-lip oysters respectively at the optimal environmental condition in hatchery.

Keywords: *Pinctada maxima,* induce spawning, fertilization, optimal environmental condition

Introduction

Mollusca is the most diverse marine phylum on earth. As a fossil record dating back almost 550 million years. They are related to well-known groups such as gastropods (snails), cephalopods (octopus), scaphopods (tusk shells) and other bivalves (clams, edible oysters and mussels) (Southgate and Lucas, 2008). *Pinctada* species (Roding) belongs to family Pteriidae and bivalves of great beauty and age. They occur in almost all the seas of the tropical and subtropical belt (Alagarswami, *et al.*, 1983b). there are 28 species of pearl oysters, the species for producing of pearls with good gem quality and most commercial value are *Pinctada maxima* (Jameson), *P. margaritifera* (Linnaeus) and *P. fucata* (Gould) (Chellam, *et al.*,1991). Pearls are the oldest gems known to man. Pearls and their shells have been used for human adornment since at least 1500BC (Strack, 2006) and the oldest found and documented pearl has been dated back to 5500BC (Charpentier *et al*., 2012).

Plate 1. Shells and Pearls of Silver lip oyster (Introduced from Indonesia, 2017) and Native gold lip oyster (*Pinctada maxima*)

Among these species, a *Pinctada maxima* (Jameson,1901) is one of four major pearl oyster species utilized by the cultured pearl industry for pearl production. Traditionally, pearls were only obtained from nature, but due to scientific advancement, vast majority of pearls today

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[1] Department of Zoology, University of Yangon

[2] Dagon University

[3] Department of Zoology, University of Yangon

are produced from nursing, nucleus inclusion, then breeding a technology dominated by Japan-based companies in South East Asia and northern Australia (Fassler, 1992; Strack, 2006; Septy, *et al* 2018). In Myanmar, *P. maxima* are rich in the banks of Mergui Archipelago in the Andaman Sea. They are considered as the grounds geographically farthest to the west, with the hundreds of islands and coral reefs offering good ecological conditions. The "Mergu Shell", as they were soon known, began to play a role on the world market and were shipped to London and Hamburg via Singapore (Southgate and Lucas,2008).

In nature, the breeding season of *P. maxima* extends from the months of September and October through to the months of April and May. Although there is variability from month to month, the primary spawning occurs from the middle of October to December. A smaller secondary spawning occurs in February and March (Rose *et al.*, 1990; Rose and Baker, 1994). In the present study, the induce spawning method was used for larval and spat oyster culture conduction in Myanmar Pearl Enterprise, Pearl Island, Myeik Archipelago with the following objectives: to study on hatchery of gold-lip oyster and silver-lip oyster *P. maxima* by induce spawning method, to determine the larval development for both gold-lip oyster and silver-lip oyster (Plate 1).

Materials and Methods

Study area and study period

The research was conducted at Myanmar Pearl Enterprise (MPE) in Pearl Island locating 11'16.2' N and 98' 13.8'E, Myeik Archipelago, Tanintharyi Region (Fig. 1). The study period was from July, 2019 to January, 2021.

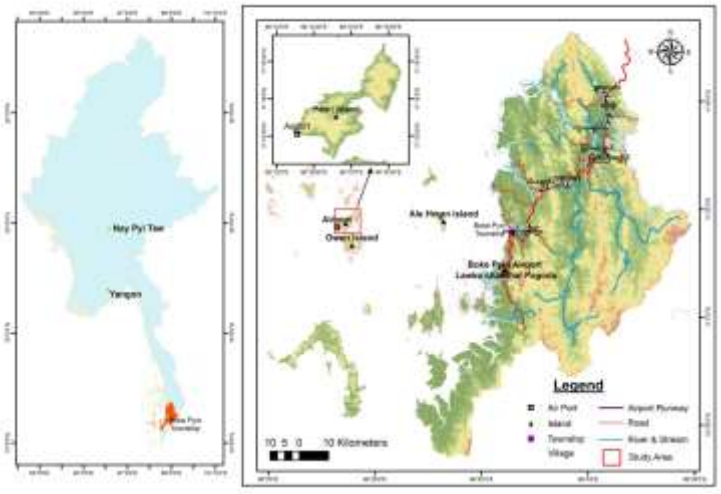

Figure 1. Location map of Pearl Island, Myanmar Pearl Enterprise, Pearl Island, Myeik Archipelago, Tanintharyi Region

Selection of Oysters Breeders

The pearl oysters were collected from selective brood stock in pearl farm. The fouling organisms from the collected oyster were removed by using a knife and clean. The cleaned oysters were put by row in the rectangular water tanks and the gonad condition were examined with naked eyes for each individual by using a shell opener which putting between two shells. If an individual which was at the suitable gonadal development, it was kept separately as the sex group for the culture. And then these selected oysters were cleaned with a brush and marking sign for each individual. After cleaning, these selected oysters were put 15 individuals per plastic

basket by separate sexes such as Male oysters, Female oyster and transferred into a 500L water tank (Plate 2).

Spawning and rearing in hatchery

A total of 21 individuals males and six females of Gold-lip oysters whereas two individuals of male and two individuals of female of Silver-lip oysters were taken for the spawning in the hatchery. These mother oysters in the blue tank were spawned by induce spawning method. This process was done by putting heater 2000 Watt for heat. Water temperature was taken to raise between 32°C to 35°C. The water temperature was checked and recorded the time of recording gonads for gold-lip oysters (Table 1)and silver-lip oyster(Table 2). Firstly, male oysters were spawned and released sperm and later the female oysters were releasing the egg. When this condition, the both sexes were kept separately into the new tank again and pouring sperm water in female spawning tank. The water sample from the spawning tank was collected using a pipette and examined the fertile larvae under the compound microscope (Plate 3).

After fertilization, the 2 days oyster larvae were moved from a larval culture tank through a various size of sieve and cleaning with seawater. After cleaning the oyster larvae were transferred to a beaker and replaced back into another clean culture tank. The seawater was exchanged in larval rearing tanks and reared for 40 days (Table 3). The stocking densities were checked for 500 L tank capacity (Plate 4). For the feeding, the five species cultured micro-algae planktons in the hatchery as the species *Chaetoceros simplex, C.calcitrans, C. gracilis, C. ceratosporum, Isochrysis galbana* fed to the cultured oyster larvae. The feeding rate was determined from six days to 20 days. In this study, the growth and developmental of oyster larvae were examined from first polar body to spat stages in the hatchery. The larvae were fed on chloramphenicol (Antibiotic) from day four to the stage before the collector hanging.

Results

There were different developmental stages of larval oyster after the fertilization in the hatchery such as cleavage stage, trochophore stage, D-shape stage, umbo stage, eye-spot stage and spat stage, etc. From fertilized eggs to D-shape stage were become within ~20 hours. The early umbo stage was within six to seven days. The umbo stages were become for 10-12 days after. The eye-sport stage was become 16-18 days and then become to the spat stage (Plate 5).

The larval development for both gold-lip and silver-lip oysters was shown in Table 4. The measurements of larvae were 80-90 µm with the mean size 87.6 µm in gold-lip oyster and 90-110 µm with the mean size 105.2 µm in silver-lip oyster on six days after fertilization. After 20 days, the size of measurement was increased to 170-260 µm with the mean size 234.4 µm whereas 180-280 µm with the mean size 243.0 µm for gold-lip and silver-lip oysters, respectively. The amount of feeding rate was 1~2% on that six and eight days of culture and then the rate became 2% on 10 days, 2~3% on 12-14 days, 3% on 16 days, and 3~4% on 18-20 days during the larval development. The measurements of larvae were 80-90 µm with the mean size 87.6 µm in gold-lip oyster and 90-110 µm with the mean size 105.2 µm in silver-lip oyster on six days after fertilization. After 20 days, the size of measurement was increased to 170-260 µm with the mean size 234.4 µm whereas 180-280 µm with the mean size 243.0 µm for gold-lip and silver-lip oysters, respectively.

Table 1. Thermal Induced Spawning for Gold lip oysters

Sr. No.	Steps	Time (26.11.19)	Duration (mins)	Remarks
1	Transfer the selected mother oysters in blue water tank	6:25 AM	5	Males-21 & Female- 6
2	Heating (32°-35°-32°C)	6:30 – 6:45 AM	15	
3	Leaving in the water tank	6:45 - 7:00 AM	15	
4	Heating (up to 35°C)	7:00 – 7:20 AM	20	Giving food-10 liters
5	Leaving in the water tank	7:20 – 7:50 AM	30	
6	Heating (up to 34°C)	7:50 – 8:10 AM	20	Giving food-15 liters
7	Leaving in the water tank	8:10 – 8:25 AM	15	
8	Heating (up to 34°C)	8:25 – 8:35 AM	10	
9	Leaving in the water tank	8:35 – 9:00 AM	25	
10	Running water started	9:00 AM		
11	Gonads leave start and pouring sperm water for fertilization	9:15 AM	15 mins after running water	
12	Finished all eggs fertilization	9:40 AM	25	

Table 2. Thermal Induced Spawning for Silver lip oysters

Sr. No.	Steps	Time (17.12.19)	Duration (mins)	Remarks
1	Transfer the selected mother oysters in blue water tank	6:15 AM	5	Males-2 & Female- 2
2	Heating (up to 32°C)	6:30 – 7:10 AM	40	Giving food- 15 liters
3	Leaving in the water tank	7:10- 7:25 AM	15	
4	Heating (up to 35°C)	7:25– 7:55 AM	20	Giving food- 15 liters
5	Leaving in the water tank	7:55 – 8:25AM	30	
6	Running water	8:25 – 8:55 AM	30	
7	Heating (up to 32°C)	8:55 – 9:20 AM	25	Giving food- 20 liters
8	Leaving in the water tank	9:20 – 9:35 AM	15	
9	Heating (up to 35°C)	9:35– 10:00 AM	25	Giving food- 20 liters
10	Leaving in the water tank	10:00 – 10:30 AM	30	
11	Running water start	10: 30 AM		
12	Gonads leave start and pouring sperm water for fertilization	10: 45 AM	15 mins after running water	
13	Finished all eggs fertilization	11 :20 AM	35 mins	

Table 3. The used of different sieve's size for cleaning oysters

Sr. No.	Gold-Lip Oyster		Silver-Lip Oyster	
	Day	Used of sieve's size (μm)	Day	Used of sieve's size (μm)
1	2	65, 40, 20	2	65, 40, 20
2	4	85, 58, 40	4	85, 58, 40
3	7	118, 85, 75	7	118, 95, 85
4	10	132, 95, 85	10	132, 95, 85
5	13	180, 118, 100, 95	13	180, 132, 118, 100,
6	16	212, 150, 132, 118	16	212,150, 132
7	20*	400, 180, 160	19	400, 160, 150, 132
8	23	400, 180, 160	22*	400, 180, 160
9	27	400, 180, 160	25	180
10	30**	180	28	180
11	33	180	31**	180
12	37	180	34	180
13	40		37	180

Remarks: * Hanging collectors start, ** Running water start

The amount of feeding rate was 1~2% on that six and eight days of culture and then the rate became 2% on 10 days, 2~3% on 12-14 days, 3% on 16 days, and 3~4% on 18-20 days during the larval development. Three individuals of abnormal larvae were recorded on 12 days in gold-lip oysters whiles non abnormal larvae in silver-lip oysters. Eight nos. of eye spot larvae were found on 18days in gold-lip oysters as compare as three nos. and 15 nos. of eye spot larvae were observed on 16 days and 20 days, respectively. The density (%) was 3.7 % for gold-lip oysters whereas 4.8% of silver-lip oysters in 500L culture blue tank on six days. But it was become 1% on 20 days for both gold-lip oyster and silver-lip oyster. During the study period as the environmental condition the air temperature was minimum 19.8°C and maximum 27°C. The water temperature was minimum 25°C and maximum 28°C. The salinity of water was 30-31 ppt and humidity 46-60%.

Discussion

Thermal stimulation has been reported as a successful means of spawning induction in all major commercial pearl oyster species (Alagarswami *et al,* 1983a, b; Alagarswami *et al,* 1989; Chellam *et al*., 1991; Rose and Baker, 1994; Southgate and Beer, 1997). In the present study,

thermal induce spawning method used for pearl oysters in hatchery. The growth of pearl oyster seeds starts from zygote, followed by larvae, then spat (Gervis and Sims,1992).

Table 4. Larval development of Gold-lip oyster and Silver-lip oysters (n=25)

No	Day	Measurement (µm)		Average size (µm)		Amount of eat (%)		No. of Abnormal larvae		No. of Eye spot larvae		Density (%) in 500L culture blue tank	
		Gold-lip	Silver-lip	Gold-lip	Silver-lip	Gold-lip	Silver-lip	Gold-lip	Silver-lip	Gold-lip	Silver-lip	Gold-lip	Silver-lip
1	6	80-90	90-110	87.6	105.2	1~2	1~2					3.70	4.80
2	8	90-110	90-110	103	107.4	1~2	1~2					2.80	2.80
3	10	100-130	110-150	116.2	130	2	2					3	3
4	12	100-160	120-190	130	154	2~3	2~3	3				2.40	2.00
5	14	110-200	120-220	169.4	183	2~3	2~3					2.20	1.60
6	16	130-210	140-240	187.2	207	3	3				3	2.10	1.40
7	18	160-250	160-270	219.8	233	3~4	3~4			8		1	1
8	20	170-260	180-280	234.4	243	3~4	3~4				15	1	1

A. Uncleaning oysters in panels

B. Cleaning oysters with a knife

C. Oysters put in tanks

D. Examination on Gonad's developmental stages

E. Cleaning oysters with a brush and marking on the shell

F. Leaves the selected oysters breeder in water tank

Plate.2 Selection of oyster breeders

A. Heating Oysters in blue tank

B. Feeding on oysters

C. Checking the water temperature

D. Record the time of releasing gonads

E. Keeping of both sexes separately into the new tank again

F. Pouring sperm water in female spawning tank

G. Taking the water sample from fertilized tank with a pipette

H. Examining samples under a compound microscope

Plate. 3 Spawning of oysters in the hatchery

A. Water from a larval culture tank through a sieve

B. Larvae cleaning with seawater

C. Water through various size of sieve

D. Oyster larvae in a sieve

E. After cleaning the larvae transfer into a beaker

F. Replaced back into another clean culture tank

Plate. 4 Cleaning and changing of water tank during the stage of D-shape oysters

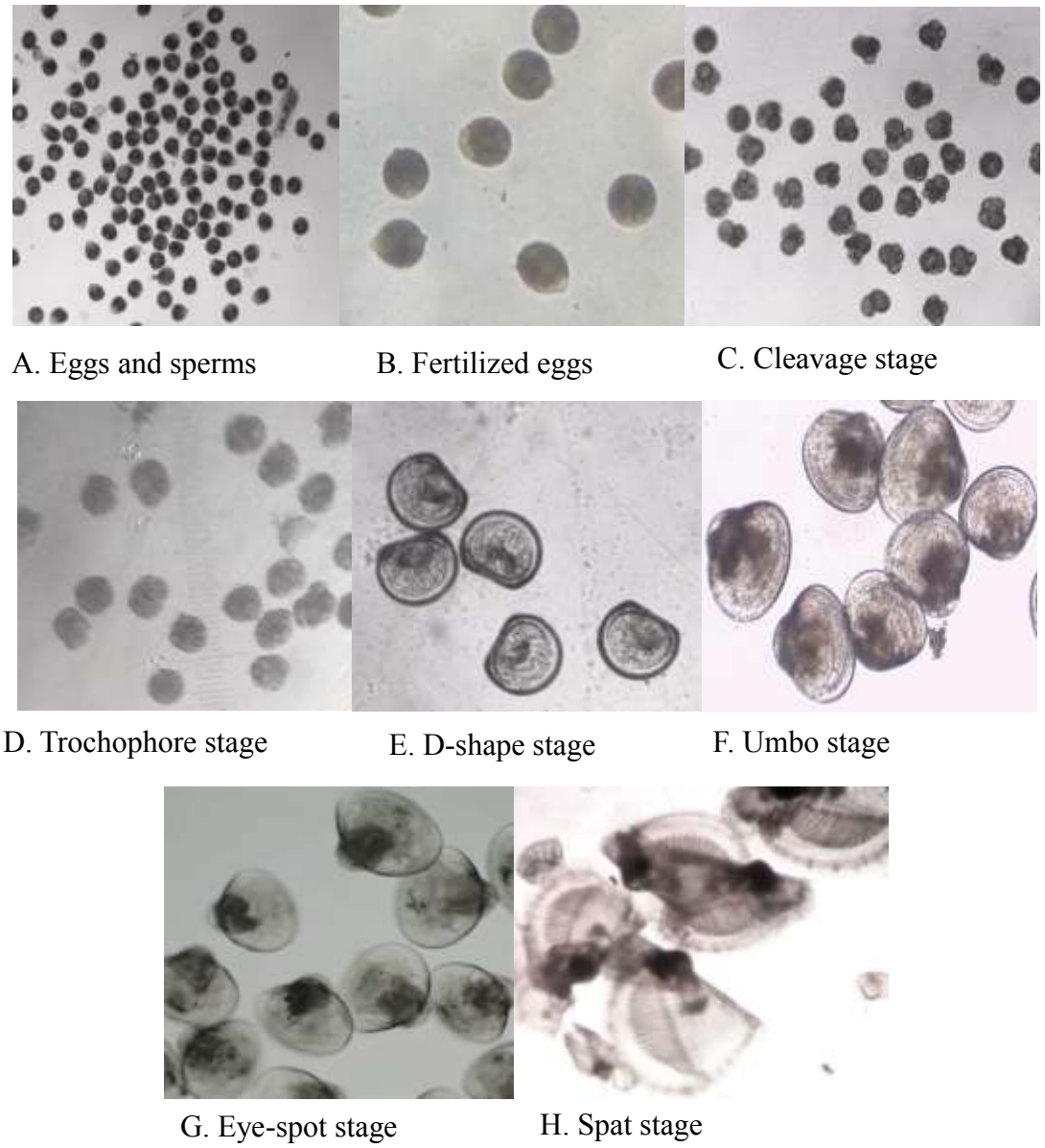

Plate. 5 Developmental stages of oyster larvae

Rose (1990) recommended that the egg density should not exceed 30/mL for Pinctada maxima. Initial larval stocking density should be no more than 5/mL and this should be reduced to 2/mL by day 10 and to 1/mL by day 14. Larval stocking density should be adjusted at water change. In the present hatchery the egg densities were 21/ml of gold lip oysters and 10/ml of silver lip oysters. Larval stocking density were adjusted at water change as the reduction of density from 3.7 % for gold-lip oysters whereas 4.8% of silver-lip oysters on six days to become the similar density 1% on 20 days for both oysters. All fertilized eggs transformed into the D-shape stage within ~20 hours. The early umbo stage was within six to seven days. The umbo stages were become for 10-12 days after. The eye-spot stage was become 16-18 days and then become to the spat stage. Thus, the duration between the day of selection for breeder oysters until the spat collection stage was prolonged for 22days.

Tranter (1958) described that spawning results from muscular contractions and oocytes are activated immediately prior to spawning in the follicle. In developmental stages of larval oysters, the extrusion of the first and second polar body occurred within 5minutes and 15-

20minutes of insemination, respectively, in Akoya pearl oysters (Wada *et al.,* 1989) whereas Doroudi and Southgate (2003) stated that first polar extrusion in *Pinctada margaritifera* was recorded after 24minutes. In the present study recorded that six different developmental stages of larval oyster after the fertilization in the hatchery namely cleavage stage, trochophore stage, D-shape stage, umbo stage, eye-spot stage and spat stage. The first and second polar body occurred within 5minutes and 15-20 minutes after fertilization take place in both gold-lip oysters and silver-lip oysters. Trochophore stage appear within 5 hours to 8 hours and measuring around 75µm. Thus, the data indicated that around the same size as compare to Saucedo and Southgate (2008) who stated that trochophore stage measure around 75µm *Pinctada maxima* and 70µm in *Pinctada margaritifera*.

According to the previous literatures stated that the growth rate of pearl oyster larvae is influenced by their surrounding environment, in particular food availability (Doroudi *et al.,*1999a; Doroudi and Southgate, 2002), food quality (Martinez – Fernandez *et al*.2006, water quality (Doroudi *et al.,* 1999b). Mills (2000) reported that growth of *Pinctada maxima* spat was optimal at a temperature of 26-29ºC and at an algal concentration of 54 cells/µL. Also, Doroudi *et al*., (1999a) reported maximum survival of 6-day-old *Pinctada margaritifera* larvae to occur within a salinity range of 26.5-33.5% and within a water temperature range of 22.5-26.5ºC. In the present hatchery, the ambient temperature 27.8ºC and an increase from 32-35ºC and the salinity of water was 30-31 ppt and humidity 46-60%. Therefore, the environmental condition was in the optimal condition for the growth of spat *Pinctada maxima* in the hatchery of the study area.

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INVESTIGATION ON MITOTIC DIVISION OF DIFFERENT TYPES OF TISSUES IN SILVER CARP *HYPOPHTHALMICHTHYS MOLITRIX* (VALENCIENNES IN CUVIER AND VALENCIENNES, 1844) AND SILVER BARB *BARBONYMUS GONIOTUS* (BLEEKER, 1850)*

Thidar Aung[1], Thawdar Aung[2]and Win Win Mar[3]

Abstract

The silver carp *Hypophthalmichthys molitrix* and silver barb *Barbonymus gonionotus* belonging to family Cyprinidae were collected from Thatyetkone Fisheries Station, Mandalay Region, during January to August, 2022 to evaluate the mitotic check of cellular process. Various colchicine concentrations (CC) were injected below the pelvic fins depend on the fish weight (1 ml/100g). Liver, oral cells, kidney, heart, gill filaments and blood cells were extracted and treated with hypotonic solution (HS) 0.56 % KCL for various durations. In silver carp, the highest frequencies of metaphase stage 100 % (n=22) in kidney treated with CC 0.50 % for 5 hrs with HS for 1 hr, 81.25 % (n=13) in liver treated with CC for 3 hrs by exposing HS for 45 mins, 75.00 % (n=18) in heart with CC for 4 hrs and HS for 1 hr, and the lowest 47.83 % (n=11) in kidney with CC for 5 hrs with HS for 1 hr 30 mins. In silver barb, the highest frequencies of metaphase stage were 88.14 % (n=52) in gill filaments treated with CC 0.10 % for 2 hrs with HS for 10 mins, 79.17 % (n=57) in kidney treated with CC 0.10 % for 2 hrs with HS for 30 mins, 88.10 % (n=37) in gill filaments treated with CC 0.05 % for 5 hrs by exposing HS for 1 hr, 30.77 % (n=32) in kidney with CC for 6 hrs and HS for 1 hr, 12.50 % (n=8) in blood cells with CC for 4 hrs 45 mins with HS for 1 hr and the lowest 4.94 % (n=4) in oral cells with CC 0.30 % for 2 hrs with HS for 2 hrs 45 mins. These results will provide the foundation of genetic assessment in mitotic cell division of freshwater fishes.

Key words: Fishes, mitotic division, colchicine, KCL, frequencies

Introduction

The aquaculture is the second sector of economic income for sustainable development of human resource management. Among the fishes, silver carp *Hypophthalmichthys molitrix* (Valenciennes in Cuvier and Valenciennes, 1844) is native to China, Mongolia and Russian Federation, and the silver barb *Barbonymus gonionotus* (Bleeker, 1850) locally known as (Nga-khone-ma-kyee) is distributed around from Viet Nam, the Mekong basin in Lao PDR, Cambodia and Thailand (Froese and Pauly, 2022 and Integrated Taxonomic Information System, ITIS, 2022).

Mitosis is a process of nuclear division in which the replicated DNA molecules of each chromosome are faithfully segregated into two nuclei and maintains the identical chromosome numbers and generates haploid or diploid cells (Iwasa and Marshall, 2018). The cytogenetic studies on silver carp *Hypophthalmichthys molitrix* locally known as (Nga-gyin-phyu) and silver barb *Barbonymus gonionotus* locally known as (Nga-khone-ma-kyee) from Thatyetkone Fisheries Station are concerning with these information's, the different methodological approaches on cytogenetic studies have been limited in various research areas. The objectives of this study were to designate the mitotic inhibitors of various cells and to investigate the frequency of mitotic cells in these fishes during mitotic divisions.

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[1] Department of Zoology, University of Mandalay
[2] Department of Zoology, University of Mandalay
[3] Department of Zoology, University of Mandalay

Materials and Methods

The present research work was conducted at Laboratory, Department of Zoology, University of Mandalay. The study period was from January 2022 to August 2022. Twenty fish samples for each species were collected from Thatyetkone Fisheries Station which is situated between 21° 59' 28.53" N, and 96° 7' 44.60" E, Patheingyi Township, Mandalay Region. The collected fishes were cultured at Laboratory, Department of Zoology, University of Mandalay. The commercial pellets were fed twice a day. The water was changed twice a week and reared in well-aerated aquarium.

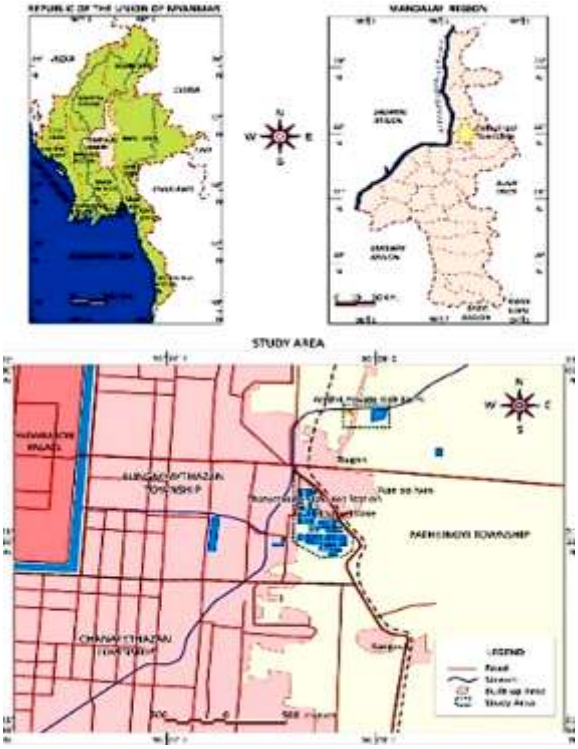


Figure.1. Study map of Thatyetkone Fisheries Station, Mandalay Region (Source: UTM)

Identification of species

The species identification was followed by Talwar and Jhingran (1991), and Integrated Taxonomic Information System (ITIS), (2022).

Injection technique

The total length and standard length of fish were measured by a plastic ruler to the nearest 0.1 cm, and their weight were recorded by digital balance to the nearest 0.01 g. The concentrations of colchicine solutions such as 0.10 % for 1 hr 30 mins and 2 hrs; 0.30 % for 2 hrs and 0.50 % for 3 hrs, 4 hrs, 4 hrs 45 mins, 5 hrs and 6 hrs were used, and injected at the base of pelvic fin that depend on the fish weight (1 ml/100g) (Plate 1, 2 B and C).

Plate 1. Injection technique

Collection of blood and tissues

The blood was collected by using a syringe, diluted with 0.56 % KCL solution and sacrificed with 10 % formaldehyde solution. Different tissues such as liver, heart, kidney, oral cells and gill filaments were also extracted from each fish (Plate 2 D).

Extraction of cells

Each tissue sample was kept in block-cup filled with 0.56 % KCL solution, minced thoroughly with glass rod and incubated for 10 mins, 20 mins, 30 mins, 45 mins, 1 hr, 1 hr 30 mins and 2 hrs 45 mins, and transferred to 6 cm glass test tube and mixed homogeneously by a Vortex mixer and centrifuged at 2000 rpm for 5 mins. The supernatant was discarded by using a pipette and the pellets were treated with 3 methanol:1 acetic acid for 15 mins. The process was repeated twice again (Plate 2 E, F, G and H).

Preparation of slide, Giemsa stain and identification

One or two drops of samples was placed onto the warm slide with a far distance, dehydrated at room temperature, stained with undiluted Giemsa stain for 10 mins, washed under running tap water, and then dried at room temperature. The permanent slides were prepared by dropping one or two drops of immersion oil, coated with Canada balsam, and checked under biological microscope (x1000) with attached camera (Plate 2 I, J, K and L).

Statistical analysis

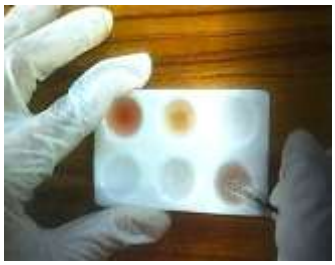
The frequency of chromosomal configurations in different stages of cells from various tissues of silver carp and silver barb were generated by Microsoft Excel, 2010.

A. Fish rearing in aquarium

B. Weighting the fish

C. Injection of the fish

D. Extraction of blood

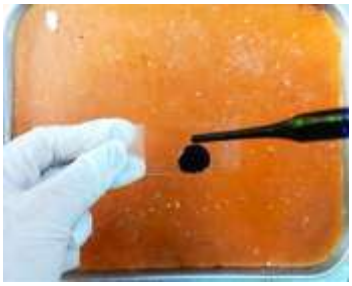


E. Mechanical dissociation of tissues

F. Homogenization of tissues

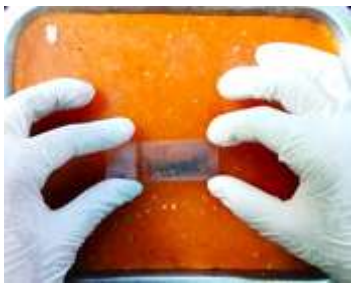
G. Centrifugation of pellets

H. Discarding the supernatant from the test tube



I. Staining on the slides

J. Washing under tap water



K. Coating with cover slip

L. Examination of microscope slide

Plate 2. Preparation of cytological process from fish

Results

The frequency distribution of mitotic division in different tissues and blood cells of silver carp *Hypophthalmichthys molitrix* and silver barb *Barbonymus gonionotus* were investigated by treating with three types of colchicine concentrations 0.10 %, 0.30 % and 0.50 % with various durations 1 hr 30 mins, 2 hrs, 3 hrs, 4 hrs, 4 hrs 45 mins, 5 hrs and 6 hrs, and then exposing with 0.56 % KCL solution for durations such as 10 mins, 20 mins, 30 mins, 45 mins, 1 hr, 1 hr 30 mins and 2 hrs 45 mins, respectively. Different types of tissues generated the different stages of chromosomal configuration containing with various frequencies of each stage (Plate 3).

Percent and frequency distribution in silver carp *Hypophthyalmichthys molitrix*

The colchicine concentration 0.50 % for duration 3 hrs with 0.56 % KCL for 45 mins generated the highest frequency of blood (100.00 %, n=7) at interphase stage followed by kidney (82.35 %, n=28), liver (54.76 %, n=23), oral cells (42.86 %, n=12), gill filaments (50.00 %, n=12) and heart (20.83 %, n = 5) for 4 hrs colchicine solution with 0.56 % KCL for 1 hr.

The highest frequency of blood cells (35.48 %, n=11) and the lowest frequency of liver (2.38 %, n=1) produced the prophase stage at 4 hrs colchicine treated with 0.56 % KCL for 1 hr. The highest prophase frequencies (oral cells, 33.33 %, n=12 and kidney, 31.88 %, n=22) were observed in colchicine solution (CS) for 3 hrs with 0.56 % KCL duration for 45 mins. The frequency of prophase stage in heart (25.00 %, n=4) was highest in CS 5 hrs with KCL duration 1 hr and the highest prophase frequency for liver (60.87 %, n=42) was observed in CS 5 hrs with KCL for 1 hr 30 mins.

The highest frequency of metaphase stage (75.00 %, n=18) in heart by treating with CS for 4 hrs with KCL for 1 hr. However, CS for 5 hrs generated the metaphase stage in oral cells (67.39 %, n=31), kidney (100.00 %, n=22), liver (80.00 %, n=44), heart (62.50 %, n=10) and gill (72.27 %, n=17) by exposing KCL for 1 hr (Fig. 2, 3, 4, 5).

Percent and frequency distribution in silver barb *(Barbonymus gonionotus)*

The highest metaphase stage of chromosomes 88.14 % (n=52) in gill filaments, followed by 74.36 % (n=29) in blood cells,62.63 % (n=62) in kidney by treating with 0.10 % CS for 2 hrs with 0.56 % KCL for 10 mins. The highest frequency of interphase stage 79.22 % (n=61) from oral cells was observed at 0.10 % CS for 2 hrs and 0.56 % KCL for 30 mins. The CS for 2 hrs with 0.56 % KCL for 10 mins generated the prophase stage 21.21 % (n=21) (Fig. 6,7).

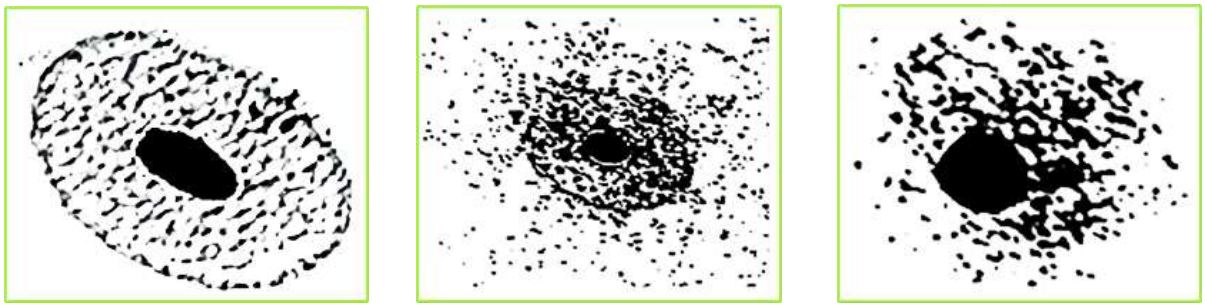
The highest frequency of interphase stages was observed in oral cells 93.82 % (n=76) and 100.00 % (n=95) in liver cells by treating with 0.30 % CS for 2 hrs with 0.56 % KCL for 2 hrs 45 mins. The lowest frequency of prophase stage 1.23 % (n=1) and metaphase stage (4.94 %, n=4) were found in oral cells (Fig. 8).

The liver cells generated the highest frequency of interphase stage 100.00 % (n=98) and prophase stage (kidney) 17.07 % (n=7) and metaphase stage from blood cells 12.50 % (n=8) by treating with 0.50 % CS for 4 hrs 45 mins with 0.56 % KCL for 1 hr. The mitotic stages of cells were not observed in oral cells and heart (Fig. 9).

The highest metaphase stage 88.10 % (n=37) in gill filaments followed by oral cells 80.36 % (n=45); prophase stage 26.22 % (n=16) in kidney cells and interphase stage 55.77 % (n=29) in heart cells by treating with 0.50 % CS for 5 hrs with 0.56 % KCL for 1 hr. The lowest prophase stage 5.36 % (n=3) was found in oral cells. Among these tissue cells, the mitotic division was not observed in blood cells (Fig. 10).

The mitotic cells division were not observed in blood cells and oral cells by treating with 0.50 % CS for 6 hrs with 0.56 % KCL for 1 hr. However, the highest frequency of interphase

stage 75.93 % (n=82) in gill filaments followed by prophase stage 59.26 % (n=16) in liver cells and metaphase stage 30.77 % (n=32) in kidney cells (Fig. 11).



A. Early interphase stage B. Middle interphase stage C. Late interphase stage

D. Early prophase stage E. Middle prophase stage F. Late prophase stage

G. Early metaphase stage H. Middle metaphase stage I. Late metaphase stage

Plate 3 The arrested stages of chromosomal configurations in mitotic cell division

Figure.2. Effect of colchicine concentration 0.50 % for 3 hrs with 0.56 % KCL for duration 45 mins on the mitotic division of different tissues *Hypophthalmichthys molitrix* (1st ring- blood cells; 2nd ring- oral cells; 3rd ring- kidney; 4th ring- liver)

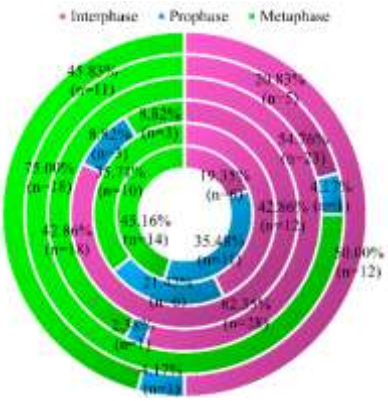


Figure.3. Effect of colchicine concentration 0.50 % for 4 hrs with 0.56 % KCL for duration 1 hr on the mitotic division of different tissues in *Hypophthalmichthys molitrix* (1st ring- blood cells; 2nd ring– oral cells; 3rd ring- kidney; 4th ring- liver; 5th ring- heart; 6th ring-gill filaments)

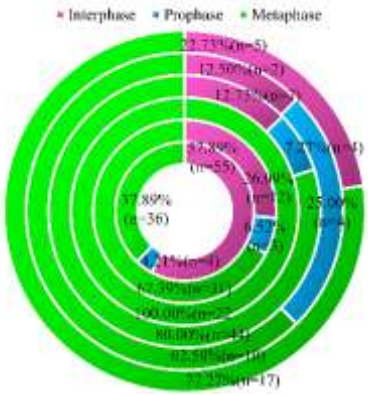


Figure.4. Effect of colchicine concentration 0.50 % for 5 hrs with 0.56 % KCL for 1 hr on the mitotic division of different tissues in *Hypophthalmichthys molitrix* (1st ring-blood cells; 2nd ring- oral cells; 3rd ring- kidney; 4th ring- liver; 5th ring- heart; 6th ring-gill filaments)

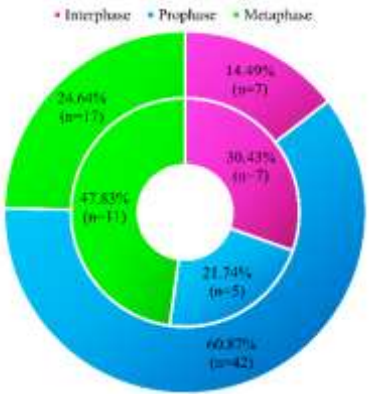


Figure.5. Effect of colchicine concentration 0.50 % for 5 hrs with 0.56 % KCL for 1 hr 30 mins on the mitotic division of different tissues in *Hypophthalmichthys molitrix* (1st ring- kidney; 2nd ring- liver)

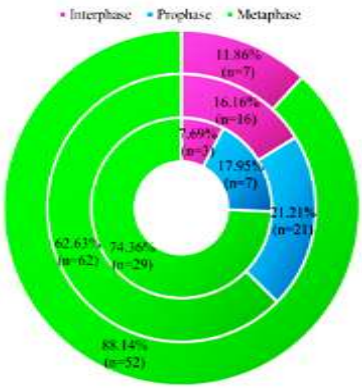


Figure.6. Effect of colchicine concentration 0.10 % for 2 hrs with 0.56 % KCL for 10 mins on the mitotic division of different tissues in *Barbonymus gonionotus* (1st ring- blood cells; 2nd ring- kidney; 3rd ring- gill filaments)

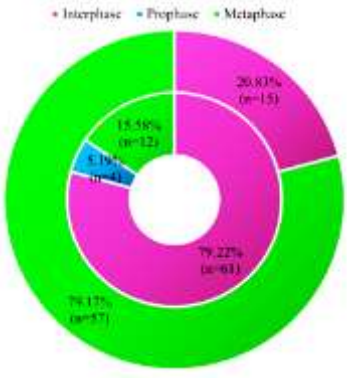


Figure.7. Effect of colchicine concentration 0.10 % for 2 hrs with 0.56 % KCL for 30 mins on the mitotic division of different tissues in *Barbonymus gonionotus* (1st ring- oral cells; 2nd ring- kidney)

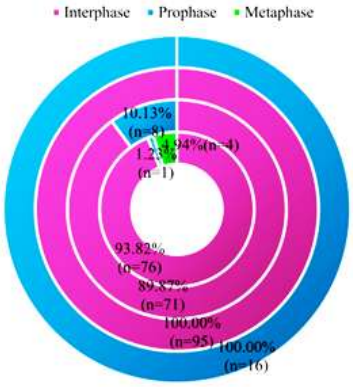


Figure.8. Effect of colchicine concentration 0.30 % for 2 hrs with 0.56 % KCL for 2 hrs 45mins on the mitotic division of different tissues in *Barbonymus gonionotus* (1st ring- oral cells; 2nd ring- kideny; 3rd ring- liver; 4th ring– gill filaments)

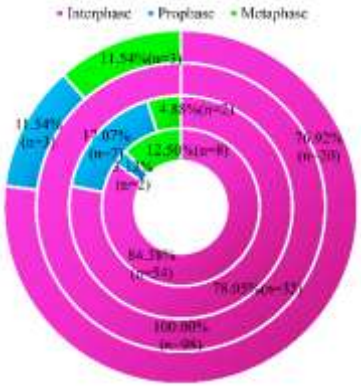


Figure.9 . Effect of colchicine concentration 0.50 % for 4 hr 45 mins with 0.56 % KCL for 1 hr on the mitotic division of different tissues in *Barbonymus gonionotus* (1st - blood cells; 2nd ring- kidney; 3rd ring- liver; 4th ring- gill filaments)

Figure.10. Effect of colchicine concentration 0.50 % for 5 hrs with 0.56 % KCL for 1 hr on the mitotic division of different tissues in *Barbonymus gonionotus* (1st ring- oral cells; 2nd ring- kidney; 3rd ring- liver; 4th ring- heart; 5th ring- gill filaments)

Figure.11. Effect of colchicine concentration 0.50 % for 6 hrs with 0.56 % KCL for 1 hr on the mitotic division of different tissues in *Barbonymus gonionotus* (1st ring- kidney; 2nd ring- liver; 3rd ring- heart; 4th ring - gill filaments)

Discussion

The mitotic division of silver carp *Hypophthalmichthys molitrix* and silver barb *Barbonymus gonionotus* were investigated by injection of different concentration of colchicine solutions at the base of pelvic fin of fish depending on the fish weight 1 ml / 100 g. Three different mitotic stages viz interphase, prophase and metaphase stages were observed in which interphase and metaphase stages were more distributed than prophase stages. Every treatment generates these stages that are depending on the colchicine concentration and hypotonic treatment with different durations.

Each stage of mitotic division is designated to the chromosomal configuration in each cell spread. In silver carp, late interphase stage of cells was more observed in colchicine concentration 0.50 % for 3 hrs with 0.56 % KCL for 45 mins. The early metaphase stage from different tissues reveals 4 hrs with 0.56 % hypotonic solution for 1 hr. When fish were treated with colchicine solution (CS) 5 hrs injection with 1 hr fixation of hypotonic solution, the early and middle metaphase stages of chromosomes were observed. The complete sets of metaphase

chromosomal configurations were observed in 5 hrs colchicine treatment with 0.56 % KCL for 1 hr 30 mins. In silver carp, the best metaphase stage was observed in liver cells 81.25 % (n=13) and 80.00 % (n=44) as well as in kidney cells 100 % (n=22) than other different stages of mitotic cells division. This is the optimum treatment for desired condensation degree of metaphase chromosomes.

These treatments were also generated the best metaphase stage in oral cells 80.36 % (n=45) and gill filaments 88.10 % (n=37) treated with 0.50 % CS for 5 hrs with KCL for 1 hr followed by kidney cells 30.77 % (n=32) in silver barb for 6 hrs with KCL for 1 hr. The condensed chromosomal configuration was observed in 0.50 % colchicine concentration for 4 hrs 45 mins duration with 0.56 % hypotonic solution for 1 hr indicating the optimal stage of metaphase check point of cells in silver barb. The middle stage of metaphase stage was observed in 5 hrs injection and hypotonic treatment duration for 1 hr. The complete metaphase stages were observed in colchicine treatment for 6 hrs and hypotonic solution treatment for 1 hr.

The silver barbs were injected with colchicine concentration 0.10 % for a duration 1 hr 30 mins and the extracted cells were treated with 0.56 % hypotonic solution for 10 mins. The insufficient amount of solution unable to disrupt the mitotic spindle formation. Although the cells become reached on the metaphase stage in mitotic division, the complete metaphase stages of cells were not observed. Thus, this treatment could not be resolved to get the optimal checkpoint of mitotic cell division. The early metaphase stage of chromosomes was observed in 0.10 % colchicine treatment 2 hrs with hypotonic treatment duration for 10 mins and 30 mins. The longer exposure to the hypotonic solution leads to the random spreading of chromosomes on the slides. When injection treatment of 0.30 % colchicine solution was for 2 hrs and 2 hrs 45 mins in hypotonic solution, the early synchronized metaphase stages of cells were randomly spread due to overtreatment duration of hypotonic solution.

These results were correspondence to Mark (2000) who reported that the longer culture is exposed to colchicine, the greater the potential number of arrested metaphases. In addition, they reported that undertreatment of hypotonic solution leads to inadequate spreading and unable to distinguish individual chromosomes as well as overtreatment in hypotonic solution leads to overspreading with rupture of the cell membrane and random loss of chromosomes (Dorathy and Mokhasi, 2013).

In this study noted that the duration of Carnoyl's fixative for 15 mins was the optimal condition for the preservation and suspension of the cells in silver carp and silver barb. The good shape of miotic chromosome spreads were observed by using pre-warmed slides and stained with undiluted Giemsa stain for 10 mins. The overstaining leads to count difficultly and identify the chromosomal configurations in every step of mitotic division. Giemsa stain was effective for getting the good shape of chromosomes in prepared slides.

Conclusion

The different degree of chromosomal configuration such as interphase stage (early, middle, late) and prophase (early, middle, late) and metaphase stage (early, middle, late) were observed in various tissues of silver carp and silver barb. The best optimization of mitotic cell division was 0.50 % colchicine solution for duration 5 hrs with 0.56 % KCL for 1 hr in silver barb but 4 hrs with 0.56 % KCL for 1 hr were investigated as the optimal duration in silver carp for karyological analysis. The present results provide the basic information of chromosomal studies on designated fish before identification of karyotypic analysis of these fish. In addition, the mechanical assessment and chemical solutions on treatment of different cells generated various kinds of mitotic division stages.

Acknowledgements

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GROWTH AND REPRODUCTIVE PERFORMANCE OF ROTIFER (*BRACHIONUS* SP.) USING DIFFERENT DIETS

May Thu Rein Oo [1], Cho Cho Thin[2], Yu Yu Aye[3], Kay Lwin Tun[4]

Abstract

One of the rotifer species, Brachionus sp. plays an important role in aquaculture as live food for in the early larval stages of many marine and brackish water fish species. It is an excellent first food for larvae because of its relatively smaller size, slow swimming speed, habit of staying suspended in the water column, and ability propagation in captivity at high density and reproductive rate. Microalgae comprise the principal food component for most cultured rotifer. Many species of micro algae may be used for cultivation of the rotifers. In the present study, population growth and reproductive capacity of rotifer Brachionus sp. was evaluated for a period of eight day cultivation under three different feeding diet such as Nannochloropsis sp., Chlorella sp. and Chaetoceros sp.. The feeding density of each algae species was maintained similar as of ($4.5x\ 10^6$ cell/ml). The maximum mean population density of rotifer was observed in Treatment I (Chlorella sp.) (118.00 ± 1.00) compared to Treatment II (Nannochloropsis sp.) (45.00 ± 1.73) and Treatment III (Chaetoceros sp.) (42.00 ± 1.00) At the eight day of culture, the number of egg bearing rotifers was significantly highest ($p < 0.05$) in those fed on (Chlorella sp.) compared to Treatment II (Nannochloropsis sp.) and Treatment III (Chaetoceros sp.). According to the present results, the marine Chlorella sp. is the best food for rotifers for the mass production.

Keywords: *Nannochloropsis* sp., *Chlorella* sp., *Chaetoceros* sp., Rotifer *Brachionus* sp.

Introduction

Live food organisms include all plants (phytoplankton) and animal (zooplankton). Phytoplanktons (microalgae) are generally eaten by zooplankton. Phytoplankton forms the basis of the food chain in early stages of life cycle due to small sizes, easy digestions and enriched in nutrients.

Microalgae are cultured intensively for direct or indirect feeding through production of zooplanktons and Artemia nauplii. Seawater was supplemented with commercial nitrate and phosphate fertilizers, and a few other essential micro nutrients, are commonly used for growing marine microalgae. Microalgae are high in nutrient, not harmful to fish, not pollute the environment, suitable with the size of fish's mouth and has a high tolerance to environmental change.

Aquaculture is experiencing significant growth worldwide, leading to an increased demand for live food biomass (Lee, 2001). Hatcheries, with remarkable advancements in larval rearing technology, require suitable culture techniques for rotifers, which are essential as larval food. The success of any hatchery system heavily relies on the availability of suitable live food organisms (Dhert *et al.*, 2001).

The live food such as Artemia, rotifers, and some microalgae are commercially used as live feeds for fish and shellfish larval management. Among the commonly used live feeds for larviculture, rotifers are excellent as the first food for larvae. The rotifer species *Brachionus* sp. plays an important role in aquaculture as live food during the early larval stages of many marine and brackishwater fish species.

Rotifers are an excellent choice for fish larvae food due to their relatively smaller size, slow swimming speed, tendency to stay suspended in the water column, and their ability to propagate in captivity at high densities and reproductive rates (Lubzens *et al*., 2001). Microalgae serve as the primary food source for most cultured rotifers, and various species of algae can be used for cultivating rotifers.The most common microalgae species are *Nannochloropsis* sp.,

[1] Department of Zoology (Fisheries and Aquaculture), University of Yangon
[2] Department of Zoology (Fisheries and Aquaculture), University of Yangon
[3] Lecturer, Department of Zoology (Fisheries and Aquaculture), University of Yangon
[4] Department of Zoology, University of Yangon

Scenedesmus sp., *Pavlova* sp., *Dunaliella* sp., *Spirulina* sp., *Chlorella* sp., *Rhodomonas* sp., *Tetraselmis* sp., *Chaetoceros* sp. and *Skeletonema* sp., etc. (Parrish *et al*., 2012).

Among them, The *Chlorella* sp. is perfect food for shrimps, and all other ornamental fish, crustacean and also serves as a food for zoo-planktons such as daphnia, moina and rotifer. It is also used in food industry, cosmetics and pharmaceutical industry (Sergejevová and Masojidek, 2011).

Nannochloropsis sp. is a single-celled marine microalga that can be used as the live feed for larvae cultivation of shrimp, fish, and shellfish. *Nannochloropsis* sp. is used in aquaculture as a valuable feed, providing polyunsaturated fatty acids, essential vitamins, and amino acids, along with energy. *Nannochloropsis* sp. has high nutrition value, and it is used widely as aquaculture hatchery industry for food of larvae and juvenile of bivalve, rotifer, as well as fish larvae (Tawfiq *et al*., 1999). *Chaetoceros* sp. can also be used as rotifer feeds since they are easy to digest and are favoured by rotifers (Sutomo, 2005).

In Myanmar, fish farmers have limited experience in culturing the marine microalgae such as *Nannochloropsis* sp., *Chlorella* sp. and *Chaetoceros* sp. which is essential for the growth and hatching of rotifer *Brachionus. plicatilis*, for marine fish and shrimp rearing. Moreover, fish farmers lack technical knowledge in breeding and larval rearing, as well as knowledge of the culture and supply of appropriate live food for the first feeding fish larvae. One of the strategies to resolve this problem is the mass production of rotifer *Brachionus s*p. using three different microalgae which will be crucial for growth and survival of fish larvae, fry, and fingerlings in aquaculture.

The present study was conducted to investigate the feeding effect of three different diets on the growth of rotifer *Brachionus* sp. under culture conditions.

Materials and Methods

The present study was conducted in the Live Food Culture Laboratory of Fisheries and Aquaculture in the Research and Innovation Center, University of Yangon *Latitude*: 16° 49' 28.76" N *Longitude*: 96° 08’. The study period lasted from April 2022 to March 2023.

The initial microalgae seeds and rotifer were supported by Live Food Culture laboratory, Asia Institute of Technology (AIT), Thailand. The cultivation of microalgae seeds and rotifer were conducted in Live Food Culture Laboratory of Fisheries and Aquaculture, Center for Research and Innovation, University of Yangon.

Preparation of apparatus for cultivation of algae and rotifer

All apparatus (beakers, bottles and sea water) were covered by aluminum foil and autoclaved at 12°C for 25 mins to avoid contaminations. The seawater was prepared using natural salt to obtain 25‰ salinity and filtered with Millipore (0.45 µm) filter paper. The seawater was sterilized in an autoclave for 121°C at 20 mins pressure lb/in^2 to avoid contamination. The solution was then kept in a dark and cold place until used.

Preparation of agricultural fertilizers media for cultivation of algae

The agricultural fertilizers such as Urea, Ammonium Sulphate, and Triple Super Phosphate (TSP) were weighed by using a digital balance and added to the beaker that contained 1000ml of distilled water. After adding the substances to the beaker, the solution was stirred by using a magnetic stirrer. The solutions were sterilized in an autoclave for 121°C at 25 mins, the solutions were then taken out when the temperature was dropped to 80°C. Then, the solutions were stored in a sterilized bottle and kept in the refrigerator to avoid contamination for further use.

Experiment I .Cultivation of microalgae for rotifer feed

The sterilized plankton culture Septicity was filled with 1200 mL of sterilized 25 ppt seawater and 300 mL of pure strain microalgae (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.) were added into each glass bottle. A total of 1ml of the agriculture fertilizers media were added to glass bottle with *Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.. Each culture bottle was sealed with aluminum foil and labeled with date and time. They were arranged on cultivation shelf and aerated with blower. All culture bottles were kept at air-conditions room at 25°C with light by fluorescent tubes. The experiments were extended for 10 days and population density of microalgae was calculated every day (Plate 1).

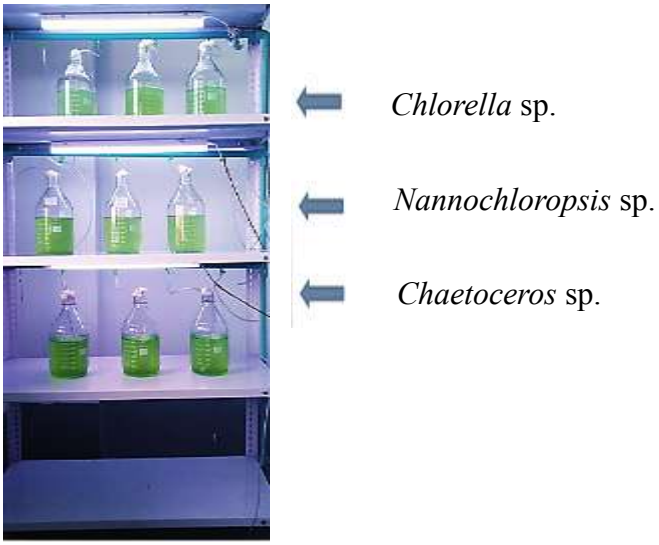

Plate 1 Cultivation of microalgae *Nannochloropsis* sp., *Chlorella* sp.,and *Chaetoceros* sp.

Determination of growth conditions and cell density of *Chlorella sp. Nannochloropsis* sp., and *Chaetoceros* sp.

The growth of *Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp. were estimated by counting cell density using hemocytometer. The subsamples of 1-mL from each bottle were collected without replacement. One drop cell suspension was placed in the central counting chamber of hemocytometer (Thoma, Germany) and covered carefully with cover glass 22 mm to prevent the formation of bubbles between the cover glass and hemocytometer. The chamber was then positioned under light microscope (CX 31, Olympus) at 100× magnification. The counting of cell density was started from the first day of culture period until the 10th days and calculated using the formula (Taw, 1990) Plate 2.

$$\text{Cell count (cells/mL) for 25 squares} = \frac{\text{total number of cells counted}}{\text{Number of blocks}} \times 4 \times 10^{6}$$

Plate 2 Neubauer hemocytometer

Experiment II Cultivation of rotifer, *Brachionus* sp.

The pure strain rotifers, (*Brachionus* sp.) were fed with three different diets namely live microalgae *Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp. were used as food to evaluate their effects on the growth of the rotifers, *Brachionus* sp..The experimental design of cultivation of rotifer was determined into three groups such as Treatment I (*Chlorella* sp.), Treatment II (*Nannochloropsis* sp.) and Treatment III (*Chaetoceros* sp.). The concentration of each algae reached to $4.5x10^6$ cells/ml, they were harvested to treat the rotifer. Three rotifer culture bottles were inoculated with *Brachionus* sp. at a density of 5 ind./ml. It was filled in 300 ml of each algal species. The salinity of culture bottles were kept 20 ppt.

Each culture bottle was labeled with the date. The culture bottles were arranged on the cultivation shelf and aerated with the blower and light by fluorescent tubes (Plate 3). The aeration and light were supplied with 24 hours. The experiments were extended for eight days and the growth of the rotifer was estimated every two days.

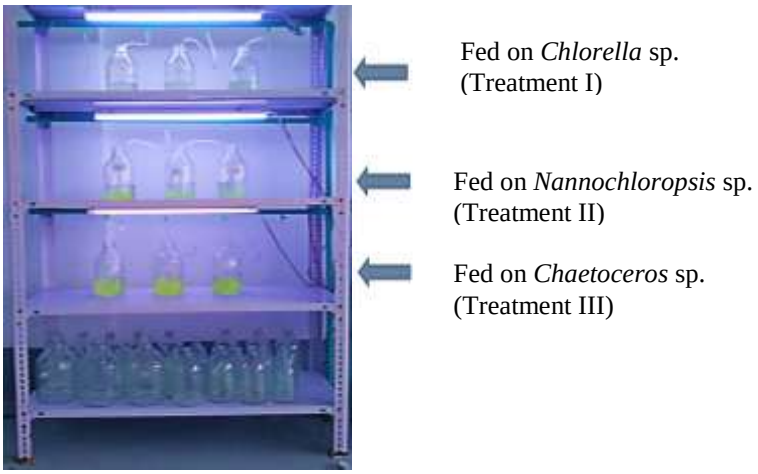

Plate 3 Cultivation of rotifer *Brachionus* sp. fed on *Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.

Determination of growth of rotifer

The concentration of rotifers was counted every two days. Based on the density of rotifers, the rotifer culture was poured through a mesh size to collect a large number of rotifers on the screen. They were then rinsed with a small amount of filtered seawater and transferred to a petri dish. From each group, a total of 1 ml of rotifers was sampled using a bulbed Pasteur pipette and placed on the Sedgewick-Rafter counting cell. The rotifers were counted under the microscope (Braley, 1994) .

In addition, number of egg bearing was recorded to assess the reproductive capacity of them treated with three different diets. The reproductive capacity of rotifer was calculated using the following formula.

$$\text{Reproductive capacity of rotifer (\%)} = \frac{\text{Number of egg bearing rotifers}}{\text{Total number of rotifers}} \times 100$$

Determination of water quality

The water quality including temperature, salinity, pH and dissolved oxygen were measured every day.

Data Analysis

Cell densities of algae and rotifers were expressed as the average number of cell ml^{-1} ± standard deviation. Cell densities of the rotifer, as well as growth curves, were measured and analyzed using a one-way analysis of variance (ANOVA). The significance of the result was determined at a p-value of 0.05. Growth curves for each treatment were generated by plotting the average cell density *vs* corresponding cultivation time. These curves were created using the EXCEL computer program.

Results

Population density of *Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp. agricultural fertilizer media

The population density of *Nannochloropsis* sp., *Chlorella* sp. and *Chaetoceros* sp.increased during the culture period. The colour of culture bottle intensified as the experiment progressed increasing from day 1 to day 10 (Plate 4).

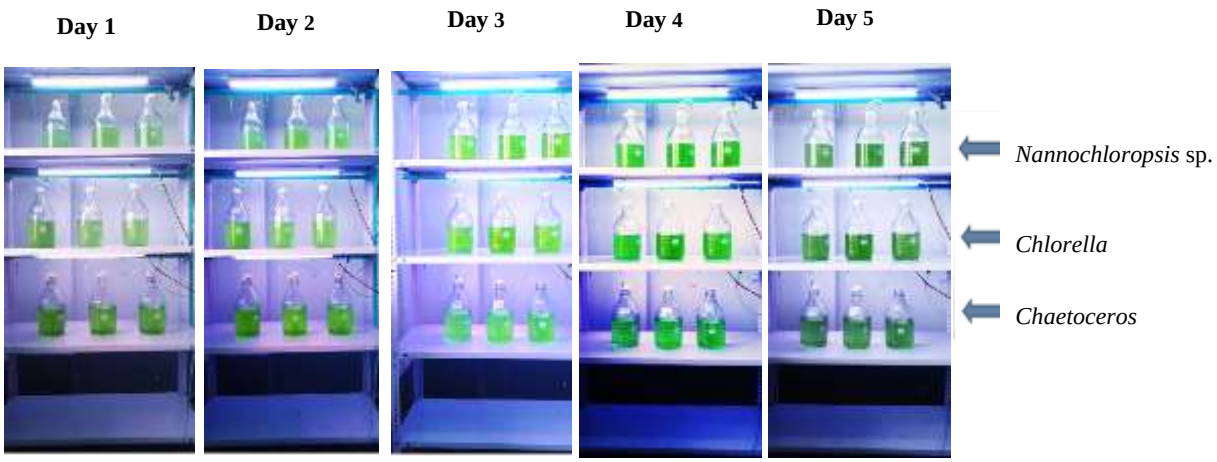

Plate 4 Cultivation of microalgae *Nannochloropsis* sp., *Chlorella* sp., *Chaetoceros* sp. (Day 1 to Day 5)

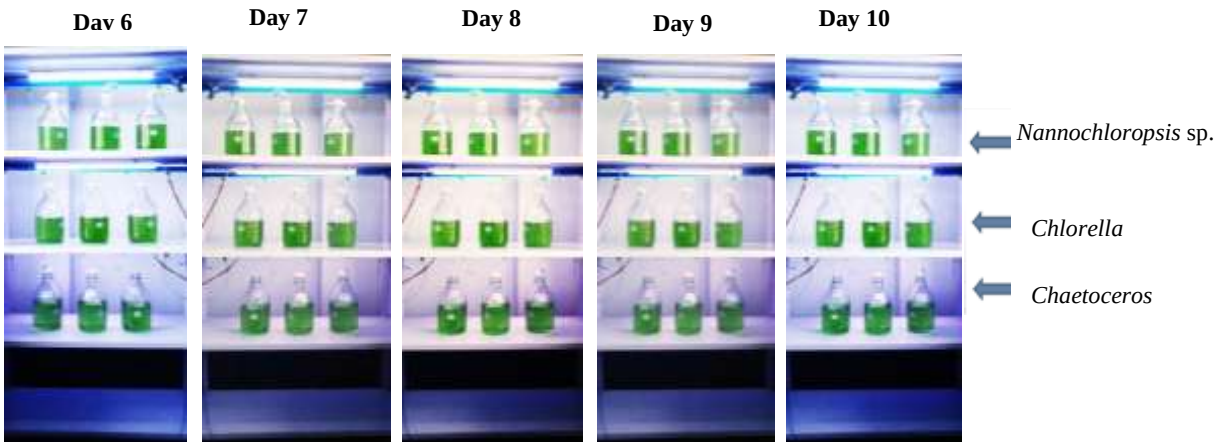

Plate 4 Cont. Cultivation of microalgae *Nannochloropsis* sp., *Chlorella* sp., *Chaetoceros* sp. (Day 6 to Day 10)

The population density of *Nannochloropsis* sp., *Chlorella* sp. and *Chaetoceros* sp. cultured with agricultural fertilizer media was shown in (Table 5). The maximum population density of *Chlorella* sp. and *Nannochloropsis* sp., were found on the fourth day of cultivation. The highest population densities of *Chaetoceros* sp. were reached in seventh day during the cultivation period. The highest population density of *Chlorella* sp. was observed in (6.83 x 10^6 cell/ml), followed by *Nannochloropsis* sp. (6.19 x 10^6 cell/ml) and *Chaetoceros* sp. (7.07 x 10^6 cell/ml) during the culture period (Table 1 and Figure 1, 2, 3,4.).The density decreased starting from 5th day in *Nannochloropsis* sp., *Chlorella* sp. While it decreased in 8th day in *Chaetoceros* sp..

Table 1. Population density of *Chlorella* sp. *Nannochloropsis* sp., and *Chaetoceros* sp. during the culture period

Time (Day)	*Chlorella* sp.	*Nannochloropsis* sp.	*Chaetoceros* sp.
Day 1	1.03±00	1±00	1.02±00
Day 2	1.76±0.23	1.5±0.1	1.33±0.05
Day 3	3.4±0.06	3±0.2	2.35±0.51
Day 4	6.83±0.06	6.19±0.36	3.72±0.15
Day 5	5.17±0.06	4.86±0.05	4.15±0.1
Day 6	4.23±0.13	4.11±0.07	5.87±0.17
Day 7	3.27±0.1	3.32±0.41	7.07±-0.26
Day 8	2.34±0.2	2.18±0.15	4.21±0.1
Day 9	1.48±0.22	1.41±0.05	2.74±0.2
Day 10	0.42±0.35	0.69±0.01	1.77±0.51

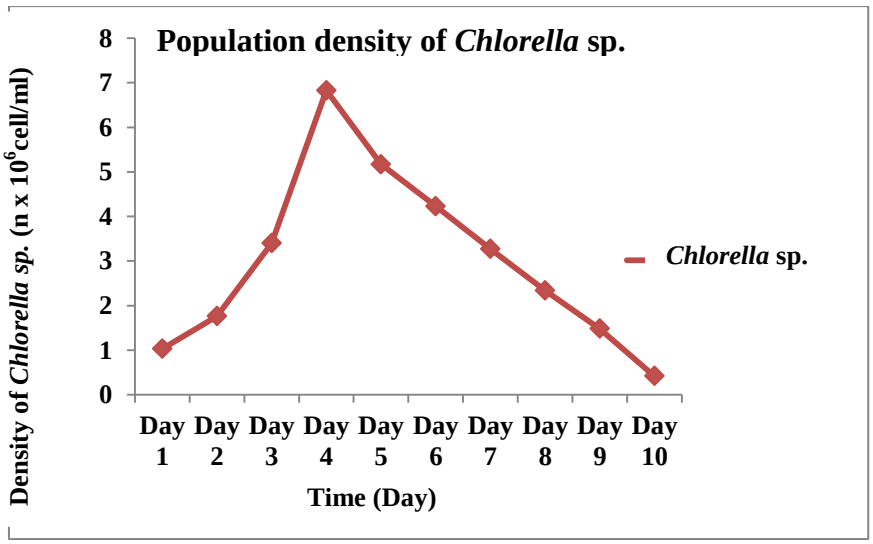

Figure. 2 Growth curve for *Chlorella* sp. cultured in agriculture fertilizer media

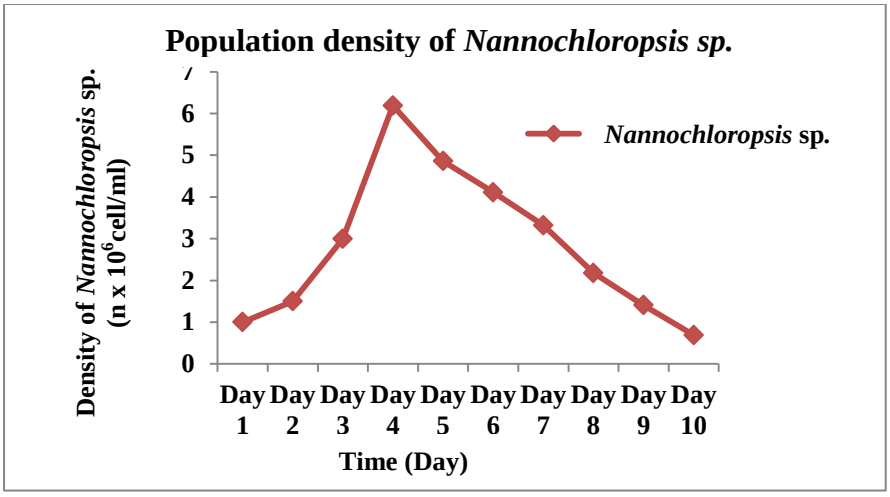

Figure 3 Growth curve for *Nannochloropsis* sp. cultured in agriculture fertilizer media

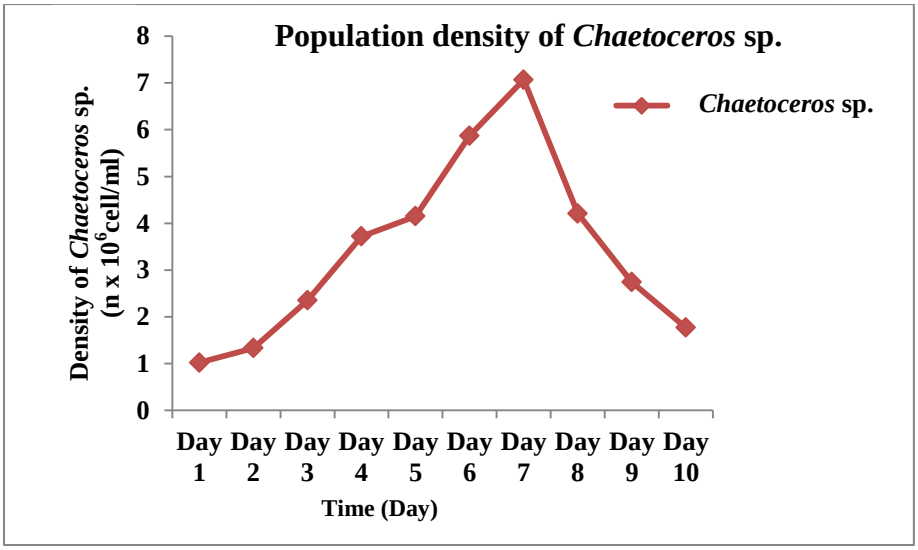

Figure 4 Growth curve for *Chaetoceros* sp. cultured in agriculture fertilizer media

Morphology of rotifer

The body of the rotifers was spherical, flattened, microscopic, multicellular, mostly aquatic organisms that are found in water. They had specialized organ systems and a complete digestive tract that included both a mouth and an anus. The rotifer's body was divided into three distinct parts: the head, trunk, and foot. The head had the corona, a rotatory organ responsible for creating whirling water movements that facilitated in the intake of small particles. The trunk contained the digestive tract, while the foot was a non-segmented, retractable ring-like structure. The rotifer observed in this study was assumed to the S Type due to its length of 130 - 210 µm (Plate 5).

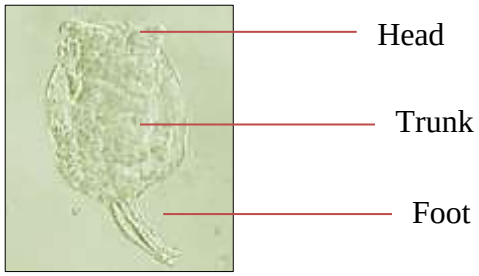

Plate 5 . Rotifer on microscope (400 x magnification)

Population density of rotifer, *Brachionus* sp. treated with three different algae

The present experiments were performed to cultivate the rotifer, *Brachionus* sp. in laboratory conditions using three different diets live microalgae (*Chlorella* sp., *Nannochloropsis sp.* and *Chaetoceros sp.*). The maximum mean population growth of the rotifer occurred on the 8th day of the culture period. The maximum mean population density of rotifer was observed in Treatment I (*Chlorella* sp.) (118.00 ± 1.00) ind./ml, followed by Treatment II (*Nannochloropsis* sp.) (45.00 ± 1.73) ind./ml and finally Treatment III (*Chaetoceros* sp.) (42.00 ± 1.00) ind./ml during the cultivation of *Brachionus* sp**.** after 8 days of the culture period (Table 2 and Figure 4).

Table 2. Population density of rotifer, *Brachionus* sp. fed on different live feeds (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.)

Time (Day)	Density of rotifer feed on *Chlorella* sp.(ind./ mL)	Density of rotifer feed on *Nannochloropsis* sp. (ind./ mL)	Density of rotifer feed on *Chaetoceros* sp. (ind./ mL)
Day 2	24.67 ± 0.58	10.33 ± 0.58	7.67 ± 0.58
Day 4	59.67 ± 1.53	20.67 ± 2.52	18.33 ± 0.58
Day 6	80.67 ± 1.15	28.00 ± 1.00	24.33 ± 4.04
Day 8	118.00 ± 1.00	45.00 ± 1.73	42.00 ± 1.00

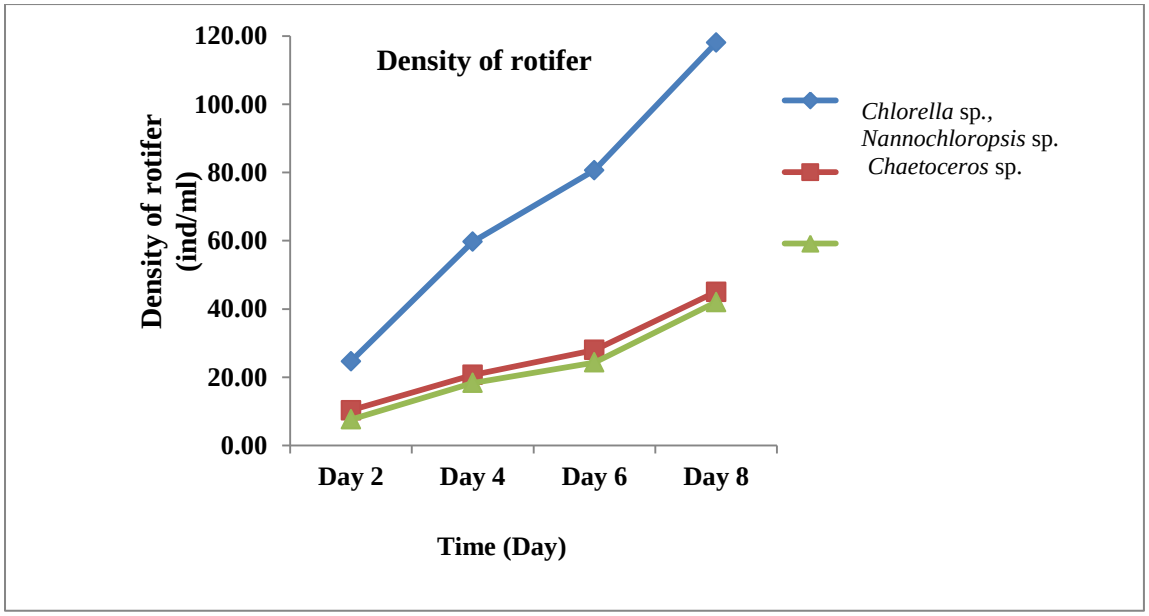

Figure. 4. Density of rotifer, *Brachionus* sp. fed on different live feeds (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.)

Number of egg bearing rotifer fed on different live feeds (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.)

The maximum number of eggs bearing rotifer was observed in Treatment I (*Chlorella* sp.) (44.33 ±2.10), followed by Treatment II (*Nannochloropsis* sp.) (11.00 ± 1.73) and finally Treatment III (*Chaetoceros* sp.) (10.00 ±1.50) in culturing *Brachionus* sp. (Table 3 and Figure 5).

Table 3. Number of egg bearing rotifer fed on different live feeds (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.)

Time (Day)	Number of egg bearing rotifer feed on *Chlorella* sp.	Number of egg bearing rotifer feed on *Nannochloropsis* sp.	Number of egg bearing rotifer feed on *Chaetoceros* sp. .
Day 2	5.67 ± 0.58	1.67 ± 0.58	0.67 ± 0.58
Day 4	16.67 ± 0.58	4.00 ± 1.00	2.00± 1.00
Day 6	24.33± 2.08	6.33 ± 0.58	4.00 ± 1.73
Day 8	44.33 ± 2.10	11 .00 ± 1.73	10.00 ± 1.50

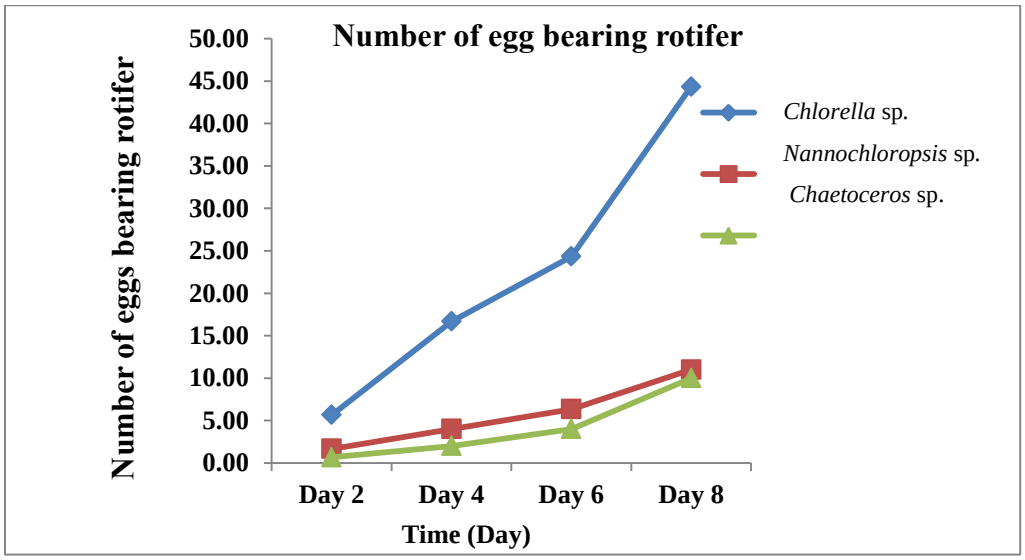

Figure. 5 Number of eggs bearing rotifer fed on different live feeds (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.)

Reproductive capacity of rotifer fed on different live feeds *Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.

The highest reproductive capacity of rotifer was observed in Treatment I (*Chlorella* sp.) (37.57%), followed by Treatment II (*Nannochloropsis* sp.) (24.44%) and Treatment III (*Chaetoceros* sp.) (23.81 %) during the eighth day culture period (Table 4).

Table 4. Reproductive capacity of rotifer fed on different live feeds (*Chlorella* sp., *Nannochloropsis* sp. and *Chaetoceros* sp.)

Time (Day)	Reproductive capacity of rotifer feed on *Chlorella* sp. (%)	Reproductive capacity of rotifer feed on *Nannochloropsis* sp. (%)	Reproductive capacity of rotifer feed on *Chaetoceros* sp. (%)
Day 2	22.97	16.13	8.70
Day 4	27.93	19.35	10.91
Day 6	30.17	22.62	16.44
Day 8	37.57	24.44	23.81

Water Parameters

The water parameters of Treatment I, Treatment II, and Treatment III were measured during the study period. The results for the water parameters were as follows: temperature ranging from 26.6 to 27.8°C, pH ranging from 7.3 to 7.8, dissolved oxygen (DO) ranging from 5.1 to 5.3, and salinity ranging from 25 to 26.2 ppt for Treatment I; temperature ranging from 26.6 to 27.8°C, pH ranging from 7.43 to 7.9, DO ranging from 5.2 to 5.4, and salinity ranging from 25 to 26.4 ppt for Treatment II; and temperature ranging from 26.6 to 27.87°C, pH ranging from 7.2 to 8, DO ranging from 5.2 to 5.5, and salinity ranging from 25 to 26.3 ppt for Treatment III, respectively. These values are presented in Table 5.

Table 5 Water Parameter during the culture period

No	Cultivation of rotifer fed on algae	Temperature °C	Salinity ppt	pH	DO
1	Treatment I (*Chlorella* sp.)	26.6 - 27.8	25 - 26.2	7.30 – 7.8	5.1 - 5.3
2	Treatment II (*Nannochloropsis* sp.)	26.6 - 27.8	25 - 26.4	7.43 – 7.9	5.2 - 5.4
3	Treatment III (*Chaetoceros* sp.)	26.6 -27.87	25 - 26.3	7.2 - 8.0	5.2 - 5.5

Discussion

The maximum population density of *Nannochloropsis* sp*.,* and *Chlorella* sp. were found on the fourth day of cultivation period in the present study. *Chaetoceros* sp. reached its maximum density on the seventh day during the cultivation period. The factors affecting the growth of microalgae in the cultivation include light intensity, dissolved oxygen, temperature and nutrients. The microalgae require nutrients for their growth because nitrogen is a major nutrient for microalgal cultivation and marine environment. The highest population density of different live feeds (*Nannochloropsis* sp*., Chlorella* sp*.,* and *Chaetoceros* sp.) for feeding rotifer *Brachionus* sp.was obtained by using cheapest agriculture fertilizer media in this experiment.

The present study was observed the variations in survival and growth rates among different live feeds. The maximum population density of rotifers was observed in Treatment I (*Chlorella* sp.) (118.00 ±1.00), compared with Treatment II (*Nannochloropsis* sp.) (45.00 ± 1.73) and Treatment III (*Chaetoceros* sp.) (42.00 ± 1.00) in culturing *Brachionus* sp. According to the present results, among three different diets, Treatment I (*Chlorella* sp.) showed significantly highest mean population growth ($p < 0.05$) at the end of the cultured period. The egg-bearing rotifers were significantly highest ($p < 0.05$) in those fed on (*Chlorella* sp.) compared to Treatment II (*Nannochloropsis* sp.) and Treatment III (*Chaetoceros* sp.) throughout the cultured period.

Alam (2004) reported that reproductive capacity of the rotifers fed on Treatment I (*Chlorella* sp.) was 38% compared to Treatment II (*Nannochloropsis* sp.) at 24%. The reproductive capacity of the rotifers fed Treatment I (*Chlorella* sp.) showed a significantly higher value ($p < 0.05$) compared to Treatment II (*Nannochloropsis* sp.) and Treatment III (*Chaetoceros* sp.). The number of eggs produced by a female is dependent on the species of food algae (Hirayama *et al.*, 1979).

Epp and Winston (1978) described that changes in pH from 6.5 to 8.5 had no effect on rotifer activity or metabolism effect on rotifer activity or metabolism. The result of the present study indicated that temperature, pH, dissolved oxygen, and salinity remained consistently stable throughout the rotifer culture period. These parameters showed no significant changes. Therefore, the water quality in the present study was considered suitable for rotifer culture.

The effects of different microalgae diets on the culture of rotifer *Brachionus* sp. indicated that the suitability of a particular microalgae species in rotifer culture largely depends on its nutritional quality. Hirata (1979) described that marine *Chlorella* sp. was considered to have better nutritional value than other species in Japan. In the present study, marine *Chlorella* significantly enhanced the population growth of rotifers."

Conclusion

The present study showed that the rotifer *Brachionus* sp. grows best when fed *Chlorella* sp.. Successful fingerling production in fish hatcheries, aimed at stocking grow-out production systems, depends heavily on having suitable zooplankton. *Chlorella* sp., serving as a marine rotifer food source, is preferred not only for its easy mass cultivation but also because it imparts beneficial nutritional characteristics to marine rotifers. This makes them excellent food for rearing various marine fish larvae. Mass-producing the rotifer *Brachionus* sp. will enhance the optimal growth and survival of fish larvae, fry, and fingerlings. The successful production of early fish and shrimp larvae in hatchery largely depend on the availability of suitable live food. The mass production of the rotifer by the use of *Chlorella* sp. will be benefitted for the production of fish and shrimp larvae in aquaculture farms.

Acknowledgement

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IDENTIFICATION OF COMMERCIALLY IMPORTANT GREEN MUSSEL FROM KYAUKPHYU, RAKHINE STATE USING MORPHOLOGICAL AND MOLECULAR APPROACH

July Maung Maung[1], Hla Htet Soe[2], Kay Lwin Tun[3]

Abstract

The Asian green mussel is widely distributed along the Indo-pacific region, spanning from Japan to New Guinea and from Persian Gulf to South Pacific Islands. The objective of this research is to identify the green mussel species through a comprehensive approach utilizing both morphological and molecular analyses, specifically targeting the mitochondrial cytochrome "c" oxidase subunit I (COI) gene sequences. The relation between shell length (mm), wet tissue weight (g), dry tissue weight (g) and sex of the green mussels were measured. Regarding sexual differentiation, high numbers of male green mussels were abundant than female green mussels. Genomic DNA was extracted using the pet NAD Nucleic Acid Co-Prep Kit, resulting in a sequence length of 660 base pairs (bp). The phylogenetic analysis of the specimens involved a comparison of their sequences with others deposited in GenBank, revealing a close clustering with *Perna viridis* species, supported by a robust bootstrap value of 100%. This discovery holds significance for taxonomic identification and contributes to the advancement of mariculture development.

Keywords: morphological, phylogenetic, *Perna viridis*, green mussel

Introduction

The Asian green mussel (*Perna* sp.) is a type of marine bivalve mollusk under the family Mytilidae. The native habitat of the Asian green mussel is in the Indo-Pacific region, which encompasses regions between Japan to New Guinea and from Persian Gulf to South Pacific Islands (FAO, 2013). Asian green mussel Locally known as Kha-Yu-Nyo generally inhabits marine intertidal, subtidal and estuarine environments, which have high salinity and receive more nutrients from land run-off (Rajagopal *et al*., 1998). Asian green mussel is able to tolerate a wide range of salinities and temperatures (Sivalingam, 1977).

Green mussel locally known as Kha-yu-Nyo) was harvested commercially as human food in Myanmar due to their dense, fast-growing, and inexpensive source of marine protein. That makes green mussel meat as an important fishery commodity. The marine mussel genera *Perna* encompass both green and brown mussels, distinguished morphologically by variations in shell color and shape. The taxonomic classification of *Perna* recognizes three species: the green mussel (*Perna viridis*, Linnaeus 1758), the brown mussel (*Perna perna*, Linnaeus 1758), and the green-lipped mussel (*Perna canaliculus*, Gmelin 1791) (Siddall, 1980; Vakily, 1989). Identifying bivalve species based on morphology proves challenging due to the extensive diversity in shell forms and sizes. Habitat-specific traits contribute to significant variations in shell shapes, complicating species identification, even within the same species (Comesana *et al*., 2001).

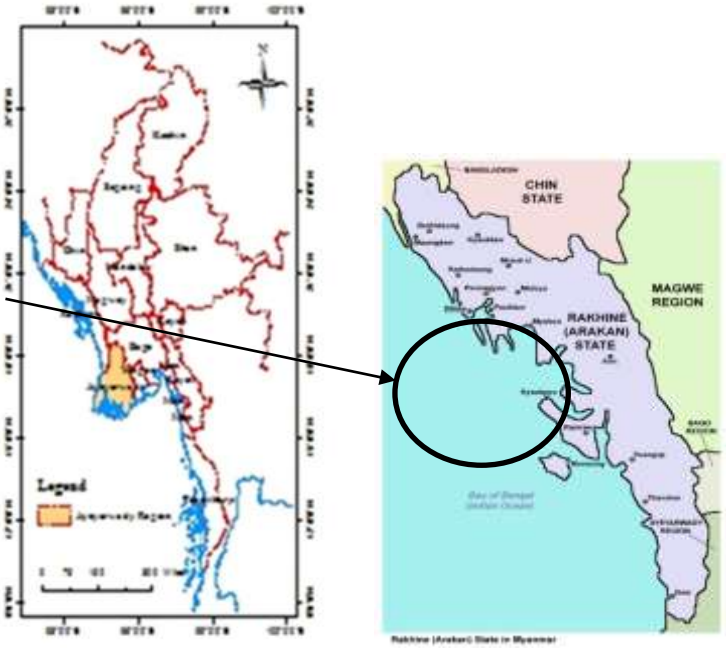
To address challenges posed by morphological characteristics, recent years have witnessed the adoption of molecular biological techniques. These methods utilize various genetic markers to enhance accuracy in species identification. In this study, the suitability of mitochondrial DNA sequencing was assessed as a fundamental requirement to advance research on the green mussel. The study aimed to explore the morphology, DNA sequencing identification and the phylogeny of the green mussel species *Perna* in Kyaukphyu, Rakhine State, Myanmar.

[1] Department of Zoology (Fisheries and Aquaculture), University of Yangon
[2] Department of Zoology (Fisheries and Aquaculture), University of Yangon
[3] Department of Zoology, University of Yangon

Materials and Methods

Sampling site and the study period

Green mussel samples were collected from the subtidal zone in Kyaukpyu Township, Rakhine State, Myanmar. The study site is positioned on the northwestern corner of Yanbye Island, bordering Combermere Bay, with coordinates at Latitude 19° 12' 58" North and Longitude 93° 43' 56" East (Fig. 1). The research was conducted from October 2022 to September 2023.



Myanmar Rakhine State, Kyaukphyu Township

Figure 1 Map showing location of Kyaukpyu Township of Rakhine State, Myanmar (www.googlemap.com)

Sample collection

The samples were collected during the low tide period, as it is the optimal time for harvesting green mussels inhabiting the subtidal zone (Plate 1). Subsequently, all collected samples were promptly transported to the Laboratory of Aquatic Bioscience at the University of Yangon following harvest. The gill tissue of ten specimens was kept at room temperature for DNA extraction.

(A) (B)

Plate 1 (A) *Perna* spp. (B) Internal organs of the studied green mussel

Morphological identification

Throughout the study period, a total of 50 green mussels were collected. In the laboratory, each mussel was measured its shell length using a vernier caliper, recorded to the nearest

millimeter. Subsequently, the mussels were opened, and the wet tissue weight was determined after removing excess water with absorbent tissue paper. Gender identification was conducted under a light microscope. The mussels were further characterized by examining morphometric features such as shell color, pallial line, posterior adductor muscle, posterior pedal retractor muscle, and shell length based on the criteria outlined by Rajagopal *et al*. (1998).

DNA extraction of the tissue samples for molecular identification

A total of ten green mussels were utilized for DNA extraction and subsequent gene sequencing. Prior to DNA extraction, the mussels were preserved in 70% ethanol, as illustrated in Plate (2A). The Pet NAD Nucleic Acids Co-Prep Kit was employed for the extraction of DNA from the gill tissue of the mussels, as depicted in Plate (2B). The DNA extraction procedure followed the guidelines provided by the Pet NAD Nucleic Acids Co-Prep Kit.

(A) (B)

Plate 2 (A) Fixation of *Perna* spp. (B) Pet NAD Nucleic Acid Co-Prep Kit

Polymerase Chain Reaction (PCR) amplification

To amplify the mitochondrial cytochrome gene, we designed universal primers targeting the bivalve COI region, namely 28S - LCO 1490 Forward (5'-ggtcaacaaatcataaagatattgg-3') and 28S - HCO 2198 Reverse (5'-taaacttcagggtgaccaaaaaatca-3') (Table 1). The PCR reaction consisted of mixing 5 µL of extracted DNA with 75.5 µL of water, 3.0 µL of each primer (25 µM), 0.5 µL of HS Taq DNA polymerase, 10 µL of 10× PCR buffer, and 8.0 µL of dNTPs (WizPure, Seongnam, South Korea). The PCR conditions involved an initial denaturation at 94° C for 5 min, followed by 35 cycles of denaturation at 94° C for 30 sec, annealing at 60° C for 30 sec, and extension at 72° C for 1 min, with a final extension at 72° C for 7 min. Subsequently, PCR products (around 700 bp) were separated and visualized via electrophoresis on a 1.5% agarose gel containing SYBR Safe DNA gel stain (WizPure, Seongnam, South Korea).

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 excised the fragment from an agarose gel using a clean scalpel. It was transferred up to 300 mg of the gel slice into a microcentrifuge tube, added 500 µl of binding buffer GP1, vortexed, and incubated at 55°C for 10-15 minutes, inverted the tube every 2-3 minutes. Next, it was applied up to 800 µl of the sample mixture from previous step into the FastGene® GP Column and centrifuged at 13,000 rpm for 30 seconds. Then, it was added 600 µl Wash Buffer GP2, and centrifuged at 13,000 rpm for 30 seconds. Wastes from the collection tube was discarded the flow-through, returned the column to the tube, and centrifuged again for 2 minutes to dry the column. Finally, 20-50 µl of elution buffer GP3 was added to the column and centrifuged at 13,000 rpm for 2 minutes to elute the purified DNA.

Table 1. Primer details for the PCR detection of the studied green mussel (Meyer, 2003)

Primers	Direction	Sequences	Tm
LCO1490	Forward	5'-ggtcaacaaatcataaagatattgg-3'	51°C
HCO2198	Reverse	5'- taaacttcagggtgaccaaaaaatca-3'	44°C

Tm = melting temperature ; LCO = (Universal Primer (forward),
HCO = (Universal Primer (backward)

DNA Data Sequence Analysis and constructing of phylogenetic tree

Purified DNA products were quantified and directly used for DNA sequencing. Sequencing reactions were performed using the DNA Engine Tetrad 2 Peltier Thermal cycler (BIO-RAD) and the ABI BigDye® Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The sequences are then aligned, edited and deleted whether there are stop codons or not by using software MEGA 11 (Tamura *et al*., 2013). The mussel's gene sequence was compared with that of other bivalve 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 "c" oxidase subunit I (COI) region. The obtained sequence was then compared to mitochondrial COI data sequences from the GenBank database, specifically those associated with the Mytilidae family. The analysis confirmed the species belonging to the genus *Perna*, including *Perna perna*, *Perna viridis*, and *Perna canaliculus*. Additionally, out-group species, *Ruditapes philippinarum*, was included in the study according to Wood *et al*. (2007).

A phylogenetic analysis of the mitochondrial cytochrome c oxidase subunit 1 gene (mtDNA COI) 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. viridis* species is compared with various species of *Perna* with reference Gen Bank samples.

Results

Sex ratio, shell length and soft tissue weight of *Perna* sp. in a natural population

The soft tissue weight of male was 12.6 ± 51 g while that of female was 15.2 ± 1.78 g. The mean length of male and female were 5.6 ± 0.9 cm and 5.8 ± 1.7 cm, respectively. The soft tissue weight and the length were not significantly different in male and female green mussels (t test, $P<0.05$).

The number of females and males *Perna* sp. collected from Kyaukphyu was shown in (Table 3). Samples contained a relatively larger number of male mussels than females throughout the study period. Significant differences in the sex ratio between male and female were not found in this study.

Table 3 The sex ratio, mean shell length, soft tissue weight of studied green mussels

Gender	Mean Length	Mean Soft Weight	Number	Sex Ratio
Male	5.6 ± 0.9 cm	12.6 ± 1.51 g	32	0.56:1
Female	5.8 ± 1.7 cm	15.2 ± 1.78 g	18	

Morphological description

The mussel had two thick, smooth elongated shells with a curved shape and posterior adductor scars extended beyond the pallial line, leaving a muscle scar in a wavier or S-shaped mark. Also, it was observed a smooth mantle edges, visible concentric growth rings, the beak

curved down (i.e., where the two valves hinge together) and a pair of hinge teeth on the left valve that interlock with a single hinge tooth on the right valve.

Posterior pedal retractor muscle and PRM pesterion retractor muscles was present. Pallial sinus was internally deep. Anterior and posterior adductor muscle or scar was generally equal in size. Umbo was posteriorly elongate and Pedal gape was present. Anterior pedal protractor muscle or scar was present where anterior pedal retractor muscle or scar was smaller in size. Periostracum was thickened in this species labial palp was unridged and CTE- ctenidium was large. Inner labial palp was ridged where foot was elongated. The body of the mussel was surrounded by a mantle. Rectum and style sac were present in this mussel. Stomach was small. According to morphological characters, the mussel was tentatively identified as belonging to the species *Perna perna* or *Perna viridis* or *Perna canaliculus.*

DNA sequencing and constructing of phylogenetic tree

The PCR analysis performed on the green mussel specimen successfully amplifies a mitochondrial DNA of the COI region approximately 700 base pairs (bp) (Figure 3). Figure 4 illustrates DNA sequences of a 660 bp fragment were obtained from the green mussel. The results of the BLAST analysis revealed that our sample is 100.00% similar to *Perna viridis* with the accession numbers KP892919, MN119648 and KY081305 (Table 2). These accession numbers with sequence submissions originated in China and India. The findings revealed that the collected sample is a *Perna viridis* species that clustered together with members of the Mytilidae family. Moreover, 99.85 % identities with the species *Perna viridis* (MH664002) from (India) and 99.70 % to *Perna viridis* (DQ343576) from (China).

Identities with the species *Perna viridis* are much lower than to 89.74 % to *Perna picta* (DQ917602), 88.17 % to *Perna canaliculus* (MG766134), 87.58 % to *Perna perna* (NC026288) and 86.77 % to *Mytilus californianus* (KF931643) respectively. The phylogenetic tree was constructed with the 9 similar sequences obtained from the GenBank database; the accession numbers are shown in Fig (5). The graphic blast visualization is shown in Figure 5. The evolutionary divergence was minimal (3.8%) among the sequences of India (Accession numbers: MH664002, JF520794 and MN119648) and Pakistan whereas the considerable (4.4%) divergence was denoted (Figure 5) between the sequence from Pakistan and Australia (Accession number DQ343576).

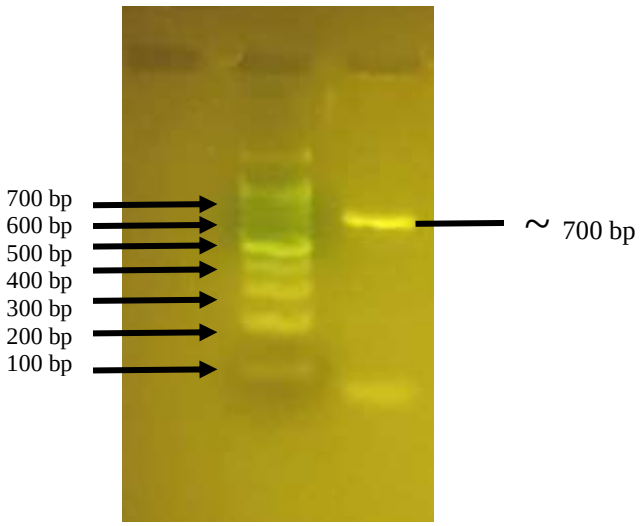


Figure 3 PCR amplification of mitochondrial DNA of the COI region of green mussels after examination with agarose gel exhibits a fragment of around 700 bp

AGCTGGGTTAATGGGGAGAAGGCTTAGGTTAATTATTCGAATTCAGCTTT……50
CTCATCCTGGGGGTAATTTTTTGAAAAATGAAAGGTTATATAATGTTGTA……100
GTAACAACTCATGCATTAGTAATAATTTTTTTTGCTGTAATGCCTTTACT……150

TATTGGTGCTTTTGGGAATTGATTACTTCCATTATGTATTGGTGGTGTTG……200
ATTTAATTTTTCCTCGTTTAAATAATTTGAGATTTTGGTTGGCACCTAAT……250
GCTTTGTACTTACTTATTTTGTCTTTTATAACGGAGAAAGGAGCTGGGAC……300
AGGTTGAACTATTTATCCACCTTTATCTTCTGGGTTGTACCATACTGGGC……350
CTGCTGTTGATATTTTGATTACGTCTTTACATTTAATTGGATTGAGTTCT……400
TTATTAGGTTCGATTAATTTTGTGAGGACTAATAAGAATATACCTACAAT……450
AAAAATAAAGGGTGAGAAATCTGAGTTGTATTTGTGGAGGATTACTGTAA……500
CCGGTGTTCTTTTAATCATTTCTGTGCCAGTTCTGGCCGGTGGGATTACT……550
ATATTGTTGTTTGATCGAAATTTCAATACTAGGTTTTTTGATCCTATTGG……600
AGGGGGAGATCCTGTTTTATTTCAGCATGTATTTTGATTTTTTGGTCACC……650
TGGAAGGTTT……660

Figure 4 Partial sequences of mitochondrial cytochrome gene of the studied mussel

Table 2 Mitochondrial cytochrome gene partial sequence identities of the studied mussel to other species in the Gene Bank

Species	Gene Bank Codes	%
Perna viridis (Linnaeus, 1758)	KP892919	100.00 %
Perna viridis (Linnaeus, 1758)	MN119648	100.00 %
Perna viridis (Linnaeus, 1758)	KY081305	100.00 %
Perna viridis (Linnaeus, 1758)	MH664002	99.85 %
Perna viridis (Linnaeus, 1758)	DQ343576	99.70 %
Perna picta (Born, 1778)	DQ917602	89.74 %
Perna canaliculus (Gmelin, 1791)	MG766134	88.17 %
Perna perna (Linnaeus, 1758)	NC026288	87.58 %
Mytilus californianus (Conrad 1837)	KF931643	86.77 %

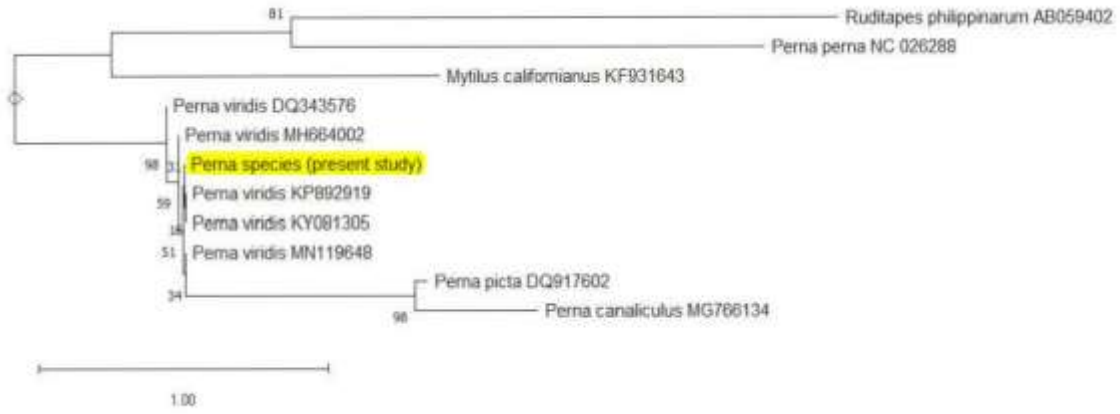


Figure 5 Phylogenetic trees are reconstructed using the Mitochondrial DNA of the COI region. *Ruditapes philippinarum* is used as out group on phylogenetic analysis

Discussion

In the present study, morphological identification along with molecular identification using the mitochondrial cytochrome gene sequencing for the identification of green mussel was conducted. Based on morphological inspection, the specimen closely resembles the Asian green mussel (*Perna* sp.) as described by Rajagopal *et al*. (2006).

Morphological characteristics, primarily focusing on shell color and shape, were examined, as outlined by Siddall (1980). *Perna* species exhibits an external shell color that is whitish under a bright periostracum, transitioning from dark brownish green anteriorly to olive-green to bright green posteriorly (Poutiers, 1998). These mussels typically feature two hinged shells connected by a posterior adductor muscle, and a robust ligament binds the two valves

together at the hinge, resulting in an equivalve shell (equally convex) with a byssal gape (Pouters, 1998). According to Siddall (1980), early larvae stages attach through proteinaceous byssal threads.

To confirm the species, DNA analysis was conducted. It was revealed that sequence identities to studied mussel species is *Perna viridis* with 100% similar to several Genebank samples (KP892919) under accession numbers MN119648 and KY081305. These sequence submissions originated in Kerala (India), Kadiapatinam (India), Zhejiang (China) and Kerala (India). The findings revealed that it is a *Perna viridis* species that clustered together with members of the Mytilidae family. The findings revealed that it is a *Perna viridis* species that clustered together with members of the Mytilidae family. Identities with the species *Perna viridis* are much lower than to 89.74 % to *Perna picta* (DQ917602), 88.17 % to *Perna canaliculus* (MG766134) and 87.58 % to *Perna perna* (NC026288). Therefore, it is highly possible that the studied mussel could not belong to species *Perna perna*. The green mussel is therefore identity as *Perna viridis*. (Linnaeus, 1758).

Bivalves commonly display an approximate balance in the distribution of males and females within populations (Gosling, 2015). Noor *et al*., (2019) conducted a study on green mussels from Pasaran Island, Indonesia, and reported morphological characteristics. At six months of age, male and female mussels exhibited shell lengths of 55.7 mm and 57.3 mm, respectively, with corresponding weights of 10.38 g for males and 9.82 g for females. In the present investigation, the mean length of males and females was 5.6 and 5.8 cm, respectively, and their soft tissue weights were 12.6 and 15.2 g, respectively. Interestingly, there were no significant differences in length and weight between *Perna viridis* populations in Myanmar and Indonesia.

Gender formation in various bivalve mollusk species is influenced by both genetic and environmental factors (Kenchington *et al*., 2002; Lee, 2015). Contradictory genetic mechanisms explanations have been proposed (Zouros *et al*., 1992; Kenchington *et al*., 2002; Yusa *et al*., 2013). Environmental factors affecting mollusk sex ratios include unfavorable habitat conditions, anthropogenic impact, food availability, temperature, and salinity (Yusa, 2007; Stenyakina *et al*., 2010; Shurova, 2013; Chelyadina, 2014). Pollutant influence may lead to masculinization in mollusk populations due to the suppression of specific genes (Ivanov, 1989). In the present study, bivalve species were collected from natural populations experiencing temperature stress and potentially reduced food availability (due to short periods of inundation during high tide). These environmental stressors may account for the observed higher male ratio in the bivalve species.

Conclusion

The identification of green mussel species in Kyaukphyu, Rakhine State, was carried out through both morphological and molecular methods. The analysis revealed that the specimens examined belonged to the species *Perna viridis*, as indicated by the sequence KY081305. The data presented here in offers valuable insights to the Department of Fisheries (DoF) regarding the taxonomy status of the species. This information is likely to be instrumental in the effective management, conservation, and sustainable utilization of green mussel resources in the future.

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SEASONAL VARIATIONS OF HEAVY METALS ANALYSIS IN SOME FISH SPECIES, CHINDWIN RIVER SEGMENT, HOMALIN TOWNSHIP

Kay Khaing Lwin[1]*, Kay Lwin Tun[2], Cho Cho Thin[3]

Abstract

Severe heavy metal contamination in freshwater ecosystems and significant health risks can result from mining activities. The current study aimed to assess the seasonal variations of heavy metal concentrations (lead, cadmium, arsenic and mercury) in fishes (*Wallago attu* and *Cirrhinus mrigala*) and their environs (water and sediment), Chindwin River segment, Homalin Township, Sagaing Region. The analysis was conducted using a Flame Atomic Absorption Spectrometer at the Universities' Research Centre of the University of Yangon and the Water Quality Laboratory in Yezin, Nay Pyi Taw. The study spanned from December 2022 to November 2023. Heavy metal concentrations were found to vary depending on the seasons (hot, rainy and cold) and species. The highest concentrations of Pb were recorded in both *Wallago attu* and *Cirrhinus mrigala* during the hot season. However, the concentrations of As, Hg, and Cd in the studied fish species were recorded within the maximum permissible limits set by the World Health Organization/Food and Agriculture Organization (WHO/FAO). These findings suggest that the fish, water and sediments in the study area may not be entirely safe from toxic metal contamination. The observed values for all tested metal concentrations of studied fish species and their environments, except for water, were below the maximum permissible limits, indicating a relatively lower risk of contamination.

Key words: Fish muscle, Water, Sediment, Metal Concentration

Introduction

Heavy metals represent a significant group of aquatic pollutants with persistent characteristics, leading to bioaccumulation within aquatic biota through the food chain. The entry of these pollutants into aqutic environments is primarily attributed to anthropogenic activities such as mining, industrial processes, and agricultural practices. Among the toxic heavy metals of concern, arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) pose severe threats to ecosystems.

Non-essential heavy metals As, Cd, Pb and Hg affect human health through food chain (Jarup, 2003). Toxic effects of Pb, Cd and Hg have been reported even at trace concentrations and they are not essential for human body (Goyer, 1995). During gold recovery Hg directly contaminates soil, water and air, finally reaching sediments where it is converted to methylmercury (MeHg), absorbed by plankton, whereby entering the food chain (Selin, 2009).Humans also get directly exposed from Hg vapor inhalation, in any case, both Hg species are highly to the nervous system (Aschner and Aschner, 1990), causing sensory and mental disturbances, motor and cognitive dysfunction, ataxia, constriction of the visual field, audition problems (Harada, 1995), as well as deleterious effects on the renal, pulmonary, cardiovascular, digestive and immune system (Bernhoft, 2012; Frodello *et al*., 2000; WHO, 2016), among others.

The Chindwin River, situated in the Sagaing Region of Myanmar, stands as one of the country's most significant rivers. The upstream areas of the Chindwin River Basin, encompassing Mahar Myaing, Htmanthi, and Hukaung valleys, boast diverse ecological conditions and habitats critical for the basin's healthy ecosystem functioning. However, these components of riverine biodiversity face threats from deforestation, mining, and illegal fishing activities. The use of chemicals in illegal mining poses additional risks to inland fishing activities.

Artisanal gold mining is said to be on the increase and it is said to be the major contributor of metals in surface and groundwater because of indiscriminate use of mercury (Hg)

[1]Department of Zoology (Fisheries and Aquaculture), University of Yangon
[2]Department of Zoology, University of Yangon
[3]Department of Zoology (Fisheries and Aquaculture), University of Yangon

and other chemicals, which are detrimental to human health during mining activities (Donkor *et al*., 2006). Small-scale gold mining all over the world is noted for its effects on water bodies through pollution of both ground and surface waters, because its activities by nature make use of a lot of water thereby seriously polluting water resources (Cunningham *et al*., 2005; Owens *et al*., 2005).

Fish is one of the indicators of heavy metals pollution in water. Due to fish consuming heavy metal toxicity affect in the human body. Fish populations are declining due to illegal fishing and gold mining. This mining activities can be caused the degradation of breeding grounds and habitat loss for aquatic animals in the Chidwin River. The fisheries and fish populations of the Chindwin River have been affected by water pollution caused or otherwise adversely affected by gold mining, logging and agricultural expansion (Win Maung, 2021).

Gold mining is one of the main occupations of the people living along the banks of Chindwin River. This mining activities contaminate the river water and cause harmful aquatic animals. As a result, the present study aims to assess and evaluate the presence and levels of specific heavy metals, specifically cadmium, lead, mercury and arsenic, in fish.

*Wallago attu (*Bloch & Schneider , 1801) , (locally known as Nga-bat), *Cirrhinus mrigala* Hamiton 1822 locally known as Nga-Gyin *)* and their surrounding environments (water and sediment) within the Chindwin River segment of Homalin Township. This research is pivotal in understanding the extent of heavy metal contamination in the Chindwin River segment of Homalin Township, providing essential insights for effective environmental management and conservation strategies. To determine the heavy metals (Pb, Cd, As and Hg) concentrations in muscle of fishes and to assess the metal contents in water and sediments of the river.

Materials and Methods

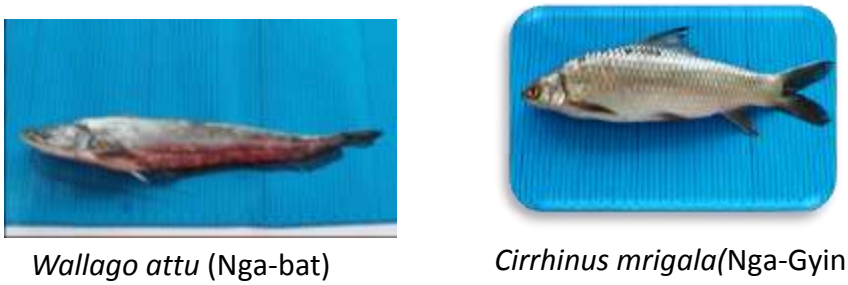
Study Area

Chindwin river segment of Myin Thar Yae Lae village, Homalin township, Sagaing Region situated between 24º 50′ 19″ N and 94º 56′ 20″ E was selected as the study area to analyze element concentrations in some fish species and their environs (water and sediment) (Fig. 6 and 7). It is used for irrigation, mining, drinking and fisheries. The quality of this ecosystem has been degrading due to gold mining and human activities. Therefore, it has been selected as a study area to investigate the heavy concentrations.

Study Period

Study period lasted from December 2022 to November 2023.

Collection of Samples



Wallago attu (Nga-bat) *Cirrhinus mrigala(*Nga-Gyin)

Plate 1. Collected species for the analysis of heavy metals

A total of 15 specimens of fish species (*Wallago attu*, *Cirrhinus mrigala*) were collected seasonally from local fishermen (Plate 1). Identification of studied fish was carried out follow after Talwar and Jhingran (1991) and Fish Base 2013.Collected specimens were washed by tap water until the contamination on the body surface was runoff. Total length (cm) and body weights (g) of specimens were measured. After that, they were dissected using stainless steel scalpels and forceps. A part of the tissue was removed and weighed. Samples were put into an oven to dry at 90°C and until reached constant weights. After that they were stored at low

temperature until digestion. Digestion of the samples carried out according to dry method by using furnace (Model-L-3383). Water and sediment samples were also collected seasonally at study site during the study period.

Sample preparation

Preparation of water and sediment

Each water sample was filtered through a 0.45 μm Whatman filter. The sample was analyzed directly.

The sediment sample was sun dried, grounded and sieved with 200 mm sieve to obtain a find powder 1.0 g of dried sediment sample in a crucible was placed in a furnace at 200°-250° C for 30 min, and then ashed for 4 hours at 480° C. Then the sample was removed from the furnace and cool down, 2mL of nitric acid was added and evaporated to dryness on sand bath. Then, 2 mL of concentrated HCl was added and transferred to furnace in which the temperature was raised slowly to 450° C and hold at this temperature for 1 hour. The crucible was then removed, cooled and 50mL of deionized water was added. The solution was filtered through 0.45μm Millipore filter paper and then transferred to 25 mL volumetric flask by adding distilled water. The digested sediment sample was analyzed for heavy metals using the Flame Atomic absorption spectrometer (FAAS) (Perkin Elmer AAanalyst 800 and Winlab-32 software) Universities' Research Centre (URC) and Water Quality Laboratory, Yezin at Nay Pyi Taw.

Preparation of fish

Tissue samples were dried to constant weight in an oven and dried samples were weight and stored in airtight containers. Digestion was conducted according to dry method. Five grams of dry sample was placed into crucible. And then transfer to a furnace (Model-L3383) and slowly raise temperature to 500° C for 2 hours. Samples were allowed to ash overnight. Once remove, samples were allowed to cool in room temperature and 5 mL nitric acid were added and swirl. After that 10mL HCl were added. The digestion was transferred to furnace and slowly raised temperature to 500° C and hold at this temperature for 1 hour. The crucible was removed, cooled and added 50mL deionized water and transferred to volumetric flask.

Chemical Analysis

The concentration of elements (Mercury, Arsenic, lead and cadmium) in muscle of studied fish species and aquatic environs of study area were analyzed tri-replicates by Flame Atomic Absorption Spectrometer (FAAS) (Perkin Elmer AAanalyst 800 and Winlab-32 software) in Universities' Research Centre (URC) at University of Yangon and Water Quality Laboratory, Yezin at Nay Pyi Taw. The results were compared with WHO/FAO maximum permissible limits and U.S. Environmental Protection Agency (EPA) guidelines.

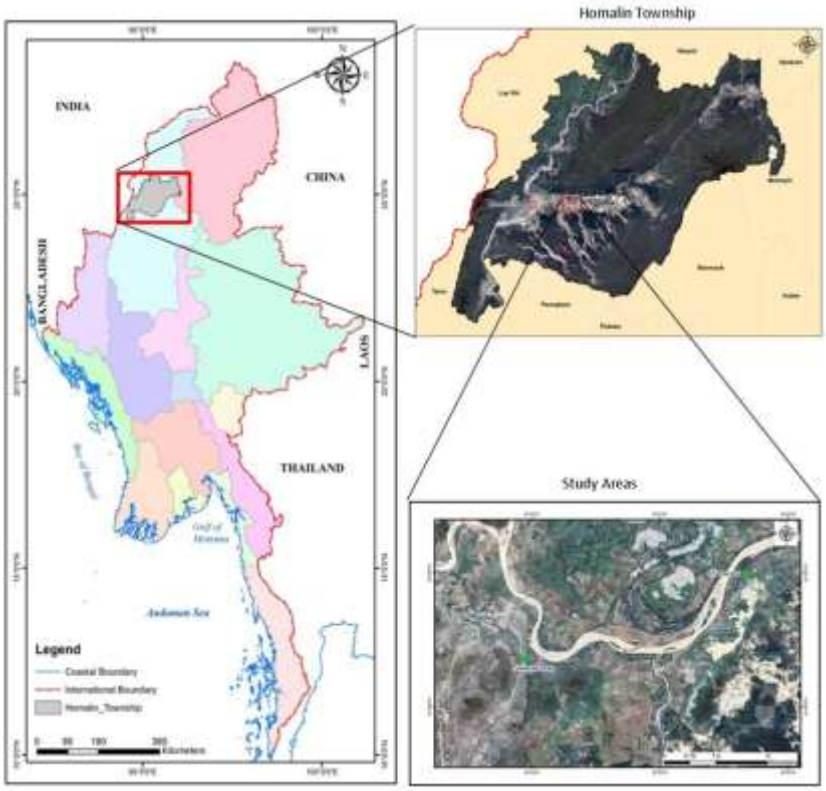


Figure. 1 Map of the study area and study site

Results

The concentrations of heavy metal (lead, cadmium, arsenic and mercury) in fish species (*Wallago attu* and *Cirrhinus mrigala*), water, and sediment in the Chindwin River segment, Homalin Township, were analyzed seasonally. Seasonal variations of test results were compared with (WHO/FAO) permissible limits for fish and water and the U.S. Environmental Protection Agency (EPA) guidelines for sediment.

The total length and weight of studied species were recorded in Table 1. The concentration of lead (Pb) was found to be highest in *Wallago attu* in three seasons. In hot season, this metal concentration of *Wallago attu* was found to be over the recommended maximum permissible limit WHO/FAO (Fig. 2).

The concentration of cadmium (Cd) in *Wallago attu* was also found to be highest in three seasons but it remained lower than the WHO/FAO permissible limits (Fig. 3).

The value of Arsenic in *Wallago attu* was not detected in three seasons throughout the study period (Fig. 4).

Lead (Pb) concentration in *Cirrhinus mrigala* was detected in three seasons but it was not over the WHO/FAO permissible limit except the hot season (Fig. 2).

The value of cadmium (Cd) in *Cirrhinus mrigala* was observed in three seasons and within the WHO/FAO permissible limits (Fig. 3).

Arsenic (As) content of *Cirrhinus mrigala* was found to be highest in rainy season but this value was not exceeded the WHO/FAO permissible limit (Fig. 4).

The concentration of mercury (Hg) in studied species (*Wallago attu* and *Cirrhinus mrigala)* was recorded in same value (0.003 mg/L) in hot season and below the WHO/ FAO permissible limits (Fig. 5). The concentrations of mercury in remaining seasons (cold and rainy) were not detected.

The value of lead contents in water was recorded to be higher than the WHO/FAO recommended limit in hot season. Cadmium, arsenic, and mercury contents in water were within the WHO/FAO maximum permissible limits (Fig. 6).

Test metal (Pb, Cd, As, and Hg) concentrations in sediment were found within the U.S. Environmental Protection Agency (EPA) guidelines during the study period (Fig. 7).

Table 1 Length and weight of studied fish species during study period

Sr. No.	Species	Season	Number	Mean Total Length (cm)			Mean Body Weight (g)		
1	*Cirrhinus mrigala*	Hot	5	21.6	±	6.10	108.88	±	115.87
		Rainy	5	18.4	±	3.86	54.18	±	28.12
		Cold	5	16.64	±	0.48	38.72	±	4.10
2	*Wallago attu*	Hot	5	37.94	±	6.02	243.32	±	123.26
		Rainy	5	17.78	±	4.06	20.32	±	8.69
		Cold	5	26.28	±	0.61	74.44	±	6.81

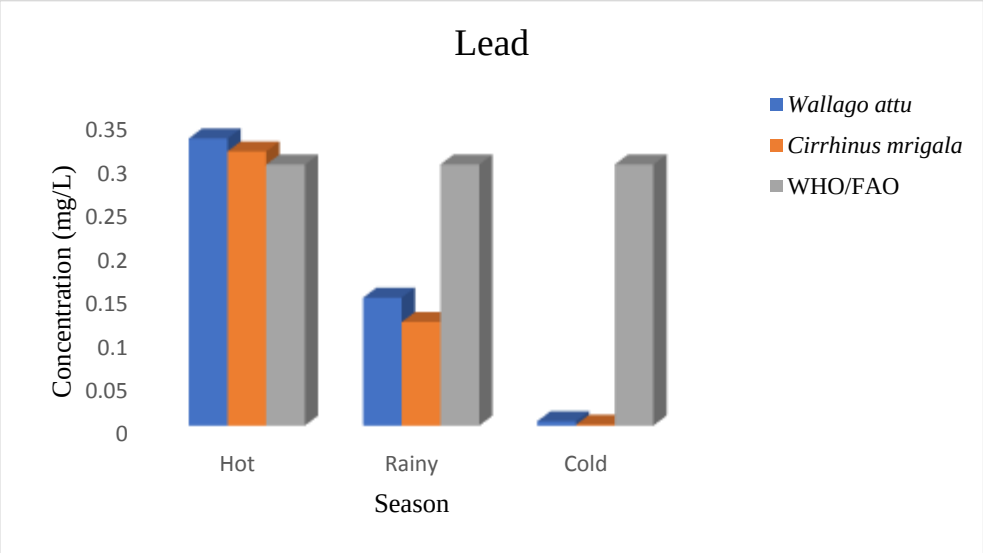


Figure. 2 Seasonal variation of lead concentration in studied fish species

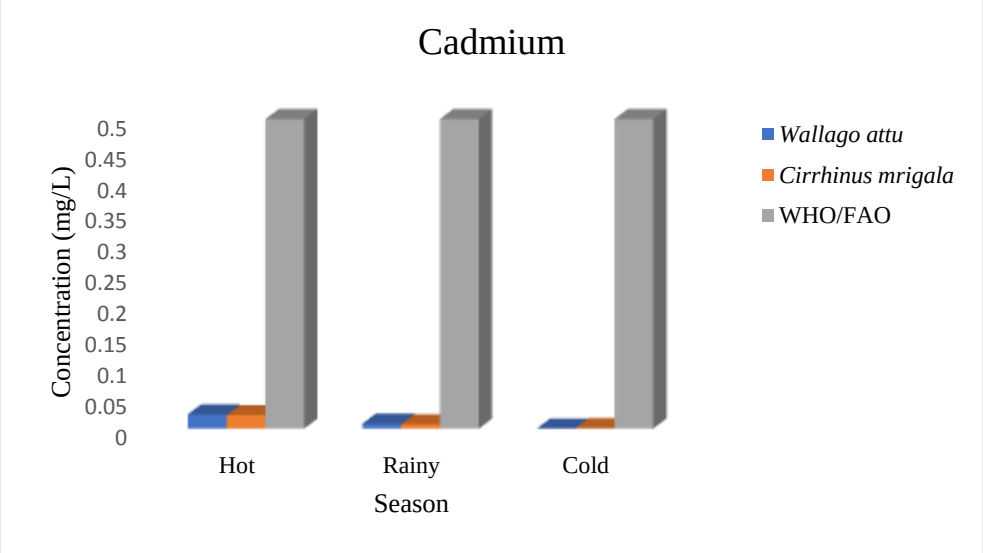


Figure.3 Seasonal variation of cadmium concentration in studied fish species

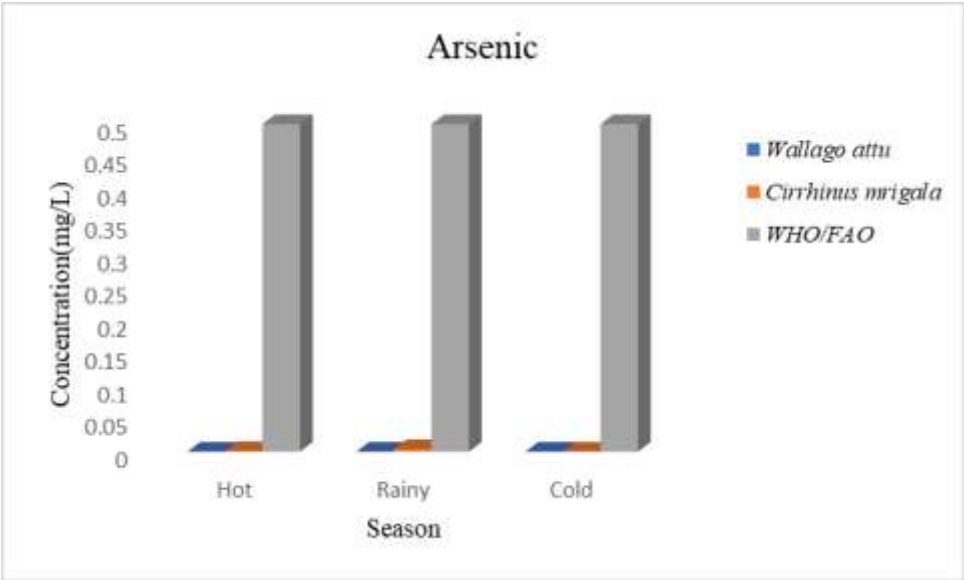


Figure. 4 Seasonal variation of arsenic concentration in studied fish species

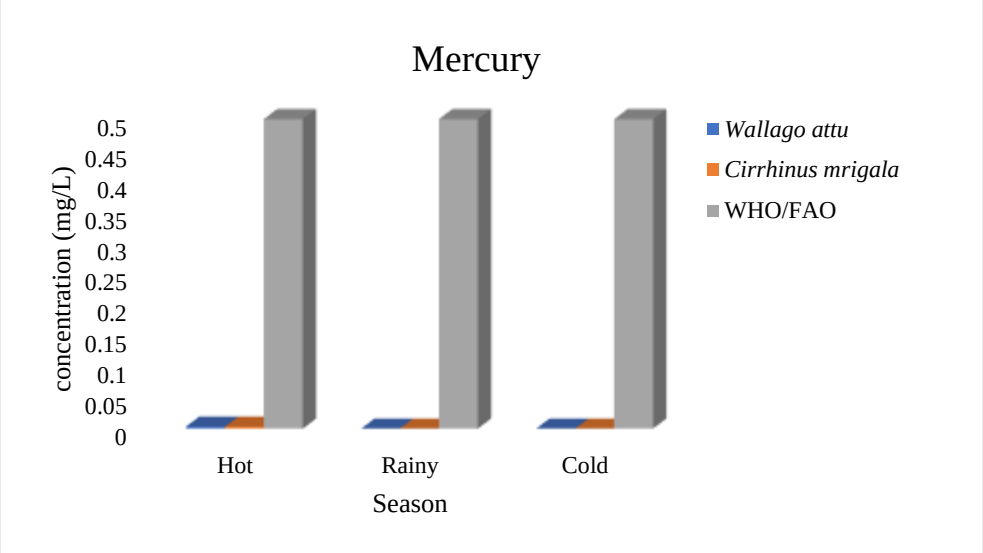


Figure. 5 Seasonal variation of mercury concentration in studied fish species

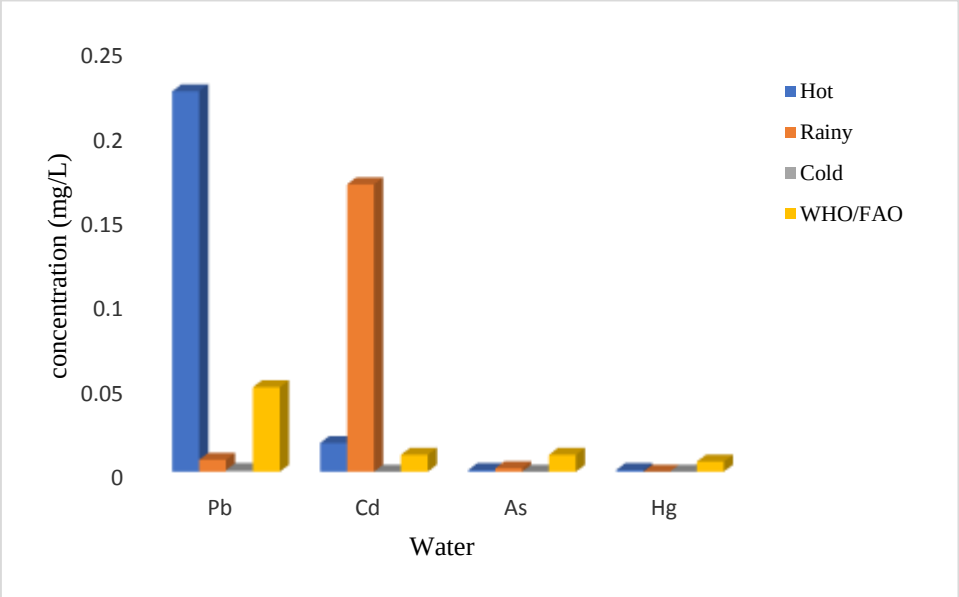


Figure. 6 Seasonal variation of metals concentration in water samples

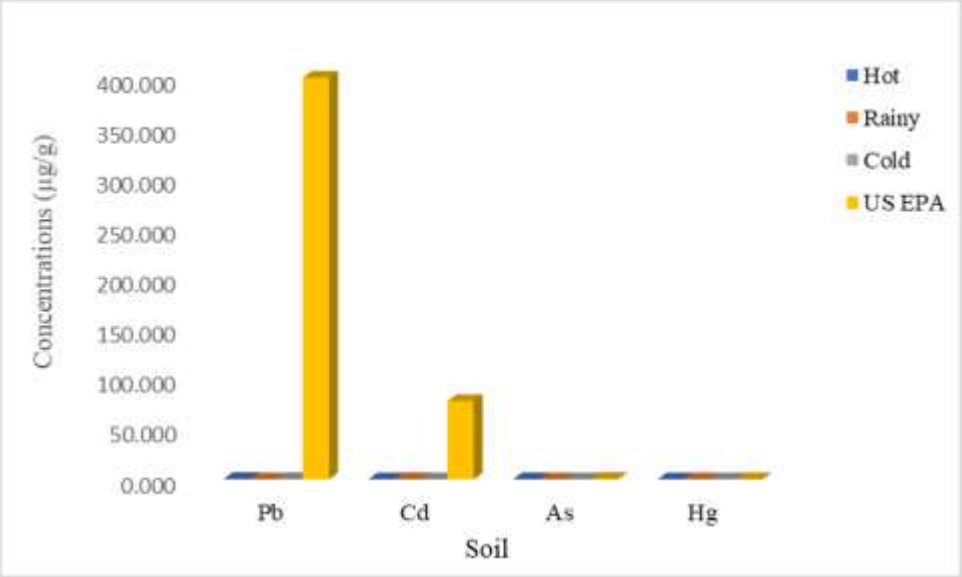


Figure. 7 Seasonal variation of metals concentration in soil samples

Table 2 Maximum permissible limit of metal concentrations (mg/L) stated in WHO and FAO guidelines and U.S. Environmental Protection Agency (EPA) (µg/g)

Sr. no.	Metal	WHO/FAO limit	WHO/FAO limit	US EPA
		Muscle	Water	Sediment
1	Pb	0.3	0.05	400
2	Cd	0.5	0.01	78
3	As	0.5	0.01	0.4
4	Hg	0.5	0.006	0.1

Discussion

The present study was aimed to investigate the concentrations of heavy metals (Pb, Cd, As, Hg) in fish species (*Wallgo attu* and *Cirrhinus mrigala*) and their surroundings (water and sediment) collected from the Chindwin River segment of Homalin Township. Moreover, the heavy metals contents found in tested samples (fish, water, and sediment) were compared WHO/FAO maximum permissible limits and U.S. Environmental Protection Agency (EPA). The levels of heavy metals were analyzed in this species due to its significance for human consumption. The gold mining activities reveals crucial insights into the differential responses of herbivorous and carnivorous fish species to heavy metal contamination.

Ahmed and Hossam (2013) reported high Cd and Pb concentrations in the muscle tissue of *Clarias gariepinus* during the summer and the lowest concentrations in winter of the study area and these concentrations in muscle were under the permitted in all area. So, the result was coincided with the current study. Moreover, Sein Moh Moh Paing (2019) found that Pb and Cd concentrations in study fish were exceeded than the maximum permissible limits in all seasons.

In the rainy season, Pb concentrtions in the muscle tissue of *Channa striata* (Nga Yant) were observed slightly than these of hot season and cold season in Hinthada Township (Aye Aye Mu, 2011). In the present results, the highest concentrations Lead and Cadmium in fish species (*Wallgo attu* and *Cirrhinus mrigala*) in hot season were found over the WHO/FAO maximum permissible limits. Cadmium concentration in *Wallago attu* was not detected in cold season during the study period.

Arsenic concentrations in *Wallago attu* were not found in all seasons although this metal concentration in *Cirrhinus mrigala* was observed in hot and rainy season. However, the concentrations of arsenic did not exceed the maximum permissible limits during study period. According to the Aye Aye Mu (2011), arsenic concentration in muscle tissue of *Lates calcarifer* were over in hot>rainy>cold season and *Channa striata* was high on rainy>hot>cold season. Chun *et al*. (2018) highlighted that arsenic concentration in the muscle tissue of their study fish were high in monsoon season. Sein Moh Moh Paing (2019) reported that arsenic concentration in all study fish species were exceeds the maximum permissible limits and guide line limit of WHO/FAO within the study period.

Regarding mercury concentrations, Khin Myint Mar (2011) reported levels exceeding the WHO(1990) maximum permissible limit in omnivorous and carnivorous fish species. In the present result, mercury contents in fish species carnivore (*Wallago attu*) and herbivore (*Cirrhinus mrigala*) were observed within the maximum permissible limits in hot season. Mercury concentration in studied fish species was not found in remaining seasons (cold and rainy).

Herbivorous fish species exhibit distinct patterns in heavy metal concentrations, with analyses revealing elevated levels of Pb and Cd Cho Cho Thin (2017) observed that lead concentration of *Labeo rohita* in rainy season in Ayeyarwady River segment of Salay environ was higher than the recommended maximum permissible limit of WHO/FAO, which is not coincided with the present findings as well. This suggested the potential contamination in their aquatic habitats, emphasizing their susceptibility to direct uptake of contaminants from polluted water and sediments due to their reliance on primary producers.

Karadede *et al.* (2004) reported that carnivorous fish, which mainly eat fingerlings, shrimp and zooplankton, are known to be active swimmers. These activities are known to accumulate high levels of heavy metals in the body. In contrast, carnivorous fish species, characterized by their dietary habits of preying on smaller organisms, display higher concentrations of Cd and Pb. This indicates the potential biomagnification of these heavy metals through the food chain, highlighting the role of predators in accumulating contaminants from lower trophic levels.

Herbivores may serve as indicators of direct contamination from mining effluents, while carnivores may reflect the biomagnification of contaminants through the food web. The presence of elevated concentrations of Pb and Cd in herbivores, along with increased levels of Cd and Pb in carnivores, emphasizes the importance of understanding the intricate pathways of heavy metal transfer in aquatic ecosystems affected by gold mining activities.

In the present results, lead concentrations of water were found to be higher than the maximum permissible limit (0.05 mg/L) in hot season. The remaining season (rainy and cold) within the WHO/FAO limit. The concentration of cadmium in water was exceeds the limits of (0.01mg/L) during rainy and hot season. Arsenic and mercury contents in water were observed to be lower than the maximum permissible limits recommended by WHO/FAO in hot and rainy seasons. Mercury concentrations in water was not detected in rainy and cold season. The obtained results of all metal concentrations (Pb, Cd, As and Hg) from sediment during the study period were observed to be lower than the U.S. Environmental Protection Agency (EPA). In the current study, the highest concentrations of heavy metals may originate from small scale gold mining and agricultural activities.

The seasonal variations of heavy metals in fish, water, and sediment further highlight the dynamic nature of contamination in response to gold mining impacts. This study indicates that the species is safe for human consumption. Although heavy metal concentrations of water over the WHO/FAO maximum permissible limits, heavy metals contents in sediment within the U.S. Environmental Protection Agency (EPA). Therefore, a regular monitoring of heavy metals level in fish, water and sediment in the study area is necessary.

Conclusion

In the current study, heavy metals concentrations in studied fish species were found to be lower than the maximum permissible limit. Base on the results, it can be concluded that the consuming the studied fish species is safe. However, heavy metals concentrations in water exceed the maximum permissible limits and heavy metals contents in sediment within the U.S. EPA guidelines. Therefore, regular monitoring of heavy metal levels in both fish and their surroundings, including water and sediment.

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I would like to express my gratitude to Professor/Head Dr. Ye Chan, University Research Center (URC) and University of Yangon, for the permission to use FAAS. I would also like to thank Daw Lay Shee Phine, Homalin Township, Sagaing Region for her invaluable contribution in providing the fish samples.

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GROWTH PERFORMANCE OF *NOTOPTERUS NOTOPTERUS* (PALLAS 1769) LARVAE USING IN THREE DIFFERENT DIETS

Saw Soe Moe Thu[1*], May Thu Rein Oo[2], Cho Cho Thin[3], Kay Lwin Tun[4]

Abstract

The present work was investigated the growth performance of post-larvae *Notopterus notopterus* by using three different diets. A total of 300 post larvae, ranging from 2.9 cm to 3.4 cm in length and weighing between 0.02 g and 0.05 g, were divided into three groups and fed with three different live feeds: Moina, Tubifex, and Rotifer. The experiment were conducted for three triplicate. The fishes accepted all types of diets. The specific growth rate (SGR) was higher in post larvae fed on Moina (6.98±0.10%), followed by Tubifex (6.92±0.04%), with the lowest SGR was recorded for Rotifer (6.74±0.44%). The highest final mean weight and weight gain were observed in post larvae fed on Moina (2.64±0.16) g, followed by Tubifex (2.54±0.06) g, with the lowest weight gain recorded for Rotifer (2.30±0.31) g. No significant difference was observed in fish fed on Tubifex and rotifer, whereas Moina-fed larvae showed a significant difference ($p < 0.05$) compared to other two groups. The survival rate on day 60 was 83% for larvae fed on Moina and 82% for those fed on Rotifer, whereas the lowest survival rate of 75% was recorded for Tubifex-fed larvae. Therefore, The larvae of *Notopterus notopterus* could be reared using different livefood to promote enhanced growth and survival rate.

Keyword : *Notopterus notopterus*; Larval rearing; Different diets; SGR; Growth and Survival

Introduction

In Myanmar, *Notopterus notopterus (locally known as Nga-pe)* is one of the famous foods for the local people and one of the most economically important freshwater fish found in Myanmar. The meat of the featherback fish is tasty and has the highest nutrient value, resulting in a high price. A biochemical study conducted on the featherback knife fish revealed that it is a rich source of protein, with a protein content of 19.8% and a lipid content of about 5.0% (Kamal *et al.*, 2007).

In recent years, aquaculture has been recognized as an important strategy for meeting the growing demands of fish protein worldwide. Larval rearing is still consider the most critical aspect, and the development of rearing technology is essential for the conservation of fish species. The successes of larval rearing depend mainly on the availability of suitable diets that were readily consumed, efficiently digested, and provide the required nutrients to support higher growth and health (Sarkar *et al*., 2006).

Diet plays a significant role in aquaculture production. Different commercial feeds, such as nursery and grow out feed of various sizes, are available in the market. However, these feeds were manufactured based on the nutrient requirements of fry or young fish and may not fulfill the requirements of fish larvae. Fish larvae cannot feed on artificial supplemented feed; they require small live foods for their nutrition. Live foods are easily digestible, protein-rich diets for fish larvae. Live zooplankton, such as cladocerans (Moina and Daphnia), brine shrimps (Artemia), tubificids (Tubifex) and Rotifer are the most widely accepted live feeds globally and play a significant role in the feeding of cultivable species of fishes and crustaceans (Morris and Mischke, 1999). They are excellent first foods for larvae due to their relatively smaller size, slow swimming speed, the habit of staying suspended in the water column, and their ability to propagate in captivity at high density and reproductive rate. The availability of a suitable larval rearing diet is important for the propagation of many aquatic species because it plays a vital role in the growth, survival, and disease resistance during early stages (Sontakke *et al*., 2019). The success of larval rearing depend mainly on the availability of optimal diets that can be easily

1. Department of Zoology (Fisheries and Aquaculture), University of Yangon.
2. Department of Zoology (Fisheries and Aquaculture), University of Yangon.
3. Department of Zoology (Fisheries and Aquaculture), University of Yangon.
4. Department of Zoology (Fisheries and Aquaculture), University of Yangon.

consumed, efficiently digested, and provided the required nutrients to support good growth, survival, and health (Giri *et al*., 2002).

Live feeds are considered a convenient and essential food source for the post larvae and fry of some cultivable fish species and are widely accepted as living capsules of nutrition. The use of an optimal live feed species is a vital role in survival and growth performance during early life stages. The aim of the present study was conducted to evaluate the growth performance and survival rates of *Notopterus notopterus* post larvae when fed three different diets. These diets are selected based on their high nutritional value, capacity to support growth in dense populations, and ease of mass production under controlled conditions.

Materials and Methods

Study Area and Study Period

The experiment was conducted at wet laboratory, Department of Zoology, Fisheries and Aquaculture, University of Yangon. Study period was lasted from November 2022 to January 2023.

Samples Collection

A total of 300 *Notopterus notopterus* post larvae were collected from Hlawga Fisheries Experimental Station to conduct the larvae diets experiment. The initial length of post larvae of *Notopterus notopterus was* 2.98±0.43 cm and their body weight was 0.03±0.01 g (Plate 1.). They were carried to the Aquatic Bioscience Laboratory, Fisheries and Aquaculture, Department of Zoology, University of Yangon for the experiment. They were divided into three groups and kept in (90cm×30cm×45cm) glass tanks through continuous aeriation for the experiment.

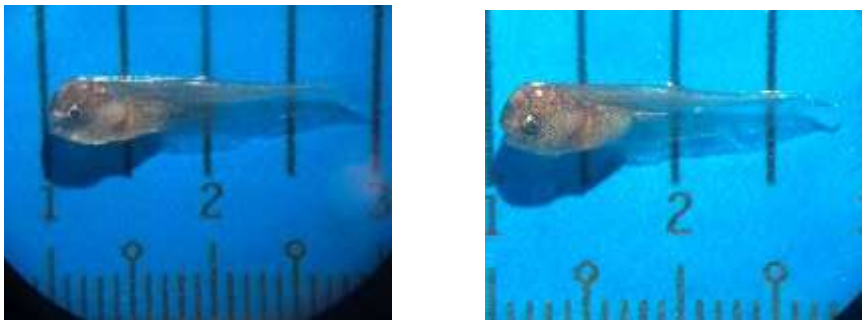

Plate. 1 Post larvae of *Notopterus notopterus* collected from Hlawga

Preparation of Diets

Three larval diets, Moina (*Moina* sp) , Rotifer (*Brachionus* sp) and Tubifex (*Tubifer* sp) were prepared. The initial seeds of Moina and Rotifer were received from the Aquatic Live Feed Laboratory, Department of Fisheries (DoF), Thaketa Township, Yangon region, while Tubifex was purchased from the local aquarium shop (Plate 2.). Mass culture of Moina and Rotifer was conducted in Live food Laboratory, Fisheries and Aquaculture, Department of Zoology, University of Yangon.

Plate 2. Preparation of diets for the post larvae of *Notopterus notopterus*

Culture of Moina

The five individuals of Moina were introduced to 1L of beaker filled with tap water. Moina was fed with 1 gram of baker's yeast during the culture period and maintained in the beaker with continuous aeration. The population of Moina in the beaker was checked every day by collecting the sub-sample 1ml of water. When the Moina population reached approximately 100-110 individuals per milliliter, they were transferred into fiber tanks with dimensions of 120cm x 120cm x 45cm for the mass production. Three fiber tanks were prepared for the mass production and Moina were treated with baker's yeast in the same ratio as described in above (1L/g). The fiber tanks was filled with 800L of tap water.

Culture of Rotifer

In order to obtain sufficient Rotifer density for the experiment five Rotifers were inoculated in three 500 mL beakers with tap water, with aeration and light availability. The Rotifers were fed daily with a mixture of algal diets (*Chlorella* sp.) at a concentration of approximately 40×103 cells/ ml. A total of 100ml of *Chlorella* sp. were added beakers of Rotifer and the density of Rotifer was checked as described in Moina everyday. When the sufficient density of adult Rotifer (10 individual/ml) was reached from the inoculated beakers, they were transferred into the 14 liters cylindrical plastic tanks for the mass culture. Rotifer were fed with 1000ml of *Chlorella* sp. per day (at $4.5x10^6$ cells ml^{-1}). The 40% of water was replaced every 5-6 days.

Preparation of Tubifex

Tubifex worms were sourced from the local aquarium shop and maintained in a 3-liter stainless steel bowl under a continuous running water system to remove dirt and debris. The worms were thoroughly rinsed before being fed to the fish larvae. They were sliced into small pieces to match the size of the fish's mouth.

Feeding Design

The larvae were feed twice a day, during the morning and evening at 09:00 am and 5:00 pm. The post larvae were treated with 5 g of three different diets. Five grams of Rotifers were equal to approximately 58,330 individuals, while Moina consisted of 12,695 individuals. The feeding trial was conducted for a period of 60 days. The fecal matters were removed by siphoning out and 80% of the water from each tank was changed everyday.

Water Quality Parameters

The water quality parameters were checked once every three days. Oxygen and temperature were measured using a DO Meter ID-150, and pH was measured using a pH meter Model TPX-999. 26.

Determination of Growth Parameters

The body weight and length of the fish were assessed at an interval of 15 days by randomly collecting 10 fish from each tank randomly. Fishes were starved overnight before being weighed on an electric balance to reduce their stress. The growth parameters such as percentage weight gain (%), specific growth rate (SGR) and survival rate (%) were calculated by using the following equations. (Cerozi and Fitzsimmons, 2017).

$$\text{Weight Gain (WG,g) \%} = \frac{Wf - Wi}{\text{Number of fish per tank}} \times 100$$

$$\text{Specific Growth Rate (SGR) \%} = \frac{Ln Wf - Ln Wi}{\text{Day of rearing period}} \times 100 \times 100$$

Where, Wf= Final weight of larvae
Wi= Initial weight of larvae

$$\text{Survival rate} = \frac{\text{Initial number of}}{\text{Final number of fish}} \times 100$$

Results

Growth Performance of Fingerling

In the present study, larval of *Notopterus notopterus* was treated using three different diets, and their growth and survival rates were calculated. During the experimental period, *Notopterus notopterus* exhibited a typical preying habit of swallowing feed with upward and downward movements, as well as good swimming behavior. No attacking behavior was observed.

The mean body length reached 4.84±0.45 cm in group I (Moina) while it was 5.03±0.10cm and 4.75±0.16 cm in group II (Tubifex) and group III (Rotifer), respectively. The final body length were 7.39±0.25cm 6.79±0.41cm and 6.76±0.34cm in group I (Moina), group II (Tubifex), and group III (Rotifer), respectively (Fig. 1). The mean body weights of the fish were 0.94±0.17g, 0.92±0.06g, and 0.82±0.05g in group I (Moina), group II (Tubifex) and group III (Rotifer), respectively. The mean body weight of *Notopterus notopterus* in group I (Moina) gradually increased during the experiment. It reached 2.64±0.16g, during 60 days of the study period. However, the mean body weights of fish in group II (Tubifex) 2.54±0.06g and group III (Rotifer) 2.30±0.31g were lower than in group I (Moina) (Fig. 2).

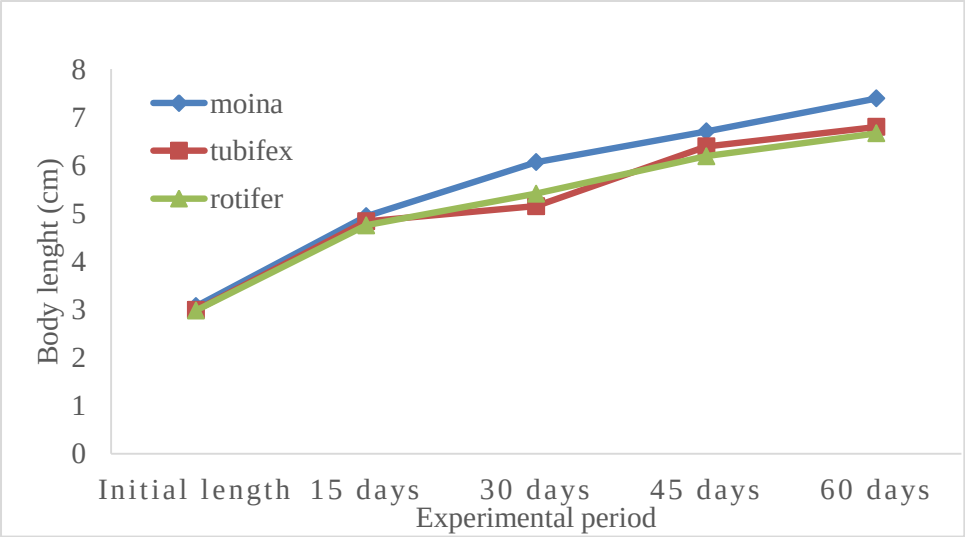

Figure. 1. Mean body length of *Notopterus notopterus* during experiment

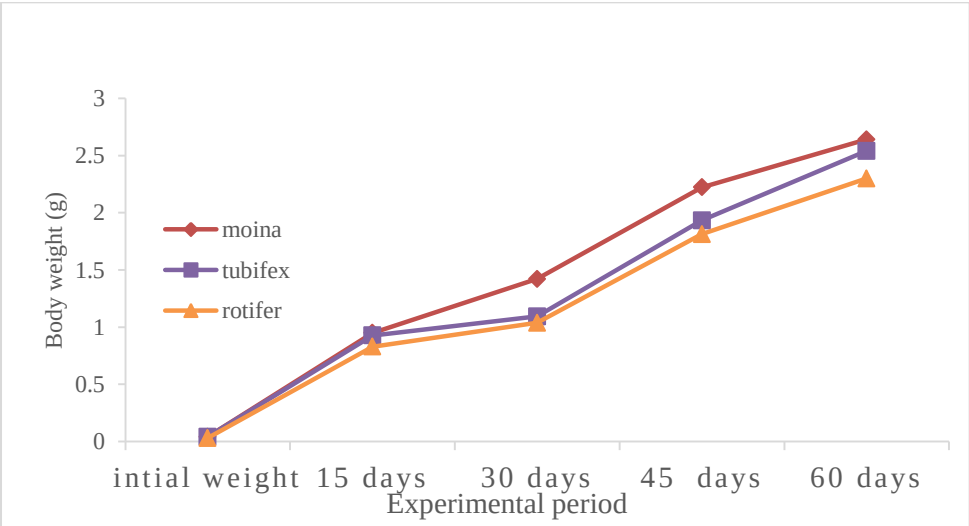

Figure. 2. Mean body weight of *Notopterus notopterus* during experiment

Specific Growth Rate and Weight Gain

The specific growth rate (SGR) was calculated to determine the growth performance during the experimental period. A high SGR was observed for larvae fed on Moina

showing an average of (6.98±0.10)g/day, followed by Tubifex (6.92±0.04)g/day while the lowest value of SGR was found in Rotifer (6.74±0.44) g/day. The highest weight gain was also found in Moina (13.96±0.20)g followed by Tubifex (13.83±0.08)g and Rotifer (13.48±0.44)g. (Table 3)

Table 3. Mean length, weight, weight gain, SGR, survival of *Notopterus notopterus* post larvae treated with three different live food diets

Parameters	Feeds		
	Moina	**Tubifex**	**Rotifer**
Initial Length (cm)	3.07±0.53	2.99±0.47	2.98±0.43
Final length (cm)	7.39 ± 0.25.	6.79 ± 0.41	6.76±0.34
Initial weight (g)	0.04±0.02	0.04±0.02	0.03±001
Final weight (g)	2.64±0.16	2.54±0.06	2.30±0.31
Weight gain (%)	13.96±0.20	13.83±0.08	13.48±0.44
SGR (%)	6.98±0.10	6.92±0.04	6.74±0.44
Survival rates(%)	83	75	82

Survival Rate

The survival rates of Notopterus notopterus post-larvae fed on three different diets during the experimental period varied from to 75% to 83% . The results indicated that maximum survivability for Moina was (83%) followed by Rotifer (82%). The lowest survival rate was recorded in Tubifex (75%).

Figure. 5. Survival rate (%) of *Notopterus notopterus* post larvae during the experiment

Water Quality

Water quality was monitored to provide an overview of changes in the experimental tanks during the study period. No remarkable variation in water quality parameter was observed. The dissolved oxygen level in the rearing tank was observed 6.23 ppm to 6.48 ppm while pH ranged from 6.6 to 7.25 and temperatures varies from 23.7 °C to 27.3°C. (Table 4)

Table 4. 6 Mean of water parameters of tanks during the experimental period

Treatment	November			December		
	Dissolved oxygen (ppm)	pH	Temperature (°C)	Dissolved oxygen (ppm)	pH	Temperature (°C)
Moina	6.48±0.02	6.86±0.01	26.3±0.02	6.34±0.02	6.6±0.02	23.7±0.03
Tubifex	6.34±0.01	7.25±0.02	26.5±0.02	6.45±0.02	7.28±0.02	24.3±0.01
Rotifer	6.23±0.02	6.6±0.01	27.3±0.01	6.28±0.02	6.6±0.02	24.8±0.02

Discussion

The featherback fish is a highly demanded freshwater fish species and therefore has excellent potential for culture (Sontakke, *et al.,* 2019). To expand featherback fish culture, understanding early larval development and feeding is imperative. However, the feeding and larval development of this fish species are poorly understood, and only a few studies have been conducted on it. Many factors are related to feeding, such as stocking density, production system, type and size of rearing tanks, size of fish, and quality and quantity of food (Mgay and Mercer, 1995).

In the present study, the growth performance of *Notopterus notopterus* larvae was evaluated using three different types of diets Moina, Tubifex and Rotifer. The study revealed differences in the growth, SGR, and survival of *Notopterus notopterus* when fed with different diets. Studies conducted so far revealed that fish larvae were generally physiologically immature, with little or no capacity to produce certain hormones and digestive enzymes. They were dependent, to a greater or lesser extent, on exogenous sources of food, including the mother and/or live food (Lam, 1994). According to Seidgar (2014), the use of live food was considered one of the prerequisites for the larval stage culture of most aquatic animals, as it provides nutritional feeding sources that are also economically cost-effective.

Common live foods used in the economic rearing of fish larvae usually include macro and microlive food, such as Moina, Daphnia, Artemia, Tubifex, Bloodworm, Mosquito larvae, and Rotifers. According to the experiment, growth parameters and weight gain were highest with live Moina (13.96±0.20)g, followed by Tubifex (13.83±0.08)g and Rotifer (13.48±0.44)g. A similar finding was observed by Sontakke *et al*., (2019), where growth parameters and weight gain were higher in Moina (22.9±0.08) g compared to live Tubifex (22.6±0.11) g treated with *Notopterus chitala*. The study indicated that the post larvae of *Notopterus notopterus* were successfully weaned onto Moina, Tubifex, and Rotifer in an experimental setting. Moina, Tubifex, and Rotifer were proven to be excellent feeds for the post-larvae rearing of *Notopterus notopterus* in terms of growth. It is interesting to note that Moina served as an excellent diet for rearing post larvae of *Notopterus notopterus.* According to Fluchter (1982), the growth rates of fish receiving these diets could be due in part to the physical properties of the feed.

During the rearing period, *Notopterus notopterus* exhibited sluggishness and showed schooling and hiding behavior within gravels in the rearing tanks. Sakar *et al*. (2006) closely examined weight gain percentages and specific growth rates of *Chitala chitala* indicated a significant difference ($p<0.05$) when using zooplankton, Daphnia, and boiled egg yolk as feed. In the present study, it was noted that Moina served as an excellent diet for rearing post-larvae of *Notopterus notopterus*. Villegas (1990) reported that Moina was known to be suitable as a feed for *Chanos chanos*. Several hypotheses have been proposed to explain the low effectiveness of a dry diet as the sole food supply for fish larvae, as different larval stages have specific nutritional requirements. The present study also confirmed the feasibility of using a Moina to feed *Notopterus notopterus* post larvae.

Therefore, Moina exhibited rapid growth rates and achieved the highest final length and weight. *Moina* sp. has several advantages as a live feed in aquaculture, with its protein content averaging around 55% compared to other natural foods such as *Daphnia magna*. In the present study, higher survival rates were observed with Moina and Rotifer (83% and 82%, respectively) compared to Tubifex (75%). This finding is consistent with Sontakke *et al*., (2019) who reported higher survival rates of *Notopterus notopterus* when fed Moina (64%) and live Tubifex (62%). Many authors had shown the importance of Moina as a live feed for fish, including similar carnivorous species such as *Seriola dumerili* (Roo *et al*., 2019), Asian sea bass fry (Vartak and Singh 2009), and *Clarias gariepinus* (Achionye-Nzeh *et al*., 2012; Chepkirui-boit *et al*., 2011; Faruque *et al*., 2010; Musa, *et al*., 2012). The impact of a Rotifer diet on pikeperch (*Sander lucioperca*) significantly improved the optimum survival rate of pikeperch larvae (Yanes-Roca *et al*., 2018).

The results of the present study showed that post larvae of *Notopterus notopterus* actively fed on Moina. According to Gopakumar *et al.*, (2012) fish larvae were typically attracted to live feed due to their movement and exhibit a preference for small sized prey that fits their mouth gape. However, the effective use of live feed mainly depends on its size and nutritional content. The highest weight gain and mean weight were observed in post larvae fed live Moina (13.96±0.20)g, making it the best live feed option. Following closely was Tubifex (13.83±0.08)g, which showed a moderate weight gain, while the lowest weight gain was recorded with Rotifer (13.48±0.44)g. On day 60, the survival rate was highest at 83% for live Moina and Rotifer, while the lowest survival rate of 75% was recorded in Tubifex-fed larvae.

Conclusion

In conclusion, the present study successfully established the reliability of rearing post-larvae of *Notopterus notopterus* throughout the experiment period using three different diets. The utilization of Moina emerges as a promising option for the early life stage of featherbacks. The study emphasized the larvae's preference for Moina, due to the movement of the prey. Potential areas for further investigation, to advance conservation and aquaculture efforts for this species, include optimizing stocking density, enhancing diet presentation, adjusting feeding levels and rearing protocols, as well as developing effective feeding strategies.

Acknowledgment

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SEASONAL VARIATION OF HEAVY METAL RESIDUES IN SOME FISH FROM RICE FISH FARM OF WEST TARPET VILLAGE, MAUBIN TOWNSHIP

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

Abstract

Rice fish farming system experimental trials in Myanmar's Ayeyarwady Delta started in 2017. Fish from rice fish farms and paddy fields are supply nourishment of local people. Bioaccumulation of heavy metals in fish causes serious threats to human when they are consumed. This study focused on the seasonal variation of heavy metal concentrations (Arsenic (As), Cadmium (Cd) and Lead (Pb) in muscle tissues of six fish species (*Catla catla, Labeo rohita, Puntius gonionotous, Puntius chola, Mystus pulcher* and *Channa striata*) cultured in the rice-fish farm and paddy fields located at West Tarpet Village, Maubin Township. Seasonal variation of metal concentrations in water and soil were also investigated separately from paddy field fish and rice-fish farm fish from June 2022 to May 2023. The levels of metal concentration varied depending on the season and the different fish species. The highest concentrations of As and Cd were found in *Puntius chola* and *Mystus pulcher* during the rainy season in paddy field. However, these values were not over the maximum permissible limit and guide line limit of WHO and FAO. The concentration of Pb in *Labeo rohita* was high during the rainy season and it exceeded the permissible limit. Cd and Pb concentrations in water samples were found to over the maximum permissible limit during the rainy season. The values of As concentrations in water samples were not over permissible limit in all seasons. The concentrations of As, Cd and Pb in soil sample were recorded beyond the acceptable limit. The values of heavy metal concentrations observed for all fish species except *Labeo rohita*, did not exceed the acceptable limits set by WHO/FAO (2011). While certain species showed elevated levels during the rainy season, all fish remained within acceptable limits set by WHO/FAO standards, highlighting the importance of ongoing environmental monitoring for sustainable aquatic ecosystems.

Keywords: rice fish farm, heavy metal, muscle tissue, soil.

Introduction

Rice fish farming system is an ecological symbiosis system where fishery is simultaneously introduced to the rice farming (Halwart, 2004). In rice fish farming system, fish would defend the crops by feeding on pests as well as and moreover facilitate the growth of rice by providing nutrients through disturbing the water and softening the sediment, leading to the limited using of pesticides and fertilizers (Xie, *et al.* , 2011).

Fish species are an important inductor of ecological health and economically important for human consumption (Zainudin, 2005). Fish accumulate contaminants directly from water and/or through the food chain. It is noteworthy that the consumption of fish can supply an additional pathway rather than rice for human exposure to pollutants from paddy fields, especially heavy metals. Heavy metal could be introduced via cultivation soil, irrigation water, pesticide application, organic and chemical fertilizers, atmospheric precipitation and industrial activities (Hu, *et al*., 2016; Chen. *et al* 2018; Sharafi *et al.*, 2019).

To increase production and productivity, application of widespread and unconscious use of chemicals cause direct environmental damage, as air, soil, and water pollution, degrading plant and animal health and existence (Kalyoncu, 2009). The indiscriminate and irresponsible use of pesticides in agriculture causes environmental problems especially to aquatic system by altering the quality of water and affecting the physiological and biochemical characteristics of non-target fish (Murty, 1986). In addition, extreme fertilizer use can pollute the groundwater with inorganic chemicals.

[1]Department of Zoology, University of Yangon
[2] Department of Zoology (Fisheries and Aquaculture), University of Yangon
[3] Department of Zoology, University of Yangon

During the manufacturing of fertilizers, varying amounts of different heavy metals are transported to fertilizers, and these are later transmitted to the soil which enters the food chain (Wei *et al*., 2020). One of the most important issues of heavy metal pollution is related to fertilizers that can flow into the food chain and cause severe risks to human health (Qaswar *et al*., 2020). Heavy metals have enticed attentions 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 *et al.*, 2017 and Rahman *et al.*, 2012).

Therefore the risk assessment of heavy metal concentration in fish for human health is important and necessary. The objective of present study was to examine the concentrations of heavy metals (As, Pb and Cd) on fish species from rice fish farm and paddy fields that are daily food of local people. Water samples from rice fish farm, reservoir and river, and soil sample from study area were also detected.

Materials and Methods

Study Area

Study area was designated at West Tarpet Village, Maubin Township, Ayeyarwady Region. It is located between 16° 43′ N and 95° 39′ E (Fig. 1 and Plate 1). A total area of 4046.85m^2 of rice fish farm and total area of 72843.3 m^2 of the paddy fields near the rice farm were chosen as study sites.

Study Period

Study period lasted from June 2022 to May 2023.

Collection of Samples

A total of six fish species (*Catala catala* (Nga-Thaing Khaung Pawa), *Labeo rohita* (Nga-Myint-chin*), Puntius gonionotus* (Nga-khone-ma-kyee), *Puntius chola (Nga-khone-ma-Myee-Ni), Mystus pulcher* (Nga- Zin-Yine) and *Channa striata* (Nga-Yant) were collected once in every season from rice fish farm and adjacent paddy fields. Collected fish specimens were identified follow by Talwar and Jhingran (1991). Water samples were also seasonally collected from rice fish farm, reservoir and river. Soil sample from this area was also collected to assess the heavy metals contents.

Sample preparation

The collected fish samples were washed by tap water, total length and body weight were measured. Muscles were removed and dried in an oven until constant weight was reached and grounded into powder. The soil sample was sun dried and grounded into powder. The fish samples were digested according to the dry method by using a furnace. Each water sample was filtered through a 0.45μm Whatman filter and the sample was analyzed directly.

Data Analysis

Heavy metals contents in muscles of collected fish species, water samples and soil samples were analyzed tri-replicates by Flame Atomic Absorption Spectrometer (FAAS) (Perkin Elmer AA analyst 800 and Winlab-32 software) in Universities' Research Centre (URC) at Yangon University. Seasonal variations of test results were compared with WHO/FAO, 2011 maximum permissible limit (MPL).

Figure. 1 Mape of study area (Google,2023)

Plate 1 Study site

Results

A total of six fish species (*Puntius gonionotus, Catla catla, Labeo rohita, Channa striata, Mystus pulcher,* and *Puntius chola*) were seasonally collected from rice fish farm and adjacent paddy fields in West Tarpet Village, Ayeyarwady Region (Fig. 1and Plate 1). The total length and total weight of collected fish species were recorded in Table 1 and 2.

The seasonal variation of arsenic concentration of fish species was shown in table 3 and Fig.2 .The highest value of arsenic concentration 0.00411mg/L was observed in muscle tissues of *Puntius chola* in during rainy season.

The seasonal variation of cadmium and lead concentration was also mentioned in Table 3and Figure 3&4. It was shown that cadmium concentrationin in muscle tissue of *Mystus pulcher* 0.077mg/L was highest than other fishes in rainy season. However , highest concentration of lead in muscle tissue of *Labeo rohita* (1.236 mg/L)was observed in rainy season. It was found that the cadmium concentrationin in *Mystus pulcher*0.077mg/L was highest among the seasons.

The seasonal variation of heavy metal concentration in water samples was presented in table 4, Figure 5, 6 and 7. The highest value of arsenic concentration in the water was 0.0069mg/L in hot season . The highest level of Cadmium concentration in the water was 0.024mg/L in rainy season. The highest level of lead concentration of water in river water was 0.215mg/L in rainy season.

In addition, the seasonal variation of metal concentrations in soil sample was also mentioned in Table 5 and Fig. 8. During the study period the highest value of arsenic, cadmium and lead concentrations of soil sample were 0.13726mg/L, 0.021mg/L and 0.498mg/L in cold season followed by hot season while those were the lowest in rainy season.

Table 1 Mean total length (cm) of studied fish species selected to test metal concentration

No.	Species	Number	Rainy	Cold	Hot
1	*Catla catla*	10	8.72 ± 0.59	15.5 ± 1.62	25.9 ± 4.82
2	*Labeo rohita*	10	11.28 ± 0.74	17.7 ± 2.88	25.9 ± 5.21
3	*Puntius gonionotus*	10	8.47 ± 1	18.7 ± 1.99	22.9 ± 2.49
4	*Puntius chola*	25	7.17 ± 1.34	7.28 ± 1.1	8.74 ± 1.86
5	*Mystus pulcher*	25	9.76 ± 1.16	12.2 ± 2.41	9.27 ± 1.4
6	*Channa striata*	20	8.27 ± 0.77	13.6 ± 2.18	15.8 ± 3.99

Table 2 Mean body weight (g) of fish species selected to test metal concentration

No.	Species	Number	Rainy			Cold			Hot		
1	*Catla catla*	10	10.64	±	1.35	44.94	±	19.9	198	±	113.6
2	*Labeo rohita*	10	18..29	±	4.93	59.12	±	36.2	185	±	114.1
3	*Puntius gonionotus*	10	10.83	±	1.51	95.3	±	32.4	159	±	59.51
4	*Puntius chola*	25	5.59	±	3.81	5.751	±	2.75	11.8	±	3.123
5	*Mystus pulcher*	25	11.42	±	4.27	18.53	±	9.87	10.5	±	4.792
6	*Channa striata*	20	6.31	±	0.99	24.54	±	10.3	42.5	±	28.48

Table 3 Seasonal variation of heavy metal concentrations (mg/L) in fish species

No.	Species	Arsenic			Cadmium			Lead		
		Rainy	Cold	Hot	Rainy	Cold	Hot	Rainy	Cold	Hot
1	*Catla catla*	0.00074	0.00047	0.00014	0.015	0.013	0.019	0.274	0.254	0.255
2	*Labeo rohita*	0.00178	0.00082	0.00004	0.017	0.011	0.018	1.236	0.231	0.486
3	*Puntius gonionotus*	0.00146	0.00082	0.00081	0.013	0.011	0.015	0.201	0.243	0.3
4	*Puntius chola*	0.00411	0.00247	0.00234	0.023	0.015	0.022	0.339	0.343	0.382
5	*Mystus pulcher*	0.00275	0.00073	0.00121	0.077	0.013	0.019	0.303	0.234	0.288
6	*Channa striatus*	0.00125	0.00126	0.00071	0.02	0.015	0.019	0.247	0.3	0.312
	MPL(WHO/FAO,2011)		0.5mg/L			0.5mg/L			0.3mg/L	

MPL = Maximum Permissible Limit

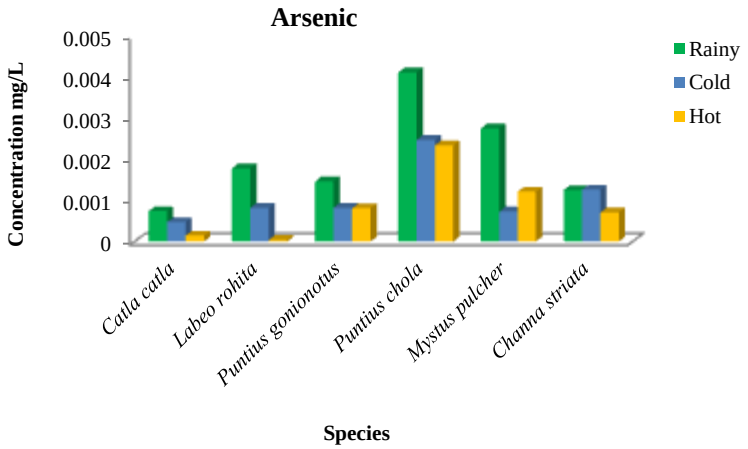

Figure. 2 Seasonal variation of Arsenic concentration in studied fish species

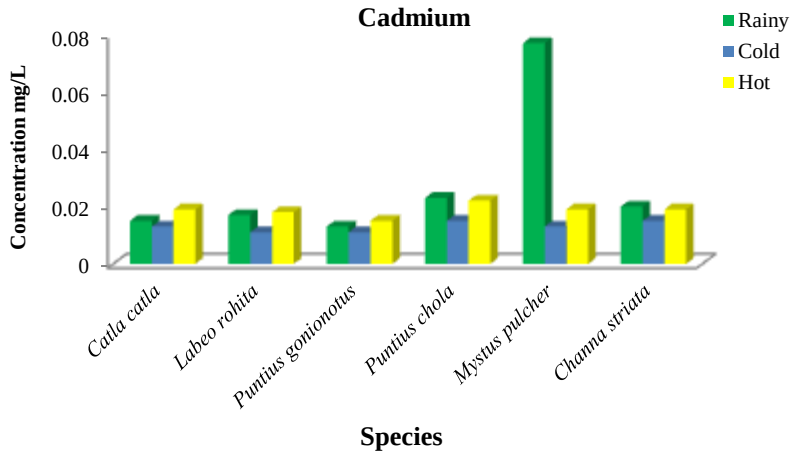

Figure. 3 Seasonal variation of Cadmium concentration in studied fish species

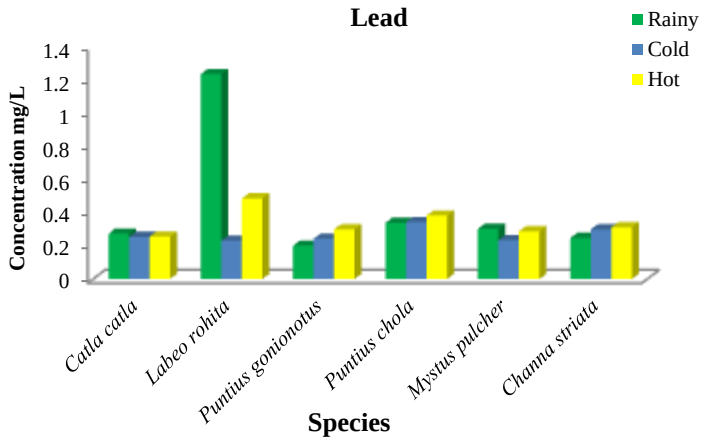

Figure. 4 Seasonal variation of Lead concentration in studied fish species

Table 4 Seasonal variation of heavy metal concentrations (mg/L) in water samples

No	Water samples	Arsenic			Cadmium			Lead		
		Rainy	Cold	Hot	Rainy	Cold	Hot	Rainy	Cold	Hot
1	rice fish farm water	0.00074	0.00099	0.00111	0.019	0.01	0.012	0.215	0.153	0.172
2	reservoir water	0.00064	0.00073	0.00107	0.012	0.01	0.014	0.208	0.192	0.197
3	River water	0.00105	0.00073	0.0069	0.024	0.01	0.012	0.173	0.209	0.215
MPL(WHO,2011)		0.01mg/L			0.01mg/L			0.01mg/L		

MPL = Maximum Permissible Limit

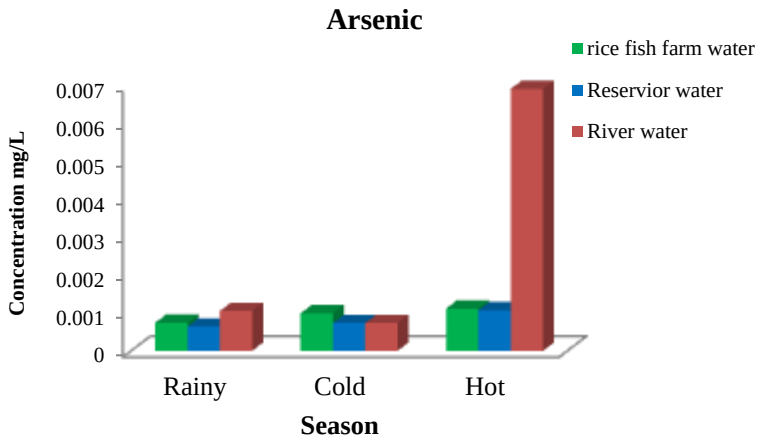

Figure. 5 Seasonal variation of Arsenic concentration in water samples of study area

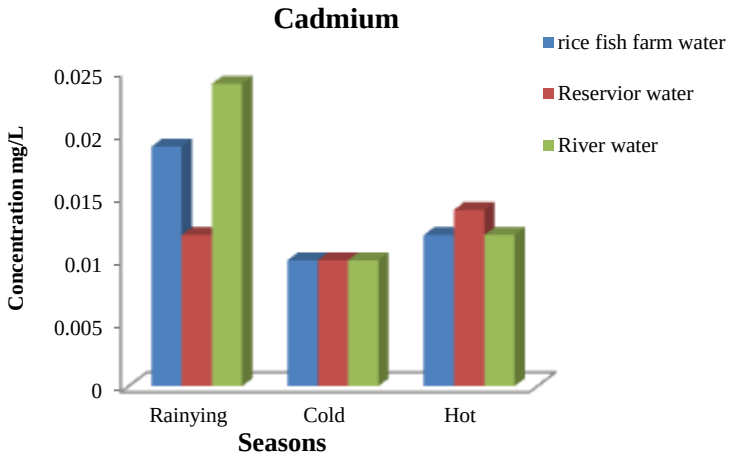

Figure. 6 Seasonal variation of cadmium concentration in water samples of study area

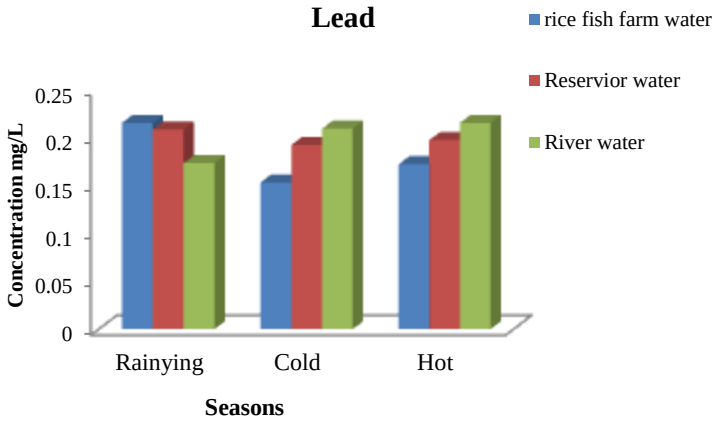

Figure. 7 Seasonal variation of lead concentration in water samples of study area

Table 5 Seasonal variation of heavy metal concentrations (mg/L) in soil sample

No.	Metals	Rainy	Cold	Hot	WHO,2007 MPL	TEC	MEC	PEC
1	Arsenic	0.0255	0.13726	0.028	0.01	9.8	21.4	33
2	Cadmium	0.016	0.021	0.019	0.01	0.99	3	5
3	Lead	0.247	0.498	0.351	0.05	36	83	130

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

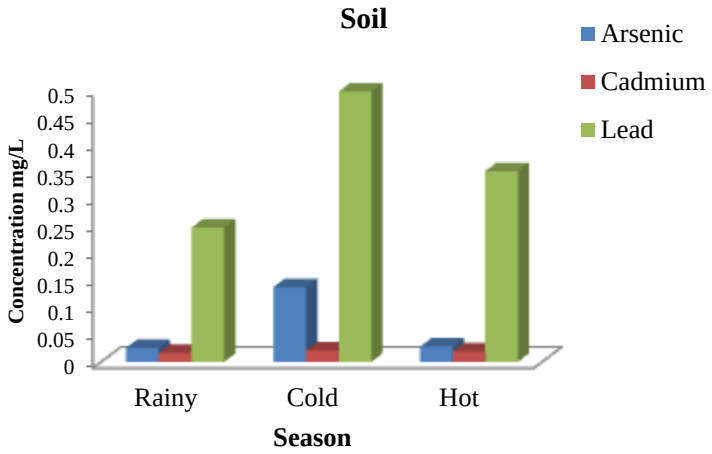

Figure. 8 Seasonal variation of metals concentration in soil samples

Discussion

A total of six fish species which included three fish species from rice fish farm and other three from adjacent paddy fields near the rice fish farm, were selected to examine the seasonal variation of heavy metal concentrations. In the present study, the highest concentration of arsenic (As) was found in *Puntius chola* (0.004 mg/L) among the six species during the rainy season. The WHO/FAO guidelines for maximum permissible limits of arsenic in fish are 0.5mg/L. The concentration levels of arsenic were found to be lower than the WHO/FAO maximum permissible limits. According to Aye Aye Mu (2011), arsenic concentration in muscle tissue of *Channa striata* was high in rainy, hot and cold season.

Sein Moh Moh Paing (2019) were also reported that As concentrations in study fish species were high in the cold season and was found to be over WHO/FAO maximum permissible limit.

In the current study, lead (Pb) concentration of *Labeo rohita* (1.236mg/L) in the rainy season was found to be the highest and over the recommended maximum permissible limit established by WHO/FAO. Cho Cho Thin (2017) reported that lead concentration of *Labeo rohita* in rainy season in Ayeyarwady River segment of Salay environ was over the recommended maximum permissible limit of WHO/FAO. Khin Thida Kyaw (2008) stated that lead of all studied fish species was the highest in the rainy season in Thi La War Fisheries of Daydayae Township, Pya Pon District, Ayeyarwady Division, which is similarly to the present study's findings as well.

In the present study, the lead concentration was found to be highest in soil and water samples. Similarly, the values observed for lead concentration was the highest in fish species

(*Labeo rohita*). Therefore, lead concentrations in *Labeo rohita* may transfer from fertilizers, soil and water due to bioaccumulation and may attain higher concentration in such manners.

In present study, cadmium (Cd) content was the highest in *Mystus pulcher* (0.077mg/L) in the rainy season. But the concentration level of this metal was found to be lower than WHO/FAO maximum permissible limits. Aye Aye Mu (2011) observed that Cd contents of *Latest calcarifer* were slightly higher in the rainy season and those of *Channa striata* were found to be not much different among the three seasons in Hinthda Township. Shakir *et al.* (2014) reported that the bottom feeder has a prolonged contact with river bed sediments; it may accidentally consume sediments when digging in search of food. In the present study, cadmium concentration in *Mystus pulcher* may attain higher concentrations in such a manner.

The concentrations of As, Cd, and Pb in studied fish species were found to be highest in rainy season in this investigation. In the study area, the rice-fish farm adjacent to the paddy fields was found to use pesticides and fertilizers. In the rainy season, the concentration of heavy metals residues from pesticides and fertilizers may come from the paddy fields and can leak into the rice- fish farm.

In the present study, arsenic (As) concentrations in river water (0.0069mg/L) was the highest in the hot season. But the result did not excess the maximum permissible limit established by WHO/FAO. Cogun *et a*l., (2006) stated that evaporation resulting from increased temperature during the dry season, the concentrations of heavy metal in the water generally higher compared to those in the wet season. Cho Cho Thin, (2017) observed that arsenic concentrations of water from Ayeyarwady River segment of Salay environ were the highest in hot season, which is similarly with the present study's finding as well.

This investigation shows the cadmium (Cd) concentration in river water was the highest in the rainy season and the result was higher than WHO maximum permissible limit. Lead (Pb) concentrations in river water (0.215mg/L) was highest in the rainy season and rice fish farm (0.215mg/L) in hot season and the obtained results exceeded the WHO maximum permissible limit. The high level of As, Pb and Cd in water samples can be related to agricultural discharge (Mason, 2002).

In the present study, all metal concentrations - lead (0.498 mg/L), arsenic (0.13726 mg/L) and cadmium (0.021mg/L) in soil samples were highest during the cold season and were observed to exceed the maximum permissible limit set by WHO/ FAO. However, the concentrations of metals in soil sample during the study period were observed to be lower than the 'threshold effect concentration' (TEC), "midpoint effect concentration" (MEC) and "probable effect concentration" (PEC).This is consistent with the previous findings of Sein Moh Moh Paing (2019), who reported that concentrations of Mg, Pb, Cd, and As in the sediment of the study area were highest during the cold season. Kalfakakour *et al*., 2000 and Rashed, (2001) observed that the heavy metal contamination of aquatic environs (water and sediment) can be affected the aquatic organism. Edogbo *et al*., (2020) observed that organic fertilizers may also contain a different concentration of heavy metals applied to fishponds and soil. Assimilation of metals by fish occurs through the ingestion of particulates suspended in the water.

Based on the results of the present study, the concentration levels of As, Cd, and Pb in soil samples, as well as lead (Pb) in *Labeo rohita*, Cd in river water exceeded the acceptable limits recommended by WHO/FAO. However, other five fish species from the study area were generally considered safe for human consumption.

In conclusion, it can be summarized that various factors, including cultivation soil, irrigation water, pesticide application, organic and chemical fertilizers, and atmospheric precipitation, may all contribute to variations in heavy metal concentrations in different fish species.

Conclusion

In the present study, the concentrations of As and Cd in all studied fish species were found to be generally lower than the maximum permissible limits set by WHO/FAO in all three seasons. However, the concentration of lead (Pb) in *Labeo rohita* exceeded the WHO/FAO limit in the rainy season, potentially due to agricultural runoff of fertilizers and pesticides. The results indicated that the concentrations of Pb and Cd in water and soil from the study area during the research period exceeded the WHO/FAO maximum permissible limits in 2011. Except for *Labeo rohita*, the other five species from the study site were below the WHO/FAO limits in 2011.

Acknowledgement

I would to express my gratitude to U Aung Kyaw, owner of rice fish farm for providing for this study and Professor/ Head University Research Center (URC) and University of Yangon for the permission to use FAAS.

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INVESTIGATION OF SOME BIOLOGICAL CONDITIONS OF *JOHNIUS COITOR* (HAMILTON, 1822) FROM GYAING RIVER SEGMENT BETWEEN GADOE AND KAWBEIN AREA

Mi Khin Sandar Win[1], Aye Aye Khaing (1)[2], Nyunt Nyunt Oo[3,]

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 study is based on 200 samples of *J.coitor* (Hamilton, 1822) collected at a monthly interval for a period of 12 months (January 2022 to December 2022). The result showed that the total length of male *J.coitor* specimens ranged from 12.00 cm to 20.00 cm and weight from 15.00 g to 70.00 g, while the female specimen ranged from total length 14.00 cm to 20.00 cm in total length and 13.00 g to 81.00 g in total weight. The length-weight relationship values of male and female *J. coitor* were significantly correlated (R^2 = 0.5036 and R^2 = 0.551, respectively). The peak value of GSI was attained in February for males and for females in October. A gradual increase in GSI value was found during the spawning period and gradually decreased during the post-spawning and resting periods. GSI values occur in an inverse relationship to those of HSI. The condition factor (K) is (0.41-0.95) in males and (0.40-0.89) in females, which is close to (1) and could be considered an indicator of good growth and feeding conditions. The result of this study contributes information on species restoration and fishery management in the Gyaing River.

Key words: length-weight relationship, GSI, HSI, and Condition factor, *Johnius coitor*,

Introduction

Fish are critical source of animal protein to the people and almost half of the total number of vertebrates in the world (Devashish *et al.*, 2006). And it has great commercial value and received special attention to scientists.

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. locally known as Thinma or (kyauk-Nga- poke thin). 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 knowledge of length-weight relationships (LWRs) has important implications in fishery biology and population dynamics. The length-weight relationship (LWR) has considerable importance in fishery research especially for the study of fish population dynamics and growth (Mathur and Bhatara, 2007), taxonomic differences, events in life history like metamorphosis, maturity (Le Cren, 1951) and to the fisheries officials in evolving effective policies for management and conservation of the resource.

The mathematical relationship between length and weight of fishes is a practical index suitable for understanding their survival, growth, maturity, reproduction and general well-being (Le Cren, 1951) and LWRs are also useful for fishery biologists for monitoring the state of health of a population (Cone, 1989).

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

[1] Department of Zoology, Hpa-an University
[2] Department of Zoology, Yangon University
[3] Department of Zoology, Hpa-an University

used in fisheries and fish biology studies. The results can be used for sustainable management and conservation of this commercially important species in this estuary.

Therefore, the objectives of this paper are

- To record and identify the study fish in the Gyaing River segment between Gadoe and Kawbein areas
- To assess the GSI, HSI, and K values of the studied fish species
- To determine the length-weight relationship of *Johnius coitor*

Materials and Methods

Study areas and Study 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, 2022 to December, 2023

Figure. 1 Topographic map of the study area (Source: Office of Land Record, Hpa-an Township, KayinState, 2022)

Specimen collection

A total of 200 specimens of *Johnius coitor* were collected and recorded from the local fisherman at the study site during the study period (January 2022 to December 2022). 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. After collection, fish were kept on ice and carried immediately to the laboratory at the Zoology Department of Hpa- an University. In the laboratory, the specimens were washed with tap water. Before weighing,

excess water from the specimens was removed with blotting paper. Fish were classified according to sex, and the total length (TL) was measured to the nearest centimeter by measuring the tip. The body weight was weighted to the nearest gram by an electric digital balance. Then, the specimens were dissected to remove the entire gonad and liver. Gonad weight and liver weight were also weighted.

(A)Fishing in study area (B) Fishing Boat (c) Fishing gear; drift gill net

Plate 1 Study area of Gyaing River segment between Gadoe and Kawbein area with operation of the fishing

(A) Measuring specimen (B) A paired of ovaries (C) Extracted testes

Plate 2 Dissected female and male of *J.coitor* showing gonads

Biological Assessment

Biological Assessment such as sex ratio, length-weight relationship, GSI, HSI, condition factor (K) were assessed by the following formula.

Sexes of the sampled fish specimens were determined after examination of the gonads. The sex ratio was calculated using the following formula;

$$\text{Sex ratio} = \frac{\text{Total number of female}}{\text{Total number of male}}$$ (Vazzoler, 1996)

Growth of fish was determined on the basis of length weight relationship. The regression method was employed with the following formula.

$$Y = a + bx$$

a = regression constant (intercept)

b = regression coefficient (growth exponent) Values of the exponent give information on fish growth (Morey *et al*., 2003). When b = 3, increase in weight is isometric. When the value of b is greater or smaller than 3, weight increase is allometric (positive if b> 3, negative b<3) (Mboru *et al*., 2010).

Gonado-somatic index (GSI) and Hepato-somatic index (HSI)

Gonado-somatic index (GSI) and hepato-somatic index (HSI) for each species were calculated according to the formula of (Le Cren, 1951) and (Lagler, *et al.,* 1962).

$$GSI = \frac{\text{Gonad weight}}{\text{Body weight}} \times 100$$

$$HSI = \frac{\text{Liver weight}}{\text{Body weight}} \times 100$$

Condition factor (K) was calculated according to the formula of (Le Cren, 1951 and Pauly, 1983)

Condition factor (K) = W x100/L^3

W= Body weight

L= Total Length

Results

Sex ratio

The monthly distribution of male and female frequencies is shown in Table 3. Out of 200 fish, 89 were male and 111 were female. The total sex ratio was found to be 1:1.2. The percentages of males and females were 44.5% and 55.5%, respectively. According to the results, the sex ratio of male: female is naturally consistent. (Table 3 & Fig. 5).

Length- weight relationship of male *Johnius coitor*

In male *Johnius coitor*, the minimum length was 12.00cm and the maximum length was 20.00cm. The total mean length was 15.70 cm. The minimum weight was 15.00g and the maximum weight was 70.00g. The total mean weight was 32.98g.The length-weight relationship was significantly correlated in *J.coitor (b = 4.7844*, R^2=0.5036, n=89) (Fig.1).

Length – weight relationship of female *Johnius coitor*

In female *Johnius coitor*, the minimum length was 14.00cm and the maximum length was 20.00cm. The mean value of length was 17.55cm. The minimum weight was 13.00g and the maximum weight was 81.00g. The mean value of total weight was 36.68g.The length-weight relationship was significantly correlated in *Johnius coitor* (b=6.1354, R^2=0.551, n=111) (Fig.2)

Correlation between GSI, HSI and K

In male *Johnius coitor*, the GSI values fluctuated throughout the study period and then increased in February as the peak GSI value (1.91%). The lowest GSI value was found in September (0.00%). But for females, the peak GSI values were observed in December (1.37%). The lowest GSI values were found in July (0.41%) A gradual increase in GSI value was found during the spawning period and gradually decreased during the post-spawning and resting periods. (Table 1&2) and (Fig. 3&4)

In male *J.coitor*, the peak HSI values were found to be 1.60% in March, and the lowest values were found to be 0.13% in August. But *J.coitor* for female the highest HSI values were observed in January (1.06%), and the lowest HSI values were found in July (0.06%) and October (0.06%) (Table 1&2) and (Fig. 3&4)

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 0.95 in males and 0.40 to 0.89 in females. The K value is close to 1, therefore males and females were in good condition in the study area (Table 1&2) and (Fig.3&4).

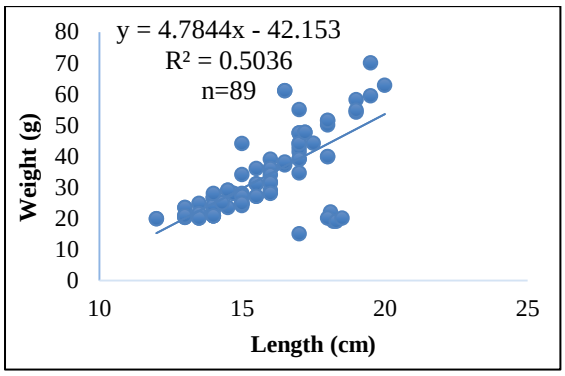

Figure. 1 Length-weight relationship of male *Johnius coitor*

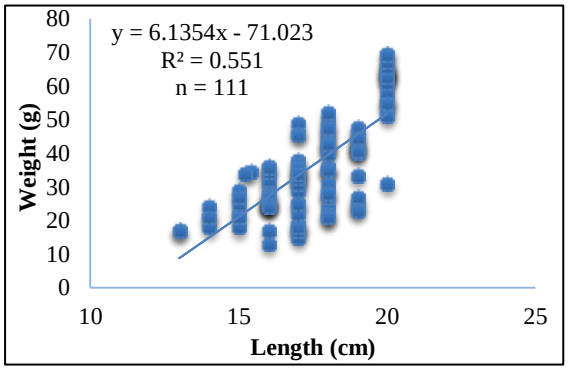

Figure.2 Length–weight relationship of female *Johnius coitor*

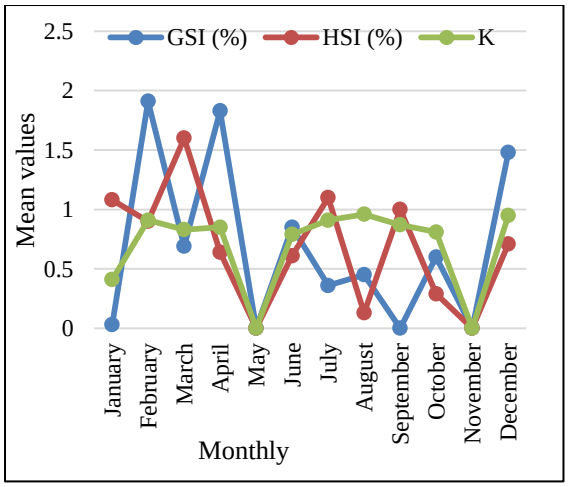

Figure.3 Monthly values of GSI, HSI, and K of male *Johnius coitor*

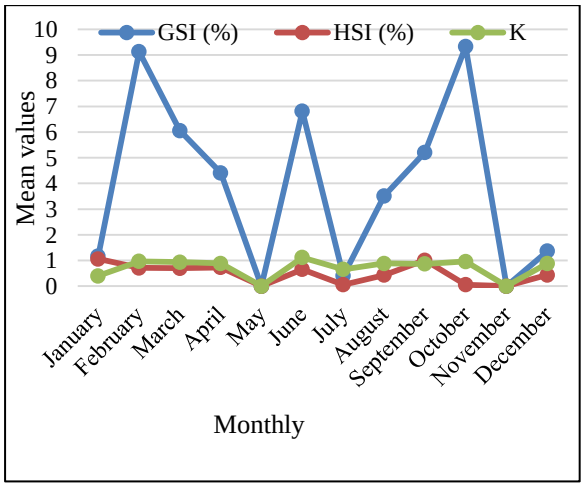

Figure.4 Monthly values of GSI, HSI, and K of female *Johnius*

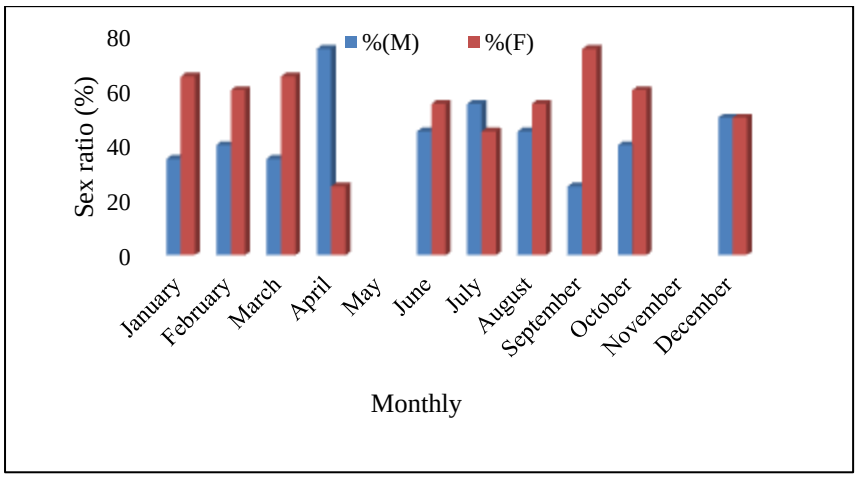

Figure. 5 Monthly sex ratio of *Johnius coitor*

Table 1.Monthly mean values of GSI%, HSI%, and K value of male *Johnius coitor*

Monthly	Total length (g)	Body weight (g)	Ovary weight (g)	Liver weight (g)	GSI (%)	HSI (%)	K value
January	18.23±0.68	26.43±17.90	0.01±0.01	0.22±0.00	0.03±0.05	1.08±0.04	0.41±0.22
February	13.50±0.83	22.13±2.37	0.43±0.25	0.20±0.05	1.91±1.06	0.90±0.17	0.91±0.14
March	17.31±1.73	43.96±11.77	0.33±0.33	0.24±0.07	0.69±0.72	1.60±0.24	0.83±0.04
April	15.33±1.42	31.44±9.28	0.60±0.48	0.19±0.08	1.83±1.30	0.64±0.25	0.85±0.07
May	-	-	-	-	-	-	-
June	15.13±0.86	27.57±4.85	0.20±0.33	0.17±0.12	0.85±1.37	0.61±0.41	0.79±0.08
July	16.05±0.99	37.95±6.59	0.17±0.49	0.04±0.06	0.36±1.02	1.10±0.11	0.91±0.06
August	16.36±1.37	41.66±11.08	0.23±0.28	0.05±0.06	0.45±0.54	0.13±0.18	0.96±0.24
September	14.36±0.37	25.80±1.94	0.00±0.00	0.01±0.01	0.00±0.00	1.00±0.22	0.87±0.05
October	17.31±1.78	43.15±13.08	0.34±0.44	0.10±0.11	0.60±0.78	0.29±0.37	0.81±0.10
November	-	-	-	-	-	-	-
December	14.07±1.58	27.97±13.58	0.39±0.22	0.15±0.07	1.48±0.89	0.71±0.36	0.95±0.18

Table 2 Monthly mean values of GSI%, HSI%, and K value of female *Johnius coitor*

Monthly	Total length (g)	Body weight (g)	Ovary weight (g)	Liver weight (g)	GSI (%)	HSI (%)	K value
January	17.08±1.54	19.62±3.56	0.28±0.64	0.01±0.01	1.17±2.63	1.06±0.04	0.40±0.10
February	15.54±1.44	37.36±11.05	3.90±3.36	0.26±0.09	9.13±6.61	0.71±0.25	0.97±0.10
March	15.45±2.21	36.01±13.70	2.02±1.11	0.22±0.09	6.06±3.18	0.70±0.33	0.94±0.11
April	15.08±0.77	30.90±6.90	1.44±0.85	0.22±0.07	4.41±2.18	0.73±0.25	0.89±0.09
May	-	-	-	-	-	-	-
June	15.36±1.17	41.40±18.31	3.24±2.91	0.34±0.29	6.81±5.47	0.66±0.43	1.12±0.42
July	16.72±1.12	29.44±10.67	0.10±0.21	0.02±0.01	0.41±0.94	0.06±0.04	0.66±0.30
August	16.76±1.03	42.35±13.27	1.50±2.03	0.25±0.30	3.51±4.84	0.43±0.20	0.89±0.07
September	17.49±1.47	46.69±11.84	2.59±2.50	0.42±0.22	5.21±5.81	1.01±0.00	0.87±0.15
October	17.58±1.26	51.72±19.88	4.70±1.99	0.46±0.35	9.33±3.79	0.06±0.05	0.96±0.36
November	-	-	-	-	-	-	-
December	14.97±1.85	0.37±0.23	0.37±0.23	0.11±0.10	1.37±0.86	0.44±0.44	0.89±0.06

Table.3. Monthly sex ratio of *Johnius coitor*

Month	Male	Female	Total	%(M)	%(F)	Sex ratio (M:F)
January	7	13	20	35	65	1:1.8
February	8	12	20	40	60	1:1.5
March	7	13	20	35	65	1:1.8
April	15	5	20	75	25	1:0.3
May	-	-	-	-	-	-
June	9	11	20	45	55	1:1.2
July	11	9	20	55	45	1:0.8
August	9	11	20	45	55	1:1.2
September	5	15	20	25	75	1:3
October	8	12	20	40	60	1:1.5
November	-	-	-	-	-	-
December	10	10	20	50	50	1:1
Total	89	111	200	44.5	55.5	1:1.2

M=male, F=female

Discussion

A total of 200 specimens of *J.coitor* (Hamilton, 1822) were studied for some biological conditions **in the** Gyaing River segment between Gadoe and Kawbein Area. The total sex ratio of male: female was 1:1.2. According to the results, the sex ratio of male: female is naturally consistent. Many researchers studied the sex ratio of *J.coitor*, (Sarkar *et al.,* 2017) studied that the sex ratio of male: female was 1:2. The present finding is in disagreement with the above authors. It may be suggested that locality, mesh size, fishing gear, and study period were different, and then it may be due to the females migrating from the estuarine to entering freshwater for breeding. Different populations inhabiting different regions show different sex ratios. (Nikolsky, 1956)

The regression coefficient values in the length-weight relationships may change with age, sex, and seasons and be related to the metabolism of each species and the environment in which they live as well (Schneider *et al.,* 2000). The previous author, Sushi K.V. *et al. (2018), who reported that the value of regression coefficient "b" was isometric b=3. In the present study, the value of the parameter "b" was 4.7844 in male and 6.1354 in female of J.coitor.* Therefore, the value of the regression coefficient "b" was a positive allometric b>3. The present finding disagrees with the previous authors. It may be suggested that there is a difference in the environment condition, the study period, and the number of studied specimens. In this present result, the value of both species 'b' was positively allomatric growth (b>3). Thus, the weight

fluctuation was higher than the length. It may be due to the majority of specimens having developed gonads. The positive allometric growth can be due to higher proficiency in feeding and better environmental conditions for survival for the species (Saikia *et al.,* 2011).

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 February for males and in December for females. The lowest GSI value was found in September for males and in July for 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.*

In the present finding, the highest HSI value of *J.coitor* was found in March for males and in January for females. The lowest HSI value occurred in August for males and in July and October for females. 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. 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. An important conclusion is that the fundamental basis for the conservation and management of fisheries resources is data from this study of some biological conditions of *Johnius coitor.* This information would be useful for conservationists and fishery biologists for sustainable fishery management and conservation in the Gyaing River.

Conclusion

Among fish, *Johnius coitor* was abundantly distributed in round year. The data on length-weight relationships is used for estimating a fish's condition factor, and these values are used for comparing the general well-being of fish. 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. This, study is suggestive to take necessary step to monitor the aquatic medium to protect the fish reproductive physiology and fish population as a whole. The findings are very important to fishery management to fix closed season and closed areas for conservation of fish population.

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REQUENCY DISTRIBUTION OF MITOTIC CELL DIVISION IN DECCAN CARP, *LABEO POTAIL* (SYKES, 1839) AND STRIPED CATFISH, *PANGASIANODON HYPOPHTHALMUS* (SAUVAGE, 1878)

Thawdar Aung[1], Thidar Aung[2], Win Win Mar[3]

Abstract

The deccan carp *Labeo potail* and striped catfish *Pangasianodon hypophthalmus* were obtained from Arrthit Private Fish Farm, Patheingyi Township to investigate the chromosomal configurations of mitotic cells division by treating with mitotic inhibitor during January to August 2022. The colchicine concentration 0.30 % and 0.50 % were injected into the musculature of both fishes depend on the fish weight ml/g. The various organs such as oral cells, gill filaments, liver, kidney and blood cells of these fishes were treated for durations 3 hrs, 4 hrs 30 mins, 5 hrs and 6 hrs in *L. potail*. However, 5 hrs, and 5 hrs 30 mins were applied in *P. hypophthalmus*. The hypotonic solution 0.56 % KCL was used to cell explosion. The highest frequency of interphase stage was observed in blood cells 93.97 % (n=187), kidney 94.21 % (n=114), 91.46 % (n=439), and prophase stage was also found in kidney 38.10 % (n=16) and blood cells 56.19 % (n=168) in both fishes. The most frequent distribution of metaphase stages was observed in the colchicine concentration 0.50 % for duration 4 hrs 30 mins with 0.56 % KCL for duration 1 hr in *L. potail* whereas 5 hrs, 5 hrs 30 mins in *P. hypophthalmus* with 0.56 % KCL for duration 1 hr. The most accelerated mitotic stage of cells such as interphase, prophase, metaphase, anaphase and telophase were observed. These results will provide not only the basic information of mitotic technique of cell division but also resolve the mitotic check point of the chromosomal configuration for other freshwater fishes.

Keywords: fishes, organs, colchicine, KCL, mitotic stages

Introduction

Labeo is a large genus having several species which are of considerable importance as an article of food. Some of the species of the *Labeo* genus are reared for ornamental purpose, some as food species, some for extracting oil and some are considered to be of medicinal value also. Among them, *Labeo potail* has good market value and high consumer preference, important fisheries and in aquaculture activities (Sarma *et al*., 2017). *Pangasianodon hypophthalmus* (Nga-tan) is one of the largest and most important inland fisheries, the Mekong River Fishery, in the world. Striped catfish is also riverine freshwater species that can be found in Ayeyarwady Basin of Myanmar (Griffiths *et al*., 2020).

Every organism generated the vital activities through the checkpoint of cellular process in different stages of cell cycle: mitosis and meiosis. Mitosis maintains the chromosome number and generates new cells for the growth and maintenance of an organism (Iwasa and Marshall, 2018). The natural resources of various kinds of the indigenous species as well as the exotic species must be characterized their phenotypic expression as well as their genotypic attributes before utilizing the species for various purposes.

The objective of this study was to investigate the chromosomal characteristics of mitotic checkpoint of cells in *Labeo potail* and *Pangasianodon hypophthalmus* (locally known as Nga-tan) from Arrthit Private Fish Farm treated with mitotic inhibitors.

[1] Department of Zoology, University of Mandalay
[2]Department of Zoology, University of Mandalay
[3] Department of Zoology, University of Mandalay

Materials and Methods

The present study was conducted at the Laboratory, Department of Zoology ,University of Mandalay.

The study period was from January to August 2022.

Forty fishes samples were collected from Arrthit Private Fish Farm which is located at 21° 59′ 50.90" N and 96° 07′ 50.23"E , Patheingyi township Mandalay, the collected fishes were reared at Laboratory, Department of Zoology, University of Mandalay (Plate 1 and 2). The fishes were fed twice a day with formulated commercial feeds. The water was changed twice a week and kept in well-aerated aquarium (Plate 2. A).

The fishes were the mean length of *Labeo potail* (n = 10) was 16.80 ± 1.09 cm, standard length 13.90 ± 3.70 cm and the body weight 10.32 ± 3.40 g. In *Pangasianodon hypophthalmus*, the mean total length of striped catfish (n =4) was 22.80 ± 1.37 cm, standard length 21.00 ± 1.19 cm and the body weight 37.90 ± 0.97 g**.**

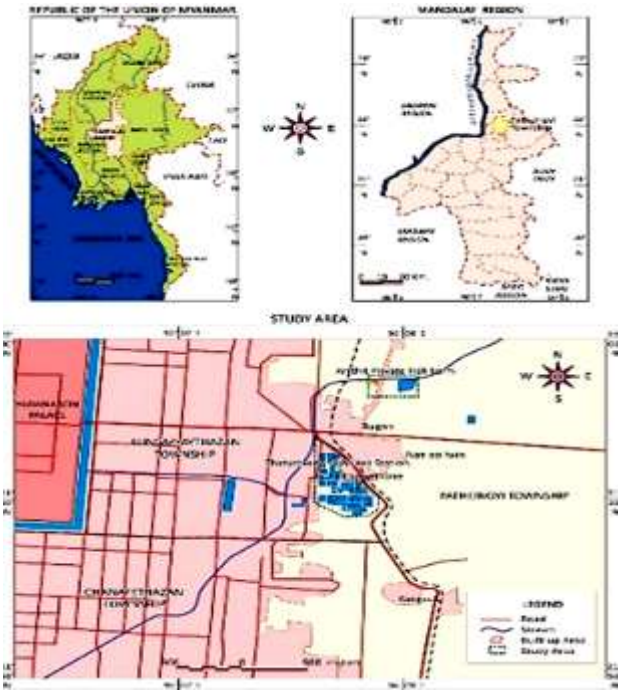


Figure.1.Study map of Arrthit Private Fish farm, Patheingyi Township, Mandalay Region (Source: UTM)

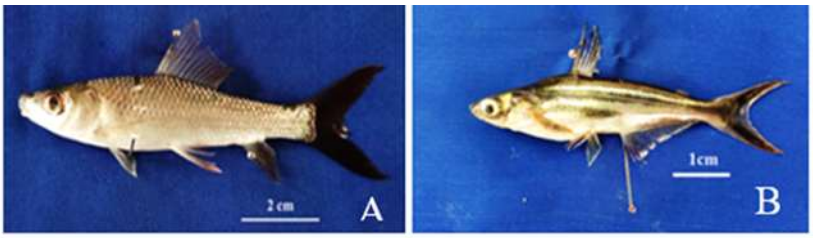


Plate 1 Lateral view of (A) *Labeo potail* and (B) *Pangasianodon hypophthalmus*

Identification of species

The identification of fish species was followed by Talwar and Jhingran (1991) and Khamees *et al*. (2013).

Injection technique

The body weight of each fish was recorded with digital kitchen scale to the nearest 0.01g. The standard length and total length were recorded to the nearest 0.1 cm by using a ruler. The concentrations of colchicine solutions 0.30 % and 0.50 % (AVI CHEM, India) were prepared and injected into the intramuscularly to fishes depending upon their weight 1ml/100 g (Plate 2.B and C).

Collection of tissues

Blood was extracted from the caudal peduncle of fish by using a syringe. The oral cells, gill,heart, liver and kidney were harvested immediately from anesthetized fish (Plate 2. D and E).

Extraction of cells

The sample tissues were incubated in 0.56 % KCL (MERCK Ltd, Munbal) for 15 mins, 20 mins, 1 hr and 1 hr 30 mins and minced with a glass rod. These samples were mixed with vortex mixer (NANOVA) by adding 3 methanol: 1 acetic acid and centrifuged (Firlabo) for

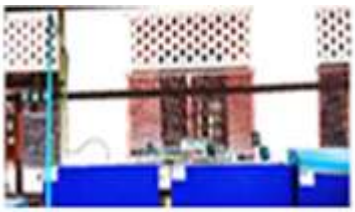
10 mins at 2000 rpm. The supernatant was discarded by using a pipette. This procedure was repeated again (Plate 2. F, G and H).

Preparation of slides

The slides were heated in the oven (Gallenhamp). One or two drops of pellets were placed onto the pre-warmed slides in a far distance and stained with undiluted Giemsa stain (AVI CHEM, India) for 10 mins and 15 mins and diluted Giemsa stain for 20 mins. The stained slides were washed under running tap water and dried at room temperature. Then, these stained slides were covered with pre-cleaned coverslips and finally coated with Canada balsam (Kanto Chemical Co., Inc, Tokyo, Japan) (Plate 2. I, J and K).

Identification of mitotic cell division

The microphotographs were recorded with a biological microscope with an attached camera (G-303P, Taiwan) (x1000). Good quality of chromosomal spreads was recorded for each slide (n = 10) and micro photographed for analysing various stages of cells in mitotic division. All recorded data was performed by Microsoft Excel 2010 (Plate 2. L).

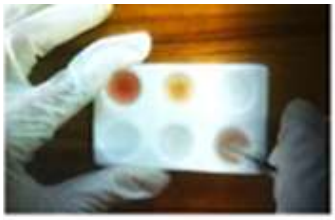


A. Fish reared in glass tank

B. Weighing the fish

C. Injection of fish

D. Extraction of blood

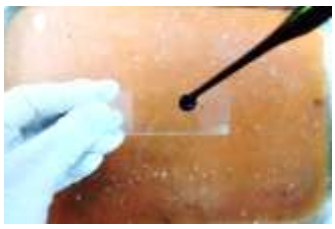


E. Mechanical dissociation of tissues

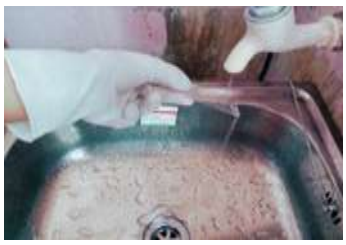
F. Homogenization of tissues

G. Centrifugation of pellets

H. Sucking the pellet from tube to drop on the slide



I. Dropping the Giemsa stain to the slide



J. Washing the slide under tap water

K. Coating with coverslip

L. Examination of prepared slides

Plate 2 Preparation of cytological process from fish tissues

Results

The concentrations of 0.30 % and 0.50 % colchicine solution were exposed to different organs such as gill filaments, oral cells, kidney, blood and liver of deccan carp and striped catfish. The optimum colchicine concentration and duration of *Labeo potail* was 0.50 % for 4 hrs 30 mins with1 hr hypotonic solution (0.56 % KCL) in gill filaments whereas 0.50 % for duration 5 hrs 30 mins with 1 hr 30 mins hypotonic solution in kidney tissues of *Pangasianodon hypophthalmus*. The good pictures of different chromosomal patterns in mitotic cell division were observed by staining the undiluted Giemsa stain for 10 mins.

Percent and frequency distribution

The different mitotic stages of cells were observed at 3 hrs treatment of colchicine concentration 0.30 % with 0.56 % KCL for duration 25 mins in blood cells, oral cells and gill filaments (Table 1). The highest percentage of interphase stage 94.22 % (n=114) were observed in the kidney cell and followed by 93.97 % (n=187) in blood cells and 81.48 % (n= 22) in oral cells for duration of 3 hrs with 25 mins in deccan carp. The most frequent mitotic stages, prophase 11.11% (n=3) and metaphase 7.41 % (n=2) were observed in oral cells. The lowest frequency of prophase stage 1.65 % (n= 2) was recorded in oral cells (Fig.2).

Colchicine concentration 0.50 % for duration 4 hrs 30 mins with 0.56 % KCL for duration 1 hr generated the interphase stage 3.33 % (n=1) in liver, 9.09 % (n=2) in kidney cells and 23.33 % (n=7) in gill filaments. In addition, the same chromosome spreads (n=2) were generated with various frequencies of prophase stages 8.33 % in oral cells, 9.09 % in kidney and 6.67 % in gill filaments expect the blood cells and liver. The highest distribution of metaphase stages was observed with different frequencies in 96.67 % (n=18) in liver, followed by 91.67 % (n= 22) in oral cells, 81.82 % (n= 18) in kidney and the lowest was found in gill filaments 70.00 % (n= 21) expect blood cells (Fig. 3).

The same frequency distribution 2.78 % (n=1) of interphase and prophase stage were observed in liver cells by rising colchicine solution for 5 hrs with the same incubation time of 0.56 % KCL. The highest percentage of metaphase stage 100.00 % (n=25) was found in gill filaments and prophase stage 11.11 % (n=2) in kidney cells. The metaphase stage was found in liver cells 94.44 % (n=34) followed by 88.89 % (n=16) in kidney cells (Fig. 4).

The highest percentage of interphase and prophase stage 61.90 % (n= 26) and 38.10 % (n= 16) in kidney cells were observed in colchicine concentration 0.50 % for duration 6 hrs with 0.56 % KCL for 1 hr. The lowest frequency of metaphase stage of chromosomes 13.33 % (n=4) was also recorded in liver cells of decan carp. Unfortunately, the distribution of metaphase stage was not found in kidney cells (Table 1 and Fig. 5). However, kidney cells operated the other mitotic stages such as anaphase and telophase in the same treatment of colchicine solution and 0.56 % KCL (Plate 3. E and F).

In *Pangasianodon hypophthalmus*, the frequency distribution of interphase stage was not observed in colchicine solution 0.50 % for duration 5 hrs in hypotonic solution 1 hr (Table 2). Prophase stage 10.34 % (n=3) was the most frequent in oral cells. Blood and kidney cells generated the prophase stage 9.09 % (n=2) and the lowest in blood cells with 4.69 % (n=3). Especially, all tissues generated the metaphase stage. The same percentage of metaphase stage was operated with 100.00 % (n=25) in liver and n= 16 in gill filaments followed by 95.31 % (n=6) in blood cells, 90.91 % (n=20) in kidney cells, 89.66 % (n=26) in oral cells (Fig.6).

When the incubation time of colchicine solution was raised up to 5 hrs 30 mins with KCL solution for 1 hr showed the distribution of interphase stage 4.17 % (n=1) and prophase stage 25.00 % (n=6) in blood cells; and 3.30 % (n=1) in gill filaments. The metaphase stage of

100.00 % (n=4) in liver, kidney (n=23) and oral cells (n=2) and 70.83 % (n=17) in blood cells were observed (Fig.7).

The incubation time of hypotonic solution 0.56 % KCL with the same treatment of colchicine duration was raised for 1 hr 30 mins resolved the highest frequency of three mitotic stages found in blood, oral and kidney cells. The highest percentage of interphase stage 91.46 % (n=439) was recorded in kidney cells and the lowest 42.27 % (n=127) in blood cells. The maximum distribution of prophase stage 56.19 % (n=168) was appeared in blood cells and the minimum 1.88 % (n=9) was found in kidney cells. The metaphase stage was recorded in four tissues expect liver cells with frequencies of 21.43 % (n=3) in oral cells, 6.66 % (n=32) in kidney cells, 2.65 % (n=4) in gill filaments and the lowest was 1.34 %(n=4) in blood cells (Fig.8).

Table 1. Percent and frequency distribution of mitotic division in *L. potail*

Duration	0.30 % Colchicine solution (3 hrs)			0.50 % Colchicine solution (4hrs 30 mins)			0.50 % Colchicine solution (5 hrs)			0.50 % Colchicine solution (6 hrs)		
	0.56 % Hypotonic solution (25 mins)			0.56 % Hypotonic solution (1 hr)			0.56 % Hypotonic solution (1 hr)			0.56% Hypotonic solution (1hr)		
Tissues/ Mitotic stages	Interphase	Prophase	Metaphase	Interphase	Prophase	Metaphase	Interphase	Prophase	Metaphase	Interphase	Prophase	Metaphase
Blood cells	93.97 % (n=187)	3.52% (n=7)	2.51 % (n=5)	0	0	0	0	0	0	0	0	0
Oral cells	81.48 % (n=22)	11.11 % (n=3)	7.41 % (n=2)	0	8.33 % (n=2)	91.67 % (n=22)	0	0	0	44.44 % (n=16)	5.56 % (n=16)	50.00 % (n=18)
Kidney	94.22 % (n=114)	1.65 % (n=2)	4.13 % (n=5)	9.09 % (n=2)	9.09 % (n=2)	81.82 % (n=18)	0	11.11 % (n=2)	88.89 % (n=16)	61.90 % (n=26)	38.10 % (n=16)	0
Liver	0	0	0	3.33 % (n=1)	0	96.67 % (n=18)	2.78 % (n=1)	2.78 % (n=1)	94.44 % (n=34)	53.34 % (n=16)	33.33 % (n=10)	13.33% (n=4)
Gill filaments	0	0	0	23.33 % (n=7)	6.67 % (n=2)	70.00 % (n=21)	0	0	100.00 % (n=25)	39.47 % (n=30)	36.84 % (n=28)	23.69 % (n=18)

Table 2. Percent and frequency distribution of mitotic division in *P. hypophthalmus*

Duration	Colchicine solution – 5 hrs						Colchicine solution – 5 hrs 30 mins					
	Hypotonic solution – 1 hr						Hypotonic solution – 1 hr 30 mins					
Tissues/ Mitotic stages	Interphase	Prophase	Metaphase	Interphase	Prophase	Metaphase	Interphase	Prophase	Metaphase	Interphase	Prophase	Metaphase
Blood cells	0	4.69 % (n=3)	95.31 % (n=6)	4.17 % (n= 1)	25.00 % (n=6)	70.83 % (n=17)	42.47 % (n=127)	56.19 % (n=168)	1.34 % (n=4)			
Oral cells	0	10.34 % (n=3)	89.66 % (n=26)	0	0	100.00 % (n=2)	64.28 % (n=9)	14.29 % (n=2)	21.43 % (n= 3)			
Kidney	0	9.09 % (n=2)	90.91% (n=20)	0	0	100.00 % (n=23)	91.46 % (n=439)	1.88 % (n=9)	6.66 % (n=32)			
Liver	0	0	100.00 % (n=25)	0	0	100.00 % (n=4)	0	0	0			
Gill filaments	0	0	100.00 % (n=16)	3.03 % (n=1)	3.03 % (n=1)	93.94 % (n=3)	89.40 % (n=135)	7.95 % (n=12)	2.65 % (n=4)			

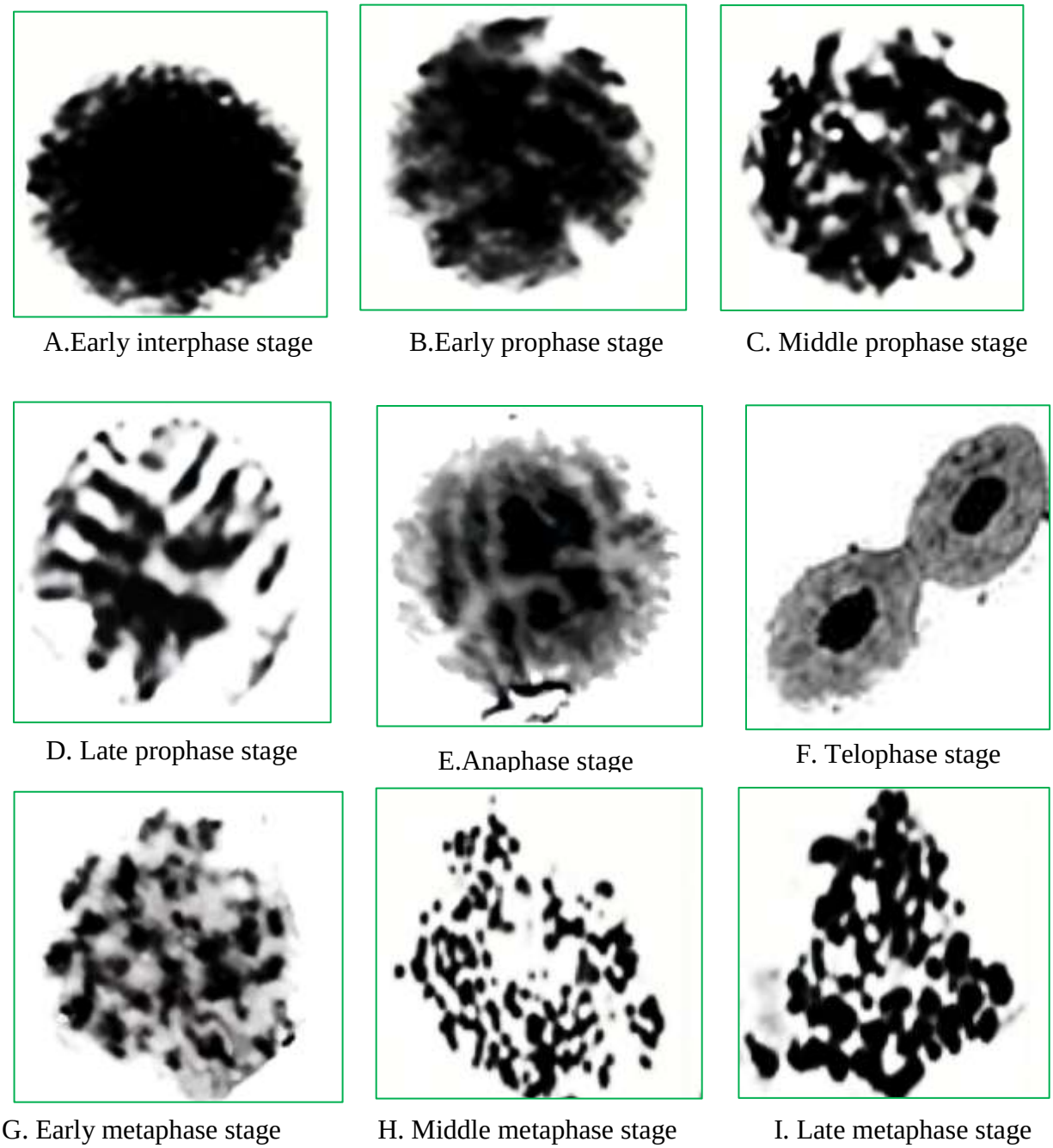


Plate 3. The arrested stages of chromosomal configurations in mitotic division (x1000)

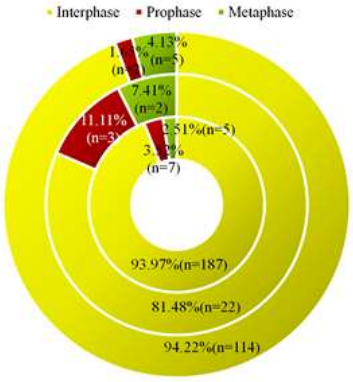


Figure.2. Effect of colchicine concentration 0.30% for duration 3 hrs with 0.56 % KCL for 25mins on the mitotic division of different tissues *Labeo potail (*1st ring- blood cells; 2nd ring- oral cells; 3rd ring- kidney)

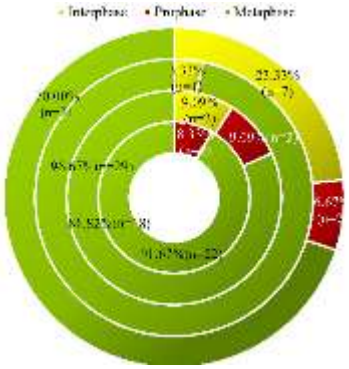


Figure.3. Effect of colchicine concentration 0.50 % for duration 4 hrs 30 mins with 0.56 % KCL for 1 hr on the mitotic division of different tissues *Labeo potail (*1st ring- oral cells; 2nd ring– kidney; 3rd ring- liver; 4th ring- gill filaments)

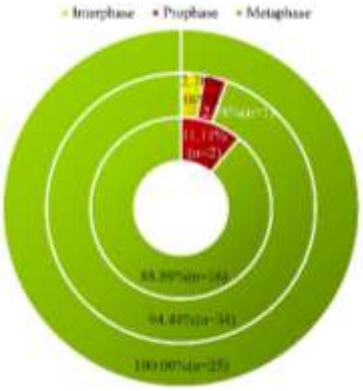


Figure.4. Effect of colchicine concentration 0.50 % for duration 5 hrs with 0.56 % KCL for 1 hr on the mitotic division of different tissues *Labeo potail (*1st ring- kidney; 2nd ring- liver; 3rd ring- gill filaments)

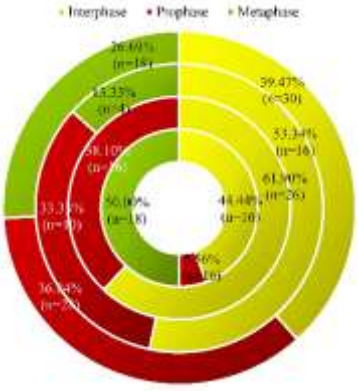


Figure.5. Effect of colchicine concentration 0.50 % for duration 6 hrs with 0.56 % KCL for 1hr on the mitotic division of different tissues *Labeo potail (*1st ring- oral cells ; 2nd ring– kidney; 3rd ring- liver; 4th ring- gill filaments)

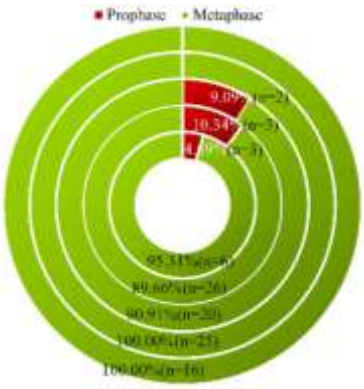


Figure.6. Effect of colchicine concentration 0.50 % for duration 5 hrs with 0.56 % KCL for 1 hr on the mitotic division of different tissues *Pangasianodon hypophthalmus* (1st ring- blood cells; 2nd ring- oral cells; 3rd ring- kidney; 4th ring- liver; 5th ring- gill filaments)

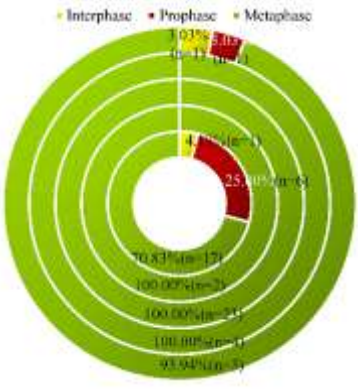


Figure.7. Effect of colchicine concentration 0.50 % for duration 5 hrs 30 mins with 0.56 % KCL for 1 hr on the mitotic division of different tissues *Pangasianodon hypophthalmus (*1st ring- blood cells; 2nd ring- oral cells; 3rd ring- kidney; 4th ring- liver; 5th ring- gill filaments)

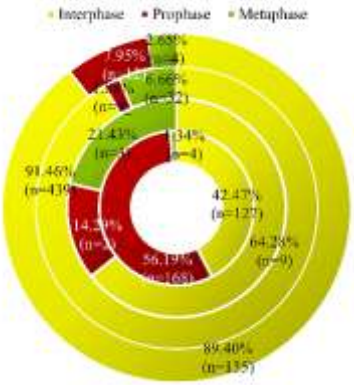


Figure.8. Effect of colchicine concentration 0.50 % for duration 5 hrs 30 mins with 0.56 % KCL for 1 hr 30 mins on the mitotic division of different tissues *Pangasianodon hypophthalmus* (1st ring-blood cells; 2nd ring- oral cells; 3rd ring- kidney; 4th ring- gill filaments)

Discussion

In the present study, the method of chromosome preparation was based on testing with different concentrations of colchicine solutions on various organs of *L. potail* and *P. hypophthalmus*. Mahfuji *et al*. (2014) described the variation of chromosomal characteristics that are largely dependent on methods of chromosome preparation, staining procedure, tissue source of the body where the dividing cells. The optimum colchicine concentration for these fishes was 0.50 %.

When the fishes were treated with colchicine concentration 0.30 % for duration of 0.56 % KCL 25 mins, the blood cells operated largely the early stage of interphase in mitotic cell division. The early interphase stage was observed in blood cells. The lower concentration with short duration of mitotic inhibitors could not be accomplished the complete chromosomal configuration. The concentration of colchicine 0.50 % for 4 hrs 30 mins with hypotonic solution at 1 hr was generated the highest frequency of late metaphase stage of chromosomes in oral cells compared with other different cells. The most frequent distribution of early interphase stage was found in gill filaments of colchicine concentration for 6 hrs and KCL for 1 hr in *L. potail.*

Therefore, the long-term treatment of colchicine duration together with the same concentration 0.50 % was used in *P. hypophthalmus*. The early metaphase stage was the maximum distribution of chromosomal stage for 5 hrs of colchicine duration and hypotonic treatment for 1 hr. Unfortunately, the same results of the early metaphase stage were also recorded by rising 30 mins duration. Therefore, the duration of colchicine solution was fixed at 5 hrs 30 mins and the incubation time of hypotonic solution was 1hr 30 mins. Then, the late metaphase and early interphase stage of chromosomal configurations were also observed in kidney cells.

The hypotonic treatment of 0.56 % KCL for 1 hr was good for explosion of nuclear membrane in kidney tissues of *Oreochromis* spp. (Win Win Mar and Thant Zin, 2020). According to the results of this study, longer treatment of 0.56 % KCL solution for 1 hr generates enough explosion of nuclear envelope and cytoplasmic membrane observed in all tissues and blood cells in *L. potail*.

However, the same technique did not support the explosion of cells from all tissues in *P. hypophthalmus* except the blood and oral cells. When the exposure time was raised up to 1 hr 30 mins, the check point of mitotic division cells was observed. Another important factor for cytogenetic analysis is Carnoyl's fixative (3 methanol : 1 acetic acid). In this study, Carnoyl's fixative was used to treat the extracted cells for 10 mins duration. The good shape of chromosomal configuration of *L. potail* and *P. hypophthalmus* were observed that is leading to count and differentiate the chromosomal structure in detail.

Therefore, the chromosomal characteristics on studied fishes could be generated and standardized by using the optimal checkpoint of mitotic cell division for their population. These

recorded data are highly recommended for further investigation on variations in cytogenetic research and designate the karyotype formula of respective species.

Conclusion

The effect of colchicine concentrations 0.50 % was better than 0.30 % with various durations 4 hrs 30 mins, 5 hrs and 5 hrs 30 mins in *Labeo potail* and *Pangasianodon hypophthalmus.* The different tissues generated the different stages, interphase, prophase, metaphase, anaphase and telophase. The highest frequency of interphase stage was observed in blood cells 93.97 % (n= 187) in *L. potail*, kidney 91.46 % (n=439) in *P. hypophthalmus* and prophase stage was also found in kidney 38.10 % (n= 16) in *L. potail* and blood cells 56.19 %(n=168) in *P. hypophthalmus*. The oral cells, kidney, liver and gill filaments of *L. potail* generated the optimal check point of metaphase stages by treating with 0.50 % colchicine concentration for 4 hr 30 mins with 0.56 % KCL for duration 1 hr, expect the blood cells, however oral cells could not be resolved in colchicine concentration 0.50 % for 5 hrs. The metaphase checkpoints of mitotic cells were more observed in all tissues and cells of *P. hypophthalmus* by treating with 0.50 % colchicine concentration for duration 5 hrs and 5 hrs 30 mins with 0.56 % KCl for duration 1 hr.

Acknowledgements

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DETERMINATION OF THE AGE OF *NOTOPTERUS NOTOPTERUS* (PALLAS, 1769) WITH REFERENCE TO THE STRUCTURAL CONFIGURATION IN SOME CALCIFIED STRUCTURES AND SCALES

Thidar Aye[1] and Win Win Mar[2]

Abstract

The growth performance and age determination of *Notopterus notopterus* from Northern Part of Meiktila Lake were investigated during December 2019 to August 2020 by using back-calculated length at age and von Bertalanffy growth curve. The total length, standard length and weight of fishes were recorded. Five different calcified structures viz. scale, otolith, vertebrae, operculum and cleithrum of fishes were applied. Annuli in these calcified structures reading revealed 4 yrs, 5 yrs and 6 yrs in which operculum reading was more precise for age determination. The relevant combination of growth constants to estimating lengths from age interpretation to the nearest asymptotic length revealed $L_{\infty=}$ 51.86, $k = 0.13$ with the growth performance index $\emptyset' = 2.542$ in female and $L_{\infty=}$ 56.36, $k = 0.12$ with $\emptyset' = 2.581$ in male. The present results will provide the precise interpretation of aging structure of local fishes as well as to give the sense of their biological aspect in aquaculture.

Key words: bronze feather fish, ages, calcified structures, growth, length

Introduction

The "Bronze feather back fish", *Notopterus notopterus* is a common fresh water fish belongs to family Notopteridae, is widely distributed in Southeast Asia. It is known as Nga-pe in Myanmar. The years of life span are 9 - 12 years, breed in stagnant or running water and delay in growth performance (Froese and Pauly, 2019).

The age determination on specific species is relatively quite different even though under the same family. The age data of a stock indicates the healthy fish stock. Age can be determined by anatomical method as well as length-frequency analysis (Morales-Nin,1992). The mechanism of annulus formation for determination of fish age is supposed by a combination of factors including temperature, feeding habits, and the reproductive cycle (Huo *et al.*, 2012).

Meiktila is one of the tropical zones where dams, lake and ponds are the main source for aquaculture research as well as small economic income for rural life process. In which, the bronze feather back is one of the commercial small income fish and readily available from local fishermen.

The purpose of this research is whether to accept null hypothesis or not:

H_0: the precision of age-length increment in *N. notopterus* could be evaluated by using all calcified structures in northern part of meiktila lake and

H_1: the precision of age-length increment in *N. notopterus* could not be evaluated by using all calcified structures in northern part of meiktila lake.

Materials and Methods

Study site

Northern part of Meiktila Lake lies between geographical co-ordinates of 20˚53′ 55.87″ N Latitudes and 95˚51′ 32.02″ E Longitudes (Plate 1).

[1] Department of Zoology, University of Mandalay
[2] Department of Zoology, University of Mandalay

Study period

The present study was commenced from December 2019 to August 2020.

Specimen collection

Fifty-three fresh samples of *Notopterus notopterus* were collected from Northern part of Meiktila Lake. These samples were weight in nearest 0.01 g (Digital Kitchen Scale) and measure their standard and total length in nearest 0.01 cm (Digital Caliper). They were divided into 10 cm equally in range according to their total length.

Plate 1. Map of Northern part of Meiktila Lake. Source: Gogle 2020

Identification of species

Identification of the species was followed those of Das *et al.*, (2012); Froese and Pauly (2019) and Muniya *et al*., (2019).

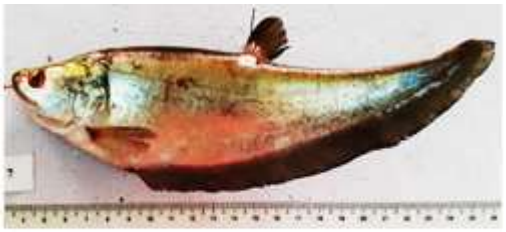


Plate 2. The bronze feather back *Notopoterus notopterus*

Age determination on calcified structures

Five calcified structures like scales, otoliths, opercula, cleithra and vertebrae were extracted from each fish. The anterior margin of the scale was checked under the light microscope (x40) and photographed to count the annuli. Left operculum and left cleithrum were photographed by hand lens (x12) to interpret the rings. Otoliths and the rings at the anterior part of the vertebrae were checked under the stereomicroscope (x40). The clear true annulus, widely spaced circuli, or one opaque and one transparent ring zone was assigned as one annulus.

Back-calculated length at age

A formula reflecting a scale- proportional hypothesis (SPH) credited to Hile (1941).

$$Li = -\left(a/b\right) + \left[Lc + \left(a/b\right)\right]\left(Si/Sc\right)$$

Where Li = back-calculated body length at age,

Lc = fish body length at capture,

Sc = Mean scale total length,

Si = mean scale length at annulus i,

a = intercept from the regression of mean scale length on body length, and

b = slope from the regression of mean scale length on body length.

The von-Bertalanffy growth curve

The relationship between fish age and calcified structures was estimated by using von-Bertalanffy growth function (Ricker, 1975) to age-at-length data using

$$Lt = L_\infty \left\{1 - e^{[-K(t-t_0)]}\right\}$$

Where Lt = expected or mean length at time t,

L_∞ = maximum asymptotic mean length,

K = measure of the exponential rate at which curve approaches,

L_∞ or a constant equal to $\frac{1}{3}$ of catabolic coefficient,

e = base of the natural logarithm (exponential),

$t_0 =$ the theoretical age at which Lt would be zero and *t* = age of the fish.

Statistical analysis

The growth performance for length-at-age and von Bertalanffy's growth formula were generated with FISAT-II (version 1.2.2, FAO).

Results

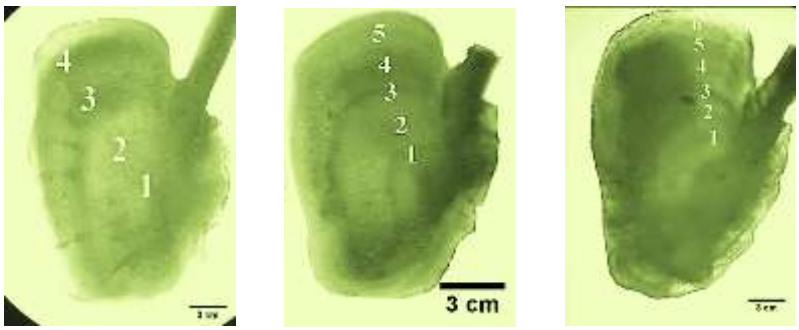
A sample of studies 53 *Notopterus notopterus* (Male = 26, Female = 27) from northern part of Meiktila Lake were taken into consideration during this research.

In female, the mean value of length and weight were 203.02 ± 28.11 mm with 54.15 ± 19.45 g at 4 yrs, 238.71 ± 23.80 mm with 101.11 ± 30.81 g at 5 yrs and 295.66 ± 34.62 mm with 204.20 ± 89.19 g at 6 yrs.

In male, the mean value of length and weight were 213.49 ± 35.57 mm with 82.39 ± 39.99 g at 4 yrs, 219.21 ± 30.40 mm with 84.22 ± 31.64 g at 5 yrs and 307.72 ± 0.00 mm with 233.30 ± 0.00 g at 6 yrs.

Age determination on calcified structures

Age for each growth mark of fish was determined by reading annulus on hard calcified structures. Annuli appeared as alternatively one broad opaque and one narrow translucent zone. The ring marks on scales were identified by the gap or broad ring between the growth annulus around the focus (Plate 3, 4, 5, 6 and 7).



A B C

Plate 3 Annuli marks in the otolith of *Notopterus notopterus*
(A) Four years, (B) Five years and (C) Six years (x40)

A B C

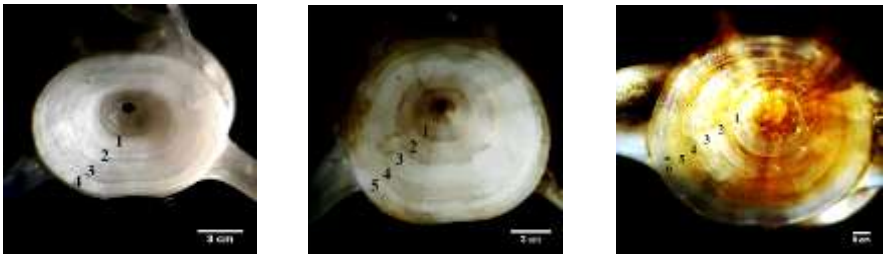
Plate 4 Annuli marks in the scale of *Notopterus notopterus* (A) Four years, (B) Five years and (C) Six years (x40)

A B C

Plate 5 Annuli marks in the cleithrum of *Notopterus notopterus* (A) Four years, (B) Five years and (C) Six years (x12)

A B C

Plate 6 Annuli marks in the operculum of *Notopterus notopterus* (A) Four years, (B) Five years and (C) Six years (x12)



A B C

Plate 7 Annuli marks in vertebrae of *Notopterus notopterus*
(A) Four years (B) Five years and (C) Six years (x40)

Age-length relationship

Length-at-age on reading calcified structures were shown by von Bertalanffy Growth curve. The maximum asymptotic length of the feather back was L_{∞} = 51.86 cm with K = 0.13 in female and the male with at L_{∞} = 56.36 cm with K = 0.12 revealed by Ford-Wolford plot (Fig.1 and Fig. 2).

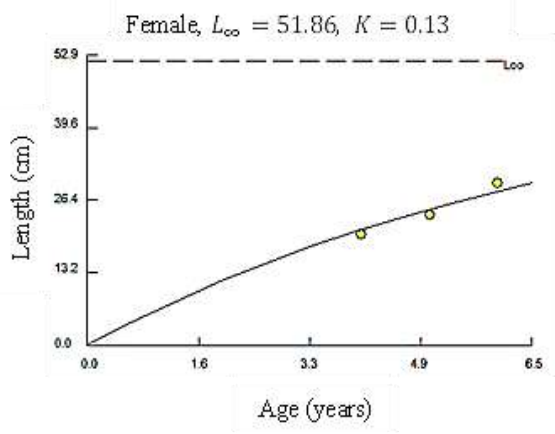


Figure.1. Age-length relationship of female male *N. notopterus*

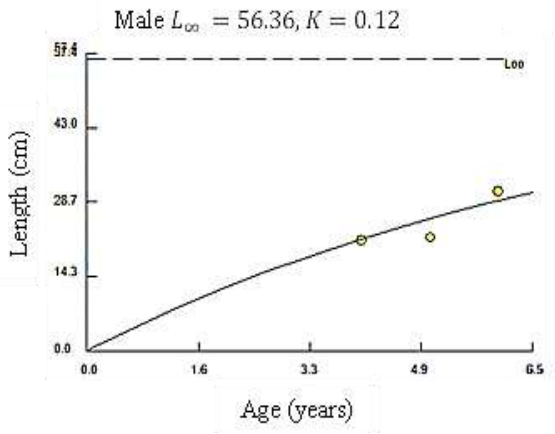


Figure.2. Age-length relationship of *N. notopterus*

The increment of growth pattern of classified structures for aging interpretation were quite different in operculum and cleithrum in both sexes. Their growth pattern showed the exponentially increased in female than male with delay growth found at 5 yrs and then increased at 6 yrs. No largely different increment in the scale, vertebrae and otolith length observed in both female and male bronze fishes (Fig. 3).

Back-calculated length

The real growth development of male and female bronze fish was determined at each age by the back-calculated length compared with the observed total length (TL) (Table 1 and Fig.4).

Growth performance index ($\emptyset'$)

The average growth performance index of female bronze feather back was $\emptyset' = 2.542$ and male with $\emptyset' = 2.581$ revealed by Ford-Wolford plot. In female, the growth performance index for vertebrae reading $\emptyset' = 2.552$ was the highest value and the lowest $\emptyset' = 2.480$ in scale reading. In male, growth performance index $\emptyset' = 2.786$ was observed in otolith reading and the lowest $\emptyset' = 2.355$ in scale reading (Table 2).

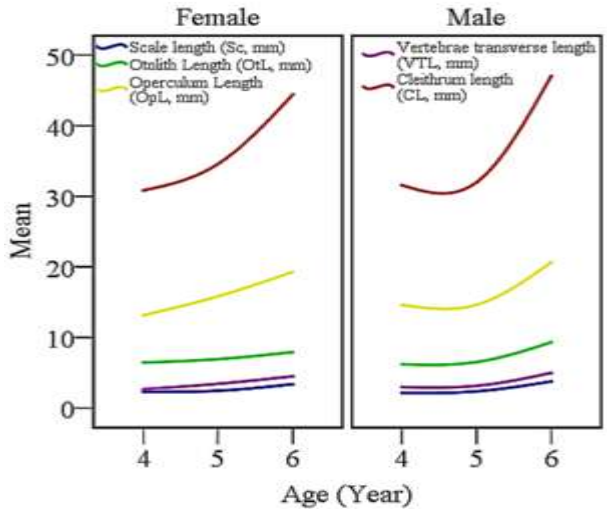


Figure. 3. Relationship of calcified structures with age of male and female *N. notopterus*

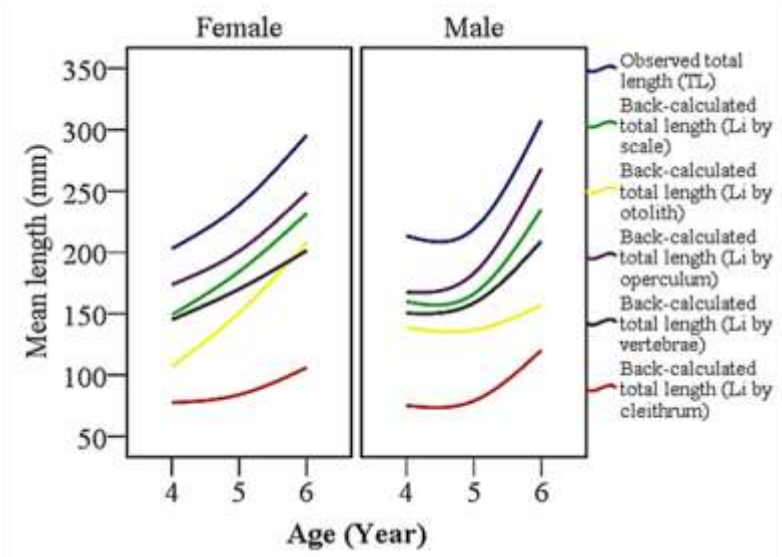


Figure. 4. Back-calculated length at age in male and female *N. notopterus*

Ford-Wolford plot of length at success at ages

The estimated parameters of the von Bertalanffy Growth formula by different calcified structures reading method in both males and females' fishes was shown in Table 2.

The growth increment of theoretical length (t_0) from both sexes revealed the negative t_0 predicted the similar growth pattern with their expected length (L_t) at each age (Table 3).

Table 1 Comparison of total length and back-calculated length of *N. notopterus* from Northern Part of Meiktila Lake. (n = number of specimens)

Items	Age (years)	Female	Age (years)	Male
Observed total length (TL, mm)	4 yrs (n = 2)	203.02 ± 28.11	4 yrs (n = 7)	213.49 ± 35.57
Back-calculated total length (Li, mm)		167.33 ± 22.16		152.47 ± 29.39
Combined Back-calculated total length (Com;Li, mm)		149.11 ± 23.82		159.73 ± 28.80
Observed total length (TL, mm)	5 yrs (n = 21)	238.71 ± 23.79	5 yrs (n = 18)	219.21 ± 30.39
Back-calculated total length (Li, mm)		199.55 ± 19.63		159.44 ± 22.75
Combined Back-calculated total length (Com;Li, mm)		184.52 ± 20.68		166.30 ± 22.53
Observed total length (TL, mm)	6 yrs (n = 4)	295.66 ± 34.62	6 yrs (n = 1)	307.72 ± 0.00
Back-calculated total length (Li, mm)		245.71 ± 31.75		228.67 ± 0.00
Combined Back-calculated total length (Com;Li, mm)		231.87 ± 33.18		235.00 ± 0.00

Table 2 Growth performance index ($\emptyset'$) of calcified structures in female and male *N. notopterus* from Northern Part of Meiktila Lake

Age characters	Female $\emptyset' = \boldsymbol{Log\ K + 2\ Log\ L_\infty}$	Male $\emptyset' = \boldsymbol{Log\ K + 2\ Log\ L_\infty}$
Average	$\emptyset'$ = Log 0.13 + 2 Log 51.80 = 2.542	$\emptyset'$ = Log 0.12 + 2 Log 56.36 = 2.581
Scale	$\emptyset'$ = Log 0.38 + 2 Log 28.20 = 2.480	$\emptyset'$ = Log 0.26 + 2 Log 29.51 = 2.355
Otolith	$\emptyset'$ = Log 0.34 + 2 Log 30.32 = 2.495	$\emptyset'$ = Log 1.27 + 2 Log 21.93 = 2.786
Operculum	$\emptyset'$= Log 0.24 + 2 Log 35.69 = 2.485	$\emptyset'$= Log 0.17 + 2 Log 41.17 = 2.460
Cleithrum	$\emptyset'$ = Log 0.21 + 2 Log 39.11 = 2.507	$\emptyset'$ = Log 0.26 + 2 Log 32.75 = 2.445
Vertebrae	$\emptyset'$ = Log 0.41 + 2 Log 29.48 = 2.552	$\emptyset'$ = Log 1.19 + 2 Log 22.20 = 2.768

L_∞ = maximum asymptotic mean length and K = measure of the exponential rate at which curve approaches

Table 3 Growth increment of aging length at designated time point of *Notopterus notopterus* from Northern Part of Meiktila Lake

Age (Year)	Female		Age (Year)	Male	
	Theoretical length -t_0	Expected length -$L_{(t)}$		Theoretical length -t_0	Expected length -$L_{(t)}$
4 yrs (N = 2)	- 3.84	33.13	4 yrs (N = 7)	- 4.00	34.78
5 yrs (N = 21)	- 4.77	37.30	5 yrs (N = 18)	- 4.13	37.52
6 yrs (N = 4)	- 6.57	41.73	6 yrs (N = 1)	- 6.58	43.90

Discussion

Utilizing the alien freshwater fishes for age interpretation relation to their growth is the base line information in fisheries biology and resource management for tropical region. Accurate determination of fish age plays a main role in understanding of growth characteristics of specific species.

Comparison of age estimation from different calcified structures on bronze feather back *Notopterus notopterus* has been proved to assign the age of fish in northern part of Meiktila Lake. Exo-skeletal elements for scale, operculum and cleithrum, and endo-skeletal for vertebrae, and bony structure of otolith were used to determine the age of fish by reading a band or a ring pattern assigned a year of fish.

The characteristics of annulus pattern on various calcified structures revealed three groups of age composition such as 4 yrs, 5 yrs and 6 yrs. The dominant age was observed at 5 yrs in pooled fish. The ring formation on each structure expressed alternately opaque and translucent as assigned as one year.

Aging pattern on growth development of fish depends on their growth histories of fish through the investigation on back-calculated method. The back calculation is based on the assumption that the growth of fish proportional to the growth of its bony structures (Tarkan *et al.*, 2006).

In this study, the mean calculated total length was higher than the back-calculated length in both sexes. The interpretation of calcified structures has shown that the variation of growth increment patterns was observed in designated age of fish group. However, the expected length (L_t) of designated age of fish indicated that not largely different in growth increment as well as their negative growth increment of theoretical values (t_0) observed in both sexes based on the interpretation of von Bertalanffy growth function.

Furthermore, the value of growth performance index ($\emptyset'$) was nearly similar value of calcified structures in female, however, a little variation was observed in male fish. In reality, the recorded growth performance index ($\emptyset'$) has shown the negative allometric growth pattern due to the growth equation in length and for the equivalent in weight von Bertalanffy used the exponent b = 3 (Bertalanffy, 1957). The present finding of growth performance index ($\emptyset'$) of fish was consistent with Pauly and Munro (1984) reported that the value of ($\emptyset'$) represents and quantifies the energetics of a given habitat or niche because ($\emptyset'$) is directly related to growth performance and metabolism and food consumption.

Above all their aging development, the growth histories of feather back *Notopterus notopterus* from northern part of meiktila lake could be assigned to the nearest growth development pattern of length by operculum reading method. Therefore, to conclude the present research was confirmed that the precision of age-length increment *N. notopterus* could not be evaluated by using all calcified structures in northern part of meiktila lake.

Conclusion

Determination of age by operculum reading method was the more precise for aging structure of bronze feather back. Three composition of age groups such as 4 yrs, 5 yrs and 6 yrs were observed in the collected fishes. The maximum values of asymptotic fish length-at-age by von Bertalanffy Growth function were reliable to acceptance the age of fish length by operculum reading method. The back-calculation of fish length-at-age was largely concerned with the operculum reading method as well as the growth performance index ($\emptyset'$). The present research was confirmed that the acceptance of alternative hypothesis H_1: The precision of age-length increment *N. notopterus* could not be evaluated by using all calcified structures in northern part of meiktila lake, and rejected the null hypothesis H_0: The precision of age-length increment *N. notopterus* could be evaluated by using all calcified structures in northern part of meiktila lake.

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CHROMOSOMAL INVESTIGATIONS ON *CYPRINUS CARPIO* LINNAEUS, 1758 AND *LABEO ROHITA* (HAMILTON, 1822) FROM MONDAING DAM, MEIKTILA TOWNSHIP, MANDALAY REGION

Me Me Thein[1] and Thant Zin[2]

Abstract

The present research was conducted to study chromosomal variations and karyogram of *Cyprinus carpio* and *Labeo rohita* from Mondaing Dam, Meiktila Township, Mandalay Region. The study period lasted from July 2022 to January 2023. For chromosome preparation, 0.5 ml of blood sample was extracted from the caudal vasculature of selected specimens and mixed with 0.5% colchicine 0.5 ml and 100% sodium chloride (hypotonic solution) 1 ml. Then, tested for different durations of 1 hour 30 minutes, 1 hour 45 minutes, 2 hours, 2 hours 15 minutes, 2 hours 30 minutes and 2 hours 45 minutes and stained with undiluted Giemsa for 15 minutes. Finally, the two slides of the treated cell suspensions were fixed in two or three drops of Carnoy's fixative solution. The optimum metaphase spreads were observed at 2 hours and 15 minutes in *C. carpio* and 2 hours and 30 minutes and 2 hours and 45 minutes in *L. rohita.* The karyotype results of two fish species indicated that the diploid number of *C. carpio* was 2n = 100 with 28 metacentric (m), 38 submetacentric (sm), 12 acrocentric (a) and 22 telocentric (te) chromosomes with the number of fundamental arms was 166. The diploid number of *L. rohita* was 2n = 50 with 16 metacentric (m), 14 submetacentric (sm), 12 acrocentric (a) and 8 telocentric (te) chromosomes with the number of fundamental arms was 82.

Keywords: chromosomes, colchicine, hypotonic solution, karyotype, fundamental arms

Introduction

Myanmar is commonly regarded as a carp country, with carps accounting for 85% of the nation's overall aquaculture production (Fishery Statistics of Myanmar, 2009-2010). The common carp (*Cyprinus carpio*, Linnaeus, 1758), locally known as (shwe war Nga gyin) is a member of the family Cyprinidae and the order Cypriniformes. It is classified into seven subfamilies, 220 genera, and around 20,000 recognized species (Howes, 1991).

A prominent member of the Cyprinidae family, *Labeo rohita (Hamiltion 1822),* locally known as (Nga myint chin) is prevalent in the natural river systems of Bangladesh, India, Pakistan, and Myanmar (Talwar and Jhingran 1991).

The study of chromosome shape, structure, disease, function, and behavior is known as cytogenetics. Chromosomes are examined during mitotic or meiotic metaphase, but some studies such as fluorescent in situ hybridization (FISH) techniques, may study interphase cells (Lawce *et al*., 2017). Chromosomal studies in fishes are restricted to about 10 % of the total fishes all over the world (Sahoo *et al.*, 2007)

Understanding of the fundamental information on cytogenetics can be used to the development of commercially economic species in the future. The investigations on the karyotypes help to study the genetic construction of aquatic animal species in their respective habitats, thus it can decide what species are relationship to each other in a correct manner. This may aid to easy the hybridization between them in the future for strain advancement (Sofy *et al*. 2008).

There are many species of fishes in Myanmar because of the abundance of rivers, lakes, ponds, streams, and dams. The study on the karyotype of fish is very restricted compared to other area of researches. So, the karyotypes of fish species still need to be studied. The study of karyotypes helps to understand the karyomorphological

[1] Department of Zoology, Meiktila University
[2] Curriculum Central Unit, Education Research, Planning and Training, Yangon

variations of fishes, to identify the nature of chromosomes and to identify species accurately. For these purposes, the present study was conducted for chromosomal investigations on *Cyprinus carpio* and *Labeo rohita* fishes from Mondaing Dam, Meiktila Township, Mandalay Region in detail with the following objectives such as to investigate the chromosomal variations in *Cyprinus carpio* and *Labeo rohita*, to examine the karyogram of these two species.

Materials and Methods

Study area for specimen collection

Fish specimen collection was conducted at Mondaing Dam situating approximately 16 km on the west of Meiktila, Meiktila Township, Meiktila District, Mandalay Region, Myanmar. It located between North Latitude 20º 48′ 0″ N and 20º 50′ 15″ N and between East Longitude 95º 41′ 15″ E and 95º 45′ 0″ E. (Fig-1).

Study period

The study lasted from July 2022 to January 2023.

Sample collection

Ten specimens each of *Cyprinus carpio* and *Labeo rohita* were collected from Mondaing Dam from local fishermen and transported to the laboratory of Zoology Department, Meiktila University and kept in a well-aerated aquarium before study.

Identification of species

The collected fish specimens were identified according to Talwar and Jhingram (1991) and Froese and Pauly (2022).

Chromosomes preparation and analyses

For chromosome preparation, 0.5 ml of blood sample was extracted from the caudal vasculature of selected specimens and put into a blood collecting tube. Blood collecting tube was shaken for about 5 minutes to prevent blood clotting. 0.5 ml of 0.5% colchicine was added to blood sample collecting tube. One ml of 100% hypotonic solution (sodium chloride) was added to colchicine treated blood sample and was shaken for 5 minutes. Blood sample treated with colchicine 0.5 ml and sodium chloride 1 ml were exposed to different durations of 1 hour 30 minutes, 1 hour 45 minutes, 2 hours, 2 hours 15 minutes, 2 hours 30 minutes and 2 hours 45 minutes respectively. Supernatant was not removed. Finally, the treated cell suspensions were fixed in two or three drops of Carnoy's fixative solution. One or two drops of treated blood sample dropped on to glass slides, made a thin blood smear and left 15 minutes to dry. Then, the slides were stained undiluted Giemsa for 15 minutes and then rinsed in tap water. When the slides dried, Canada balsam was added and covered with cover slip. Chromosomes preparation was conducted according to Arsham *et al.* 2017. Chromosome metaphase spreads were examined under a microscope (Primostar, equipped with Axiocam ERc 5 s digital camera) with an oil immersion lens at 1000 magnification. The number of chromosomes were determined. Karyotyping was conducted according to Levan *et al.,* (1964). The homologous pairs were arranged based on the centromere position.

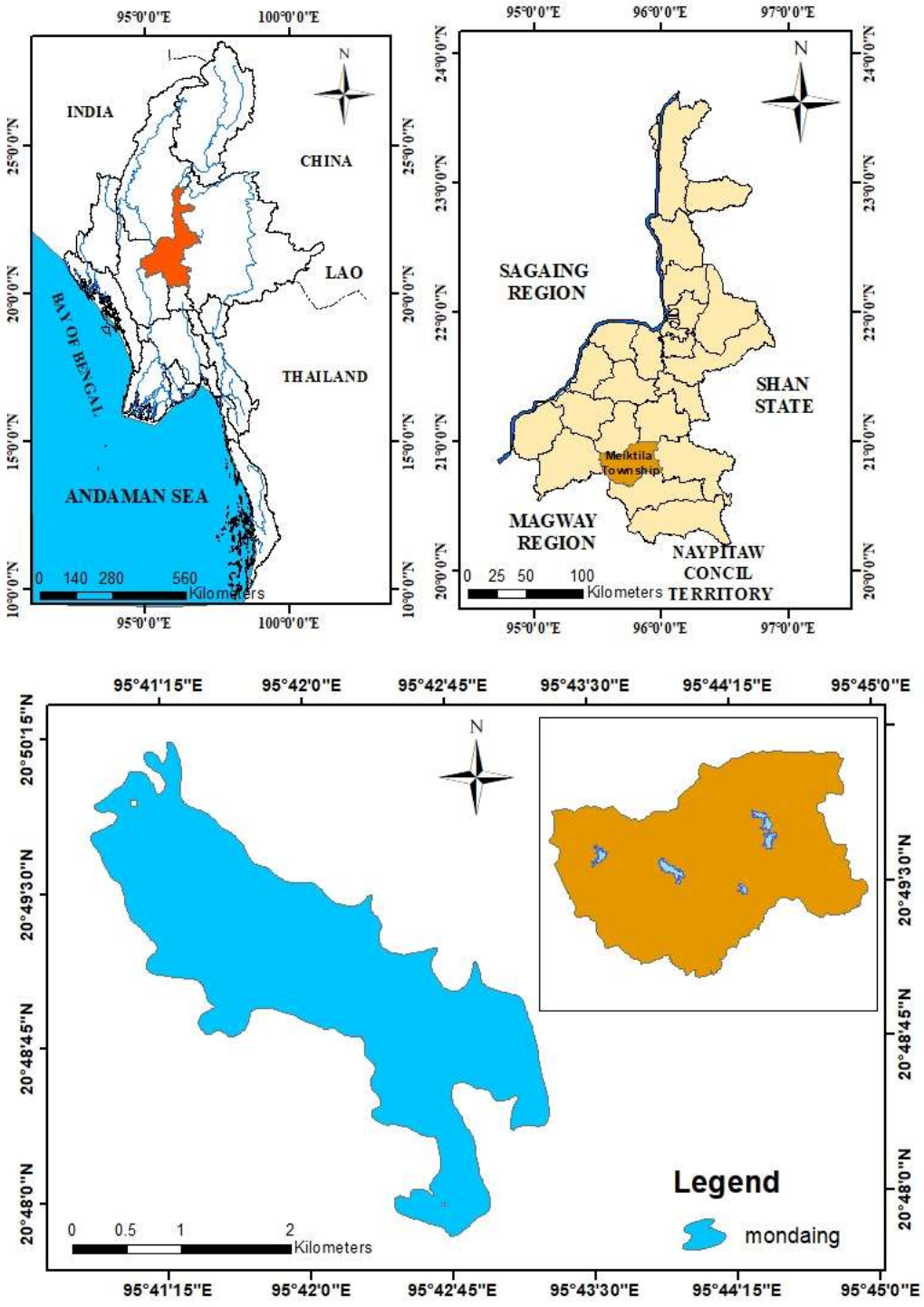


Figure. 1. Location map showing Mondaing Dam, Meiktila Township, Mandalay Region

Results

Morphological characteristics of studied fish species

Cyprinus carpio

Common name - Common carp

Vernacular name - Shwe War Nga Gyin

Body is stout, slightly compressed. Head is moderate, triangular; snout obtusely rounded. Mouth is small, oblique and protrusible; lips are thick and fleshy. Barbels are two pairs. Colour is usually olivaceous, with silvery or golden sides. Fins are yellowish, reddish, or golden. *Cyprinus carpio* is omnivorous and mostly bottom feeder (Plate 1A).

Labeo rohita

Common name - Rohu

Vernacular name - Nga myit chin or Nga gyi myet san ni

D = III 14, C = 22-24, A =I 7, Pc =17, Pv =9, LLS =42, LLS/A =7, LLS/B =6

Body is moderately elongate and devoid lateral lobe. Eyes are large. Mouth is small and inferior; lips are thick and fringed. Barbels are a pair of small maxillary barbels concealed in lateral groove. Color is bluish along back, with a reddish mark on each scale during breeding season; eyes are reddish. Fins are greyish or dark; pectoral fins are dusky. Rohu is a bottom feeder (Plate 1B).

(A) *Cyprinus carpio* (B) *Labeo rohita*

Plate 1. Studied fish species collected from from Mondaing Dam

Chromosome counts and karyotype

In the chromosome counts of *Cyprinus carpio*, the diploid number of 50 chromosomes with 24.3% (f = 17), 56 chromosomes with 12.9% (f = 9), 78 chromosomes with 1.4 % (f = 1), 88 chromosomes with 4.3 % (f = 3), 98 chromosomes with 18.6 % (f = 13) and 100 chromosomes with 38.6 % (f = 27) were observed. The karyotype formula was m = 28, sm = 38, a = 12, te = 22 with number of fundamental arms (NF) 166. As a result, the optimum metaphase spreads were observed at 2 hours and 15 minutes (Table 1 and 2; Figure 2 & Plate 2).

In the chromosome counts of *Labeo rohita*, the diploid number of 20 chromosomes with 8.6% (f = 6), 24 chromosomes with 4.3% (f =3), 25 chromosomes with 21.4% (f = 15), 26 chromosomes with 5.7% (f = 4), 28 chromosomes with 2.9% (f = 2), 38 chromosomes with 2.2% (f =2), 48 chromosomes with 8.6% (f =6), 50 chromosomes with 44.3% (f = 31) and 54 chromosomes with 1.4% (f =1) were found. The karyotype formula was m = 16, sm = 14, a = 12, te = 8 with number of fundamental arms (NF) 82. In consequence, the optimum metaphase spreads were observed at 2 hours and 30 minutes and 2 hours and 45 minutes (Table 3 and 4; Figure 3 & Plate 3).

Table 1. Frequency and percentage of different chromosome counts in *Cyprinus carpio*

Number of Chromosome counts	Frequency (f)	Percent	Cumulative percent
50	17	24.3	24.3
56	9	12.9	37.1
78	1	1.4	38.6
88	3	4.3	42.9
98	13	18.6	61.4
100	27	38.6	100.0
Total	70	100.0	

Table 2. Karyotype of *Cyprinus carpio*

Diploid chromosome number (2n)	Karyotype formula				Number of fundamental arms (NF)
	m	sm	a	te	
100	28	38	12	22	166

m = metacentric, sm = submetacentric, a = acrocentric, te = telocentric

Table 3. Frequency and percentage of different chromosome counts in *Labeo rohita*

Number of Chromosome counts	Frequency (f)	Percent	Cumulative Percent
20	6	8.6	8.6
24	3	4.3	12.9
25	15	21.4	34.3
26	4	5.7	40.0
28	2	2.9	42.9
38	2	2.9	45.7
48	6	8.6	54.3
50	31	44.3	98.6
54	1	1.4	100.0
Total	70	100.0	

Table 4. Karyotype of *Labeo rohita*

Diploid chromosome number (2n)	Karyotype formula				Number of arms (NF)
	m	sm	a	te	
50	16	14	12	8	82

m = metacentric, sm = submetacentric, a = acrocentric, te = telocentric

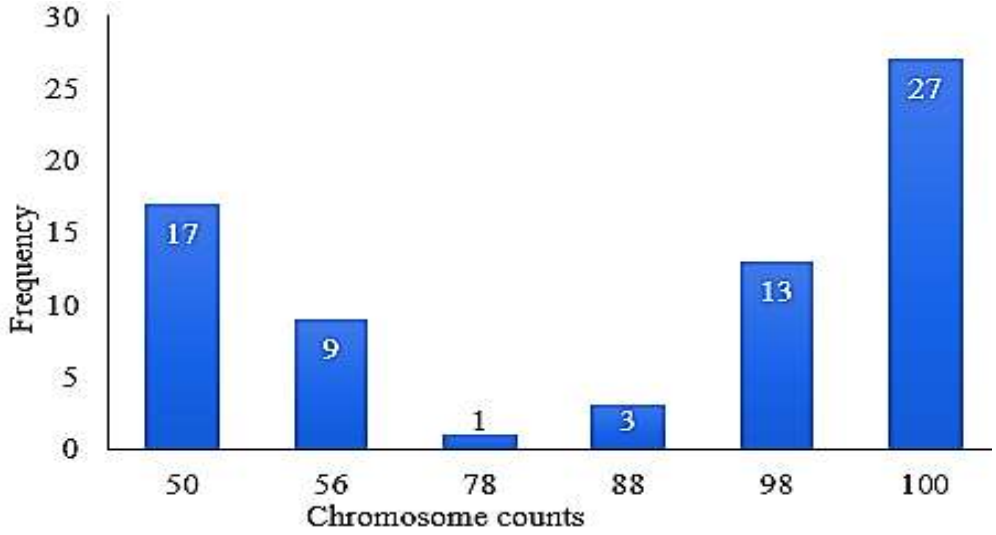


Figure. 2. Frequency of different chromosome counts in *Cyprinus carpio*

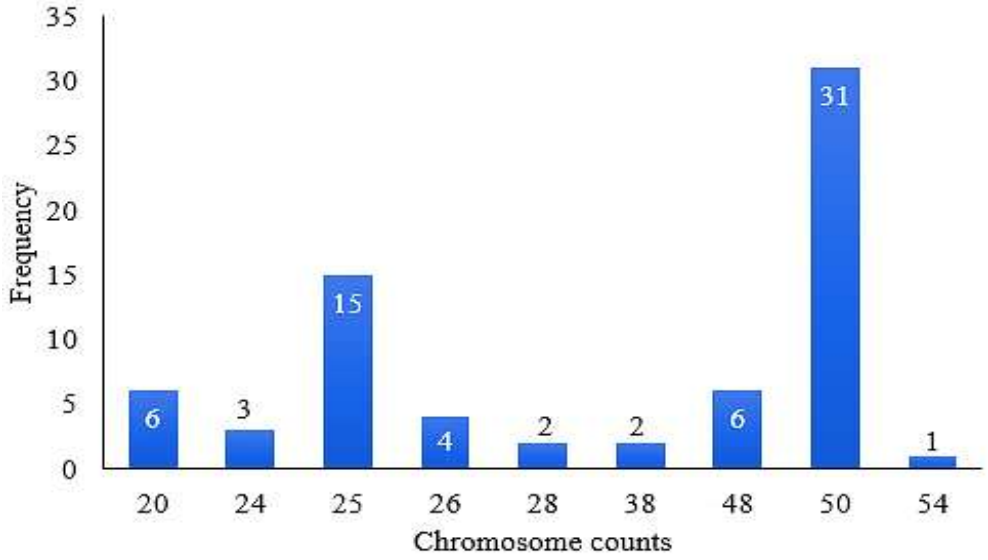


Figure. 3. Frequency of different chromosome counts in *Labeo rohita*

(A)

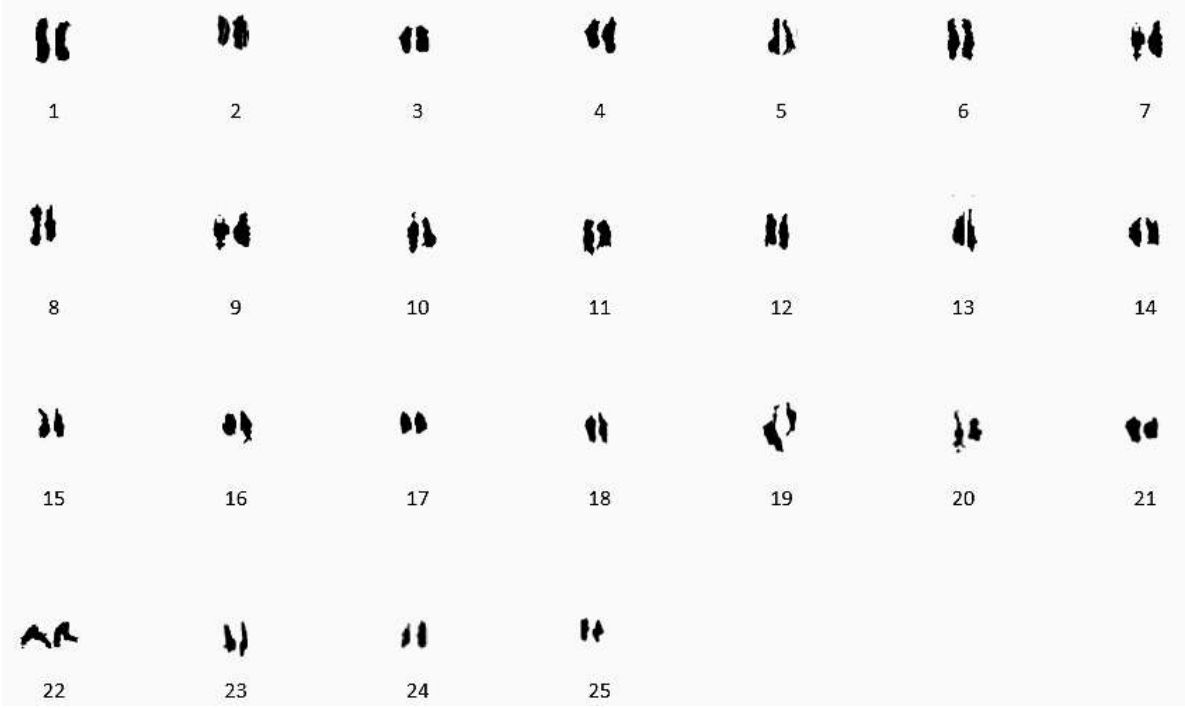
(B)

Plate 2. (A) Chromosomal spread of *Cyprinus carpio*

(B) Karyotype (28 m, 38 sm, 12 a, 22 te)

m=1-14, sm=15-33, a=34-39, te=40-50

(A)



(B)

Plate 3. (A) Chromosomal spread of *Labeo rohita*,
(B) Karyotype (16m, 14sm, 12a, 8t)
m=1-8, sm=9-15, a=16-21, te=22-25

Discussion

The species *Cyprinus carpio* and *Labeo rohita* are major carps in the aquaculture of Myanmar. In the present study, specimens of these two species were collected from Mondaing Dam during July 2022 to January 2023.

The results revealed that the diploid chromosome number of common carp was to be 2n = 100 frequency 27 with 38.6 percent. The karyotype formula of *C. carpio* are comprised of 28 m, 38 sm,12 a, 22 te (NF = 166). From a tetraploid origin, *C. carpio* is a teleostean species. Carp contains a great number of very small chromosomes; it has been moderately well examined by the cytogenetic researchers. Anjum (2005) described karyotype of *C. carpio* 2n = 100 (16 m, 34 sm, 50 a). And also, Salish and Majeed (2012) reported the karyotype of *C. carpio* as 2n = 100 (22 m, 32 sm, 12 te, 34 a). Similarly, the present studied carp species had the same number of 2n = 100 diploid chromosomes. Nevertheless, the morphologies of the investigated karyotypes varied somewhat from earlier researches. Cucchi and Baruffaldi, (1990) stated that bony fish possess relatively small size and many chromosomes leading to technological challenges for karyological research that are not found in other vertebrates. Chromosomes are organized according to their size and shape. In the present study, chromosomes in *C. carpio* were tiny and numerous, making it difficult to separate and arrange in homologous pairs.

In *L. rohita*, the diploid number was found as 2n = 50 chromosomes with the chromosome counts 50 frequency 31 with 44.3 percent. The karyotype consisted of 16 m, 14 sm, 12 a, 8 te (NF = 82). According to the previous literatures such as Win Mar *et al*. (2011) have been studied the karyotype formula 2n = 50 (10 m, 16 sm, 24 a) (NF = 76), 2n = 50 (36 m, 12 sm, 2 st) (Mahfuj *et al*., 2013), 2n = 50 (32 a, 4 st, 6 sm, 8 m) (Bhatanagar *et al*., 2014), 2n = 50; NF = 80; 8 m,14 sm, 8 a, 20 te) (Getlekha, *et al*., 2022). So, the present result of diploid number in *L. rohita* 2n = 50 was a conformity with above reports.

Buth *et al*., (1991) stated that the total chromosome number in cyprinids is wide-ranging between 42 and over 200. In the present study, the number of metacentric, submetacentric, acrocentric and telocentric chromosomes varied a little compared by previous literatures. The number of chromosomes ranged was from 50 to 100 in *Cyprinus carpio* whereas *L. rohita*, the number of chromosomes ranged from 20 to 54. These variances might be the result of chromosomal overlaps and loss during preparation, and staining and the various factors such as centromere inversions, amount of hypotonic solution, colchicine, and duration of time for preparation of chromosomes.

According to Sarasan *et al*., 2019, acrocentric chromosome is readily be mistaken as telocentric chromosome if they are highly constricted which is caused by excessive exposure to colchicine. Colchicine and hypotonic solution (NaCL) were crucial to produce the optimum chromosomal spreads in preparation of chromosomes. Colchicine 0.5 ml, 100% hypotonic solution (sodium chloride) 1 ml and undiluted Giemsa stain for 15 minutes gave the best result for present study.

The karyotype analysis is a key step towards improving the stock by polyploidy manipulation, hybridization and related generic engineering (Tan *et al*., 2004). Thus, like other animals, karyotype of aquatic species will be needed to study thoroughly.

The present research of cytogenetic investigation on *C. carpio* and *L. rohita* revealed variations in karyomorphology between the two species and there was no previous study in this field of research from fishes of Mondaing Dam. Thus, these are the first data of cytogenetic study of two fish species in this area and hope to provide basic information for future cytogenetic analyses of other Myanmar freshwater fishes.

Conclusion

The karyotype of two fish species described the diploid number of *Cyprinus carpio* 2n = 100 with 28 metacentric (m), 38 submetacentric (sm), 12 acrocentric (a) and 22 telocentric (te) chromosomes and the number of fundamental arms was 166. The diploid number of *Labeo rohita* 2n = 50 with 16 metacentric (m), 14 submetacentric (sm), 12 acrocentric (a) and 8 telocentric (te) chromosomes and the number of fundamental arms was 82. In *Cyprinus carpio*, optimum chromosome metaphase spreads were observed at 2 hours 15 minutes and in *Labeo rohita*, optimum chromosome metaphase spreads were observed at 2 hours 30 minutes and 2 hours 45 minutes.

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PREVALENCE OF SOME INSECT SPECIES ON THE PLANTATIONS OF MAIZE (*ZEA MAYS* L., 1753) IN MIN HLA TOWNSHIP,MAGWAY REGION

Win Ei Khaing[1], Daw Maw[2], Hnin Hnin Wai[3]

Abstract

Maize is among the world's three most important cereal crops. It is Myanmar's second most important crop with more than one million acres planted annually. A total number of (3210) individuals was accounted for 18 species of insects, distributed among 17 genera 14 families, and six orders, and collected from maize plantations, Min Hla Township, Magway Region from January to December 2022. Among the recorded species, 13 species were identified as pests and the other five species as beneficial or predatory insects. In the present study, the maximum number of species recorded in order Hemiptera was represented by five species (33.33%) while, the minimum number of species Orthoptera, Neuroptera, and Hymenoptera recorded only one species (6.67%) respectively from study site I (Malun Village). In study site II (Pan Taw Pyin Village) the maximum number of species recorded in order Hemiptera was represented by seven species (38.88%), while the minimum number of species in Orthoptera, Neuroptera, and Hymenoptera recorded only one species (5.56%) respectively. In study site I, the maximum number of individuals was recorded in the order Lepidoptera represented by (739) individuals (51.71%), while the minimum number of individuals in Orthoptera (21) individuals (1.47%) were recorded during the study period. The maximum numbers of individuals were collected in order Lepidoptera was represented by (815) individuals (45.76%), the minimum number of individuals were collected in Orthoptera (8) individuals (0.45%) were recorded in study site II. During the study period, Lepidopterans species are predominant on maize plantations in two study sites.

Keywords: Maize, Insects, Beneficial, Pest species, Lepidopterans species

Introduction

Maize (Zea *mays* L.) has a tropical origin and is traditionally grown in monsoon. Maize, a kind of cereal crop (family: Poaceae) and commonly known as corn in Myanmar. Maize is the second most harvested crop in Myanmar after paddy. Maize is mostly grown in Shan State and other mountainous regions in the country. In Ayeyarwady, Mandalay, Magway, and Bago regions, which grow winter maize, and in Shan, Kachin, and Kayin States which grow monsoon maize. Among them, Shan state is the major maize growing area covered with 38%, and 44% of the total production comes from this state. Also, The Magway Region includes the major maize area (Chiang Mai University, 2021).

There are two purposes of maize production such as grain and fodder purpose. These grains are used for human consumption, Corn is sold as a fresh vegetable or is canned or frozen and is also used as dairy and poultry feed in Myanmar. On the other hand, maize is also grown as a fodder crop, which is used for cattle cake. The grain also is processed into a growing number of food products, including corn flour, corn oil, corn syrup, and many other by-products. It is a very important animal feed and is heavily used in the production of cellulosic ethanol, a biofuel. (Ferreira *et al*., 2002).

Maize grains have high nutritive value containing 66.2% starch, 11.1 protein, 7.12% oil, and 1.5 minerals. There are many insects, pests, and diseases of maize crop. It can cause damage to the yield of maize. Insect infestation is one of them. Insects are the most diverse group of animals; they include more than a million species and represent more than half of all known living organisms. The total number of extant species is estimated at between six and ten million,

[1] Yenanchaung University
[2] Yenanchaung University
[3] Pakkoku University

potentially over 90% of the animal life forms on earth are insects. Insects may be found in nearly all environments (Gullan and Cranston, 2010). Jordan and Verma (2010), opined that compared with beneficial insects, injurious insects are very numerous. People have been interested mainly in two categories of insects; harmful and beneficial species. These two categories comprise only a few thousand of the millions of insect species. The beneficial species are seen as friends by humans while the harmful species are seen as enemies.

Myanmar is an agricultural country, and agriculture is the backbone of its economy. The agriculture sector contributes to 37.8 percent of gross domestic product (GDP), accounts for 25 to 30 percent of total export earnings, and employs 70 percent of the labor force. For successful production of the crops, there is a need to investigate the insect pests that destroy crops at all growing stages (FAO, 2017).

In Magway Region, the economy is mainly based on agriculture. For successful production of the crops, there is a need to investigate the insect pests which destroy crops at all growing stages. There is no previously recorded insect species on maize plant in Min Hla Environs. Thus, the present study has been conducted on some insect species on maize plantations at Pan Taw Pyin and Malun villages, in Minhla Township, Magway Region.

Thus, the present study was conducted with the following objectives:

- to record and classify the insect species on maize plantation in the study area
- to examine the species composition and relative abundance of recorded insect species
- to determine the insects which are either pests or beneficial on maize plantations in the study area

Materials and Methods

Study Area

Min Hla Township is in Thayet District, Magway Region, of central Myanmar, on the West bank of the Irrawaddy. It has an area of 2371.4 square kilometers. Pan Taw Pyin and Malun are villages of Min Hla Township. It is situated between 19º57' 56.81" to 19º58' 14.53" N and 95º 01'47.10" to 95º02' 34.80" E (Fig-1).The plantation of maize in the study area was mentioned in plate 1.

Source: *Google Earth* (2022)

Figure 1. Map of the study area and location of study sites

Plate 1. The plantation of maize in the study areas

Study Period

The study was carried out from January 2022 to December 2022.

Collection of Specimens

Specimens were collected during the daytime, once a week for one year. Some were easily collected by hand-picked and others were by insect net. Photographic records of fresh specimens were taken.

Preparation of the specimens

The collected specimens were transferred into the killing bottle which contained cotton wool soaked with chloroform vapor. Some were preserved in small glass bottles, containing 70 percent alcohol with glycerin for later examination (Revel, 2017). Under each specimen, there is a label bearing the name of the species, locality, and date of capture and it is transferred into the insect box.

Identification of specimens

The identification was carried out according to Borror and Delong (2005), Gullan and Cranston (2010), Awasthi (2016), and Debbie (2018).

Data Analysis

$$\text{Species composition} = \frac{\text{Number of a species}}{\text{Total number of all species}} \times 100$$

$$\text{Dominance index} = \frac{\text{Number of individuals of each species}}{\text{Total number of individuals of all species}} \times 100$$

Abundance categories based on index value are: Rare species (0.1-2.0), Uncommon (2.1-4.0), Frequent (4.1-6.0), Common (6.1-8.0), Abundant (8.1-above) (Kumar and Sivaperuman (2005)

Results

A total of 18 insect species belonging to 17 genera, 14 families, and six orders were recorded from January 2022 to December 2022 (Table -1). The recorded species were shown in plate.2.

Order-wise species composition of insect recorded on maize plantation from study site I

Among the 15 insect species, the order Hemiptera was included five species (33.33%), followed by Coleoptera with four species (26.66%), three species (20%) in Lepidoptera while, Orthoptera, Neuroptera, and Hymenoptera were recorded only one species (6.67%) respectively.

Order-wise species composition of insect species recorded on maize plantation from the study site II

Among the 18 insect species, the highest number, seven species (38.88%) in the order Hemiptera, followed by Coleoptera with five species (27.78%), three species (16.66%) in Lepidoptera while Orthoptera, Neuroptera, and Hymenoptera were recorded only one species (5.56%) respectively (Fig.2).

Occurrence of insect species individuals on maize plantation in two study sites

Total number of (3210) individuals of insects were recorded from two study sites throughout the one-year survey.

During the study period, a total number of (**1429**) individuals was accounted for **15** species of insects, distributed among **14** genera, **11** families, and **six** orders and collected from maize plantation in study site I (Malun Village) (Table.4).

A total number of (**1781**) individuals accounted for **18** species of insects, distributed among **17** genera, **14** families, and **six** orders and collected from maize plantation in study site II (Pan Taw Pyin Village) (Table. 5).

Beneficial and pest insect species from the study sites

Among the total of 18 insect species recorded, five species were observed as beneficial insects while the other 13 species represented pests (Table .3) (Fig. 2).

Order-wise numbers and percentage of individuals were recorded in the study site I .

Out of six orders, the order Lepidoptera was represented by (739) individuals (51.71%), followed by Hemiptera (404) individuals (28.27%), Coleoptera (213) individuals (14.91%), Neuroptera (29) individuals (2.03%), Hymenoptera (23) individuals (1.61%), Orthoptera (21) individuals (1.47%) were recorded in study site I (Table .4) (Fig .3).

Order-wise numbers and percentage of individuals were recorded in the study site II.

Out of six orders, the order Lepidoptera was represented by (815) individuals (45.76%), followed by Hemiptera (498) individuals (27.96%), Coleoptera (393) individuals (22.07%), Hymenoptera (48) individuals (2.69%), Neuroptera (19) individuals (1.07%), Orthoptera (8) individuals (0.45%) were recorded in study site II (Table .5) (Fig .3).

Table. 1 Insect species on maize plantation recorded from the study area from January 2022 to December 2022

No	Order	Family	Species	Common name
1	Orthoptera	Acrididae	*Schistocera nitens*	Large gray bird grasshopper
2	Hemiptera	Alydidae	*Leptocorisa oratoria*	Rice ear bug
3		Coreidae	*Cletus punctiger*	Squash bug
4		Miridae	*Creontiades pallidus*	Sheddeer bug
5		Cercopidae	*Callitettix versicolor*	Sugarcane spittle bug
6		Aphididae	*Rhopalosiphum maidis*	Corn leaf aphids
7		Pentatomidae	*Nezara viridula*	Green stink bug
8		Pentatomidae	*Bagrada hilaris*	Bagrada bug
9	Neuroptera	Chrysopidae	*Chrysoperla carnea*	Green lacewing
10	Coleoptera	Chrysomelidae	*Aulacophora foveicollis*	Red pumpkin beetle
11		Coccinellidae	*Menochilus sexmaculatus*	Six spotted zigzag
12		Coccinellidae	*Micraspis discolor*	Ladybird beetle
13		Coccinellidae	*Henosepilachna sumbana*	Cucurbit ladybird
14		Anthicidae	*Anthelephila caeruleipennis*	Ant-like beetle
15	Lepidoptera	Crambidae	*Spoladea recurvalis*	Beet webworm moth
16		Noctuidae	*Spodoptera litura*	Cotton leafworm
17		Noctuidae	*Spodoptera frugiperda*	Fall armyworm moth
18	Hymenoptera	Apidae	*Apis florea*	Dwarf honey bee

Table 2. Order-wise distribution of species composition of insects on maize plantation in two study sites during the study period

No	Order	Number of Families	Number of Genera	Number of Species	Composition of species (%)
1	Orthoptera	1	1	1	5.56
2	Hemiptera	6	7	7	38.88
3	Neuroptera	1	1	1	5.56
4	Coleoptera	3	5	5	27.78
5	Lepidoptera	2	2	3	16.66
6	Hymenoptera	1	1	1	5.56

Table 3. Status of insect species from two study sites during the study period

No	Scientific name	Common name	Status
1	*Schistocera nitens*	Large gray bird grasshopper	Pest
2	*Leptocorisa oratoria*	Rice ear bug	Pest
3	*Cletus punctiger*	Squash bug	Pest
4	*Creontiades pallidus*	Sheddeer bug	Pest
5	*Callitettix versicolor*	Rice spittle bug	Pest
6	*Rhopalosiphum maidis*	Corn leaf aphid	Pest
7	*Nezara viridula*	Green stink bug	Pest
8	*Bagrada hilaris*	Bagrada bug	Pest
9	*Chrysoperla carnea*	Green lacewing	Beneficial
10	*Aulacophora foveicollis*	Red pumpkin beetle	Pest
11	*Menochilus sexmaculatus*	Six spotted zigzag	Beneficial
12	*Micraspis discolor*	Ladybird beetle	Beneficial
13	*Henosepilachna sumbana*	Cucurbit ladybird	Pest
14	*Anthelephila caeruleipennis*	Ant-like beetle	Beneficial
15	*Spoladea recurvalis*	Beet webworm moth	Pest
16	*Spodoptera litura*	Cotton leaf worm	Pest
17	*Spodoptera frugiperda*	Fall armyworm	Pest
18	*Apis florea*	Dwarf honey bee	Beneficial

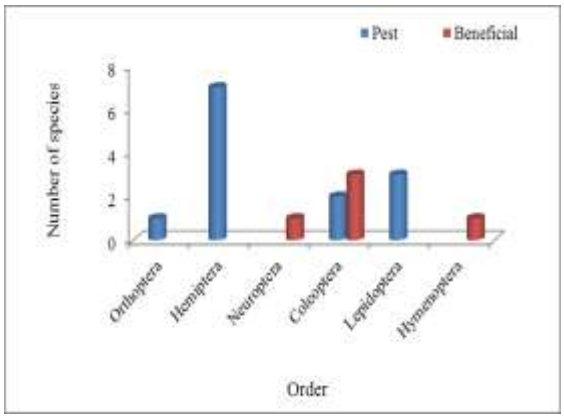


Figure. 2. Order-wise relative number of species on beneficial and pest species recorded

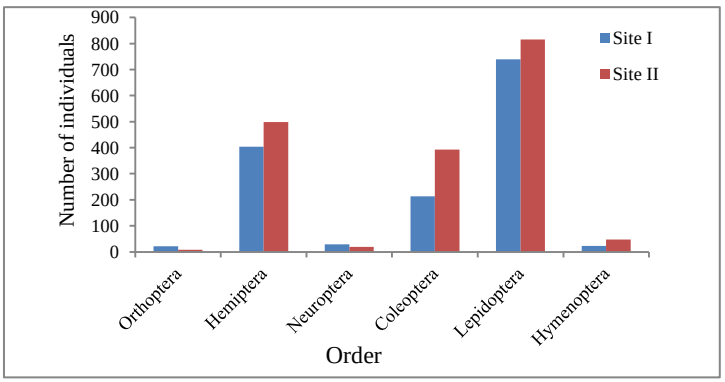


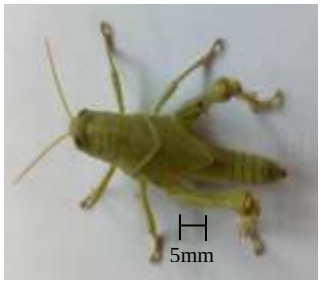
Figure. 3. Order-wise relative number of individuals of Site I and II .

Table 4. Monthly occurrence of insect species and number of individuals on maize plant recorded from the study site I during the study period

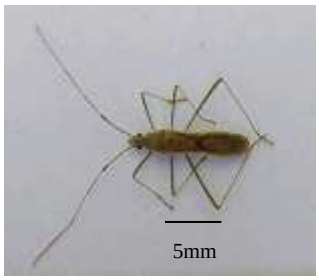
No	Scientific name	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep	Oct	Nov	Dec	TNI	DI	Categories
1	*Schistocera nitens*	**1**	**2**	**1**	**0**	**3**	**4**	**2**	**3**	**1**	**0**	**0**	**4**	21	1.469	Rare
2	*Creontiades pallidus*	**2**	**0**	**3**	**1**	**0**	**2**	**0**	**3**	**3**	**1**	**0**	**2**	17	1.189	Rare
3	*Callitettix versicolor*	**5**	**9**	**5**	**7**	**4**	**7**	**8**	**12**	**5**	**7**	**4**	**7**	80	5.598	Frequent
4	*Rhopalosiphum maidis*	**8**	**7**	**9**	**5**	**9**	**11**	**11**	**10**	**13**	**8**	**11**	**12**	114	7.977	common
5	*Nezara viridula*	**1**	**0**	**0**	**6**	**15**	**9**	**4**	**6**	**0**	**6**	**15**	**9**	71	4.968	Frequent
6	*Bagrada hilaris*	**4**	**6**	**10**	**13**	**14**	**5**	**9**	**11**	**13**	**10**	**13**	**14**	122	8.537	Abundant
7	*Chrysoperla carnea*	**2**	**2**	**5**	**1**	**0**	**4**	**2**	**2**	**4**	**1**	**0**	**6**	29	2.029	uncommon
8	*Menochilus sexmaculatus*	**2**	**12**	**11**	**6**	**7**	**7**	**5**	**7**	**9**	**9**	**8**	**7**	90	6.298	common
9	*Micraspis discolor*	**2**	**0**	**3**	**0**	**2**	**0**	**2**	**3**	**4**	**0**	**1**	**0**	17	1.189	Rare
10	*Henosepilachna sumbana*	**0**	**1**	**2**	**1**	**0**	**2**	**2**	**4**	**6**	**1**	**0**	**2**	21	1.469	Rare
11	*Anthelephila caeruleipennis*	**11**	**5**	**11**	**8**	**7**	**6**	**9**	**8**	**5**	**3**	**8**	**4**	85	5.948	Frequent
12	*Spoladea recurvalis*	**2**	**5**	**3**	**1**	**8**	**7**	**10**	**11**	**13**	**11**	**12**	**14**	97	6.787	common
13	*Spodoptera litura*	**16**	**14**	**17**	**13**	**15**	**16**	**8**	**12**	**13**	**13**	**15**	**16**	168	11.756	Abundant
14	*Spodoptera frugiperda*	**25**	**36**	**45**	**37**	**42**	**49**	**35**	**39**	**46**	**37**	**38**	**45**	474	33.170	Abundant
15	*Apis florea*	**1**	**1**	**2**	**0**	**2**	**3**	**2**	**3**	**4**	**0**	**2**	**3**	23	1.609	Rare
		82	100	127	99	128	132	109	134	139	107	127	145	1429		

Table 5. Monthly occurrence of insect species and number of individuals on maize plant recorded from the study site II during the study period

No	Scientific name	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep	Oct	Nov	Dec	TNI	DI	Categories
1	*Schistocera nitens*	1	1	1	0	1	0	1	1	1	0	1	0	8	0.4491	Rare
2	*Leptocorisa oratoria*	2	4	2	3	4	3	5	2	2	3	6	3	39	2.189	Uncommon
3	*Cletus punctiger*	0	1	0	2	4	7	0	2	8	2	5	7	38	2.133	Uncommon
4	*Creontiades pallidus*	2	0	3	1	2	2	0	3	3	1	0	2	19	1.066	Rare
5	*Callitettix versicolor*	4	6	10	6	8	8	6	8	11	8	7	12	94	5.277	Frequent
6	*Rhopalosiphum maidis*	7	8	9	11	13	17	12	9	17	11	13	17	144	8.085	Abundant
7	*Nezara viridula*	1	0	0	6	9	13	4	6	0	6	5	9	59	3.312	Uncommon
8	*Bagrada hilaris*	3	7	9	10	7	10	9	8	16	10	6	10	105	5.895	Frequent
9	*Chrysoperla carnea*	0	2	3	0	3	2	0	2	3	3	0	1	19	1.066	Rare
10	*Aulacophora foveicollis*	4	3	6	6	8	7	8	5	11	6	9	7	80	4.491	Frequent
11	*Menochilus sexmaculatus*	8	11	13	9	12	7	9	7	10	9	12	7	114	6.401	Common
12	*Micraspis discolor*	3	0	3	0	2	0	2	3	3	0	2	0	18	1.011	Rare
13	*Henosepilachna sumbana*	2	3	5	2	4	6	4	6	2	2	5	6	47	2.639	Uncommon
14	*Anthelephila caeruleipennis*	9	6	12	9	10	17	11	12	13	8	10	17	134	7.524	Common
15	*Spoladea recurvalis*	4	6	9	5	7	9	6	8	10	6	8	9	87	4.885	Frequent
16	*Spodoptera litura*	10	14	19	12	11	13	11	12	14	8	9	16	149	8.366	Abundant
17	*Spodoptera frugiperda*	45	43	53	50	46	54	45	51	55	40	45	52	579	32.509	Abundant
18	*Apis florea*	2	3	5	3	4	6	4	3	5	3	4	6	48	2.695	Uncommon
		107	118	162	135	155	181	137	148	184	126	147	181	1781		



A. *Schistocera nitens*

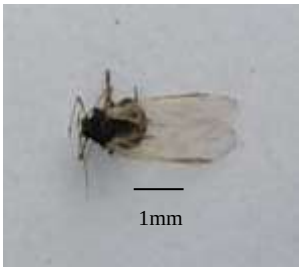


B. *Leptocorisa oratoria*

C. *Cletus punctiger*

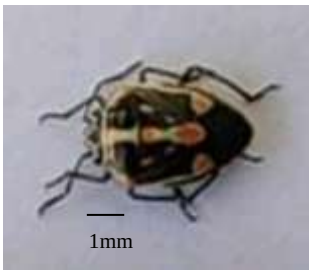
D. *Creontiades pallidus*

E. *Callitettix versicolor*

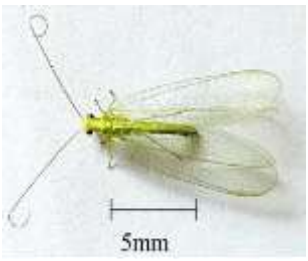


F. *Rhopalosiphum maidis*

G. *Nezara viridula*



H. *Bagrada hilaris*



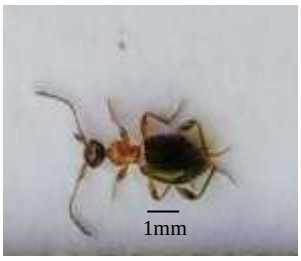
I. *Chrysoperla carnea*

J. *Aulacophora foveicollis*

K *Menochilus sexmaculatus*

L. *Micraspis discolar*

M. *Henosepilachna sumbana*



N. *Anthelephila caeruleipennis*

O. *Spoladea recurvalis*

P. *Spodoptera litura*

Q. *Spodoptera frugiperda*

R. *Apis florea*

Plate 4. Recorded insect species of Order Orthoptera, Hemiptera, Neuroptera, Coleoptera, Lepidoptera and Hymenoptera

Discussion

Ferreira *et al*., (2002) observed that maize plants were attacked by 140 species of insect pests causing a varying degree of damage. However, only about a dozen are quite serious. In the present study, 13 species of pest were identified on maize plants at Pan Taw Pyin and Malun Villages in Minhla Township during study period.

Braley, (2021) concluded that maize crops often have high levels of beneficial insects (predators and parasitoids) that may be harmed by insecticide applications. Beneficial organisms should be one tool used in integrated pest management (IPM) program. In the present study, five species of beneficial species were recorded on maize plantations during study period. Utilizing multiple pest management options can also help farmers save money, as they rely less on pesticides, which is especially important in the face of pesticide resistance.

Chowdhury, (2015) described that coccinellids (*Menochilus sexmaculatus* and *Micraspis discolor*) are key predators that are conserved and augmented in agricultural ecosystems, to achieve biological control of pests. In the present study, these two species are recorded from two study sites during study period.

Alam *et al*., (2014) discussed that the abundance of several insect pests was influenced by different growth stages of maize. The insect pest species, cutworm, maize stem borer, corn leaf aphid, maize borer, earworm and birds differed significantly at different growth stages of maize.

Khan *et al*., (2022) stated that pests occur at levels of economic injury including: *Ostrinia fiirnacalis, Spodoptera exigua, Spodoptera litura,* and *Helicoverpa armigera*. In the present study, pests occur at levels of economic injury including: *Nezara viridula, Spodoptera litura, Spoladea recurvalis,* and *Spodoptera frugiperda.* It is similar mentioned Liao *et al*., 2010. The pests that rarely occur at economic injury levels included: *Cletus punctiger, Aulacophora foveicollis, Bagrada hilaris, Rhopalosiphum maidis,* and *Henosepilachna sumbana.*

El-Heneidy and Abbas (2005) described that lady beetles mainly occurred in September and October in maize fields of Sindh Agriculture University, Tandojam. But in the present study *Menochelus sexmaculatus,* and *Micraspis discolor* mainly occurred in nearly throughout the years.

Maureen *et al*, (2006) described those seed corn maggots, seed corn beetles, corn flea beetle, billbugs, armyworm, corn leaf aphid and fall armyworms are fully occurring in maize plantations in the United States of America. In the present study, Rice ear bug, Green stink bug, Red pumpkin beetle, Beet webworm moth, corn leaf aphid, cotton leaf worm and fall armyworm are recorded. Therefore, these insect pests are similar occurrences in maize plantations during the study period.

Liao *et al*., 2010 mentioned that many hemipterans species are economically significant pests of important agricultural crops. *Callitettix versicolor* is one of the species harmful to agricultural crops and causes severe economic damage to rice and maize in China, India, Malaysia, Myanmar, Thailand and Vietnam. In the present study, this species was recorded on maize plantations during the study period.

Pannuti *et al*., 2015 described that *Spodoptera frugiperda* (Fall Armyworm) is a new pest in Africa, attacking maize, but can also feed on other crops. This species seemingly displays a very wide host range, with over 80 plants recorded. Due to its polyphagous behavior, high voracity, ability to form large populations, and high dispersion rate, this species is considered a cosmopolitan pest, one of the most destructive in America.

The most frequently consumed plants are maize and sweet corn. In the present work, fall armyworms damaged maize plantations in the study sites throughout the study period. Many factors limit maize production; insects and mites being among the most important. Lepidopteran pests are the most damaging insects of maize worldwide.

Thus, there is a need to control the pests to safeguard the crop yield. It is therefore suggested that, awareness should be given to the cultivators on the chemical method of controlling pests by

using pesticides have an effect on not only the pests but also the beneficial insects that thrive in the area and the health of human beings as well, so that cultivators should avert to more eco-friendly biological means of controlling insect pests to maintain successful harvest.

So In the present study, insects on maize plantations are classified as either pests or beneficial insect species during the study period. The present topic could be touched upon fulfilling the gap in Entomological research.

Conclusion

A total of 18 insect species belonging to 17 genera, 14 families, and six orders were recorded on maize plantation of Min Hla Environs. Thirteen species of insects were recorded as the insect pests and five species of insects were collected as the beneficial insects. Total number of (3210) individuals of insects were recorded from the study area throughout the one-year survey. Hemipterans species and Lepidopterans individuals are predominant on maize plantations in two study sites. Since Lepidopteran species inhabit agricultural lands, they are of economic importance. This research will contribute some information concerning pests and beneficial species observed in maize fields and improve the way of biological control of insect pests in agriculture.

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PREVALENCE AND HISTOLOGICAL CHANGES OF MYXOSPOREAN PARASITES IN THE KIDNEYS OF *CIRRHINUS MRIGALA*, *LABEO ROHITA* AND *LABEO CATLA* (HALMILTON, 1822) FROM ALONE FISH CULTURE POND, MONYWA TOWNSHIP

Khin Mar Lwin[1], Khine Mi Mi Zaw[2], Khin Mi Mi Oo[3]

Abstract

Myxosporean parasites were important disease in culture fish species due to decrease fish production. Myxosporean infection in the kidney of three carp species, *Labeo rohita*, *Cirrhinus mrigala* and *Gibelion catla* was conducted from Alone fish culture pond, Monywa Township from June 2019 to May 2020. Amounted to 14 species of myxosporean parasites were observed. The total 90 fish specimens of three carp species, *Cirrhinus mrigala*, *Labeo rohita*, and *Gibelion catla* were studied for the prevalence of myxosporean parasites and histological changes in the kidneys of these species. Seven species of *Myxobolus*, and one species of *Thelohanellus* in *Cirrhinus mrigala*, three species of *Myxobolus* and one species of *Thelohanellus* in *Labeo rohita* and two species of *Myxobolus* in *Gibelion catla* were observed. The prevalence of myxosporean parasites of three carp species, *Cirrhinus mrigala*, *Labeo rohita* and *Gibelion catla* were found 83.33 %, 53.33 % and 13.33 % respectively. Histological changes were studied in infected kidneys of three carps, the large amount of matured myxosporean parasites were observed in the epithelial cells of renal tubules, in the blood corpuscle, and glomerulus of kidney. Among these three carp species, *Cirrhinus mrigala* was infected by more diverse species of myxosporean parasites and appeared more susceptible than the other two carps.

Key words: Three carp species, kidneys, myxosporean parasites, prevalence, histological changes

Introduction

Myxosporean parasites are abundant and diverse group of parasites and they cause diseases in a large variety of economically important fishes in both the wild and aquaculture fisheries industries. They have been also found in platyhelminthes, reptiles, amphibians, mammals and were also detected in fecal sample of human beings (Boreham *et al.*, 1998). They are multicellular organisms and consisting of one to twelve polar capsules. Several myxosporean infections of cultured fish were reported to be pathogenic. Most notorious is the whirling disease of trout, manifected by skeletal deformites, which is also claimed to have been introduced with rainbow trout into South Africa (Van Wyth, 1968). In farmed carp, *Myxobolus* spp. caused locomotory disturbances coupled with emaciation, and sunken eyes in brain infections (Dykova *et al.*, 1986). Members of phylum Myxozoa cause some of the most common and important parasitic diseases of fishes. Several species are known to cause serious losses in pisiculture (Lom and Dykova, 2006).

The protozoan parasite *Myxobolus* is often seen in the fingerlings causing morbidity and mortility in the carp polyculture system (Mukherjee *et al*., 2000). Parasites and diseases are one of the limiting factors in aquaculture, especially in the farms where fish are usually cultured in high density in restricted water body because fish pathogens can easily be transmitted among fish (Moe Kyi Han, 2006).

Myxozoans that play a role in cause diseases of commercially important fish. *Myxobolus* species is usually causes cutaneous myxosporidiasis in common fishes (Szczepanik *et al.*, 2010). In farm carp, *Myxobolus* spp. caused locomotory disturbances coupled with emaciation and sunken eyes in the cases of brain infection, anemia, hemorrhagic dropsy and mortality in the cases of heavy cardiac infection and circulatory disfunction in infection at the base of the gill lamellae respectively (Dykova *et al.*, 1986).

[1]Department of Zoology, Kalay University
[2] Department of Zoology, Kalay University
[3] Department of Zoology, Kalay University

In fish, the kidney is the major site of blood production. Kidney disease is one of the most puzzling fish diseases. Several myxosporean species inhabiting the kidney and urinary tract have proved to be highly pathogenic in farmed fish. The previous researchers described about the various kinds of kidney diseases. The two most important examples being the agent of proliferative kidney disease in salmonids (Clifton-Hadley *et al*., 1984; and *Sphaerospora renicola* in carp (*Cyprinus carpio*) (Dykova and Lom, 1986, 1988).

Various histopathological changes of myxosporean infected fish was described by some previous researchers. The principal pathological response is one of chronic inflammation which, in renal tissue, results in the marked proliferation of the interstitial haemopoietic tissue and a reduction in the number of glomeruli and excretory tubules. These elements are dispersed by the hyperplastic interstitial tissue, but also exhibit degenerative changes during the course of the disease. Vascular pathology, with possible haemoglobin crystallisation, has also been reported (Clifton-Hadley *et al*., 1987).

Pond fish cultures play an important role for protein food sources. In Monywa environs, there are many fish farmings and *Cirrhinus mrigala*, *Labeo rohita* and *Labeo catla* have been produced by polyculture system. Parasitic disease can be lossed to fish production. The infected fish may influence the quality of fish. Myxosporean parasites not only infect the kidneys but also the gills, the infection, clumps the gill filaments leading to the lost of gaseous exchange surface area, thus stunting the growth and resulting in the decline in the quality of fish and economic lost. Thus, there is a requisite to investigate myxosporean infection in the fishery sector. Therefore, study of myxosporean parasites in the kidneys of major carp as Cirrhinus mrigala (Hamilton , 1822) Locally Known as Nga Gyin, Labeo rohita(Hamilton , 1822) Locally Known as Nga Myint Chin and Labeo catla (Hamilton , 1822) Locally Known as Nga Thaing. *Cirrhinus mrigala*, *Labeo rohita* and *Labeo catla* was carried out from Alone fish culture Pond. The objectives of the present study are as follows:

- to investigate the prevalence of myxosporean parasites in the kidney of three carp species from Alone fish culture pond
- to examine the histological changes in the kidneys of infected fishes

Materials and Methods

This research work was conducted at the Department of Zoology,Monywa University. The study period was from June 2019 to May 2020.

Alone fish culture Pond, located in the Western part of Monywa Township (Plate 1).

Specimen collection and examination of myxosporean parasites

A total of 90 specimens of three carp species (30 specimens of each) were collected from Alone fish culture Pond, located in the Western part of Monywa Township (Plate 1).used for the investigation of myxosporean parasites. Then fish were dissected and the kidneys were removed and fixed in 10% formaldehyde solution and carried to the laboratory of Zoology Department, Monywa University. The kidneys of fishes were squashed with a drop of water on the slide by forceps and covered with a cover slip and immediately examined thoroughly for the myxosporean parasites under the microscope with digital camera image analyzer DP-12 Olympus. Fresh spores were measured according to Lom and Arthur (1989) and Lom and Dykova (1992). Morphometric measurements of fresh spores were taken in μm although lateral view of spore could not be recorded. The microphotographing was also carried out on the fresh spores.

(Source: Google Earth, 2019)

Plate 1. Map of sample collected area (Alone fish culture pond)

Identification and classification of myxosporean parasites

Myxosporean parasites were identified according to Lom and Dykova (1992), (2006), Kaur and Singh (2008), (2009), (2010), (2011), and Szekely *et al.* (2009). The spores (Myxosporean) were classified using the practical key given by Lom and Dykova (1992).

Preparation and examination for histological study

Tissue samples of kidney were studied for histological effect at tissue level. For histological study, myxosporean infected kidneys of the three carp species, *Cirrhinus mrigala*, *Labeo rohita* and *Labeo catla* were fixed in 10% formaldehyde solution and embedded in paraffin wax. Sections of 5µm thicked were cut and stained with hematoxylin and eosin. And then, myxosporean uninfected kidneys were also processed for the histological slides. All of these histological slides were examined under light microscope and photomicrographs were taken with digital camera attached to Meiji Biological Microscope (MT 4300H). The method used in this study was according to the histological method of Cambell (cited by Kolmer *et al*., 1969).

Data analysis

The prevalence rates were calculated follow after Margolis *et al*. (1982):

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

Results

The total 90 fish specimens of three carp species,*Cirrhinus mrigala*, *Labeo rohita*, and *Labeo catla* were studied for the prevalence of myxosporean parasites and histopathological changes in the kidney of these species. Seven species of *Myxobolus* and one species of *Thelohanellus* in *Cirrhinus mrigala*, three species of *Myxobolus* and one species of *Thelohanellus* in *Labeo rohita*, and two species of *Myxobolus* in *Labeo catla* were observed (Plate 2).

The comparison of the shape and dimensions of *Myxobolus* spp. and *Thelohanellus* spp. in three carp species is presented in Table 1. The largest spore length, 13.1 µm, was recorded in *Thelohanellus* sp. infecting *Labeo rohita* (Table 1).

The prevalence of myxosporean parasites in the kidneys of *Cirrhinus mrigala* is described in Table 2. The highest prevalence of infection was observed in *Myxobolus* sp.7, with a prevalence of 23.3%, while the lowest prevalence was found in *Thelohanellus* sp., at 10%.

The prevalence of myxosporean parasites in the kidneys of *Labeo rohita* is described in Table (3). The highest prevalence of infection was observed in *Myxobolus* sp.2, with a prevalence of 16.7%. The lowest prevalence was found in *Myxobolus* sp.7, with a rate of 6.7%, where 2 individuals were infected out of 30.

The prevalence of myxosporean parasites in the kidneys of *Labeo catla* is described in Table (4). Only two *Myxobolus* species, *Myxobolus* sp.1 and sp.2, were found in the kidneys of this fish. The prevalence of infection in *Myxobolus* sp.1 and sp.2 was 10% and 6.7%, respectively.

When comparing the prevalence of infection by myxosporean parasites in the examined fish, the highest prevalence was found in *Cirrhinus mrigala* at 83.3%, with 25 out of 30 individuals infected (Table 5).

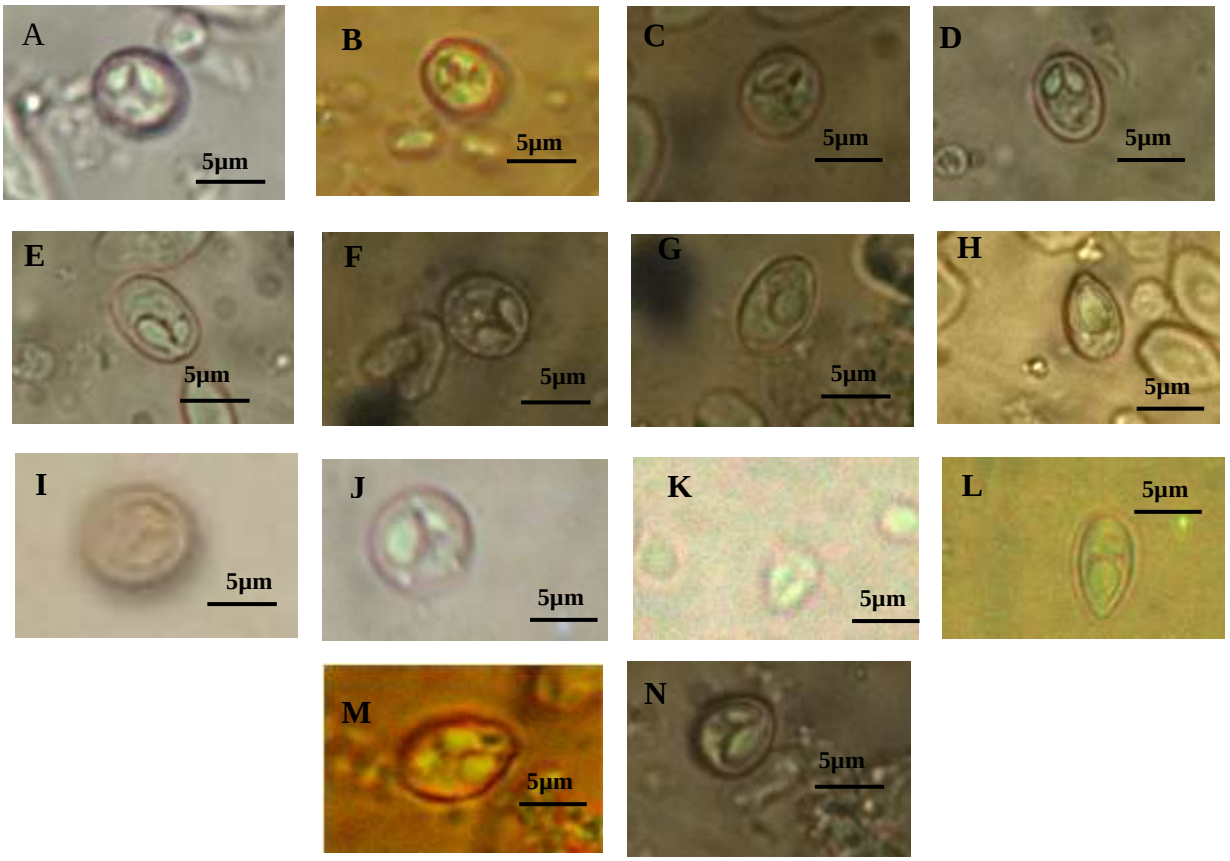

Plate 2 Myxosporean parasites found in the kidney of three carp species (A to H myxosporean parasites of *Cirrhinus mrigala*; I to L myxosporean parasites of *Labeo rohita*; M and N myxosporean parasites of *Labeo catla*)

Table 1 Comparison of shape and dimension of *Myxobolus* spp. and *Thelohanellus* sp. of three carp species (measurements are described in µm)

Sr. no.	Parasite	Host	Infected organ	Spore shape	Spore length	Spore width	Large		Small	
							Polar capsule length	Polar capsule width	Polar capsule length	Polar capsule width
1	*Myxobolus* sp. 1	*Cirrhinus mrigala*	Kidney	Rounded	8.33	8.33	5.00	3.33	3.33	2.22
2	*Myxobolus* sp. 2	*Cirrhinus mrigala*	Kidney	Pyriform	11.87	7.33	6.15	3.69	3.69	2.46
3	*Myxobolus* sp. 3	*Cirrhinus mrigala*	Kidney	Ovoidal	8.7	6.8	4.44	1.66		
4	*Myxobolus* sp. 4	*Cirrhinus mrigala*	Kidney	Subspherical	11.67	8.34	4.44	2.222		
5	*Myxobolus* sp. 5	*Cirrhinus mrigala*	Kidney	Elongated	12.22	8.33	6.11	3.33	3.33	2.22
6	*Myxobolus* sp. 6	*Cirrhinus mrigala*	Kidney	Spherical	11.11	10.0	5.0	2.77		
7	*Myxobolus* sp. 7	*Cirrhinus mrigala*	Kidney	Ellipsoidal	10.55	7.33	5.55	3.33	2.77	1.66
8	*Thelohanellus* sp.	*Cirrhinus mrigala*	Kidney	Tear- shaped	11.11	7.22	6.66	5.00		
9	*Myxobolus* sp. 1	*Labeo rohita*	Kidney	Subspherical	10.17	5.82	3.75	2.5		
10	*Myxobolus* sp. 2	*Labeo rohita*	Kidney	Subspherical	10.6	8.75	5.0	3.7	2.5	1.25
11	*Myxobolus* sp. 3	*Labeo rohita*	Kidney	Pear shaped	7.5	3.75	3.75	1.7		
12	*Thelohanellus* sp.	*Labeo rohita*	Kidney	Tear-drop shaped	13.1	6.8	6.25	3.7		
13	*Myxobolus* sp. 1	*Labeo catla*	Kidney	Ovoidal	4.92	3.69	1.85	1.54		
14	*Myxobolus* sp. 2	*Labeo catla*	Kidney	Ovoidal	7.38	4.92	3.69	2.46	2.46	1.85

Table 2 Prevalence of myxosporean parasites in the kidney of *Cirrhinus mrigala*

Sr. no.	Parasite	Examined fish	Infected fish	Prevalence (%)
1	*Myxobolus* sp.1	30	6	20
2	*Myxobolus* sp.2	30	6	20
3	*Myxobolus* sp.3	30	4	13.33
4	*Myxobolus* sp.4	30	4	13.33
5	*Myxobolus* sp.5	30	5	16.67
6	*Myxobolus* sp.6	30	5	16.67
7	*Myxobolus* sp.7	30	7	23.33
8	*Thelohanellus* sp.	30	3	10

Table 3 Prevalence of myxosporean parasites in the kidney of *Labeo rohita*

Sr. no.	Parasite	Examined fish	Infected fish	Prevalence (%)
1	*Myxobolus* sp.1	30	6	20
2	*Myxobolus* sp.2	30	5	16.67
3	*Myxobolus* sp.3	30	2	6.67
4	*Thelohanellus* sp.	30	6	20

Table 4 Prevalence of myxosporean parasites in the kidney of *Labeo catla*

Sr. no.	Parasite	Examined fish	Infected fish	Prevalence (%)
1	*Myxobolus* sp.1	30	3	10
2	*Myxobolus* sp.2	30	2	6.67

Table 5 Total prevalence of myxosporean parasites from two carp species

Sr. no.	Host	Examined fish	Infected fish	Prevalence (%)
1	*Cirrhinus mrigala*	30	25	83.33
2	*Labeo rohita*	30	16	53.33
3	*Labeo catla*	30	4	13.33

Histopathological effects in the Kidneys of three carp species

In *Cirrhinus mrigala*, any changes were not occurred in the uninfected normal kidney (Plate 3 A). However, in serious infected fish, necrotic lesions were more distinct among the renal tubules (Plate 3 B). Some glomeruli were cluster, shrinkage and atrophy (Plate 3 C). The spores were accumulated and dispersed in the kidney tubules (Plate 3 D). The mature myxosporean parasites liberated from the plasmodium were observed (Plate 3 E).

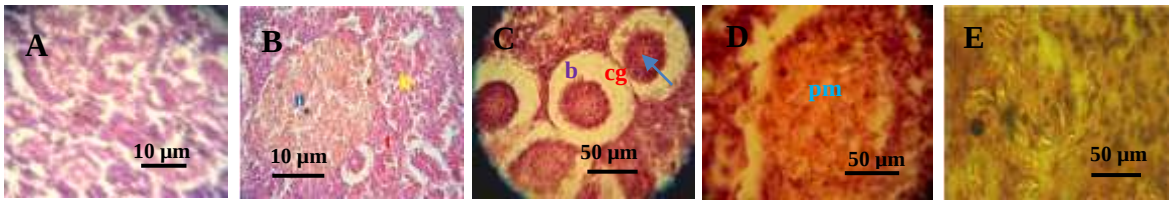

Plate 3 Histological changes in the Kidneys of *Cirrhinus mrigala*

In *Labeo rohita*, the kidneys infected with myxosporean parasites showed that the renal tubules were shrinkage and lumen was occlusion (Plate 4 A). The glomerulus was shrunk into atrophy (Plate 4 B). The accumulation of inflammatory cell was observed among the renal tubules (Plate 4 C). In serious infected kidney, the large amount of mature myxosporean parasites was observed in the epithelial cells of renal tubules, in the blood corpuscle of kidney tissue and glomerulus (Plate 4 F). In *Labeo catla*,the kidney tubules were Necrosis and distortion (Plate 5 A), the glomerulus were atrophy of and necrosis found around the glomerulus (Plate b B), the glomerulus were Fusion (Plate 5 C) and the lumen in the renal tubule were degeneration and closing (Plate 5 D).

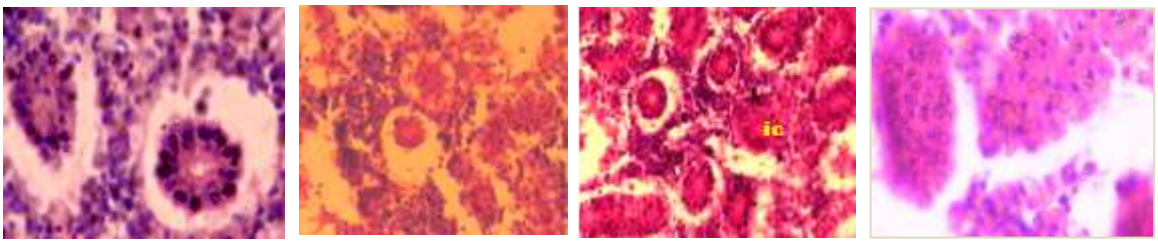

Plate 4 Histopathological changes in the kidney of *Labeo rohita* by myxosporean parasites

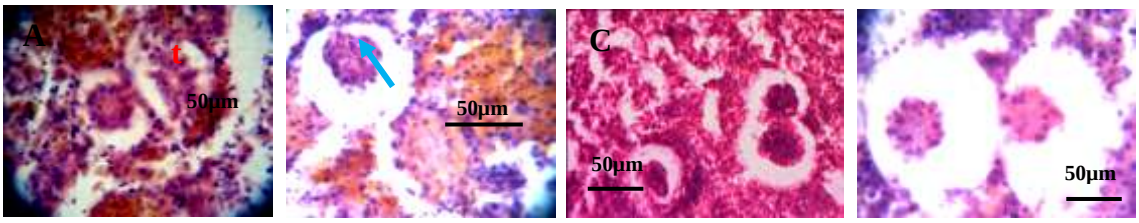

Plate 5 Histopathological changes in the kidney of *Labeo catla* by myxosporean parasites

Discussion

Comparison of myxosporean parasites from polyculture carp species, *Labeo rohita*, *Cirrhinus mrigala* and *Labeo catla* were studied. The total of 90 specimens (30 specimens in each fish species) was examined. In *Cirrhinus mrigala* eight species of myxosporean parasites (seven *Myxobolus* spp. and one *Thelohanellus* sp.) were occurred. In *Labeo rohita*, four species of myxosporean parasites (three *Myxobolus* spp. and one *Thelohanellus* sp.) were found. In *Labeo catla*, two species of myxosporean parasites (two *Myxobolus* spp.) were observed. But the shapes and dimensions of spores were not similar between these species. It is concluded that *Cirrhinus mrigala* more diversed species of myxosporean parasites and more infected than the others two carps. But the polyculture fish host, different myxosporean parasites were observed in the present study.

In *Cirrhinus mrigala*, of prevalence of *Myxobolus* sp.1, 20 %, *Myxobolus* sp.2, 20 %, *Myxobolus* sp.3, 13.33 %, *Myxobolus* sp.4, 13.33 %, *Myxobolus* sp.5, 16.67 %, *Myxobolus* sp.6, 16.67 %, *Myxobolus* sp.7, 23.33 % and *Thelohanellus* sp. 10 % were observed. In *Labeo rohita*, *Myxobolus* sp.1, 20 %, *Myxobolus* sp.2, 16.67 %, *Mxobolus* sp.3, 6.67 % and *Thelohanellus* sp., 20 % were found. In *Labeo catla*, *Myxobolus* sp.1, 10 % and *Myxobolus* sp.2, 6.67 % were observed.

The prevalence of *Myxobolus* sp.7 (23.33 %) was predominant agent in *Cirrhinus mrigala. Myxobolus* sp.1 and *Thelohanellus* sp. (20 %) were predominant agent in *Labeo rohita* and *Myxobolus* sp.1 . (10 %) was predominant agent in *Labeo catla* during the present study. Lom and Dykova (1992) stated that all functions of the kidney are fluid balance, waste excretion and blood cell production become inexorably compromised and the glomerulus is the main component of the renal corpuscle and it composed of blood capillary loops. The blood is selectively filtered as it flows through the glomerular capillaries.

Pa Pa Win (2007) reported that the histological changes in the kidney of *Cirrhinus mrigala*, the mature spores of *Myxobolus* spp. are attached to the lumen of renal tubule and convoluted tubule of kidney. In the kidney of host fish heavily infected plasmodia containing mature spores of myxosporean parasites destroyed the glomerulus and interstitial cells. Early developmental stage of myxosporean parasites were also observed in kidney interstitial cell and glomerulus. In kidneys of host fish, numerous spores and large plasmodium of myxosporean parasites were not found. In the present study, the observations of histological changes in the kidney of *Cirrhinus mrigala* were

agreed with Pa Pa Win (2007). But in the present study, numerous spores were distributed and large plasmodium of myxosporean parasites also observed.

Moe Kyi Han (2006) reported that the histological changes in the kidneys of *Labeo rohita* were early developmental stages of parasites, damages in glomerulus and renal tubules, developmental stages of myxosporean parasites were found in the lumen of renal tubules. In the present study, the histological changes in the kidneys of *Labeo rohita* were observed as deformities; dilation of glomerulus, shrinkage of collecting duct, degeneration of lumen in the renal tubules, hypertrophy and hyperplasia of glomerular cells, swelling of glomerular epithelial cells and *Myxobolus* sp. was found in the blood corpuscles.

Moser and Kent (1994) recorded that affected myxosporean parasites in *Parvicapsule* sp. (coho salmon) are dark and lethargic and postmortem examination reveals renal hypertrophy and hemorrhage beneath the renal corpusles. Spores and other developmental stages occupy the epithelium and lumina of the renal tubules. The infection is associated with severe dilation and necrosis of the renal tubules. In the present study, the histological changes in the kidneys of *Labeo catla* were as cluster and atrophy of glomerulus, fusion of glomerulus more occurred.

The myxosporean infected kidneys of *Cirrhinus mrigala* and *Labeo rohita* the distortion were more occurred and the parasite spores were more incident than *Labeo catla*. Large necrotic lesions, spores and other developmental stages occupy the epithelium and lumina of the renal tubules were found in the kidney tissue of all infected fish hosts.

Bruno *et al*. (2006) described that myxosporidian infections can cause all categories of regressive and progressive pathological changes in the host including atrophy, dystrophy, hypertrophy, hyperplasia, necrosis and inflammation. Similarily most myxosporidian species cause minimal tissue damage, relatively few species are known to cause serious or fatal infections. Pathological changes similar to severe cases, led to nephrosis and necrosis of the tubular epithelium. Desser *et al*. (1983) and Supamattaya *et al*. (1991) also noted vacuolation of renal tubule epithelial cells in fish infected with myxosporean parasites, *Sphaerospora angulata* and *S. epinepheli* respectively.In the present study, myxosporean parasites were found within the blood corpuscles of kidney tissues.

The three studied fishes were cultured species and of economic importance. The enhancements of fish production are depended on healthily growth fish; there is need for adequate knowledge of parasites that infect them. The present described species of 14 myxosporean parasites were different in size and shape. The mix myxosporean infected fish was mostly found in fish culture pond. Among the three carp species, *Cirrhinus mrigala* was the most infected mix myxosporean parasites in fish culture pond. The fish parasites and diseases are the most important problem due to decrease fish production and death. The myxosporean parasites are one of the most important in fish. These parasites were incident in all parts of the body of fish host. Therefore, this research is needed to carry out for studying myxosporean parasites as well as disease of fish.

In conclusion, three carp species of fish hosts from Alone fish culture Pond, Monywa Township were parasitized by 14 species of myxosporean parasites. The fish hosts could be threated to their health by these parasites and would lead a condition to loss of the fish population. It should carefully consider managing fish health, to control or reduce measure for the parasites infection and to conserve fish fauna. The information in this work is expected to be useful for local consumers and further researchers.

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MORPHO-MERISTIC CHARACTERIZATION AND KARYOMORPHOLOGICAL VARIATIONS IN *CHANNA PUNCTATA* (BLOCH, 1793) FROM MEIKTILA LAKE, MEIKTILA, MANDALAY REGION

Myo Myo[1] and Win Win Mar[2]

Abstract

Assessment on the variations of morpho-meristic and cytotaxonomic characteristics due to the variation of karyo-morphology in some *Channa punctata* from Meiktila Lake, Mandalay Region was conducted during July 2022 to January 2023. Blood samples were treated using 0.50 % colchicine solution and incubated with the saturated NaCl for various durations: 1 hr 15 mins, 1 hr 30 mins, 1 hr 45 mins, 2 hrs, 2 hrs 15 mins, 2 hrs 30 mins, 2 hrs 45 mins, 3 hrs, 3 hrs 15 mins and 3 hrs 30 mins, respectively. A drop of each sample was put onto the slide and stained with undiluted Giemsa stain. The best optimal checkpoint for metaphase chromosomes was at 2 hrs 30 mins and were not clearly discerned on morphometric changes. Model numbers of metaphase chromosomes were octaploidy 2n = 8X = 128 having 3 submetacentric (sm) + 6 acrocentric (ac) + 7 telocentric (t) with fundamental arm number (NF = 304). Thus, *C. punctata* from Meiktila lake possess the diverged karyotypic patterns indicating that they are asymmetric species and can be used as cytogenetic marker for their population.

Keywords: *Channa punctata*, morpho-meristic, cytotaxonomic, octaploidy, asymmetric

Introduction

Myanmar's fishery sector plays an important role in Myanmar's economy and culture, and provide at least 60 % of Myanmar's animal protein consumption. Presently, 36385 valid species in which 300 new species in 2022 are reported in the Eschmeyer fish catalogue and new ones are discovered yearly, mainly from the tropical and subtropical areas (Fricke *et al.*, 2022). In addition, Froese and Pauly (2022) reported that among 1135 species, only 24 species of fishes are still remained to confirm their species status in fishery resource management.

To designate the species, not only the taxonomic aspect but also cytogenetic analysis is critically important in every research area. For that reason, understanding on the genome function is incomplete without the basic knowledge of genome organization at the chromosome level. Such chromosome-scale genome assemblies provide new opportunities for both cytogenetic and genome research (Sharakhova and Trifonov, 2021).

Channa punctata locally known as (Nga-yant-panaw) in Myanmar is distributed throughout India, Iran, Afghanistan, Bangladesh, Malaysia, Myanmar, Nepal, Pakistan, Sri Lanka and Thailand. In order to validate, the karyo-morphology of *Channa punctata* from Meiktila Lake, the present research was conducted with following objectives: to collect, identify and record the *C. punctata* in Meiktila Lake; to investigate the effect of mitotic inhibitor on metaphase checkpoint of the species identified; and to confirm through the erected hypothesis: H_0 : Karyomorphology cannot be referred as cytogenetic marker in the morpho-meristic variations in *C. punctata* and H_1 :Karyomorphology can be referred as cytogenetic marker in the morpho-meristic variations in *C. punctata.*

Materials and methods

Study area and study site

The present study was conducted at Meiktila Lake for fish specimen collection, locating in the center of Meiktila town between North latitude 20˚50′ 0″ and 20˚56′ 0″N and East longitude between 95˚49′ 30″ E and 95˚52′ 0″E (Plate 1).

[1] Department of Zoology, Kalay University
[2] Department of Zoology, University of Mandalay

Study period

This study was conducted from July 2022 to January 2023.

Sample collection

Fifteen fishes of *Channa punctata* were collected from Meiktila Lake. After identification, the fish specimens were kept in separate aquaria at Department of Zoology, Meiktila University.

Plate 1 Location map of Meiktila Lake, (Source: Google Earth 2022)

Identification of species

The identification of collected fish species *Channa punctata* was done according to Tawlar and Jhingran (1991) and Froese and Pauly (2022).

Preparation of solution

Stock solution 0.50 % of colchicine, the saturated hypotonic solution NaCl, and Carnoy fixative (3 methanol:1 acetic acid) were prepared. Giemsa stain was also prepared by mixing 0.78 g mixed with 500 mL methanol and 500 mL glycerol.

Preparation of mitotic division

One milliliter of blood samples were extracted from caudal vasculature by using syringe and placed into 10 mL anti-coagulant tube, treated with 0.50 % colchicine solution and then incubated with saturated hypotonic solution NaCl at room temperature for various durations: 1 hr 15 mins, 1 hr 30 mins, 1 hr 45 mins, 2 hrs, 2 hrs 15 mins, 2 hrs 30 mins, 2 hrs 45 mins, 3 hrs, 3 hrs 15 mins and 3 hrs 30 mins. The blood sample mixtures were vigorously shaken for 5 mins and fixed with 1 mL Carnoy's fixative solution, and stored at room temperature.

Slide preparation and staining

One mililiter of Carnoy fixative was added to the treaded cells and mixed thoroughly by shaking the tube back and forth. One or two drops of sample solution were placed on a clean slide and dried at room temperature. The slides were immersed in undiluted Giemsa stain for 15

mins and destained by rinsing with distilled water, and then dried overnight. Permanent slides were made by mounting Canada balsam and covered with cover slip.

Examination on microphotograph

Microphotographs were taken with Olympus CX-21FS1 camera attached to microscope (x1000) with Magvision camera attachment software.

Karyological study

The metaphase chromosome spreads (n = 8 - 10) from each specimen were examined by using immersion oil. The recorded michrophotographs were generated with SmartType3 (SDB-459) and arranged by decending order according to their size. The chromosomal numbers were recorded by Image.J (1.52a, USA).

Characteristics of chromosome patterns were classified according to Levan *et al.* (1964). The fundamental arm numbers (NF) were assessed and also the metacentric and sub-metacentric chromosomes were designated as biarmed chromosome, and acrocentric and telocentric chromosomes were denoted as uniarmed chromosome.

Statistical Analysis

All the data recorded of *Channa punctata* was analyzed by Microsoft Excel 2019.

Results

A total of 15 specimens of *Channa punctata* were collected from Meiktila Lake and identified the species confirmation. Their morpho-meristic characterization and karyo-morphological variations were investigated in detailed studies (Table 1).

Description of *Channa punctata* (Bloch, 1793)

Common name - Spotted snakehead,

Vernacular name - Nga-yant-pa-naw

The body is elongated and slightly rounded. Eyes are moderate and mouth is large. Pectoral fin is extended to anal fin. Pelvic fin is about 75 % of pectoral fin length. Caudal fin is rounded. Pre dorsal scales 13 - 14 and lateral line scales 37 - 42 present. Body color varies from black to light green on dorsal side and flanks; ventral side is white to pale yellow, sometimes with a reddish tinge; several dark blotches are on flanks; numerous black spots with a reddish tinge and paired fins are pale orange (Plate 2).

Plate 2 Lateral view of *Channa punctata*

Karyomorphological analysis of Snakehead *Channa punctata*

The blood samples of study fish species were treated with 0.50 % colchicine solution to block the mitotic check point of metaphase chromosomes and incubated with saturated NaCl solution at room temperature for various durations. The optimal check point of metaphase spread chromosomes was observed at 2 hrs 30 mins. The shape and size of chromosomes were identified by staining with undiluted Giemsa. The unique chromosomal characteristics and the unusual characters of chromosome spread were observed (Plate 3).

Percent and Frequency Distribution

The highest percentage of chromosome counts was 128 (54.67 %) with 88 frequencies followed by 126 (14.67 %) with 22 frequencies, 125 (12.00 %) with 18 frequencies, 129 (7.33 %) with 11 frequencies, 127 (6.0 %) with 9 frequencies and the lowest percent was found in 130 (5.33 %) with 8 frequencies (Table 2 and Fig. 1).

Karyotype

A total of 150 metaphase chromosome spreads of snakehead *Channa punctata* was 2n = 8X = 128, autotetraploid having 3 submetacentric (sm) + 6 acrocentric (a) + 7 telocentric (t) with constant fundamental arm number NF = 38 and the advanced fundamental arm number (NF) was 304. The distinct chromosomal patterns were found in chromosome set number 3 with extra-rectangular pattern and 6 with ring-shaped chromosome. Asymmetric chromosomal shape and size appeared in each set of chromosomes. The V - shaped chromosomes were observed in chromosome set number 1, 3, 4, 5 and 7 (Table 3, Plate 3)

The secondary constriction of chromosomes was found in chromosome number 1, 2, 3 and 4. The J - shaped chromosomes were found in chromosome set 1 and 7. The rod-shaped chromosomes were observed in 6, 7, 8, 10,11 and 12. The triangular patterns were found in chromosome set number 10 and 12. The last chromosome set number 16 represents the male sex character of spotted snakehead (Table 3, Plate 3).

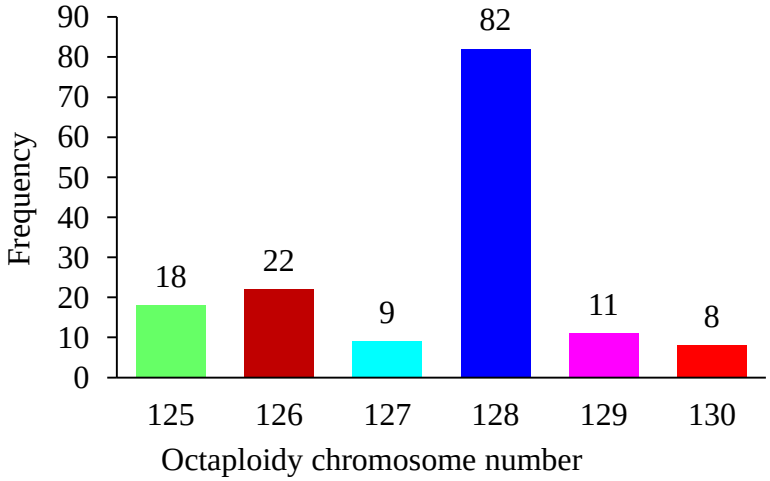

Figure.1. Frequency distribution of octaploidy chromosome number in *Channa punctata*

Table 1. Meristic counts of *Channa punctata* (n = 15) from Meiktila Lake

Meristic characters	Numbers
Dorsal fin rays (DF)	33-34
Pectoral fin rays (PecF)	17-19
Pelvic fin rays (PelF)	4-5
Caudal fin rays (CF)	13-15
Anal fin rays (AF)	22-24
Scales above lateral line (SaLL)	4.5
Scales below lateral line (SbLL)	8.5-9.5
Lateral line scales (LLS)	37-42
Pre dorsal scales (PDS)	**13-14**

Table 2.Percent and frequency distribution of octaploidy *Channa punctata* in Meiktila Lake

Chromosome counts	Frequency	Percent	Valid percent	Cumulative percent
125	18	12.00	12.00	12.00
126	22	14.67	14.67	26.67
127	9	6.00	6.00	32.67
128	82	54.67	54.67	87.33
129	11	7.33	7.33	94.67
130	8	5.33	5.33	100
Total	150	100	100	

Table 3. Karyotypical analysis of Channa punctata (NF = fundamental arm numbers)

Species	Chromosome sets				Chromosome types				NF	
	Haploid number (n)	Diploid (2n)	Ploidy (> 2n)	Chromosome numbers	Metacentric (m)	Sub-metacentric (sm)	Acrocentric (ac)	Telocentric (t)	Constant	Advanced
C. punctata	16	32	8X	128	0	3	6	7	38	304

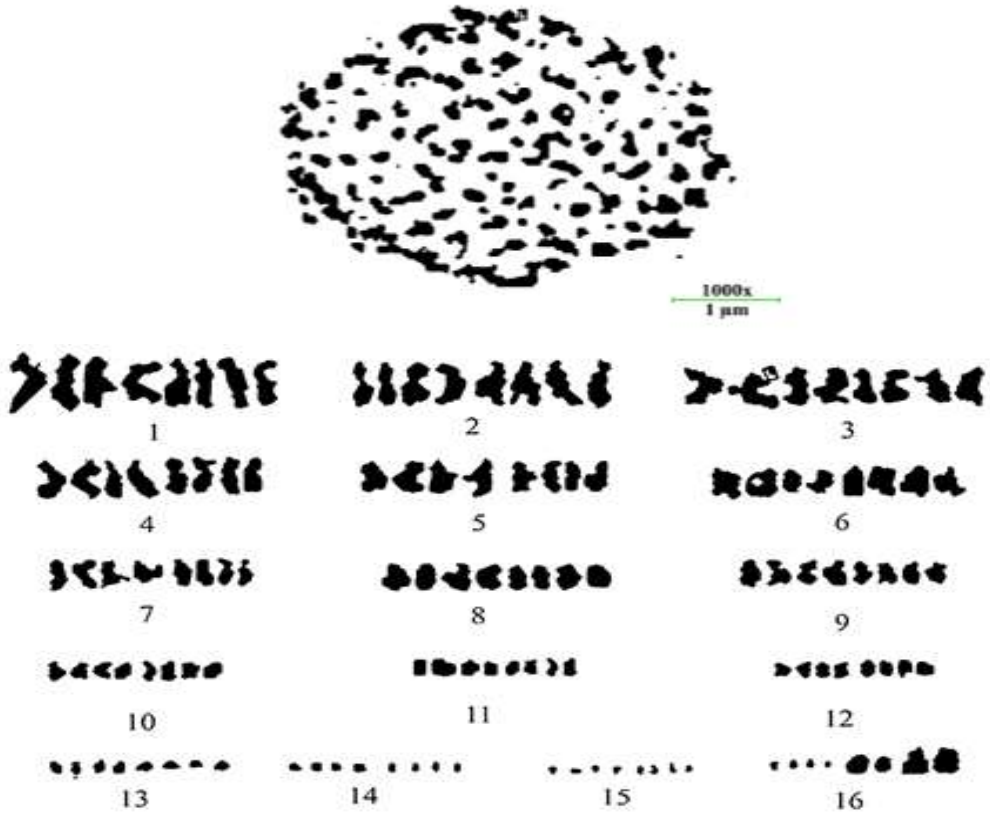

Plate 3. Metaphase chromosome plate (Upper) and karyotype (Below) of *Channa punctate* (1000x).

Discussion

The morpho - meristic characterization and karyo-morphological variations of *Channa punctata* from Meiktila Lake were investigated from July 2022 to January 2023. Some of morphological characters of *Channa punctata* are consistent with Talwar and Jhingran (1991), Plamoottil (2017), Widodo *et al.* (2020), Paunikar and Panwar (2021), and Froese and Pauly (2022) whereas the meristic counts of *Channa punctata* such as the pelvic fin rays (PelF), dorsal fin rays (DF) and pre dorsal scales (PDS) were different.

In nature, every organism has its own unique karyotype. However, there was lack of phylogenetic analysis in *Channa punctata* including morphological and cytogenetic data (Naorem and Bhagirath, 2006). With respect to the cytogenetic data, Kumar *et al.* (2019) suggested that the process of evolution in *Channa punctata* is complicated due to the polyploidization, duplication, rearrangement, fusion and loss of chromosomes. However, there is no evidence of a fixed pattern found in *Channa punctata*. Furthermore, Lawce and Brown (2017) reported that chromosome number can vary due to a number of errors in cell division, that is, during meiosis, fertilization, or mitosis.

In this study, *Channa punctata* are asymmetrical species having more acrocentric and telocentric chromosomes with variable chromosomal characteristics. Thus, the cytotaxonomic study on *Channa punctata* could be based not only on their centromeric position but also on their size and morphology of chromosomes. The best optimal check point of metaphase chromosomes was observed at a duration of 2 hrs 30 mins compared to other durations. The highest percentage

of chromosome counts 128 (54.67 %) with 88 frequencies and the lowest percentage of chromosome counts 130 (5.33 %) with 8 frequencies in *C. punctata*.

The karyological formula of *Channa punctata* was 3 sm + 6 ac + 7 t with fundamental arm number (NF) = 304 indicating that the fish possesses the autotetraploid 2n = 8X = 128 instead of constant diploid n = 16.

To sum up, the study on the morpho-meristic characters and karyograms revealed variable morpho-meristic characters with the divergence of karyotypic patterns in *Channa punctata* from Meiktila Lake. Therefore, the present karyomorphological results revealed the diverged karyotypic patterns indicating that *C. punctata* is asymmetric species and the present karyomorphological results can be referred as cytogenetic marker for *Channa* species.

Therefore, the erected hypothesis, H_1 is accepted and H_0 is rejected. The alternative hypothesis – "karyomorphology can be referred as cytogenetic marker in the morpho-meristic variations in *Channa punctata*" is accepted. The null hypothesis – "karyomorphology cannot be referred as cytogenetic marker in the morpho-meristic variations in *Channa punctata*" is rejected.

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DETERMINATION OF ANTIMICROBIAL ACTIVITY ON BACTERIAL ISOLATES FROM SELECTED YOGURT SAMPLES

Win Thi Thi Han[1], Thida San[2], Nyein Nyein Hlaing[3]

Abstract

Yogurt is among the most popular fermented foods in the world. In the present study, ten samples of yogurt from South Okkalapa Township and North Dagon Township were collected. The study period was lasted from January 2022 to August 2022. This study was conducted at the Microbiology Laboratory, Department of Zoology, Dagon University. In the present study, the objectives were to enumerate and identify bacterial isolates and access their antimicrobial activities. The enumeration of total bacteria count in ten yogurt samples was duplicated carried out. The maximum bacteria count was found in sample code YgI of South Okkalapa Township at 9.62×10^8 cfu/ml and the minimum count in sample code YgA of South Okkalapa Township was 5.32×10^6 cfu/ml. This research was used streak plate method for isolation on MRS agar for their colony morphology and biochemical characters. According to the colony morphology, gram-staining and biochemical tests, six bacteria genera were identified as *Leuconstoc* (14.81%), *Lactococcus* (18.52%), *Pediococcus* (3.71%), *Clostridium* (18.52%), *Lactobacillus* (33.33%) and *Streptococcus* (11.11%). All of the isolated bacteria did not show antimicrobial activity against tested targets *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* bacteria. Yogurt is good for health because it contains beneficial live bacteria so it should be consumed every day.

Keywords: Yogurt, colony forming unit, bacteria

Introduction

Yogurt is a dairy product produced by lactic fermentation of milk (Hui, 1992). Any sort of milk may be used to make yogurt, but modern production is dominated by cow milk. It is the fermentation of the milk sugar (lactose) into lactic acid that gives yogurt its gel-like texture and characteristics tang (Davis, 1974). It is a widely consumed as functional food due to its good taste and nutritional properties (rich in potassium, calcium, protein and vitamin B) and excellent vehicle to deliver probiotics to consumers (Reid *et al*., 2003). Regular consumption of yogurt is thought to be beneficial in the strengthening of the immune system, improvement in lactose digestion, blood glucose management (Yadav *et al*., 2007).The reduction of constipation, diarrhea, colon cancer, inflammatory bowel disease and allergies (Adolfsson *et al*., 2004).

Yogurt is a fermented dairy product and have highly digestible proteins. Fermented dairy products, also categorized in functional foods group, are considered to have functional properties because of its enhanced nutritional values and the presence of probiotics (friendly bacteria). Among fermented dairy product, the most important fermented food is yogurt.Therefore, yogurt bacteria are very important to human nutrition. In addition, having antimicrobial activity increases the importance of yogurt bacteria (Suskovic *et al*., 2010).

[1] Department of Zoology, Dagon University
[2] Department of Zoology, Dagon University
[3] Department of Zoology, Dagon University

Lactic acid produced on fermentation of lactose contributes to the sour taste of yogurt by decreasing its pH and enables the formation of the characteristic texture by acting on milk proteins (Guarner *et al*., 2005).

One of the most dairy products for the delivery of viable *Lactobacillus* sp. cells is yogurt (Analie and Bennie, 2001). Viable bacteria in yogurt are believed to actively enhance health by improving the balance of microflora in the gut (Fuller, 1989 and Fuller, 1992). Due to this yogurt by itself has been recognized as a healthy food by virtue of the beneficial action of its viable bacteria that compete with pathogenic bacteria for nutrients and space

In Myanmar, yogurt is the most popular dairy products and sold in many areas. People widely consumed it as soft drink. Therefore, the present study was undertaken with the following objectives :

- to enumerate of bacterial isolates in yogurts
- to identify the isolates their specific genus levels
- to access the antimicrobial activities of isolates

Materials and Methods

Study sites

A total of ten yogurt samples were purchased from South Okkalapa and North Dagon Townships in Yangon Region.The samples were analyzed carried to the Microbiology Laboratory, Department of Zoology, Dagon University.

Study period

The study period was from January, 2022 to August, 2022.

Materials and Methods

The materials used for research were yogurt samples.The apparatus and equipment used for the laboratory work were autoclave, water distiller, hot air oven, incubator, analytical balance, stirrer hotplate, refrigerator, compound microscope, vortex mixer, biosafety cabinet, colony counter, various kinds of glassware, pipettes, inoculation nichrome wire loop, aluminium foil, sterilized screw-cap bottle, microscope glass slides, disposable gloves, masks, sterilized cotton and cotton buds.

The culture media and biochemical test media used in bacteriological study consisted of Lactobacillus Man Rogosa Sharpe (MRS) Agar (M641), Triple Sugar Iron (TSI) Agar, Simon's Citrate Agar,Methyl-Red Voges-Prokauer (MR-VP) Broth, Urea agar, Sulphide Indole Motality (SIM) Medium, Geltinase, Methyl Red solution,∝-naphthol solution, 40%KOH solution containing 0.3% creatine, 3% H_2O_2 and Kovac's reagent. The test bacteria, Gram-negative bacteria *Escherichia coli*, Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*.

A total of ten yogurt samples were purchased from South Okkalapa Township and North Dagon Township and brought to the laboratory for isolation and identification of bacteria.

Preparation of samples

1mL of each yogurt sample was serially diluted with 9ml of distilled water by using a vortex mixer.

Enumeration of total bacteria

Serial dilution and pour plate method were used to enumerate the total bacteria from various collected materials (Dubey and Maheshwari, 2002). The suspension was diluted into 1:10 serial dilutions to the seventh dilution level. 1 ml of suspension from each dilution was inoculated onto MRS agar by using pour plate method. The petri dish cultures were inoculated at 35˚C for 24-48 hrs in an anaerobic jar. Subsequently, the number of growing colonies on each plate was counted for estimation of the number of total viable bacteria. The colony numbers only between 30 and 300 in each plate were used to calculate the colony forming unit (cfu/ml). Then the number of colonies was multiplied with the dilution factor and the bacteria counts in cfu/ml were calculated (Dubey and Maheshawri 2002).

Isolation and identification of bacteria

Streak plate methods were used to obtain a pure culture of bacteria. According to streak plate method, one loopful of bacteria from a colony on the plate from pure plate method was streaked onto the surface of MRS agar. Then, these plates were incubated at 35˚C for 24-48 hrs and the colonies growing on the surface of the culture plates were examined for its purity by detailed characterization. Only the pure colonies growing the streak line without coalescing with another colony was picked out to examine or store as a stork culture for further studies. The isolated bacteria were characterized by observing colonial morphology, Gram staining, catalase test, motility test and some biochemical characteristics (Bisen and Verma, 1998),

All isolates were subjected to the following standard biochemical tests described using dehydrated media. These tests included (i) triple sugar iron (TSI) test, (ii) citrate utilization test, (iii) methyl-red (MR) test, (iv) Voges-Proskauer (VP) test, (v)gelatinase test, (vi) indole formation test, (vii) H_2S production, and (vii) catalase 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 standards described in Bergey's Manual for Determinative Bacteriology (Breed *et al*., 1994), Cowan and Steel's Manual for Identification of Medical Bacteria (Cowan, 1975).

Antimicrobial activity

A perpendicular streak method was used for determining antimicrobial activities of each isolate on MRS agar (Egorov, 1987). The target bacteria were cross streaked as single lines on solidified MRS media in a petridish and were incubated at 35℃ for 24-48hrs. The isolated bacteria were then cross streaked perpendicular to the original streaks of bacteria isolates (Dubey and Maheshwari,2002).

The different test as target microorganisms used in this study were Gram-negative bacteria *Escherichia coli*, Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis.*

Results

Bacteria counts of the yogurt samples

The value of the standard viable total plate count of bacteria in the sample code YgA from South Okkalapa Township was 10.65×10^6 cfu/ml, YgB from North Dagon Township was 7.36×10^7 cfu/ml, YgC from South Okkalapa Township was 14.48×10^8 cfu/ml, YgD from North Dagon Township was 19.76×10^7 cfu/ml, YgE from South Okkalapa Township was 10.30×10^7 cfu/ml, YgF from North Dagon Township was 10.84×10^8 cfu/ml, YgG from South Okkalapa Township was 15.75×10^8 cfu/ml, YgH from North Dagon Township was 8.80×10^8 cfu/ml, YgI

from South Okkalapa Township was 19.23×10^8 cfu/ml and YgJ from North Dagon Township was 2.11×10^8 cfu/ml were found in present study (Table 1).

Cell morphology, Gram staining reaction and Biochemical reactions of the isolates bacteria

A total of 27 isolates were isolated from ten yogurt samples. Among them, 15 isolates Gram positive bacilli (rod) shaped bacteria (55.56%) were most abundant, 8 isolates cocci (spherical) shaped bacteria (29.63%) were second abundant and 4 isolates coccobacilli (oval) shaped bacteria (14.81%) were abundant and when grow onto De Man, Rogosa and Sharpe (MRS) agar (Table 2).

The biochemical properties of the isolates were designated six genera: *Leuconostoc* sp., *Lactococcus* sp., *Pediococcus* sp., *Clostridium* sp., *Lactobacillus* sp. and *Streptococcus* sp. (Table 3).

Identification of isolates

According to the biochemical tests, six genus bacteria namely *Leuconstonc* sp., *Lactococcus* sp., *Pediococcus* sp., *Clostridium* sp., *Lactobacillus* sp. and *Streptococcus* sp. were identified. Among them, *Leuconstoc* sp. was isolated in the samples codes YgA, YgC and YgE. *Lactococcus* sp. also isolated in the sample codes YgC, YgD, YgG and YgI. *Pediococcus* sp., was only found in the sample code YgE. *Clostridium* sp. was found in the sample codes YgB, YgE, YgF and YgH. *Lactobacillus* sp. was found in the sample codes YgB, YgC, YgE, YgF, YgG, YgH and YgI. *Streptococcus* sp. was isolated from the sample codes YgG, YgH and YgJ (Table 4 and Fig 1). Morphological features and some biochemical characteristics of isolated bacteria from the yogurt samples are shown in Fig.2.

Composition of identified bacteria isolated from yogurt samples

Among ten samples of yogurt, *Leuconstoc* spp., *Lactococcus* spp., *Pediococcus* spp., *Clostridum* spp., *Lactobacillus* spp. and *Streptococcus* spp. were selected 14.81%, 18.52%, 3.71%, 18.52%, 33.33% and 11.11% respectively (Table 4).

Antimicrobial activity

All of the isolates did not show antimicrobial activity against tested targets *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* bacteria .

Table 1. Total bacteria count of yogurt samples from different markets

Sr No	Sample code	Single bacteria count	Duplicate bacteria count	Total bacteria count	Average bacteria count(cfu/ml)
1	YgA	2.07×10^6	8.58×10^6	10.65×10^6	5.32×10^6*
2	YgB	3.96×10^7	3.40×10^7	7.36×10^7	3.68×10^7
3	YgC	9.30×10^8	5.18×10^8	14.48×10^8	7.24×10^8
4	YgD	8.20×10^7	11.60×10^7	19.76×10^7	9.90×10^7
5	YgE	6.50×10^7	3.80×10^7	10.30×10^7	5.15×10^7
6	YgF	6.45×10^8	4.39×10^8	10.84×10^8	5.42×10^8
7	YgG	10.75×10^8	5.00×10^8	15.75×10^8	7.87×10^8
8	YgH	4.50×10^8	4.30×10^8	8.80×10^8	4.40×10^8
9	YgI	9.58×10^8	9.66×10^8	19.23×10^8	9.62×10^8**
10	YgJ	1.22×108	0.89×108	2.11×10^8	1.06×10^8

Note: counts on MRS agar

** = maximum

* = minimum

Table 2. Number and composition of types of isolates from the yogurt samples

Isolate code	Number of Gram positive, rod shaped isolates	Number of Gram positive, cocci shaped isolates	Number of Gram positive, coccobacilli shaped isolates	Total
YgA	-	-	2	2
YgB	3	-	-	3
YgC	1	1	1	3
YgD	1	1	-	2
YgE	2	1	1	4
YgF	2	-	-	2
YgG	2	2	-	4
YgH	2	1	-	3
YgI	2	1	-	3
YgJ	-	1	-	1
Total	15	8	4	27
%	55.56	29.63	14.81	100

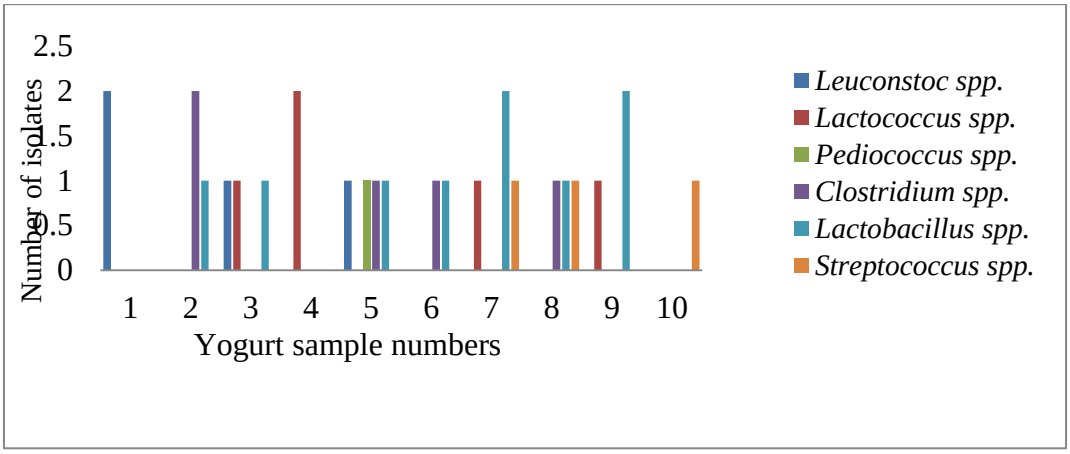


Figure.1 Number of isolates from the yogurt samples

Table 3. Biochemical reactions of the isolates from the yogurt samples

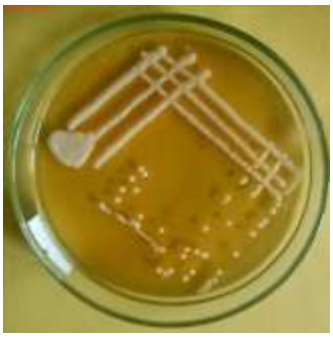
Sr No.	Isolate code	TSI			Cit	MR	VP	Gel	Urea	SIM			Ca	Tentative genera
		B	S	H_2S						Indo	Motile	H_2S		
1.	YgA-2	A	K	-	-	+	-	-	+	-	-	-	-	*Leuconstoc* sp.
2.	YgC-3	A	A	-	-	+	-	-	+	-	-	-	-	*Lactococcus* sp.
3.	YgE-1	K	A	-	-	+	-	-	+	-	-	-	-	*Pediococcus* sp.
4.	YgF-2	K	K	-	-	-	-	-	+	-	-	-	-	*Clostridium* sp.
5.	YgI-1	A	A	-	-	-	-	-	-	-	-	-	-	*Lactobacillus* sp.
6.	YgJ-1	K	A	-	-	-	-	-	+	-	-	-	-	*Streptococcus* sp.

(+) = positive reaction, (-) = negative reaction

S = Slant, B = Butt, G = Gas, Cit = Citrate utilization, MR = Methyl Red, VP = Voges Proskauer, Gel = Gelatinase, Urea = Urease test, SIM=Sulphide Indole Motility, Indo = Indole, M = Motility, Ca = Catalase, A = Acid, K = Alkaline, TSI = (Triple Sugar Iron)

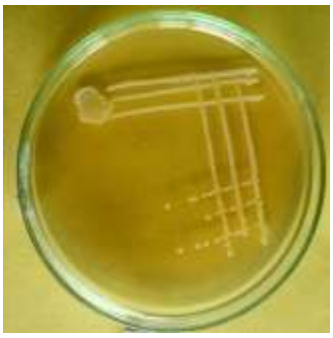
Table 4. Number of isolates identified from the yogurt sample

Sample code	**Identified bacteria**						**Total**
	***Leuconstoc* spp.**	***Lactococcus* spp.**	***Pediococcus* spp.**	***Clostridium* spp.**	***Lactobacillus* spp.**	***Streptococcus* spp.**	
YgA	2	-	-	-	-	-	2
YgB	-	-	-	2	1	-	3
YgC	1	1	-	-	1	-	3
YgD	-	2	-	-	-	-	2
YgE	1	-	1	1	1	-	4
YgF	-	-	-	1	1	-	2
YgG	-	1	-	-	2	1	4
YgH	-	-	-	1	1	1	3
YgI	-	1	-	-	2	-	3
YgJ	-	-	-	-	-	1	1
Total	4	5	1	5	9	3	27
%	14.81	18.52	3.71	18.52	33.33	11.11	100

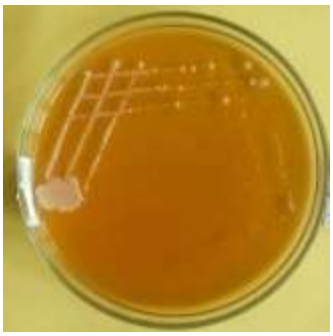


A. *Leuconstoc* sp. colonies on MRS agar

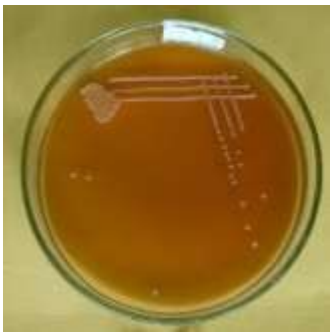
B.*Lactococcus* sp. colonies on MRS agar



C.*Pediococcus* sp. colonies on MRS agar



D. *Clostridium* sp. colonies on MRS agar



E. *Lactobacillus* sp. colonies on MRS agar

F.*Streptococcus* sp. colonies on MRS agar

G. Biochemical reactions of *Leuconstoc* sp.

H. Biochemical reactions of *Lactococcus* sp.

I. Biochemical reactions of *Pediococcus* sp.

J. Biochemical reactions of *Clostridium* sp.

K. Biochemical reactions of *Lactobacillus* sp.

L. Biochemical reactions of *Streptococcus* sp.

Figure.2 Morphological features and some biochemical characteristics of isolated bacteria from the yogurt samples

Discussion

In the present study, the maximum bacteria count was found in sample code YgI of South Okkalapa Township at 9.62×10^8 cfu/ml and the minimum count in sample code YgA of South Okkalapa Township was 5.32×10^6 cfu/ml. Abrar *et al*., 2009 reported that the total bacterial counts of the registered and non-registered samples were in the range of 3.0×10^3 - 9.0×10^4 cfu/ml and 8.2×10^4 - 28.4×10^5 cfu/ml, respectively. Compare with these statements, according to the result of present study, colony forming unit was a more higher and this might be due to different selective agar used in this research and also to temperature and environment conditions of yogurt production sites and markets.

Anwarul Hasan *et al*., 2016 stated that the yogurt samples collected from different district of Bangladesh showed a large number of total bacterial counts. Among the ten samples total viable bacterial count (TVBC) ranged from 1.72×10^2 to 5.04×10^8 cfu/ml. Therefore, the present study is similar to the total bacterial count of his finding.

In the present study, a total of twenty seven isolates were obtained from the ten yogurt samples. All isolates were characterized as being identified at the six genus level but not at the species level because their biochemical reactions and morphological changes were complicated. The isolates were grouped into six genera of bacteria based on the results of morphological, physiological and biochemical characterization. The isolates were identified as *Leuconstoc* spp., *Lactococcus* spp., *Pediococcus* spp., *Clostridium* spp., *Lactobacillus* spp. and *Streptococcus* spp.

Isolation of bacteria is also possible from other substrates like traditional fermented foods, beverages and sourdough. Generally, bacteria genera identified in the present study were comparable to that of other studies. Sawsan *et al*., 2010 reported that the genera *Lactobacillus, lactococcus* and *Pediococcus* had been isolated from raw cow milk, white cheese and rob in Sundan. Vuysta and Vancanneyt, 2007 also reported that the genera *Leuconstoc, Streptococcus, Pediococcus and Lactobacillus* were isolated from borde and shamita. Mayeux *et al*., 1962 stated that *Lactobacillus plantarum, Lactococcus lactic ssp. lactis, Lactobacillus delbrueckii subsp lactis, Leuconstoc lactis and Leuconstoc citreum* were identified in South African traditional fermented milks. Furthermore, Ei Thandar Khaing (2021) described the occurrence of *Lactobacillus xylosus, Leuconstoc citreum, Streptococcus acidominimus, Lactobacillus plantarum, Lactococcus lactis and Pediococcus petosaceus* in yogurt of Yangon area.

Calculations for percentage of occurrence regarding bacterial isolates consisted of bacilli, cocci and coccobacilli groups present in all samples: high occurrence at 55.56% in rod-shaped groups, 29.63% in cocci groups and coccobacilli groups were low in occurrence at 14.81% in yogurt samples. The result indicated that bacilli groups were dominant.

In this study, *Lactobacillus* spp. (33.33%) was the most abundant and *Lactococcus* spp. (18.52%) and *Clostridium* spp. (18.52%) were the second most abundant in equal numbers. *Leuconstoc* spp. (14.81%), *Streptococcus* spp. (11.11%) and *Pediococcus* spp. (3.71%) were found. The present findings indicated that the geneus *Lactobacillus* was the most abundant among all isolates.

Lianou *et al*., 2016 state that lactic acid bacteria are the major microbes found in yogurt and dairy fermentation although a diverse range of other organisms used in other fermentation processes. Among the lactic acid bacteria, *Lactobacillus*, *Streptococcus, Lactococcus* and *Leuconostoc* are most frequently found in fermented dairy foods, either as starter cultures or as naturally occurring members of the raw material. However, some fermented foods, especially

yogurt and other fermented milk products, may also contain added probiotic species of *Bifidobacterium* and *Lactobacillus.*

Antimicrobial activity is one of the most important selection criteria for probiotics. Bacteriocins can be broken down by some proteolytic enzymes leading to a loss in their antimicrobial activity. In the present study, twenty seven isolates were tested for their antimicrobial effects on one gram-negative and two gram- positive bacteria. The experimental results of the current research showed that all bacteria isolates were unable to inhibit the target test organisms of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. This might be due to different concentration of isolates and test organisms. Another reason may be due to temperature and time.

Sanders *et al*., 2014 reported that five European Union member states currently have national guidelines or recommendations that include yogurt with live bacteria. Nonetheless, Ebner *et al*., 2014, Rezac *et al*., 2018, Hill *et al*., 2017, Sanders *et al.*, 2014 and Bell *et al*., 2018 also stated that there appears to be emerging interest in including fermented foods as part of dietary guidelines. While the US dietary guidelines, as well as national recommendations from other countries, recommend the consumption of yogurt for its nutrient content. Therefore, yogurt should be consumed for health.

Conclusion

In vitro results showed that maximum bacteria count 9.62×10^{8} cfu/ml was found in the yogurts samples. A total of six bacteria genus *Leuconstoc*, *Lactococcus*, *Pediococcus*, *Clostridium*, *Lactobacillus* and *Streptococcus* were isolated and identified from the yogurt samples. Among fermented dairy product, one of the most important fermented food is yogurt. Therefore, yogurt bacteria are very important to human nutrition. In addition, having antimicrobial activity increases the importance of yogurt bacteria. Lactic acid produced on fermentation of lactose contributes to the sour taste of yogurt by decreasing its pH and enables the formation of the characteristic texture by action on milk protein. This findings indicate that the isolates are able to survive in the stomach and intestine of human. Based on the overall results, the bacteria isolates found in the yogurt sample should be suitable to supplement in normal diet in humans.

Acknowledgments

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THE GAMETOGENIC CYCLE OF HARD CLAM, *GELOINA EXPANSA* (MOUSSON, 1849) IN SHWE THAUNG YAN, AYEYARWADY REGION*

Wint Yee Paing[1]

Abstract

A total of 692 clams (319 were males and 373 were females) were analyzed. Six different gonad development stages in both males and females were identified. The average gonads socratic index (GSI) value of males was high in May and November (2.02 ±0.6 and 1.88 ±0.7). In females, the maximum GSI value was observed in May and November (1.80 ±0.6 and 1.75 ±0.5). The monthly changes in the gonadosomatic index and the microscopic characteristics of ovaries showed that *G. expansa* was recorded as two times spawning periods, the majority of spawning periods were recorded in the summer months of May, June, and July, and the second spawning (minor spawning) took place in or around November. The present results indicated that *G. expansa* is dioecious and gametogenic development was synchronous between the sexes. Temperature is the major factor in controlling the reproductive cycle in *G. expansa*.

Keywords: *Geloina expansa*, gonadosomatic index, spawning periods.

Introduction

The mud clam *Geloina expansa* from the family Corbiculidae lives semi-infaunally on the soft sediment that is accumulated around the roots of the mangrove trees and spends a considerable portion of its life exposed to air in mangrove swamps where salinity fluctuates greatly (Ingole *et al*., 2002). The coastal area of Shwe Thaung Yan (study area) is located in the western part of the Ayeyarwady Division and about 48 km west far from Pathein. This coastal region is known for its extensive local fisheries and mudflat and mangrove forests, which are home to a vast variety of naturally occurring crustaceans. Despite being a naturally occurring bivalve, the clam is harvested for its edible qualities and has not been commercially farmed.

Reproduction is an important aspect of the life history of any species and having an understanding of reproductive processes is central to the management of any commercial fishery (Barber and Blake 2006). Generally, the reproductive cycle of marine invertebrates, mainly bivalves is mostly influenced by adjacent environmental parameters, and their gonads could vary from place to place over a year (Drummond *et al*., 2006). Inter-area differences in reproductive cycle and breeding patterns have been noticed in bivalve communities and the differences emerge to be linked with variations in food and temperature (Delgado and Camacho, 2005).

Histological preparation of gonads on reproductive cycle studies of clams to date is the most reliable where detailed information can be obtained (Hartati *et al.,* 2005). However, the gonad in Corbiculidae is difficult to distinguish especially in the females during the off breeding season (Morton, 1982). The production of this species from its natural habitat in this area is still unknown and unreported. A poor understanding exists of the underlying mechanisms that influence *G. expansa* reproduction, as well as the potential contribution of these factors to the ongoing clam supply. A thorough understanding of these elements is necessary for the efficient management of this significant marine resource. The goal of this study was to highlight the key elements of the reproductive biology of *G. expansa* is a determining component of the population dynamics in bivalves. Detailed knowledge of these factors is required for the effective management of this important marine resource.

*Special Award (2023)

[1] Department of Marine Science, Pathein University, Myanmar

Materials and Methods

Study area and sampling procedures

Shwe Thaung Yan coastal region (Lat. 17˚ 05' 54" N and Long. 94˚ 28' 52" E) is located in the western part of Ayeyarwady Division and about 48 km west from Pathein. The present study was conducted along the U-To tidal creek. The map of the sample collection area is shown in Figure 1. Physico-chemical properties of adjacent estuarine water such as temperature, salinity, pH, and dissolved oxygen were measured and recorded using a thermometer, pH meter, and refractometer during sampling.

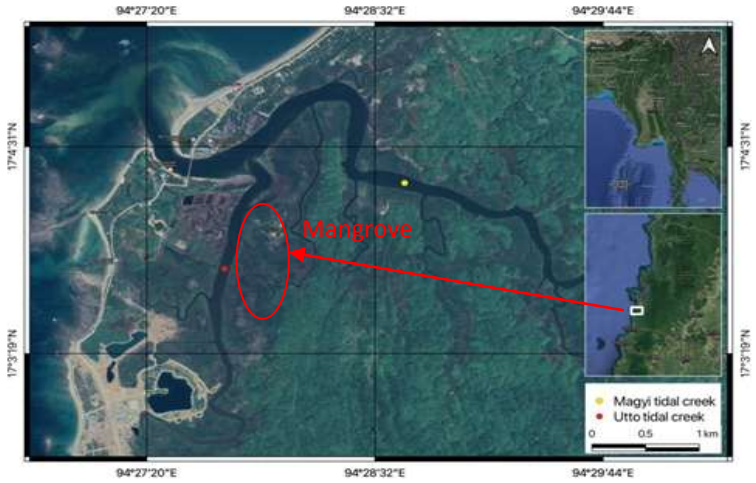

Figure 1. Map showing the sample collection area.

Clam samples were collected monthly by random sampling method from mangrove areas of the Shwe Thaung Yan region between May 2021 and April 2022. During the present study, a total of 692 clam samples (319 males and 373 females) ranging in size from 34.93 mm to 87.10 mm total length and 32 to 149.3 g body weight were examined. The shell was opened by inserting a paring knife between the valves to cut the anterior and posterior adductor muscles. The soft tissues were shucked and the sexes were recorded based on the color of the gonad. The gonad in females is black and in males is creamy white (Fig. 2). When in doubt, a gonadal smear was examined under the microscope for sperm or eggs.

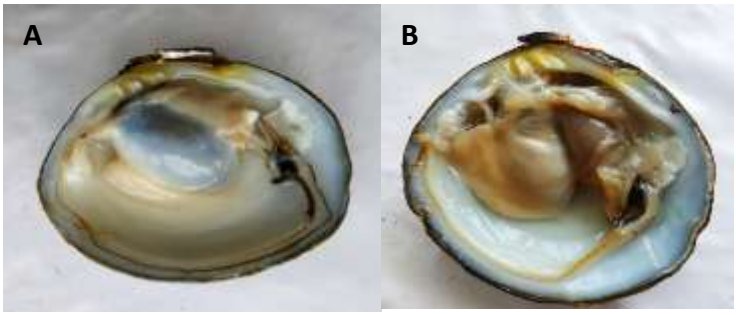

Figure 2. Anatomy of male and female *G. expansa*. A. Female; B. male.

After the samples were dissected to examine sex, the gonads were fixed in 10% buffered formalin for 48 hours and then the gonadal tissues were then embedded in paraffin blocks. After embedding the tissues, the paraffin blocks were trimmed to facilitate accurate sectioning. The blocks were then sectioned at 5 to 7 µm thickness using a microtome. The sections were then mounted on slides and dried overnight in an incubator at around 40˚C. Staining was performed using Haematoxylin and Eosin through standard methods. Histological slides were examined under the microscope, and photographed. Gonadal maturation stages were classified based on the

classification system reported by Gimin (2005). The stages of gonad development were classified as primordial, developing, maturing, partially spawned, and spent.

The gonadosomatic index (GSI) was calculated using the formula given by Banerjee (2004).

$$GSI = \frac{\text{weight of gonad}}{\text{weight of fresh meat}} \times 100$$

Results

Environmental Parameters

In the present study, the environmental parameters showed small differences among monthly measurements of salinity and DO for water quality (Table 1). The lowest temperature (27˚C) was recorded in August 2021 and the highest (28.5˚C) in May 2022. Salinity at Shwe Thaung Yan fluctuated between 17 and 32 ‰. The lowest salinity was recorded in November 2021 and the highest in May 2021. The pH of the water was highest in February, and March 2022 (5.3) and lowest in August 2021 (3.3). The pH of the seawater at the sampling site did not show any seasonal fluctuation. The minimum and maximum values for dissolved oxygen (DO) were recorded (4.2 mg/L) in April and (5 mg/L) in October respectively.

Table 1. **The monthly variations of environmental parameters (temperature, salinity, pH, and dissolved oxygen) in the Shwe Thaung Yan region from May 2021 to April 2022**.

Months	Parameters			
	Salinity (‰)	Temperature (°C)	pH	Dissolved oxygen (mg^{-1})
May 2021	30	28.5	4.2	4.5
June	20.5	27.3	4.1	4.9
July	21	27.6	4	4.7
August	22.5	27	3.3	4.5
September	18	28	4	4.6
October	18.5	28.5	4.2	5
November	17	28.2	4.3	4.9
December	18	26.5	4.6	4.9
January 2022	22.5	28.3	5	4.8
February	24	27.5	5.3	4.6
March	29.5	28.2	5.3	4.4
April	30	28	4	4.2

Gonad development stages

Six stages of gonad development were identified in *G. expansa* both for males and females are primordial, developing, maturing, partially spawned, and spent (Figs. 3 and 4, A-F). The colors of gonads were grayish in females, and whitish in males, and no hermaphrodite was found in *G. expansa* at Shwe Thaung Yan. Different gonad development stages for both sexes are summarized in Table 2.

Reproductive cycle of *G. expansa*

The reproductive cycle of *G. expansa* from Shwe Thaung Yan mangrove areas is presented in Figures 6 and 7. Most of the sampling months were predominated by maturing and ripe individuals. Only the immature stage (Stage I) occurred mainly from December to April. The maturing stage (Stage III) was encountered in almost all months and the percentage occurrence of ripe individuals (Stage IV) peaked in October followed by a decrease in July and December.

The partially spawned stage (Stage V) started to be found in June, July, November, December, and January with the maximum percentage occurrence in November. The spent stage (Stage VI) was observed from June to July and from November to January, and the maximum percentage was recorded in January.

Table 2. Description and criteria for the gonadal stages of *G. expansa*

Gonadal stages	Histological characteristics	
	Males	**Females**
Primordial	The Gonad area is fully covered by the connective tissue and present in the lumen (Fig 3, A).	Fully covered by connective tissue and less empty acini appeared (Fig 4, A).
Developing	Acinus forming stage, intensive spermatocytes,and spermatogonia stages. (Fig 3, B).	Acinus is visible and the diameter increased. (Fig 4, B).
Maturing	Ascinus reaches to maximum size and visibility of spermatozoa (Fig 3, C).	Filled with packed oocytes and nucleus was able to be seen (Fig 4, C).
Ripe	Stripes of spermatozoa occupy almost the whole area of the follicle leaving a very narrow strip along the periphery for the spermatogonia (Fig 3, D).	A small number of oocytes are still attached to the follicular wall by a thin stalk. Most oocytes are polygonal (Fig 4, D).
Partially Spawned	The number of spermatozoa decreases in some follicles. Spermatids occupy the area vacated by the spermatozoa (Fig 3, E).	Some follicles contain mature ova. Follicles reduce in size, particularly in those devoid of ova (Fig 4, E).
Spent	Most of the follicles are empty. Empty follicles with irregular and elongated shapes. Phagocytes are active (Fig 3, F).	Follicles are empty and the follicular wall collapses in places. Some residual mature oocytes are still present in the empty lumen (Fig 4, F).

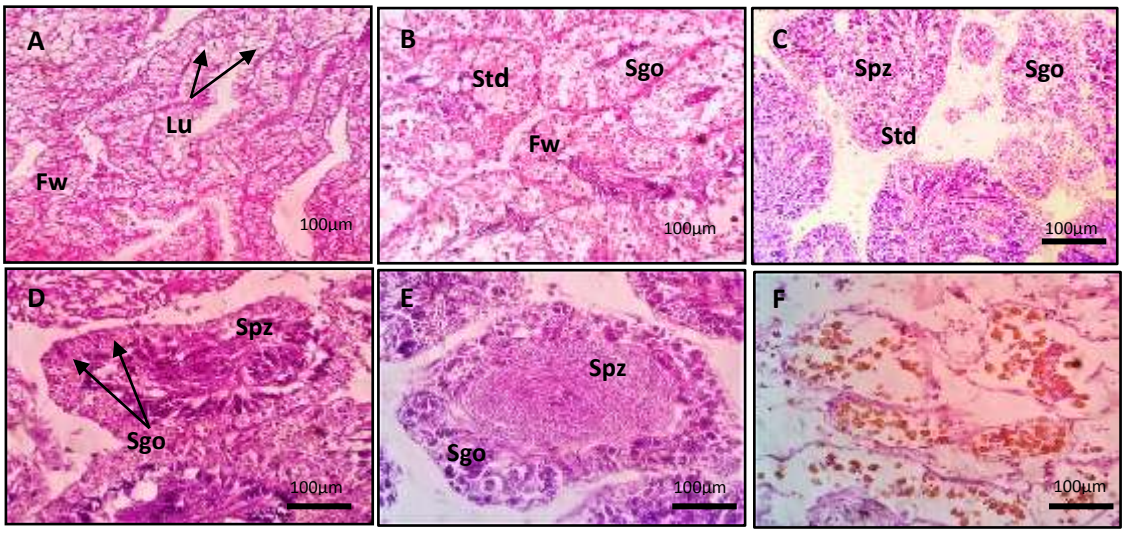

Figure 3. Gonad development stages in females of *G. expansa* (A) Primordial, (B) Developing, (C) Maturing, (D)Ripe, (E) Partially Spawned, (F) Spent. FW=follicular wall; L=lumen; DO= degenerating oocytes; sgo=spermatogonium; std=spermatid; spz=spermatozoa; -spermatozoa about to release from the follicle; MO= developing oocytes.

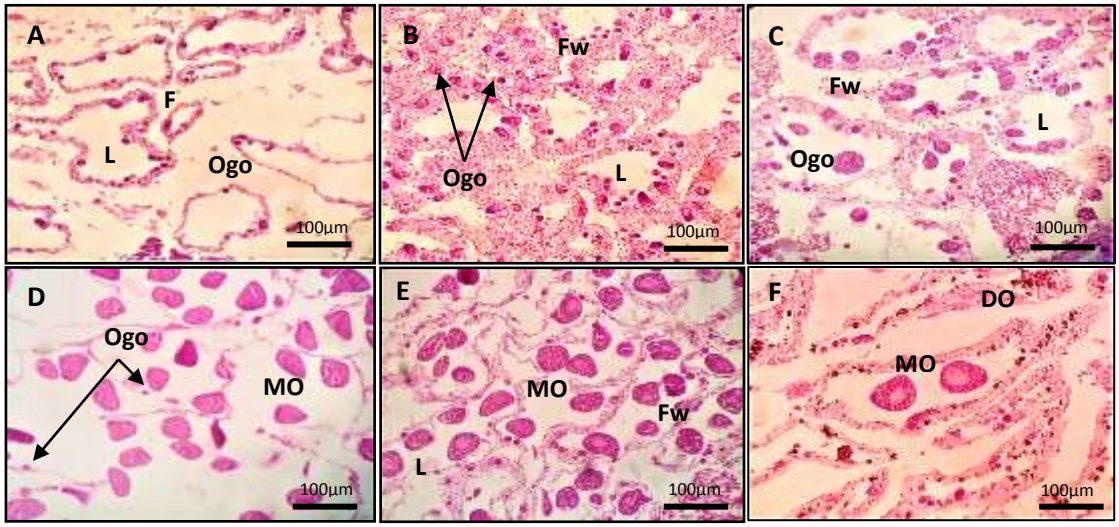

Figure 4. Gonad development stages in females of *G. expansa* (A) Primordial, (B) Developing, (C) Maturing, (D)Ripe, (E) Partially Spawned, (F) Spent. FW=follicular wall; L=lumen; DO= degenerating oocytes; sgo=spermatogonium; std=spermatid; spz=spermatozoa; - spermatozoa about to release from the follicle; MO= developing oocytes.

Gonadosomatic index (GSI)

The result of the monthly trend of the Gonadosomatic Index of males and females was expressed in Table 3 and Fig. 5. An increase in GSI values indicates the development of gonads. It is observed that the highest average GSI values were obtained in May 2021 and November 2021. The value continuously decreased from June to October and reached its peak in November. After that, it decreased from December to February 2022 and increased again in March 2022 and April 2022. It is observed that the lowest average GSI values were obtained in August and September. The monthly average GSI values of males were always higher than those of females in all months.

Table 3. Monthly average GSI values of *Geloina expansa.*

Sex	Months											
	May 2021	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan 2022**	**Feb**	**Mar**	**April**
Male	2.02 ±0.6	1.01 ±0.2	0.83 ±0.6	0.54 ±0.3	0.71 ±0.6	0.97 ±0.4	1.88 ±0.7	1.34 ±0.2	1.15 ±0.8	1.02 ±0.6	1.34 ±0.2	1.40 ±0.6
Female	1.80 ±0.6	0.86 ±0.1	0.72 ±0.4	0.49 ±0.2	0.62 ±0.2	0.85 ±0.3	1.75 ±0.5	1.24 ±0.3	1.06 ±0.2	0.92 ±0.6	1.21 ±0.3	1.22 ±0.6

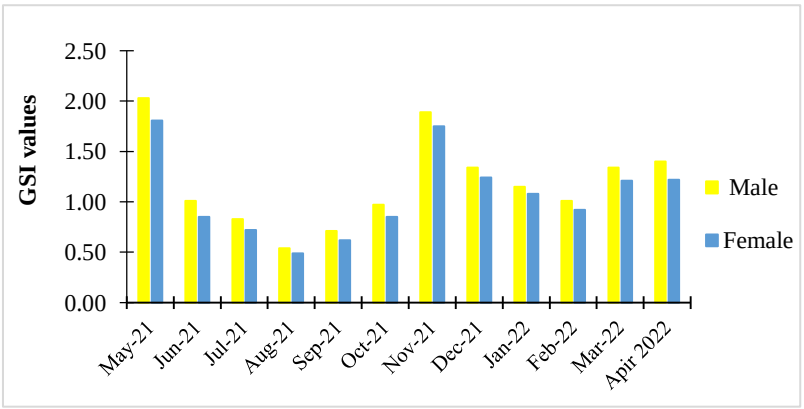

Figure 5. Monthly average GSI values of males and females of *Geloina expansa.*

Relation between Temperature and gonad maturity stages of *G. expansa* in Shwe Thaung Yan mangrove areas

The seasonal variations in water temperature exhibited a gradual increase from the beginning of the post-monsoon months to reach a peak at the end of the pre-monsoon season. A decrease in the temperature during the monsoon season into the post-monsoon season was evident (Fig 6 and Fig 7). At the Shwe Thaung Yan region, gametogenesis of *G. expansa* peaks when temperatures are lowest (Fig 6 and Fig 7). Thus temperature appears to affect reproductive patterns in this clam, at least for part of the year. Water temperature in the present study showed considerable variation with values of 25.5- 28.5 which influenced bivalve reproduction.

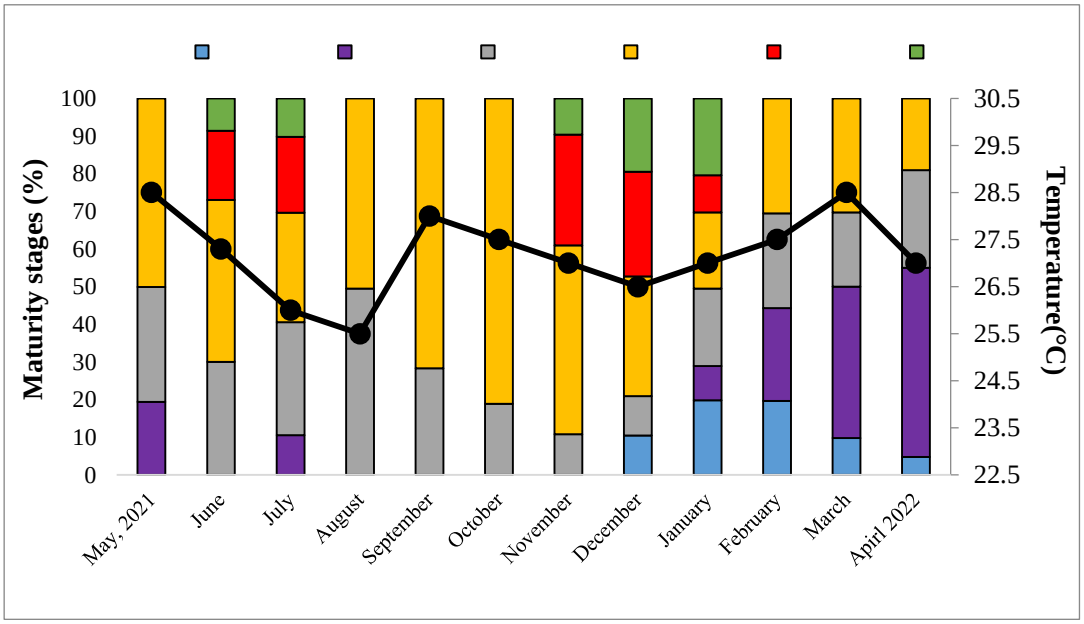

Figure 6. Relation between water temperature and gametogenesis of male *Geloina expansa* in Shwe Thaung Yan mangroves areas.

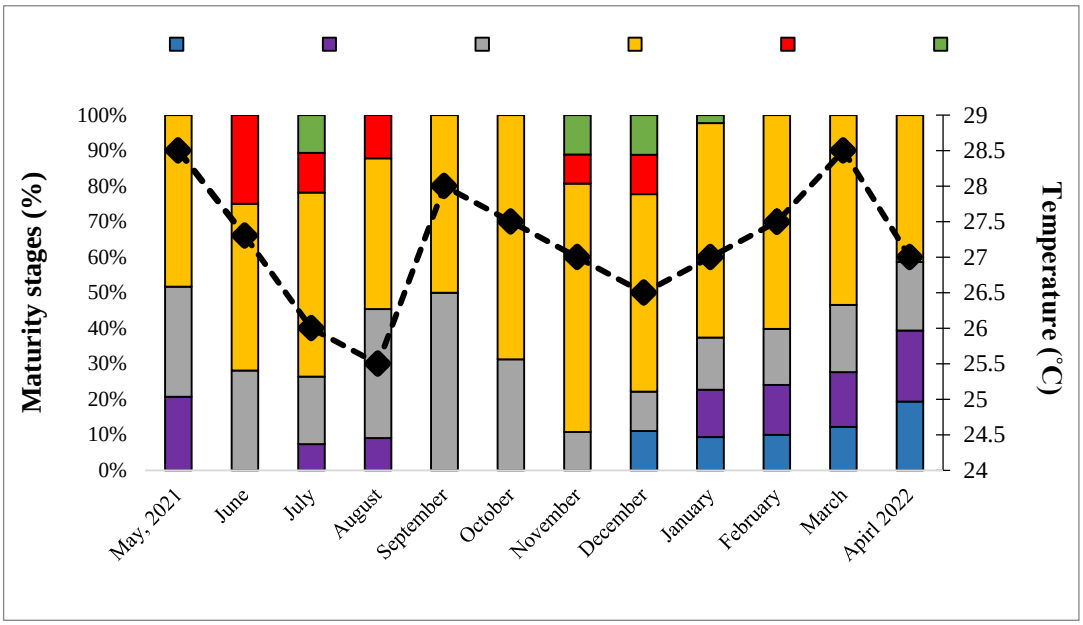

Figure 7. Relation between water temperature and gametogenesis of female *Geloina expansa* in Shwe Thaung Yan mangroves areas.

Discussion

The process of reproduction is the generation of new individuals that have the potential to become members of the population (Clemente and Ingole 2009). In the present study, the maturity stages of gonads were studied on a morphological basis as well as microscopically. Based on the microscopic examination followed by Gimin 2005, the maturity stages of *G. expansa* were recorded and categorized into six stages. From the results of the monthly percentage distribution of male maturity stages of *G. expansa,* most of the sampling months were predominated by maturing and ripe individuals.

In general, the *Geloina expansa* population from Shwe Thaung Yan mangrove areas was continuously breeder as confirmed by both GSI and histological data. However, the population showed some seasonality in spawning intensities. Continuous breeding with different spawning intensities is typical for tropical bivalves (Gimin, 2005). In the present study, the population in Shwe Thaung Yan mangrove areas had two major spawning periods i.e., a short breeding season in November and an extended season occurring between May, June, and July. This gametogenic pattern is similar to the *P. erosa* population in Hong Kong and Chareao island, Indian mangroves which is a seasonal breeder with an extended single breeding period (Morton, 1982, Clemente and Ingole 2007). Gimin (2005) suggested that the spawning season of *P. erosa* is restricted to around 4 months beginning from June to early October, with major events occurring during August-September. It is well known that the duration of the spawning period varies in a species occurring in different parts of its geographic range.

Idris *et al.* (2017) described that from March to November, the gonads were maturing and the spawning started. Gonads were observed to be spawned in September and continued throughout the year becoming less in percentage. They also stated that the spawning season of *P. erosa* in the mangrove site of Kelulit River, Miri, Sarawak, Malaysia was thus characterized by all-year-round spawning, although only in September spawning occurrence maximum frequency.

The gonadosomatic index (GSI) has been used in this study to follow the gametogenic cycle. This index increased before spawning due to the gametogenic development that produced an increase in the size of the gonad, which resulted in a rapid increase of the index throughout the spawning season. In the present study, the monthly gonadosomatic index value of *G. expansa* in the present study showed that it ranged from 0.54 to 2.02 in males and from 0.62 to 1.80 in females. The monthly average GSI values of males were always higher than those of females in all months.

According to Idris *et al*. (2017), the GSI showed a seasonal trend along the year, with high values related to mature individuals while the fall of values was due to spawning activity. In the present study, the GSI values for pooled *G. expansa* were higher in May and November and were lower in June, July, August, September, October, December, and January. Clemente and Ingole (2009) observed that the GSI values decreased from August to September due to the spawning period.

Temperature has been a major factor in controlling the reproductive cycle in various bivalves (Delgado and Camacho, 2005). Many bivalves require a minimum threshold temperature for activation of the oocyte growth phase and although oogonia can develop below this threshold level further differentiation only takes place at warmer temperatures. In temperate areas, temperature plays a very important part in triggering bivalve gametogenesis and spawning (Cross *et al*., 2012; Galimany *et al.,* 2015). At the Shwe Thaung Yan region, gametogenesis of *G. expansa* peaks when temperatures are lowest. Thus, temperature appears to affect reproductive patterns in this clam, at least for part of the year.

Besides that, the changes in water salinity affect a wide variety of biochemical, and physiological processes and growth in marine bivalves. In the present study, spawning occurred immediately when salinity dropped drastically in June 2021 despite large numbers of ripe

individuals. Although spawning in November 2021 did not correspond to the lowest salinity, indicated that low salinity did not correspond with the spawning phase.

Conclusions

In the present study, some reproductive parameters such as the maturity stages with histological analysis, and gonadosomatic index (GSI) of *G. expansa* were observed from May 2021 to April 2022 in Shwe Thaung Yan mangrove areas. From the result of this study, it was concluded that *G. expansa* was recorded as two times spawning periods, the majority of spawning periods were recorded in the summer months of May, June, and July and the second spawning (minor spawning) took place in or around November. In addition to the monthly occurrence of GSI values, the peak GSI values were recorded in May and October for both males and females, thus it was the spawning period seemed to occur in June, July, and November. These results revealed that *Geloina expansa* is dioecious and gametogenic development was synchronous between the sexes. Temperature is the major factor in controlling the reproductive cycle in *G. expansa*.

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POPULATION PARAMETERS OF ASIATIC HARD CLAM, *MERETRIX MERETRIX* (LINNAEUS, 1758) IN YE ESTUARY, SOUTHERN MON COASTAL AREA

Zarni Ko Ko[1]

Abstract

The population parameters, growth, mortality, and exploitation rates of Asiatic hard clam *Meretrix meretrix* were investigated in Ye estuary between May 2022 and January 2023. Monthly shell length frequency data of *M. meretrix* were analyzed for estimation of population parameters such as asymptotic length (L_{∞},), growth coefficient (K), and recruitment pattern to calculate the status of the stock. The asymptotic length (L_{∞}) was 40.95 mm and the growth coefficient (K) was 3.51 per year. The growth performance index (ϕ') was 2.68. The total mortality rate (Z) was estimated by length-converted catch curve at 6.71 per year, fishing mortality (F) at 3.79 per year, and natural mortality (M) at 2.92 per year. The recruitment pattern was continuous with one major peak event per year. The exploitation level E of *M. meretrix* was 0.56. Virtual population analysis estimated that the maximum fishing mortality of *M. meretrix* is collected between the mid-length 36 mm and 39 mm with the maximum F value (5.84 per year). The value of exploitation level E = 0.56 which pointed slightly overfishing condition (E > 0.50) for *M. meretrix* in Ye estuary during the present study.

Keywords: Growth and mortality, exploitation and recruitment, *Meretrix meretrix*, Ye estuary, Southern Mon Coastal Area.

Introduction

Many bivalve species have important economic roles including fisheries. Bivalves provide humans with food and decoration. A large number of different species of molluscs are eaten worldwide, either cooked or raw. Clams are commercially exploited and shipped as part of the international trade in shellfish, other species are harvested, sold, and consumed locally in tropical countries (Babaei *et al.,* 2010).

Mon coastal area is one of the most densely populated states in Myanmar. Fish and fish products are an important part of the diet in Myanmar and Mon coastal area is the main role of the fishery sector for local and export for earning foreign currency. In the Mon coastal area, Ye estuary areas such as Zeephyuthaung and Asin villages support a rich fishery in varied intensities, constituting Bombay duck, anchovy, mackerel, threadfin, and clam.

The Asiatic hard clam *(*Meretrix *meretrix)* was widely distributed in the Indo-Pacific region. This clam species was an active burrower and a suspension-feeding species that grew well in intertidal areas with muddy or silty substrate types such as mangroves and estuaries, making it easily accessible to glean. The clam is a commercially important species in coastal areas of South and Southeast Asia collected by artisanal fishermen either for consumption or direct selling in markets (Sienes *et al*.,2022).

The most commonly utilized bivalves for food include clams (Veneridae), sea mussels (Mytilidae), and edible oysters (Ostreidae) in the present day. Clams are considered to be nutritious and delicious and are fished in considerable quantities in some coastal areas. They are exploited in large quantities by traditional methods and sold live and dried in markets for human consumption in Ye estuary. Clams and other bivalves of their kind are usually handpicked in shallow waters at low tides in the Ye estuary area. In the present study area, most of the villagers depend on clam fishery for their livelihood. This fishery is the main job for the villagers, especially women whose incomes depend on those fisheries.

[1] Department of Marine Science, Pathein University

The bivalve fisheries have been studied near Myeik coastal areas such as Nat-eain-kan, Pyin-bu-nge, and Ma-san-pa (Phyu *et al*., 2019). However, there were no studies on the population dynamics of the hard clams in the Mon coastal area. In the present investigation, 741 specimens of the hard clams were recorded. The hard clams were recorded as ranging from 17.6 mm to 39.8 mm in shell length. Asiatic hard clam *(Meretrix meretrix)* was one of the most important bivalve species in the present study area. Therefore, the population dynamics were estimated to manage the hard clams' biomass in the present study.

Materials and Methods

Study site and Sample Collection

The study was conducted in Ye Estuary area, Mon State with coordinate 15°13'N 97°34'E during the period from May 2022 to January 2023 (Figure 1).

Monthly sample collection was conducted from May 2022 to January 2023 in the intertidal and subtidal zone of Ye estuary which has a sandy-muddy substrate. Shell length (the maximum distance between the anterior and posterior margins of the shell) was measured to the nearest 0.1mm using Vernier callipers in field trips.

Population Growth Parameters

The parameters of the von Bertalanffy Growth Function (VBGF), the asymptotic length (L_∞), and growth curvature (K) were analyzed by the method of ELEFAN-I and FAO-ICLARM Stock Assessment Tools-II (FiSAT-II) (Pauly, 1984).

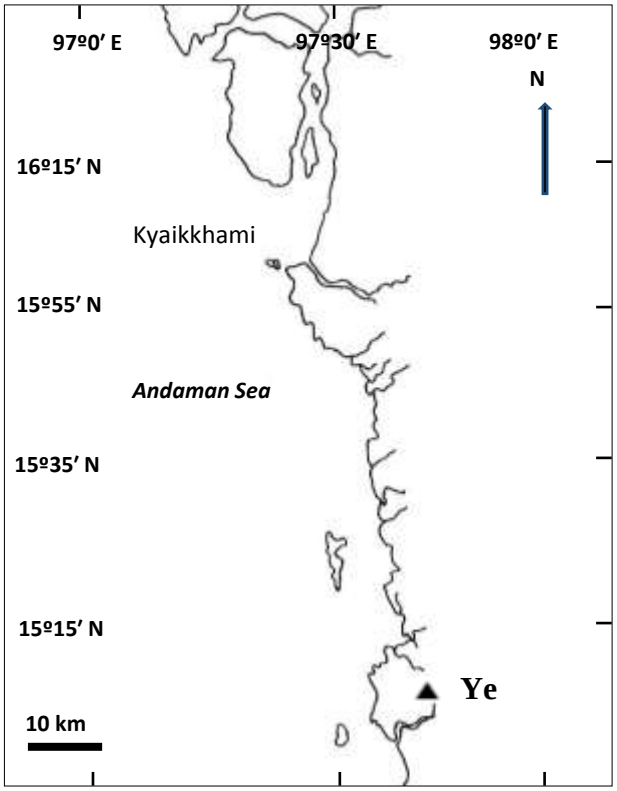

Figure 1. Map showing the present study site of the Ye Estuary area.

The resultant values of growth parameters (L_∞, K, t_0) were substituted in the von Bertalanffy growth equation: $\mathbf{L_t = L_\infty (1\text{-}e^{-K(t-t_0)})}$

Where L_t is the length at age t, L_∞ is the asymptotic length that is the mean length fish would reach if they were to grow indefinitely; K is the growth coefficient or the rate at which L_∞ is approached and t_0 is the age of the fish at zero length.

The resultant L_∞ and K were used to calculate the growth performance index (ø′) using Pauly and Munro, 1984's equation: $\boldsymbol{ø' = 2 \log L_\infty + \log K}$

Mortality Parameters and Exploitation Rates

The length-converted catch curve was utilized for the calculation of the instantaneous annual mortality rate (Z) (Pauly, 1984). The natural mortality (M) was calculated by Pauly's empirical equation: $\boldsymbol{\text{Log } M = - 0.0066 - 0.279 \log L_\infty + 0.6543 \log K + 0.4634 \log T}$

where T = the mean annual water temperature °C, which is assumed to reflect the sea surface temperature in the survey area (Pauly, 1984). In this study, the mean annual temperature of the sea surface was considered as 16.6°C.

The fishing mortality (F) was calculated by subtracting the natural mortality from the instantaneous annual mortality: $\boldsymbol{F = Z - M}$ (Appeldoom, 1984) (as cited in Pauly, 1984). The exploitation rate (E) was calculated using the following formula (Gulland, 1985): $\boldsymbol{E = F/Z}$ (as cited in Pauly, 1984).

Recruitment Pattern and Virtual Population Analysis

Recruitment patterns were obtained by projecting the length frequency data on the time axis using the estimated values of the growth parameters using the ELEFAN I programme. The length frequency data were used to carry out virtual population analysis (VPA) using the FiSAT (FAO-ICLARM Stock Assessment Tools) as explained in detail in the software computer package. The values of L_∞, K, and F, a (constant) and b (exponent) for the species were used as inputs to VPA analysis (Pauly, 1984).

Results

Length Frequency Distribution

The shell length frequencies of 741 *Meretrix meretrix* which ranged from 17.6 mm- 39.8 mm were analyzed. The maximum number of clams was found distributed in the 29 to 31 mm size group (22.7 %) followed by the 32 to 34 mm size group (19.3 %). Therefore, the clam's distribution indicated that the size group of 29 to 31 mm was predominant in the natural habitat during the present study period. The remaining clams were distributed from 26 to 28 mm (18.8 %), 23 to 25 mm (11.9 %), and 35 to 37 mm (11.6 %) in Table. 1 and Figure. 2.

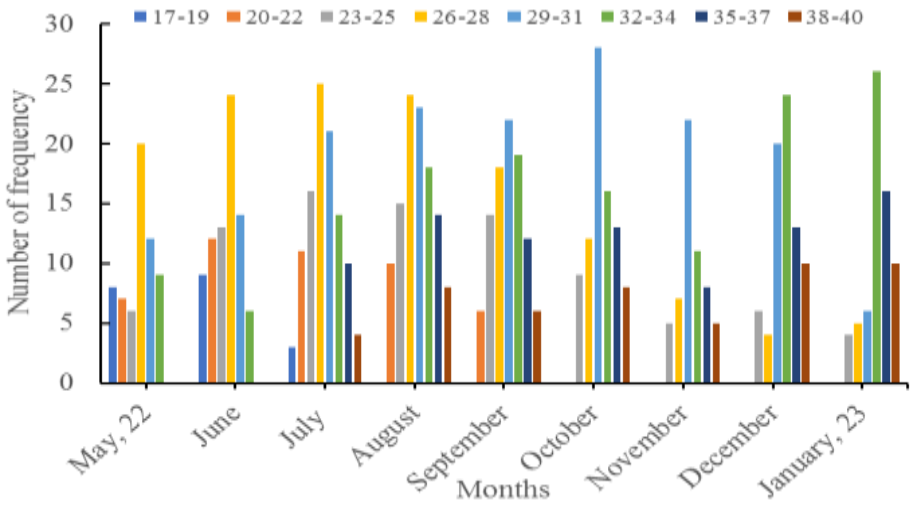

Figure 2. Monthly shell length frequency distribution of *Meretrix meretrix* during the present study.

Table 1.Monthly shell length frequency distribution of *Meretrix meretrix* during the present study.

Shell length (mm)	May, 22	June	July	Aug	Sept	Oct	Nov	Dec	Jan, 23
17-19	8	9	3						
20-22	7	12	11	10	6				
23-25	6	13	16	15	14	9	5	6	4
26-28	20	24	25	24	18	12	7	4	5
29-31	12	14	21	23	22	28	22	20	6
32-34	9	6	14	18	19	16	11	24	26
35-37			10	14	12	13	8	13	16
38-40			4	8	6	8	5	10	10
Total	62	78	104	112	97	86	58	77	67

Growth Parameters and Age

Based on shell length-frequency data, ELEFAN-I estimated growth parameters such as asymptotic length (L_∞) and annual growth coefficient (K) for *Meretrix meretrix* were 40.95 mm and 3.51 per year, respectively (Figure 1 (A and B)). The growth performance index or phi prime value (ø′) for *M. meretrix* recorded from Ye estuary was 2.68 (Table 2). In the present, it was estimated that *M. meretrix* attains a length of 33.87 mm, 39.73 mm, 40.74 mm and 40.91 mm at the end of 0.5, 1.0, 1.5, and 2 years of life its lifespan, respectively (Figure 1 (C)).

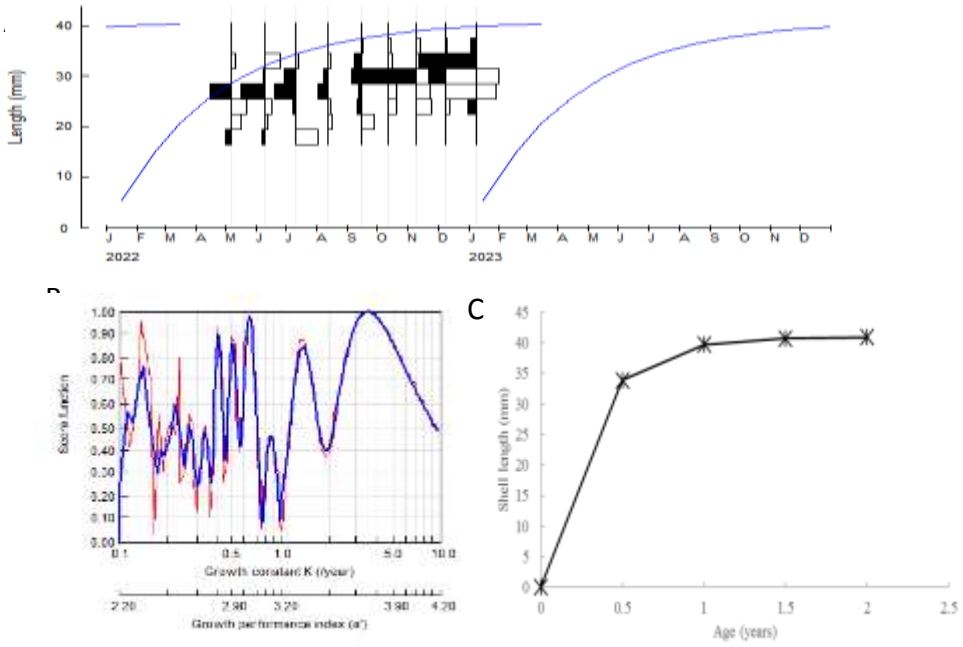

Figure 3 (A-C). A) Restructed length frequency distribution with growth curves superimposed (L_∞ = 40.95 mm and K = 3.51per year) of *M. meretrix*, B) Estimation of K of *M. meretrix* and C) Plot of age and growth of *M. meretrix* based on computed growth parameters during the present study.

Mortality parameters and Exploitation rate

The estimated value of the total mortality coefficient (Z) was found as 6.71 per year using the length-converted catch curve method (Figure 4). The value of natural mortality (M) and fishing mortality (F) was estimated as 2.92 per year and 3.79 per year, respectively (Table 2). The estimated value of exploitation rate E was 0.56 which indicated the overexploitation conditions (E>0.5) (Table 2).

Recruitment Pattern and Virtual Population Analysis

The recruitment pattern of *Meretrix meretrix* was continuous throughout the year with one major peak. The percentage of the recruitment varied from 1.97% to 17.77% during the study period. It showed that the species peaked in August (Figure 5). The structured population analysis indicated the maximum and minimum fishing mortality was estimated as 5.8400 per year and 0.3289 per year for the mid-lengths of 36 mm and 18 mm, respectively. The fishing mortality was relatively higher over the mid-length size of 33 mm (Figure 6).

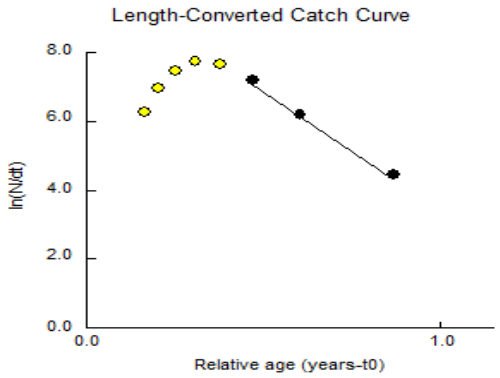

Figure 4. Length converted catch curve of *M. meretrix* during the present study.

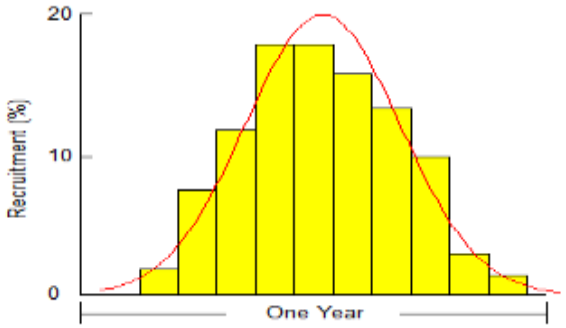

Figure 5. Recruitment pattern of *M. meretrix* during the present study.

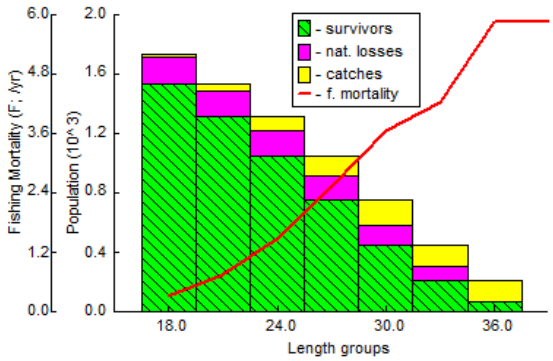

Figure 6. Virtual population analysis of *M. meretrix* during the present study.

Table 2. Growth, mortality and exploitation parameters of *Meretrix meretrix* in Ye estuary

Parameters	L_∞ (mm)	K (yr^{-1})	ø′	Z (yr^{-1})	M (yr^{-1})	F (yr^{-1})	E	Recruitment
Results	40.95	3.51	2.68	6.71	2.92	3.79	0.56	Unimodal

Discussion

The present investigation was carried out on the population dynamics of *Meretrix meretrix*, which is a commercially important bivalve species along the Ye estuary, Mon coastal area. The present observed shell length (17.6 mm- 39.8 mm) of *M. meretrix* was higher than the previous results; 24.15- 37.29 mm (Sienes *et al*., 2019) and 17.2- 39.1 mm (Sienes *et al*., 2022). The length of *M. meretrix* ranges from 11 to 46 mm recorded from Tanjung Balai, North Sumatera (Desrita *et al*., 2019) and 20 to 46 mm from Bancaran village waters, Bangkalan (Rohmah and Muhsoni, 2020). Few areas recorded higher lengths of *M. meretrix*, such as the southwest coast of Maharashtra, 21 to 55 mm (Sawant and Mohite, 2013); Mumbai waters, 38 to 57 mm (Sharma *et al*., 2005); and Kandleru Estuary, 39 to 63 mm (Thangavelu *et al*., 2008). The high length ranges of *M. meretrix* were recorded as 22.5 to 82.5 mm from the Moheskhali channel of Bangladesh (Amin *et al*., 2009) and 14.6 to 91 mm from Korampallam Creek, Tuticorin (Narasimham *et al*., 1988). The maximum size range of *M. meretrix* recorded from Telik Marudu, Malaysia was 18.3 to 101.7 mm (Admodisastro *et al*., 2021). The variation in *M. meretrix* size range might be due to the changes in biotic-abiotic factors, availability of food, geographical distribution, climate changes, and fishing efforts (Jayawiekrma and Wijeyaratne, 2009).

The estimated asymptotic length (L_∞) of 40.95 mm recorded in the present study was similar to the previous result from Panguil Bay (Sienes *et al*., 2022). The present L_∞ value was higher than the Asahen river (36.76 mm) and Batubara (39.90 mm) in the Tanjung Balai region, North Sumatra (Desrita *et al*., 2019). In the present estimation, the L_∞ value was obtained as 40.95 mm, which is lower than the earlier reported from Panguil Bay, 44.5 mm (Jimenez *et al*., 2009); from Tanjung Balai, 45.15 mm (Desrita *et al*., 2019); from Bancaran village water, 51.1 mm (Rohmah and Muhsoni, 2020); from the southwest coast of Maharashtra, 58.80 mm (Sawant and Mohite, 2013); from Kambu River estuary, 58.91 mm (Bahtiar *et al*., 2022); from Moheskhali channel of Bangladesh, 81.4 mm (Amin *et al*., 2009); from Korampallam Creek, 99.1 mm (Narasimham *et al*., 1988); and Telik Marudu, 107.63 mm (Admodisastro *et al*., 2021). The asymptotic length must be varied due to the variation in geographic distributions, fishing pressure, and local environmental situations (Gurjar *et al*., 2021). Similarly, the high fishing pressure and environmental conditions change found in the present study area. Therefore, the L_∞ value of the present study was different from the previous results.

In the present estimation, the K value was obtained as 3.51 per year, which was higher than earlier reports from different areas of the world (Table 3). The value of the growth coefficient (K) was associated with the mortality rates (as cited in Takar *et al*., 2022). Therefore, the K value was found that the high value was due to the high fishing activity in the present study area. Moreover, the present recorded growth performance index (ø′) for *Meretrix meretrix* was 2.68. It was higher than the earlier reported from the Moheskhali channel of Bangladesh (Amin *et al*., 2009) while lower than from Telik Marudu (Admodisastro *et al*., 2021) and from Panguil Bay (Sienes *et al*., 2022) (Table 3). The growth performance index indicated that high growth values showed a better and faster growth curve (Nadeem *et al*., 2017). Therefore, the present result showed that the growth rate of *M. meretrix* was good and fast.

The present estimation showed growth attained by *M. meretrix* of 33.87, 39.73, 40.74, and 40.91 mm at the end of 0.5, 1.0, 1.5, and 2.0 years respectively. From the southwest coast of Maharashtra, India, *M. meretrix* attained the length of 30, 42, and 45 mm at the end of one, two, and three years (Sawant and Mohite, 2013) and from Panguil Bay, Philippines, the species attained the length of 24.45, 32.84, 38.99, and 40.47 mm at the end of one, two, four and six years, respectively (Sienes *et al*., 2022). The present result was more similar to the study

conducted on Panguil Bay than on the southwest coast of Maharashtra, India, which might be attributed to the study area.

In the tropical regions where age structure data was not easy to determine, then the length converted catch curve method was used to determine the mortality with the length frequency distribution data. In the present study, the total (Z), natural (M), and fishing (F) mortality rates were estimated at 6.71, 2.92, and 3.79 per year, respectively. The mortality parameters of *M. meretrix* from other parts of the world are shown in Table 3. It was observed that overall values were lower than the present results. When the exploitation rate was greater than 0.5 it could be assumed that the clam stock from the areas was overexploited. Therefore, the present exploitation rate (0.56) showed that the clam stock was slightly overexploited condition. Moreover, the earlier estimated results from Jimenez *et al*., (2009), Admodisastro *et al*., (2021), Sienes *et al*., (2022), and Bahtiar *et al*., (2022) were similar to the present study (Table 3). However, the previous report from Amin *et al*., (2009) and Rohmah and Muhsoni (2020), found that the clam stock was underexploited (Table 3).

Table 3. Comparison of the growth, growth performance index, mortality, and exploitation parameters with previous studies from different regions of Asia.

L_∞ (mm)	K (yr^{-1})	ø′	Z (yr^{-1})	M (yr^{-1})	F (yr^{-1})	E	Locations	Literature
81.4	0.97	2.07	2.63	2.61	0.02	0.01	Bangladesh	Amin *et al*., 2009
51.1	1.10	-	3.21	1.69	1.52	0.47	Indonesia	Rohmah and Muhsoni, 2020
58.91	1.10	-	4.75	1.59	3.16	0.67	Indonesia	Bahtiar *et al*., 2022
107.63	0.47	3.736	2.65	0.78	1.87	0.70	Malaysia	Admodisastro *et al*., 2021
44.5	0.80	-	4.13	1.60	2.53	0.61	Philippines	Jimenez *et al*., 2009
40.95	0.71	3.07	3.18	1.22	1.96	0.62	Philippines	Sienes *et al*., 2022
40.95	3.51	2.68	6.71	2.92	3.79	0.56	Ye Estuary	Present study

Like other tropical bivalves, *Meretrix meretrix* could spawn throughout the year under favourable conditions due to minimal fluctuations in environmental parameters (Admodisastro *et al*., 2021). The one recruitment peak observed in the present study coincided with the unimodal result obtained from the assessment of *M. meretrix* in Panguil Bay (Jimenez *et al*., 2009) and the southwest coast of Maharashtra (Sawant and Mohite, 2013) while contrasting the bimodal result obtained from the assessment of species in Panguil Bay (Sienes *et al*., 2022).

The virtual population analysis showed that younger individuals of snubnose emperor were more susceptible to natural mortality caused by predation, pollution, or disease than fishing mortality. In the present study, high fishing mortality was found at the mid-lengths of 33 mm. The fishing mortality began to dominate at the length ranging from 26 mm, with the maximum value at a size of 36 mm. Therefore, the dominance due to fishing operations starting at 26 mm in size was an indicator that that species was caught after starting in the spawning activities for at least one time.

Conclusion

This study was the first report on the population dynamics of *Meretrix meretrix* from Ye estuary, southern Mon coastal area. The present study showed that the high fishing mortality and exploitation rate of *M. meretrix* stock indicating that it was overfished. The current study demonstrated *M. meretrix* population in Ye estuary was fast growing and recruitment happened throughout the year with unimodal conditions. Thus, the present study was the baseline information on the population dynamics for the sustainable utilization and conservation of *M. meretrix* in the Ye estuary. Moreover, it would form the basis for the scientific community and

conservation decision-makers to manage and reach a sustainable harvest of this resource with optimal exploitation.

Acknowledgements

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COMMERCIAL FISHES AND FISHERY OF THAE-CHAUNG AREA, SITTWE TOWNSHIP, RAKHINE STATE

Myo Min Tun[1]

Abstract

A total of 21 species (18 genera) belonging to 15 families, and 6 orders of collected commercial fishes were identified from fish landing sites of Thae-Chaung Area, Sittwe Township, Rakhine State from October 2022 to September 2023. The order of Perciformes was abundant possessing families, genus and species. Among them, the seabass, *Lates calcarifer* is exported to foreign countries. The species *Stolephorus commersonnii* (Nga-ni-tu) is a very popular dry fish species in Myanmar. The most widely used gears in the study area include cast nets, drift gill nets, bag nets, trammel nets, seine nets, man push nets, upright fish traps and lone line. Gill nets were larger in size and mesh size and higher in price and catch composition than other gears such as traps, surrounding nets, and hooks and lines. Thae-Chaung has an economy based on catching fish and producing dry fish.

Keywords: Diversity, Fishes, Fishing gears, Rakhine State, Sittwe Township, Thae-Chaung area, Uses.

Introduction

Myanmar is endowed with natural resources including rich and various aquatic fauna and flora due to its diversified and favourable climate, topography and habitats in the region (Hla Win *et al.* 2008). Fishes occur in lakes, streams, estuaries and oceans throughout the world. In most species of fish, all individuals live entirely either in fresh or in marine waters. Over 225 species are diadromous, regularly living part of their lives in lakes and rivers and part in oceans (Nelson *et al*. 2016). Many freshwater and marine species are also common in brackish water estuaries. In the oceans, the vast majority of fish are coastal or littoral. Fisheries and fishery resources for a country or region should be managed taking account of the social and ecological characteristics of the region or country. A unique characteristic of Rakhine fisheries is the high variety of fishery resources being exploited by wide-ranging fishing methods. This diversity shows that the country is bestowed with good natural resources and is rich in traditionally developed fishing gear. On the other hand, diversity is a major cause of complications in management and adjustment. Management measures produce different effects depending on the social and ecological characteristics of each fishing sector or area. A measure that has produced excellent results in one area can cause a negative impact on another area. Fine-tuned research on the impact of a measure is necessary. This study examined the status of fisheries and identified the commercial fishes of Thae-Chaung Area, Sittwe Township, Rakhine State.

Materials and Methods

Fish specimens were collected in fish landing sites of Sittwe environs, Rakhine State from October 2022 to September 2023. The location of the study area is shown in Figure 1. Color patterns and measurements of the specimens were recorded immediately after collection. For later studies, the fresh and intact specimens were carefully chosen to take photographs using a digital camera. Some samples were preserved in 10% formaldehyde seawater solution for identification and carried out in the laboratory, Department of Marine Science, Sittway University. The field visit was undertaken for a year and relevant data was obtained from a random sample of 20 fishermen and owners which interviewed in the study area. Interviews

[1] Department of Marine Science, Sittway University

contained questions on a day fishing activity, time and duration of fishing trip, characteristics of fishing method, catch size and composition, consumption and sale of fish etc. The identification was mainly based on FAO identification sheets and catalogues for fishery purposes. The classification system of sampled species was adopted by Carpenter (1988), Cohen *et al.* (1990), Collette and Cornelia (1983), Day *et al.* (1878), De Burin *et al.* (1995), Fischer and Whitehead (1981), Hla Win *et al.* (2008), Jayaram (1984), Khin Maung Aye *et al.* (2006), Lal Mohan (1984), Mckay (1992), Motomura (2004), Mya Than Tun (2001), Rainboth (1996), Russell (1990), Saski (2001), Su Su Hlaing (2010), Tint Swe (2011) and Ye and Kevern (2011).

Results and Discussion

Commercial fishes of Thae-Chaung Area, Sittwe Township, Rakhine State

A total of 21 species (18 genera) belonging to 15 families, and 6 orders of commercial fishes collected from Thae-Chaung Area, Sittween Township, Rakhine State have been recorded. The percentages of the orde-rwise in the study area are shown in Figure 4. The order of Perciformes is abundant and the second is the Scombriformes.

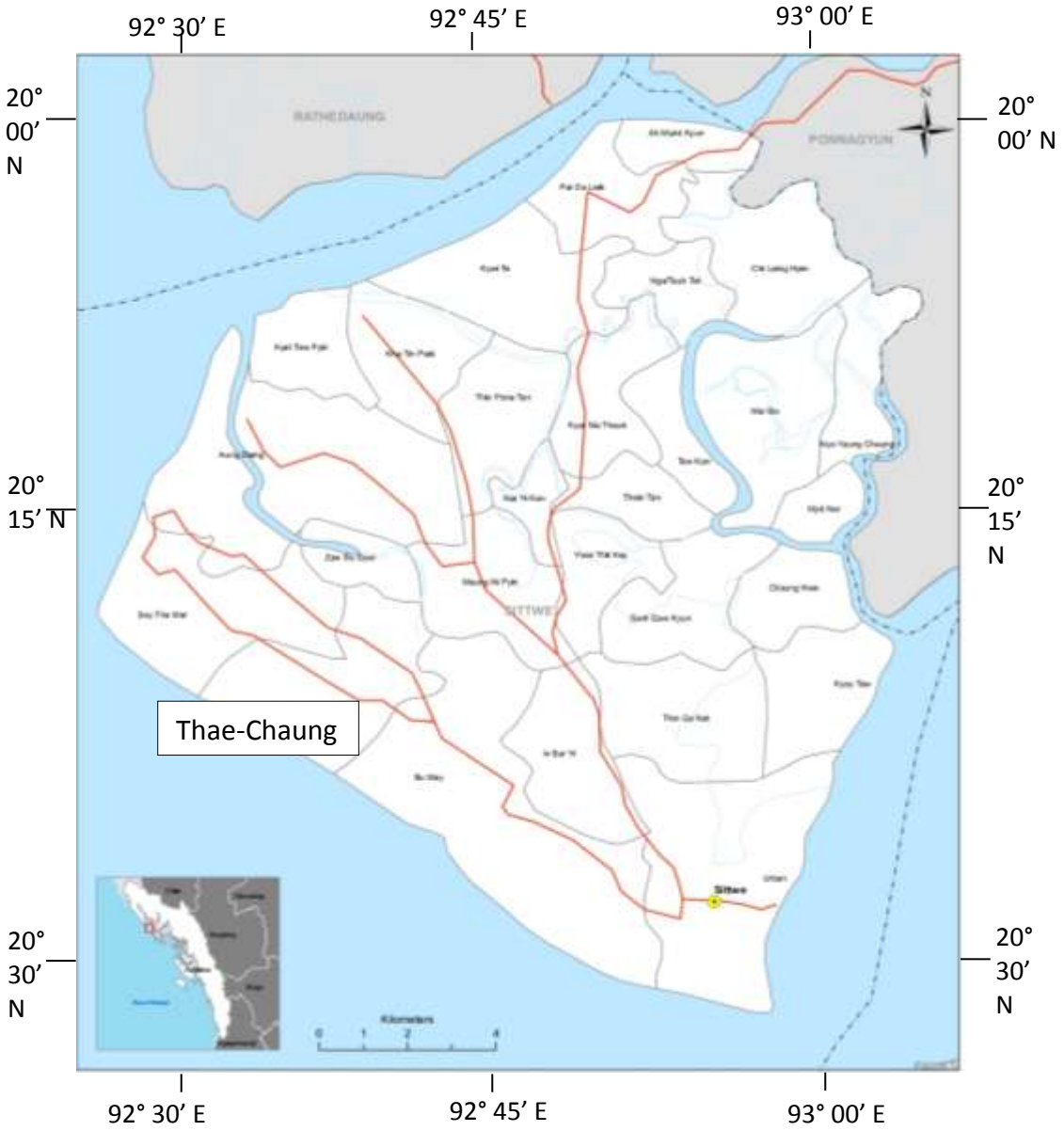

Figure.1. Map showing the sample collection sites of the study area.

Descriptions of commercial marine fishes in Thae-Chaung Area, Sittwe Township, Rakhine State

***Congresox talabon* (Cuvier, 1829) (Fig. 2A)**

Diagnostic Features: Body robust and eel-shaped; mouth very large, with gape reaching well beyond eye; dorsal fin inserted before gill-openings, pectoral fins relatively small, about 4 times in head length; lateral line pores before level of anus 41 or 42; dorsal fin rays before anus 70-75; dorsal fin inserted gill-openings; body is silver or yellow colour.

***Congresox talabonoides* (Bleeker, 1853) (Fig. 2B)**

Diagnostic Features: Body robust and eel-shaped; mouth very large, with gape reaching well beyond eye; dorsal fin inserted before gill-openings, pectoral fins relatively small, about 4 times in head length; lateral line pores before level of anus 41 or 42.

***Tenualosa toli* (Valenciennes, 1847) (Fig. 2C)**

Diagnostic features: Body fusiform; belly with fairly sharply keeled scutes. Dorsal fin origin a little before midpoint of body. Pelvic fins below anterior part of dorsal fin. Upper jaw with distinct medium notch when see from front: maxilla reaching to vertical from eye center or beyond. Gill rakers fine and numerous. Top of head without frontoparietal areas. Caudal fin larger than head. The back of the body greenish blue, flanks silvery.

***Sardinella gibbosa* (Bleeker, 1849) (Fig .2D)**

Diagnostic Features: Total number scutes 32 to 34. Vertical striae on scales not meeting at center, numerous small perforations on hind part of scale. A golden mid-lateral line down flank; dorsal and caudal fin margins dusky; a dark spot at dorsal fin origin. Lower gill rakers 45 to 59.

***Stolephorus commersonnii* Lacepede, 1803 (Fig. 2E)**

Diagnostic Features: Belly slightly rounded with 0-5 small needle-like pre-pelvic scutes. Maxilla tip pointed, reaching to or a little beyond hind border of pre-operculum, the latter convex, rounded. Small teeth on hyoid bones. Isthmus muscle tapering evenly forward. Body light transparent fleshy brown with a pair of dark patches behind occiput, followed by a pair of lines to dorsal fin origin. Bears a silver stripe on flanks.

***Mugil cephalus* Linnaeus, 1758 (Fig. 2F)**

Diagnostic features: Body rather stout, head broad and flattened on top. Adipose tissue cover eye; Lower lip very thin; a large symphysial knot front of lower jaw. First dorsal fin originated nearer snout tip than tail base. Dorsal, pectoral and pelvic fins had distinct axillary scale. Caudal fin forked. All soft rays are branched and segmented. Dark green on dorsal and silvery on lateral and ventral.

***Scomberoides tala* (Cuvier, 1832) (Fig. 2G)**

Diagnostic Features: Body oblong to elliptical, strongly compressed; snout pointed and nape slightly concave. Mouth large; eyes with well-developed adipose lid; teeth in jaw small. Two dorsal fins not widely separated; pectoral fin laced high; caudal fin deeply fork. Body bluish dorsally, white ventrally; vertically oblong black blotches, distal half of dorsal fin lobe abruptly and heavily pigmented; anal fin lobe usually immaculate.

***Drepane punctata* (Linnaeus, 1758) (Fig. 2H)**

Diagnostic Features: Color generally silvery with greenish tinge above. Pectoral fins long and pointed and having 4-11 vertical gray spots on the upper half of the sides and generally 8 dorsal spines.

Harpadon nehereus (Hamilton, 1822) (Fig. 2I)

Diagnostic features: Body elongate and compressed. Eyes small, mouth very wide armed with slender, recurved and pointed teeth. Lower jaw longer than upper jaw. Dorsal fin followed by a conspicuous adipose fin; pelvic fins are very long. Caudal fin trilobed, scales restricted to posterior half of the body. Lateral line extending into pointed medium lobe of caudal fin. Head, back and sides, light grayish.

Lates calcarifer (Bloch, 1970) (Fig. 2J)

Diagnostic Features: Body elongated or oblong, compressed, usually with concave dorsal profile with a deep caudal peduncle. Mouth is moderate or large, jaws equal or with lower longer than upper. Dorsal fin is either partly or wholly separated having a very deep notch almost dividing spiny from soft part of fin; pectoral fin short and rounded; anal fins are rounded and caudal are fork.

Lethrinus miniatus (Linnaeus, 1758) (Fig. 2K)

Diagnostic Features: Body moderately deep, its depth 2.4-2.8 times in standard length; head length 0.9-1 times in body length; dorsal profile near eye slightly convex; snout moderately long; its dorsal profile slightly concave snout angle relative to upper jaw between 50° and 65°; interorbital space convex to flat; posterior nostril an oblong longitudinal opening, closer to orbit than anterior nostril; eye situated close to dorsal profile; lateral teeth in jaws conical; pelvic-fin membranes between rays closest to body usually with dense melanophores; no scales on cheek; Colour of body silvery or yellowish, base of scales often black; base of pectoral fin red; 2 red spots often on upper rim of eye; lips reddish; fin pale or reddish.

Lutjanus johnii (Bloch, 1792) (Fig. 3A)

Diagnostic Features: Dorsal profile of head steeply sloped. Preorbital width equal to eye diameter or larger. Preoperculuar notch and knob poorly developed. Scale rows on back parallel to lateral line. Center of each scale often with a reddish-brown spot, giving an overall appearance of series of horizontal lines on side of body. Generally yellow with a bronze to silvery sheen, shading to silvery white on belly and underside of the head. A large black blotch mainly above the lateral line below the anterior dorsal-fin rays.

Lutjanus malabaricus (Bloch & Schneider, 1801) (Fig. 3B)

Diagnostic Features: Body compressed, additionally head length is two-thirds of body length. Snout lacrimal, and lower jaws naked. Mouth is terminal and fairly large having thick jaws. Dorsal fins single extending towards the caudal peduncle. The first part of dorsal is spines and the latter is soft rays. Pectoral fins are falcate and longer than pelvic fins. Color is dusky grayish and all fins with golden yellow.

Eleuthronema tetradactylum (Shaw, 1804) (Fig.3. C)

Diagnostic Features: Scale rows above lateral line 9-12, below 13-15; vomer with deciduous tooth plates on both sides except in juveniles; posterior part of maxilla deep, 3-4% of standard length; short tooth plate extension onto lateral surface of lower kaw, 7-9% of SL. Colour of upper sides of head and trunk with slight darkish silver tinge, becoming lighter in lower sides; anterior margins of first and second dorsal fins blackish, remaining parts translucent and slightly blackish respectively.

***Johnius coitor* (Ham, Buch, 1822) (Fig. 3D)**

Diagnostic Features: Body elongated and moderately compressed. Snout is swollen. Mouth is superior. Upper jaw is reaching to below the middle of eye. Swimbladder is hammer-shaped with 13 pairs of arborescent appendages. Dorsal fin is long divided by notch. Pectoral fin moderate. Caudal fin is rhomboid. Scales are cycloid. Light golden yellow with a purplish sheen. Soft part of dorsal, anal and caudal fins is grey borders.

***Auxis thazard* (Lacepede, 1800) (Fig. 3E)**

Diagnostic Features: Robusted body, elongated and rounded; teeth small and conical, in a single series; pectoral fins short, but reaching past vertical line from anterior margin of scales area above corselet; a large single-pointed (interpelvic process) between pelvic fins; which is well developed and narrow in its posterior part; a strong central keel on each side of caudal fin base between 2 smaller keels. Colour of back bluish, turning to deep purple or almost black on the head; a pattern of 15 or more narrow, oblique to nearly horizontal, dark wavy lines in scales area above lateral line; belly white; pectoral and pelvic fins purple, inner sides black.

***Scomberomorus guttatus* (Bloch & Schneider, 1801) (Fig. 3F)**

Diagnostic Features: Body elongated and strongly compressed. Snout pointed; mouth rather large; teeth present; lancet-shaped, laterally compressed. Bluish on back and silvery sides with several longitudinal rows of round dark brownish spots; first dorsal fin membrane black and posteriorly white. Two dorsal fins not widely separated; pectoral fins placed high; caudal fins deeply forked; lateral line almost straight to below middle of second dorsal fin and gently bent to the middle of caudal peduncle; finlets present.

***Rastrelliger faughni* (Matsui, 1967) (Fig. 3G)**

Diagnostic Features: Body oblong slightly compressed and deep. Snout pointed; eyes with broad anterior and posterior lids; lower jaw slightly the longer; bluish green back, and silvery sides and below; dorsal fin yellow tipped with black; some longitudinal black strips on body. Two widely separated dorsal fins; dorsal spines weak and receivable into a groove; pectoral fins placed high; caudal fins deeply forked; finlets present behind second dorsal and anal fins; two small keels present on each side of caudal peduncle.

***Rastrelliger kanagurta* (Cuvier, 1817) (Fig. 3H)**

Diagnostic Features: Body elongated, deep, and slightly compressed. Snout pointed. Eyes with broad anterior and posterior lids. Mouth rather large. Two widely separated dorsal fins. dorsal spines weak and receivable into a groove; pectoral fins placed high; caudal fins deeply forked; finlets are behind second dorsal and anal fins. Two small keels on each side of caudal peduncle. Lateral line very slightly curved. Colour bluish green back and silvery sides and below. Dorsal fin yellow tipped with black, caudal stained with black at its extremely, pectoral yellow.

***Pampus argenteus* (Euphrasen, 1788) (Fig. 3I)**

Diagnostic Features: Body very deep, oval and compressed. Head short. Mouth terminal. Eyes are large. Operculum absent. Dorsal and anal fins much elevated anteriorly with concave external margins. Pectoral fins rather pointed. No pelvic fins. Caudal fin deeply forked. Scales are small. Colour grey above and merging it silvery white to belly. Dorsal and anal grayish minutely dotted with black. Caudal and pectoral yellowish white also minutely dotted with black.

***Arius maculatus* (Thunberg, 1792) (Fig. 3J)**

Diagnostic Features: Head shield somewhat rugose; deep and long median fontanelle groove; Body shape lateral, fusiform; scale embedded or partially/completely absent; caudal fin forked; in addition to closure of the esophagal passage, considerable reduction of the palatine teeth is observed in sexually active males.

Thae-Chaung fishing village, Sittwe Township is situated on the Bay of Bengal in Lat. 20° 09´ N and Long. 92° 50´E. It is with an economy based on catching fish and producing dry fish. Situated on the Bay of Bengal at the mouth of the Kaladan River, Sittwe occupies the eastern side of a hilly ridge affording shelter from the southwest monsoon. Originally s small fishing village, Sittwe was catapulted into modernity when the British moved the capital of Myanmar here in the early 19th Century. Sittwe is at the confluence of the Mayyu, Kaladan and Lemro Rivers, all navigable. The present study was studied for the diversity of commercial marine fishes in Thae-Chaung Area, Sittwe Township, Rakhine State. A total of 21 species (18 genera) belonging to 15 families, 6 orders were identified.

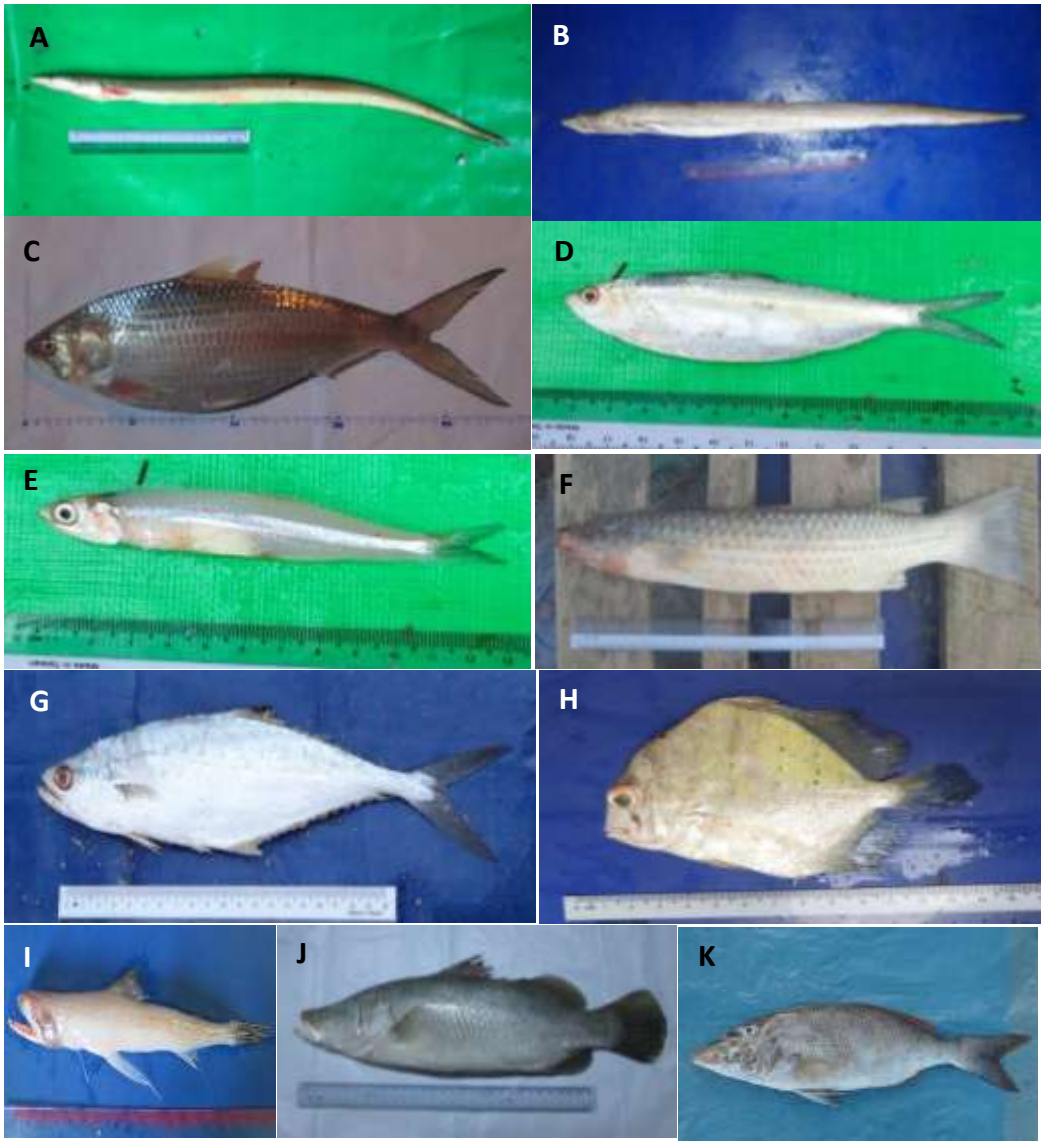

Figure. 2. Commercial fishes of the study area. A) *Congresox talabon*, B) *Congresox talabonoides*, C) *Tenualosa toli*, D) *Sardinella gibbosa*, E) *Stolephorus commersonnii*, F) *Mugil cephalus*, G) *Scomberoides tala*, H) *Drepane punctata*, I) *Harpadon nehereus*, J) *Lates calcarifer*, K) *Lethrinus miniatus*.

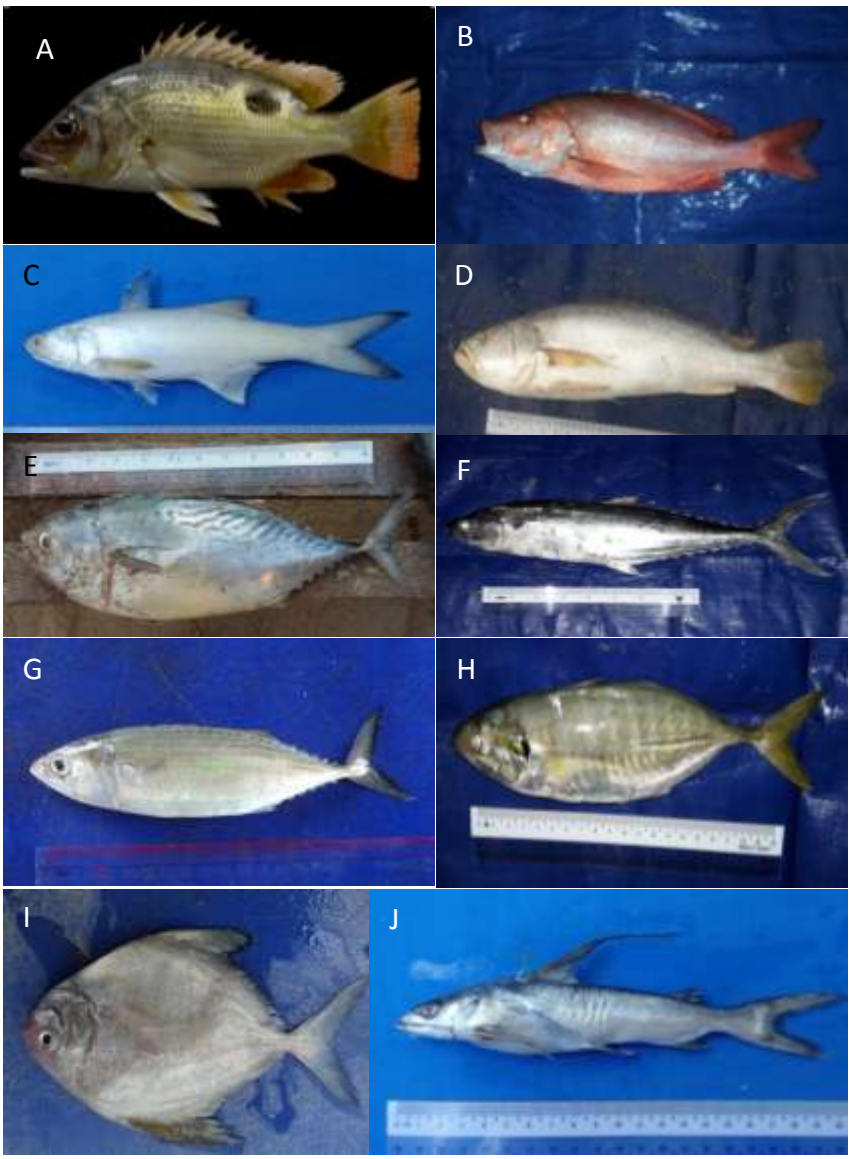

Figure. 3. Commercial fishes found in the study area. A) *Lutjanus johnii*, B) *Lutjanus malabaricus*, C) *Eleuthronema tetradactylum*, D) *Johnius coitor*, E) *Auxius thazard*, F) *Scomberomorus guttatus*, G) *Rastrelliger faughni*, H) *Rastrelliger kanagurta*, I) *Pampus argenteus*, J) *Arius maculatus*.

The identification of fishes is based on morphological distinctive characteristics because the morphological characteristics were adjustable in different species. Especially, the classification of species was based on distinctive characteristics such as body shape, counting spine and rays of fins and color pigmentation pattern.

The order of Siluriformes has only one family, 1 genus and 1 species. Most members of Perciformes are marine fish (Nelson, 2006). The family Scombridae (4 species) is most commonly found in the study area. All these species are very commercial species in this area and Myanmar. Among them, the seabass, *Lates calcarifer* is exported to foreign countries.

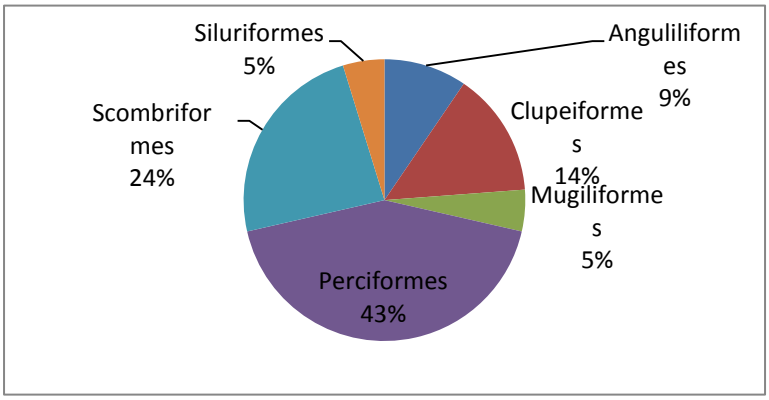

Figure 4. Percentages of the orderwise in the study area.

The meats of *Auxis thazard*, *Scomberoides tala* and *Scomberomorus guttatus* fishes are commonly taken for dry products in this area. The fishes of *Lates calcarifer*, *Lutjanus malabaricus*, *Johnius coitor*, and *Pampus argenteus* are commonly frozen species for restaurants in our country. The species *Stolephorus commersonnii* (Nga-ni-tu) is a very popular dry fish in Myanmar.

Different types of fishing gear were used in different seasons for fishing of the study area. The most widely used gears in the study area include cast nets, drift gill nets, bag nets, trammel nets, seine nets, man push nets, upright fish traps and long line. Gill nets were larger in size and mesh size and higher in price and catch composition than other gears such as traps, surrounding nets and hooks and lines (Table. 1). Among them, gill nets, seine nets, long line and surrounding nets are commonly used for commercial fishing and man push net and upright fish trap for subsistence fishing.

Gear also includes harvesting organisms when no particular gear or boat is involved. Fishing gears and methods is based on the principles of how the fish or prey are captured and to a lesser extent, on the gear construction or gear materials used (Nedelec, 1990).

Nyein Aye Hsan (2020) studied that the assessment of fish species availability and fishing gear used in Sittway environs. She examined 49 species of fish and 19 types of fishing gear during her study period. She found that the most dominant species of fish was 18 species in order Perciformes and gill net was the dominant gear in this area.

Table. 1. Various types of Fishing gear in Thae-Chaung Area, Sittwe Township

Sr No.	Common Name	Local Name
1.	Cast net	Kun
2.	Drift gill nets	Ah-nu-myu-pike/ Pin-lal-tan-pike/ Nga-tha-lauk-pike/ Yu-za-narr-pike
3.	Bag nets	Arr-htauk-pike (small/large)/ Wine-pike
4.	Trammel nets	Thone-htat-pike
5.	Seine nets	Kan-nar-swel-pike
6.	Man push nets	Yinn-tann
7.	Upright fish trap	Nga-zin-yine-pone
8.	Long Line	Nga-myar-chite

Win Cho Cho Tun (2022) also studied 58 species of marine fish in Myoma Market, Sittway Township. She described the two species of cartilaginous fishes and 56 species of bony fishes in the study period. She also found the order of Perciformes was the largest group in the study site.

Conclusion

This information will serve as baseline data for carrying out further study on ecology, conservation, sustainability and management of marine fisheries resources of Rakhine Coastal Water. Fishery management is important among citizens, fishers, fisheries processing and distribution sectors, administration and scientists. It will enable all stakeholders to fully understand not only the unique characteristics of the fishery resources and the regime shift but also the need for appropriate resources management.

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POTENTIAL IMPACTS OF MARINE DEBRIS ON NESTING GREEN SEA TURTLES AT THAMEEHLA ISLAND, MYANMAR*

Ko Myint[1] and Phone Maw[2]

Abstract

This study conducted on Thameehla Island, a protected and historically significant sea turtle nesting ground during 2023 to January 2024. The study aims to investigate the impact of increasing marine debris pollution on Green Sea Turtle (Chelonia mydas) nesting behaviors and site selection. The island, located in Ngaputaw Township, Ayeyarwady Region and no human settlement on the island, resulting in less human disturbance to sea turtle nesting compared to surrounding regions. However, the island faces challenges such as beach erosion, and reduced nesting success due to the increasing marine debris issue. The research involved collecting marine debris samples along turtle tracks and on the nesting, area using a quadrat sampling method. Statistical analysis, employing the R software package was conducted. A total of 327 debris pieces across two nesting beaches including all study tracks with and without nests, were gathered. The five most abundant categories were piece of plastic (25%), foam (16%), bottles (15%), net fragments (9%), and rope pieces (8%). Remarkably, marine debris was more abundant in areas with turtle nests compared to those without nests. Statistical tests (p-values of 0.008 and 0.004) indicated a significant correlation between marine debris and nesting success or behavior. The findings strongly support the rejection of the null hypothesis, suggesting that increased marine debris adversely affects Green Sea Turtle nesting. The study concludes that removing marine debris from nesting beaches could potentially enhance nesting habitats, nesting success and contribute to the conservation of sea turtle populations.

Keywords: Marine debris, Green Sea Turtle, nesting behavior, beach erosion, and conservation

Introduction

This study was carried out on the nesting beaches of Thameehla Island, a protected area designated as Thameehla Kyun Wildlife Sanctuary since 1970 during 2023 to January 2024. The island is formerly recognized as Diamond Island due to its diamond shape and currently known as Leik Kyun, reflecting its significance as a nesting site for sea turtle (Leik). The island is situated in Ngaputaw Township in the Ayeyarwady region and holds historical importance as a sea turtle nesting ground. The Green Sea turtle species (*Chelonia mydas*) has been nesting on the island year-round since the British colonial era to the present day. In the past, several thousands of sea turtle came to nest on this island (Maxwell, 1904). Thameehla Island is a major nesting site of green turtles in Myanmar (Maung Maung Lwin & Khin Myo Myo, 2003). Green turtle is the dominant species in Thameehla Island (Ko Myint, 2007 and Maung Maung Lwin, 2009, 2010). However, nesting sea turtle population and number of nests are decreasing with alarming rate year by year likely attributed to factors such as sea turtle bycatch and the degradation of nesting habitats including erosion and pollution (Ko Myint, 2007), (Maung Maung Lwin, 2009), (Limpus, 2012). Presence of large debris on a beach could interrupt nesting activities by turtles causing false crawls. Frequent abortion or disruption on nesting attempts by leatherback turtles was observed in a beach in Gabon in Central Africa where active industrial logging caused accumulation of logs on the beach. Additionally, nest placement may be affected by debris which could affect hatching success (Hays and Speakman, 1993).

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[1] Department of Environment and Water Studies, University of Yangon

[2]Department of Fishery, Sea Turtle Research and Conservation Station, Thameehla island, Ngaputaw Township in Ayeyawady Divison

Many sea turtle researchers reported that coastal and marine pollution due to marine debris and plastic pollution can pose negative effects on marine ecosystem and marine species. Marine debris has been identified as one source of habitat degradation and threat to coastal and marine species (Laist, 1997) including sea turtles (Fujisaki, and Lamont, 2016). Marine debris can result from various human activities, such as intense development and increased recreational use of coastal habitats, commercial fisheries, and use of other ocean-based resources by rapidly growing human populations, and natural events such as sea currents and tropical weather systems (Ribic et al., 2010). Marine debris enter into the ocean environment by ocean currents for long distances and then deposited on coastlines or ocean floors (Sheavly and Register, 2007).

Nesting Behaviors of sea turtles

Generally, sea turtles will return to the beach where they were born to nest, natal homing (FAO, 2021). Females lay their eggs high up on the beach usually adjacent to or within vegetated strand. No parental care is exercised. The complete nesting process of the turtle can be divided into eight stages. Stage 1: Emerging from the sea and selecting a course to nest (Note: Sea turtle is very sensitive and may return to the sea without nesting if they are being disturbed). Stage 2: Selecting a nesting site above the high tide level. Stage 3: Clearing the site with sweeping motions of the front and sometimes hind flippers to enclave herself in the body pit. Stage 4: Excavating the egg chamber with her rear flippers to a suitable depth, Stage 5: Laying egg. Stage 6: Filling, covering and packing the nest cavity with sand. Stage 7: Filling of the body pit and concealing of the nest site and Stage 8 Return to the sea (Ali et al., 2004). Generally, the entire nesting process of a sea turtle can range from one to three hours for completion without any disturbances during nesting (https://oceanservice.noaa.gov).

Aim and objectives

The aim of this study is to understand the potential impacts of marine debris on nesting behaviors and nesting site selection along the nesting paths of Green Sea Turtles at Thameehla Island. The specific objectives of the study are to identify and quantify the marine debris, and analyzing with its composition on the study turtle tracks and nest areas and to investigate the behavioral responses of nesting Green Sea turtles especially in selecting of nesting sites, achieving of nesting success in the presence of marine debris along their nesting paths/routes.

Materials and Methods

Study Area

Thamihla Island is a protected area, formerly known Diamon island, currently known as Leik Kyun, situated at coordinates 15^0 51′ 46.75″ N and 94^0 16′ 43.88″ E in Ngaputaw Township, Ayeyarwady Region (Figure 1). The island was established in 1970 as a Wildlife Sanctuary, positioned near the mouth of the Pathein River. The island has been historically significant as the country's foremost sea turtle nesting site (Maung Maung Lwin & Khin Myo Myo, 2003). The Island has two prominent sea turtle nesting beaches, known as Sattha-Phyu and Then-Ban, which have served as crucial study sites over the years. Sea turtles nest throughout the year on the Thameehla island (Ko Myint, 2007), (Maung Maung Lwin, 2010). Many species inhabit the surrounding waters as their feeding habitat. Historically, two sea turtle species, namely *Chelonia mydas* (Green Sea turtle) and *Lepidochelys olivacea* (Olive Ridley Sea turtle), nested on this island. However, since 2015, Olive Ridley turtle nesting was absent, and currently, only the *Chelonia mydas* (Green Sea turtle) nest on the island year-round. There is no human settlement on the island, resulting in less human disturbance to sea turtle nesting compared to

surrounding regions. However, the island faces the problem with increasing marine debris pollution affecting the sea turtle nesting beaches and the environment. This can lead to the beach erosion, decline in the number of nesting sea turtles and hatching success.

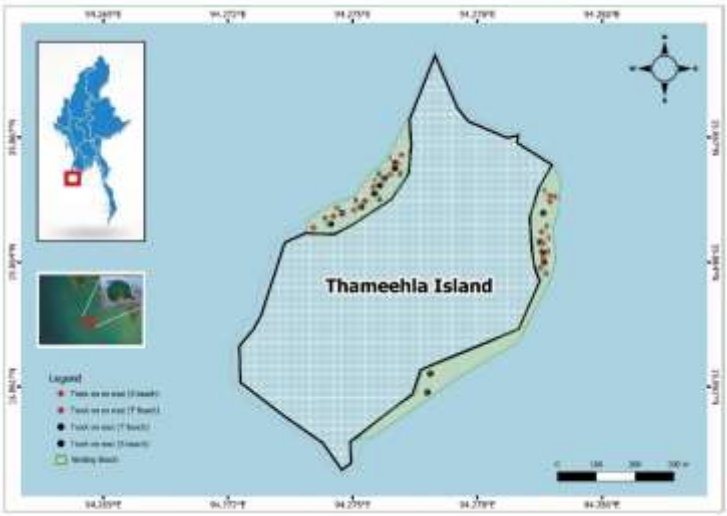


Figure 1. Location map of sampling area on both nesting beaches (Statha-Phyu and Than-Ban)

Identification of Marine Debris

Marine debris refers to any persistent, manufactured, or processed solid material discarded, disposed of, or abandoned in the marine and coastal environment. It consists of a wide range of items, including but not limited to plastics, metals, glass, rubber, paper, textiles, discarded-fishing gears, and abandoned vessels. Marine debris can originate from various sources, such as land-based activities, shipping, aquaculture, recreational activities, and natural events (NOAA, 2013), and (Ondara and Dhiauddin, 2020).

In this study, marine debris were categorized as main categories, sub-categories and detail categories based on their characteristics such as plastic, glass, rubber, textiles and wood, utilizing the field guide provided by the NOAA in 2013 and Ondara and Dhiauddin, 2020 contributed to this categorization (Figure 4).

Observation on nesting turtles and selection of turtle tracks

During the survey period, three nesting turtles were observed to indicate their nesting behavior including nest site selection and reaction to the presence of marine debris along their nesting paths during nighttime. Turtle tracks selected randomly with nest or without nest to collect the debris samples on the study beaches. Marine debris survey on the sea turtle tracks with sampling was carried out at morning time to ensure the tracks' freshness and provide a clear view of any marine debris present on the tracks Specifically, tracks left by turtles on the beach the previous night, whether with nests or without nests, were chosen for debris sampling (Figure 3 A,B, C).

Sampling Turtle Tracks

Marine debris survey was carried out at total number of 28 fresh turtle tracks on the two nesting beaches of Thameehla Island. Specifically, on Thanban nesting beach, 6 fresh turtle tracks with nests and 6 fresh turtle tracks without nests were randomly surveyed while on the Sathaphyu nesting beach, 8 fresh turtle tracks with nests and 8 fresh turtle tracks without nests were examined during the survey period. The GPS coordinates of each individual turtle track were recorded at all locations (Table 1).

Table 1. GPS coordinates of sampling turtle tracks on two nesting beaches at Thameehla island.

Track No. (Satthaphyu beach) with nest	Latitude	Longitude	Track No. (Satthaphyu beach) with no nest	Latitude	Longitude
1	15.866378	94.276033	1	15.864825	94.274052
2	15.866266	94.276014	2	15.865109	94.27454
3	15.866008	94.275759	3	15.865239	94.275025
4	15.865838	94.275667	4	15.86547	94.275304
5	15.865645	94.275527	5	15.865799	94.275503
6	15.864918	94.274463	6	15.866114	94.275904
7	15.865339	94.275199	7	15.86657	94.276174
8	15.865183	94.274747	8	15.866356	94.276066
Track No. (Thanban beach) with nest	**Latitude**	**Longitude**	**Track No. (Satthaphyu beach) with nest**	**Latitude**	**Longitude**
1	15.864	94.27961	1	15.865592	94.279736
2	15.86424	94.27957	2	15.865457	94.279747
3	15.86448	94.27951	3	15.864729	94.279553
4	15.86518	94.27958	4	15.864458	94.279581
5	15.860866	94.276796	5	15.864109	94.279578
6	15.86132	94.27687	6	15.86386	94.279518

Sampling Method

Samples of marine debris were collected along the fresh turtle tracks using a quadrat sampling method which was adapted from the quadrat sampling technique employed in the Census of Marine Life investigation (http://www.coml.org). The survey was performed during the early morning to identify unique and fresh characteristics of turtle tracks. Older turtle tracks were excluded from the sampling procedure. A quadrat is constructed measuring 1 meter on each side (1 m^2) by using polyvinyl chloride (PVC) pipes (Figure 2B). Five quadrats were randomly positioned along the turtle track to gather marine debris samples. Two quadrats (Q1 &Q2) were placed on the ascending track, one (Q3) on top of the track where turtle nests were located, and the remaining two quadrats (Q 4 & 5) were placed on the descending tracks (Figure 2A, 3C). Any marine debris larger than 2.5 cm in size within the quadrat were collected and recorded, then carefully sorting through each individual quadrat and then it was done by taking a photograph of each quadrat for future analysis.

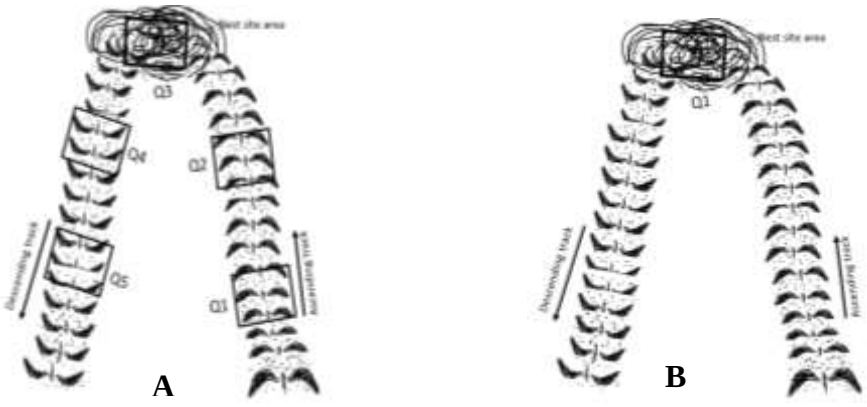


Figure 2. A. Marine debris quadrat sampling along turtle tracks
B. Marine debris quadrat sampling only on the nest area

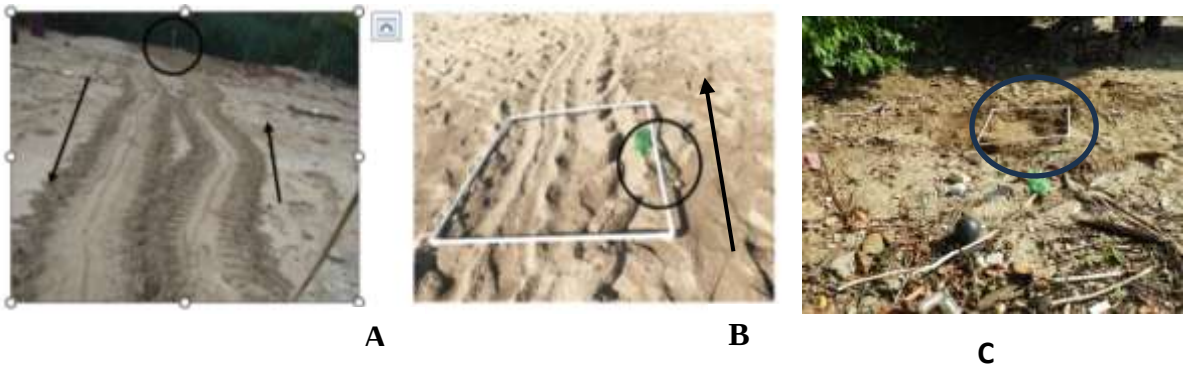


Figure 3. A. fresh turtle track with nest
B. Quadrat sample of marine debris on the ascending track
C. Quadrat sample of marine debris on the nest area

Figure 4. Identification of collected marine debris samples

Data Analysis

R, a free statistical software package, was used for data analysis and transformation to find the p-value for the test statistics. It can be compiled and runs on a wide range of UNIX platforms as well as Windows and MacOS. R is available as source code under the terms of the Free Software Foundation's GNU General Public License [The R Project for Statistical Computing]. This paper uses R version 4.1.2 (2021-11-01) - "Bird Hippie" Copyright © 2021 The R Foundation for Statistical Computing Platform: x86_64-w64-mingw32/x64 (64-bit). In this way strong statistical evidence was obtained to provide evidence for the null hypothesis testing. P-values for the t-test were obtained for both marine debris on turtle tracks with nest and without nests, while the marine debris on nest area on the turtle track with nests and without nests during the study period after intelligent calculation of all necessary steps using the R language.
Null Hypothesis (H_o): Nesting success or nesting behavior will not be disrupted by increased levels of plastic debris or pollution at the sea turtle nesting site.
Suppose the alpha level is 0.05 for a tailed test.
To compare marine debris on all study turtle tracks with nest and the track without nest, and marine debris on nest area on the turtle tracks with nests and without nests, paired t-test can be used. With the following factors, two-sample independent t-test was used:
(1) the individual in one sample must be totally unrelated to the individuals in the other sample.,
(2) the sample size is less than 30.

Results

Identification of Marine Debris

Marine debris were mainly categorized based on their characteristics such as plastic, glass, rubber, textiles and wood, utilizing the field guide provided by the NOAA in 2013 and Ondara, and Dhiauddin in 2020 contributed to this categorization. The 14 sub-categories of those group are classified such as Plastic (real plastic, foam plastic, fishing related plastic, juice related plastic, medicinal, beauty, foot wear and other), glass (glass), textile (textile), metal (metal), rubber (tyre), wood (abandoned vessel/boat, construction materials). The 39 detail categories were identified based on their specific characteristics (table 2).

Table. 2. Marine debris identification based on characteristics of collected materials from the study area.

Group	Main Category	Categories
Plastic	Plastic	Bottles, bottle caps, Pic of plastic (Soft)/fragments, Pic of plastic (hard), bags, boxes, sheets/packaging, cups
	Foam plastic	Pic of foam, plates, cups, box
	Fishing related Plastic	Pic of net, pic of rope, floats/ball
	Juice	Straw
	Medicinal	Syringes, tablet package, box/containers, package
	Beauty	Box, containers
	Foot wear	Slipper/shoes
	Other	Smoke Litter, lime box, toys, Packaging (coffee mix, arginimoto), Personal use (e.g., tooth brush, tooth paste container)
Glass		Bottles, cups
Textiles		Cloth, Shoes
Metal	Tin &containers	Tin containers
Rubber/tyre		Sipper, Pic of bicycle or others
Wood	Broken wood or load	Pices related to fishing boats, and construction

Number of marine debris collected from all-study turtle tracks

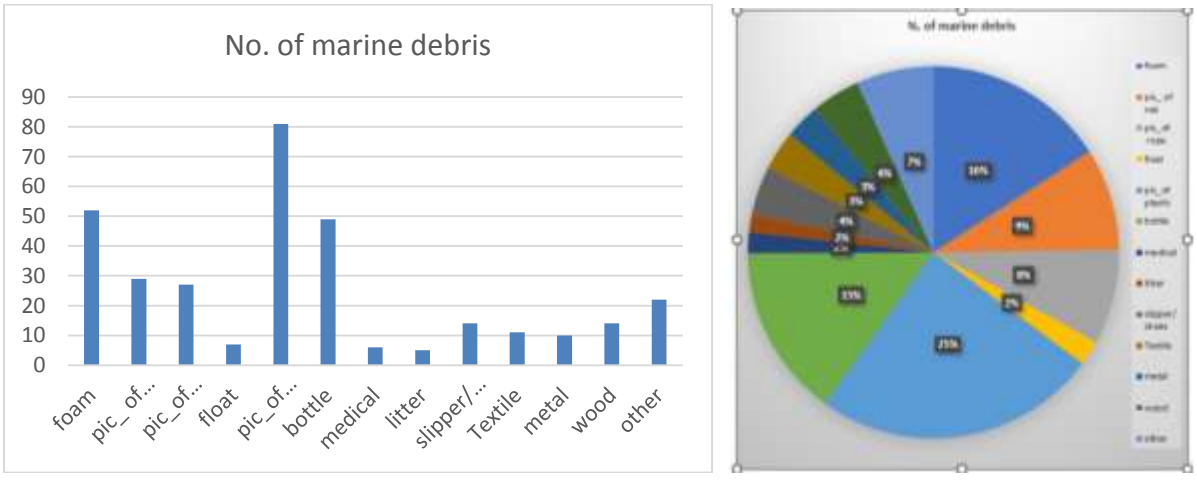
A total of 327 pieces of marine debris were collected from all sampling turtle tracks from two nesting beaches in the study area. Among these, 141 debris items were found along tracks with nests, while the remaining 186 debris items were collected from tracks without nests (Table 3). The findings indicated the presence of marine debris along every turtle track with a nest, average 10.07 pieces (n=14) while the turtle track without nest found an average of approx..13.2857 pieces of debris (n=14). The study found that the quantity of marine debris on turtle tracks without nests are larger than the amount on tracks with nests.

The focus of marine debris collection was on the turtle nest areas along the tracks. A total of 129 debris pieces were collected from the sample turtle tracks within the study area (Table 3). Sampling collection efforts included both the nest areas on tracks with nests and those without nests, assessing for a comparison of debris abundance between the two. Specifically, 48 debris pieces were collected from nest areas on tracks with nests, while 81 pieces were obtained from nest areas on tracks without nests. The findings indicated the presence of marine debris on nest area in every turtle track with a nest, average 3.4286 pieces (n=14) while the turtle track without nest found an average of approx. 5.79 pieces of debris (n=14). The finding showed that the quantity of debris items found in each nest area of turtle tracks without nests is approximately twice as high as that in every nest area of turtle tracks with nests within the study area.

During the study, marine debris collected was classified into 13 main categories, including foam, pieces of net, pieces of rope, floats, pieces of plastic, bottles, medical waste, smoke litters, slippers or shoes, textiles, metal, wood, and others (Figure 5A). Among these, the five most abundant categories were identified, with pieces of plastic, the most abundant at 25%, followed by foam at 16%, bottles at 15%, pieces of net at 9%, and pieces of rope at 8%. While, the medical waste, smoke litters, and floats were among the least categories, each representing 2% of the collected debris (Figure 5B). The study revealed that the main categories of marine debris such as plastic fragments, foam plastic, bottles, fishing nets, and ropes, mainly consist of plastic and materials associated with fishing.

Table 3. Summary of marine debris distribution on sea turtle tracks and nest areas with nests and without nests in the study beaches.

	foam	pic_ of net	pic_of rope	float	pic_of plastic	bottle	medical	litter	slipper/ shoes	Textile	metal	wood	other	Total
Debris on the tracks with nest	23	22	12	0	41	20	0	1	3	4	4	8	3	141
Debris on the tracks without nest	29	7	15	7	40	29	6	4	11	7	6	6	19	186
Total- Debris on all study tracks	52	29	27	7	81	49	6	5	14	11	10	14	22	327
Debris on the nest area with nest	9	8	5	0	11	6	0	1	0	1	2	4	1	48
Debris on the nest area without nest	12	3	6	2	14	12	4	3	6	5	4	3	7	81
Total- Debris on all nest areas	21	11	11	2	25	18	4	4	6	6	6	7	8	129



A **B**

Figure 5. A. No. of marine debris collected from the sampling tracks
B. Composition of marine debris (%) collected from the sampling tracks

Nesting Turtle Behavior

According to literature, sea turtle lay eggs on the sandy beach and avoid any disturbance on the beach. They are highly sensitive to any disruptions or pollution when they arrive at the beach for the nesting process. In this study, it was observed that Green sea turtles excavated their nest holes more frequently and spend a longer duration compared to the normal nesting process (one to three hours). Some turtles experienced difficulties nesting and altered frequently nest sites probably due to the presence of marine debris and vegetation on nest area, then returned to the sea without nesting (Table 3).

Table 3. **Summary of nesting turtle behavior associate with the presence of marine debris on their nesting paths in the study area**

Date of observation	Nesting turtle species	No. of track	Beach and GPS coordinates	Tag number	Time taken for nesting process	Nesting behavior associated with presence of marine debris along the nesting paths/tracks
May 24, 2023	*Chelonia mydas*	Track No.2 with-no nest	Satha phyu 15.865109 N 94.27454 E	MM-2740	45-minutes	-Unsuccessful nesting attempt -Searching for suitable nest sites, but unable to find a suitable location -After attempting to dig nest holes in two different spots among marine debris and vegetation, -Then returned to the sea without successfully nesting -Found bottle, foam and pic of rope in every nest sport
May 25, 2023		Track No. 3 with nest	Satha phyu 15.865645 N 94.275527 E		5-hours	-Successful nesting attempt -Searching for suitable nest sites, -After attempting to dig nest holes in five different spots among marine debris and vegetation, -Successful of nesting -Then returned to the sea -Found bottle, foam, slippers, smoke litters and pic of rope in nest sports
October 16, 2023		Track No.6 with nest	Satha phyu 15.864918 N 94.274463 E	MM 3033/ MM 3034	3:30 hours	-Successful nesting attempt -Searching for suitable nest sites, -After attempting to dig nest holes in two different spots among marine debris and vegetation, -Successful of nesting -Then returned to the sea -Found bottle, caps, float, foam, slippers, and pic of net in nest sports
October 17, 2023		Track No.7 with-no nest	Satha phyu 15.86657 N 94.276174 E	MM 3027/MM 3028	1:30 hours	-Unsuccessful nesting attempt -Searching for suitable nest sites, but unable to find a suitable location -After attempting to dig nest holes in three different spots among marine debris and vegetation, -Then returned to the sea without successfully nesting -Found pic of foam, pic of plastic, cloths, slipper and pic of rope in nest attempting areas

The data analysis revealed that in comparison of marine debris on the turtle tracks with nests and without nests in the study area, the p-value is 0.008, which is less than the alpha level of 0.05 that we have chosen. This means that we can reject the null hypothesis. There is sufficient evidence to reject that nesting success or nesting behavior is not affected by increased levels of plastic debris or pollution at Green turtle nesting process.

For the data analysis in comparison of marine debris on the nest areas on the turtle tracks with nests and without nests, the p-value is 0.004, less than our chosen alpha level of 0.05. On the basis of this result, we can reject the null hypothesis. There is strong evidence to reject the null hypothesis that we have chosen to test, suggesting that there is enough evidence to support the alternative hypothesis *Ha* that nesting success or nesting behavior is affected by increased levels of plastic debris or pollution at Green turtle nesting process. It can be seen that the null hypothesis can be rejected as all p-values for all statistical significance are less than the alpha level of 0.05.

Discussion

The study found that the prevalence of marine debris was higher in areas with turtle nests compared to those without nests. It was suggested that presence of marine debris on both nesting turtle routes and turtle nesting sites can disturb nesting processes and reduce the nesting success. Study on nesting behavior and response of green sea turtles to the presence of marine debris on nesting routes and nesting sites observed that Green sea turtles excavated their nest holes more frequently and spend a longer duration compared to the normal nesting process. Some turtles experienced difficulties nesting and altered frequently nest sites probably due to sensitive to disturbances caused by marine debris when they search for nesting sites at the beach for nesting. Regard on these matters, Laurence et al., 2008 stated that the presence of large debris on a beach could interrupt nesting activities by turtles causing false crawls. Additionally, nest placement may be affected by debris which could affect hatching success (Hays and Speakman, 1993). In addition, (Bourgeois, 2009), (Witherington et al., 2011). reported that the presence of large debris on a sandy beach could alter the spatial distribution of sea turtle nests by influencing turtle nest site selection.

During the study, marine debris collected was classified into 13 main categories, among these, the five most abundant categories were identified, with pieces of plastic, the most abundant, followed by Styrofoam, bottles, pieces of net, and pieces of rope, mainly consist of plastic and materials associated with fishing will have negative impact on nesting beach and nesting process of sea turtles. Nelms et al., (2016) identified the risks that plastics pose towards sea turtles under the headings of ingestion, entanglement, obstacles, impacts on nesting beaches and ecosystem effects. Triessnig et al., 2012 described that marine debris is an indicator of habitat quality for sea turtle nesting sites. The study suggest that removal of marine debris may open nesting habitat that was previously unavailable for sea turtle nesting.

For the data analysis in comparison of marine debris on the nest areas and on the turtle tracks with nests and without nests, the p-values (0.008 and 0.004) are less than our chosen alpha level of 0.05. On the basis of this result, we can reject the null hypothesis as all p-values for all statistical significance are less than the alpha level of 0.05. There is strong evidence to reject the null hypothesis that we have chosen to test, suggesting that there is enough evidence to support the alternative hypothesis *Ha* that nesting success or nesting behavior is affected by increased levels of marine debris at Green turtle nesting process.

Conclusion

There were 13 different kinds of marine debris were recorded on the turtle tracks and nest area. Among these, the most abundant categories were pieces of plastic, the most abundant, followed by Styrofoam, bottles, pieces of net, and pieces of rope. Notably, the prevalence of marine debris was higher in areas with turtle nests compared to those without nests. Statistical analysis indicates a rejection of the null hypothesis, as all p-values for statistical significance were below the predetermined alpha level of 0.05. There is strong evidence to reject the null hypothesis that we have chosen to test, suggesting that there is enough evidence to support the alternative hypothesis *Ha* that nesting success or nesting behavior is affected by increased levels of marine debris at Green turtle nesting process. As a final conclusion, the removal of marine debris on the sea turtle nesting beach may open nesting habitat that was previously unavailable for sea turtle nesting.

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QUALITY ASSESSMENT AND TREATMENT OF DOKHTAWADDY (MYITNGE) RIVER WATER NEAR MANDALAY ENVIRONS

Wint Thuzar Than[1], Soe Win[2]

Abstract

Myanmar has a favorable situation of fresh water resources and adverse environmental effects degrade the strength of river water quality when utilizing natural resources. Measurements of aquatic biota in terms of water quality parameters have been essential for the assessment of river health. Some anthropogenic activities have a potentially negative cumulative impact on rivers and wetlands water quantity and quality, hence negatively impacting river-dependent people. This research will be emphasized on the evaluation of quality of Dokhtawaddy (Myitnge) river water near Mandalay Region. For this purpose, Dokhtawaddy river water samples were selected from four different points [the upstream (near Ta-Lin-Gyi village and near Shwesaryan Pagoda) and downstream (near Myitnge Bridge) places of Mandalay Industrial Sewage Ditch] in the middle of Dokhtawaddy (Myitnge) river near Mandalay environs. River water samples were collected seasonally (January, April, July and November during 2019 -2020). To evaluate the river water quality, its physico-chemical characteristics such as pH, color, conductivity, turbidity, total alkalinity, total hardness, calcium hardness, chemical oxygen demand (COD), biochemical oxygen demand (BOD) and traces of minerals like chloride, iron, manganese, sulfate, arsenic, copper, cyanide and lead were determined. In addition, its biological characteristics in terms of probable coliform count and *Escherichia coli* count (*E.coli*) were also examined. To reduce the contaminants, conventional water treatment methods such as plain sedimentation, filtration through the medium consisting of sand, gravel, broken bricks, rice straw, cotton fiber, rice husk char and charcoal. It was then again filtered with secondary filtration using sand column and rice husk char column. Effort of these stepwise treatment processes clearly shows that traces of contaminated ions, BOD and COD values were reduced within the desirable values. Benefits of this research would be helpful for the knowledge of simple water treatment technology in rural communities along the river bank of Dokhtawaddy (Myitnge) near Mandalay environs.

Keywords: Dokhtawaddy river**,** water quality, treatment technology, contaminant ions, filtration

Introduction

Some of the anthropogenic activities, the developments of industry, agricultural production and ever intensive urbanization have led to the pollution of natural flow regime of water bodies. The discharging of degradable wastewater in natural water bodies result to deteriorate the river water quality generally and difficult to meet satisfactory situation becomes growing.

Myitnge river, one of the surface water resources in Upper Myanmar, plays an important role in transportation, agriculture, domestic and industrial purposes. It is one of the largest tributaries on left bank of Ayeyarwady river and it originates from Mount Loi Swang at an elevation of 1460 m on the northern Shan Plateau and joins the Ayeyarwady river about 15 km southwest of Mandalay. River basin area is 34800 km^2 and it covers from Mandalay division near the confluence of the Ayeyarwady river to the north-west part of the Shan state. The river flows in a generated direction of north-east to south-west. It longs about 530 km and its tributaries are Zawgyi, Panlaung and Nantalan rivers. The Mytinge river basin covers the northwest part of the Shan state and its location is approximately between the latitude 20 51' to 23 48' N and the longitude 96 23' to 98 22' E (Su Su Hlaing *et al*, 2019).

On the other hand, water quality assessment is the process of overall evaluation of the physical, chemical and biological nature of the water. The quality of surface water is affected by the hydrochemical changes that are indicative of the climate and environment changes such as

[1] Environment and Water Studied Department, University of Yangon
[2] Industrial Chemistry Department, University of Mandalay

increased precipitation, evaporation, domestic and industrial activities, agriculture and breeding, human and animal consumption (Zhang *et al*., 2019).

However, an altered flow regime related to hydropower production along the Myitnge river could be threatening critical processes downstream in the Myitnge river and the receiving Ayeyarwady river. Hydropower projects can disrupt the natural flows and freshwater ecosystem. The magnitude, frequency, duration, and timing of flow regime and their sediments can be changed due to dam operation (Su Su Hlaing *et al*, 2019).

Recently, pollution has become a serious concern for human life due to the industrial burst in the world and the rivers are the main choices to hold and bear the responsibility of pollutants, especially in the developing countries (Ebrahimi *et al*.,2015). To supply the enough clean water for the demand of upper tropical regions in Myanmar, the pollutants from the river water must be reduced to the acceptable quality. The aim of this study is to evaluate Dokhtawaddy (Myitnge) river water quality to meet the supply of enough clean and safe water for the demand of domestic and irrigation water uses of upper tropical regions.

- To analyze the quality of Myitnge river water (i.e., physicochemical and biological characteristics).
- To remove the undesirable contaminants from river water for clean and safe purpose
- To find the effective surface water treatments for contributing the rural community in Mandalay environs.

Materials and Methods

Sample Collection

Dokhtawaddy (Myitnge) river water has been used by the local people who live in villages near the river bank for domestic purposes. In this research, Dokhtawaddy (Myitnge) river water sample was collected from selected four different sampling points from the upstream (points A and B) and downstream (points C and D) places of Mandalay Industrial Sewage Ditch for evaluating water quality. The selected sampling sites are marked as Point A (near Ta-Lin-Gyi village, 22º 00' 09“ N and 96º 04' 02" E), Point B (near Shwesaryan Pagoda, 21º 50' 17" N and 96º 12' 50" E), Point C (upstream of Myitnge Bridge, 21º 50' 33" N and 96º 04' 03" E) and Point D (downstream of Myitnge Bridge, 21º 50' 40" and 96º 04' 09" E) located in Myitnge Township, Mandalay Region. The water quality parameters were determined by quarterly in a year from January, 2019 to October, 2020 and water samples were collected by 2 feet deep and 20 feet apart from the river bank. The clear sampling plastic bottles of (1) liter capacity each were rinsed three times with river water, filled and screwed tightly with caps. The samples were onsite tested at the river bank around sampling sites.

The physicochemical characteristics of collected water samples from the above sources were evaluated and the results are shown in Tables (1) to (4). The location of four sampling sites is shown in Figure (1).

Analysis of Collected Water Samples

The collected water sample from the selected points of Points A, B, C and D were analyzed quarterly in a year (January, April, July and October) by their physical, chemical and biological parameters. Those properties of water quality parameters such as pH was measured by pen-type pH meter (pH-2016), turbidity by Lovibond Turbicheck Water Testing Turbidimeter (Model SN13/45043), GMBH, Germany, color by using Lovibond Tintometer, conductivity by Milwaukee Portable Conductivity and TDS meter (Model SM 301 EC meter). The amount of total alkalinity, total hardness, calcium hardness, magnesium hardness, some minerals ions such as chloride, iron, manganese, sulphate, arsenic, copper, cyanide and lead present in collected water sample were determined by exact Micro 20 + Spectrophotometer with blue tooth SMART only, 525 nm + 638 nm wavelength (S/N: M 20 BTA 00009 HACH test kit).

Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were determined by HACH Sension 378 HANNA HI 839800 COD Reactor. Microbiological characteristics of collected river water before and after treatment such as total coliforms and *E.coli* were determined by agar plate method.

Figure (1) Sampling Sites of Dokhtawaddy (Myitnge) River at Point (A) near Ta-Lin-Gyi Village, Point (B) near Shwesaryan Pagoda and Points (C and D) near Myitnge Bridge

Treatment of River Water Sample

Preparation of Filter Media for Water Treatment

Sand, gravel and broken bricks used as filter media in a filter column were purchased from 'Tharaphu' Construction Shop, (73) Street, near Mandalay University Campus, Mahar-Aung Myae Township. Before using, sand was washed eight times with water to remove adhering dust and impurities. It was dried under sunlight in a clean dry place and then 3000 g were weighed. Uniform sizes of about 0.2 inches diameter of gravels were washed five times with water, dried under sunlight and then 1500 g were weighed. Bricks were crushed into uniform size of about 0.3 inches diameter, washed five times with water to remove coloring impurities, dried and then 1500 g were weighed. Charcoal and rice husk char were purchased from Phayar Gyi Market, Chan-Aye-Thar-Zan Township. Charcoal was crushed into uniform size of about 0.2 inches diameter, washed five times with water, sun dried and then 1000g were weighed. Rice Husk Char was washed five times with water, drained, dried under sunlight at clean place, and then 1000 g were weighed.

Straw was obtained from Pathein-Gyi Township, Mandalay. It was thoroughly washed with water to remove soil residue and boiled in water at 100 ºC for three hours and then drained, sun dried in a clean place. About 100 g of this dried straw was weighed.

Cotton fabric used for the separation of each filter media was boiled with water at 100˚C for 2 hours and then washed with water. After washing, the cotton fabric was dried under sunlight. The Figures of prepared filtered media were shown in Figure (2).

Experimental Procedure for Water Treatment Process

The experimental water treatment includes plain sedimentation and a follow up aeration, primary filtration and secondary filtration. These sequences of treatment were conducted from

water sample of Point (D) during October, 2020. The sequences of groundwater treatment processes were carried out and described below.

Aeration

Water sample after 24 hours plain sedimentation was aerated by air diffuser [model RS-9800 (Aquarium3 in 1) from Zhongshan Electrical Product, China] under sunlight for about 12 hours. The contact time of air and water support the effective removal of unwanted gases.

Primary Filtration Column

River water sample after plain sedimentation was passed through a filter column. A glass column of 6 inches square and 25 inches in height was used as filter column. Each of 4-inches thick layers of rice straw, broken bricks, gravel, sand and charcoal [Figure (2)] were used as filter media and this media were separated by cotton fiber [Figure (3)]. The column was connected by inlet and outlet pipes and the influent was allowed to flow down by gravity. The physicochemical characteristics of filtrate were analyzed and the results are recorded in Table (5).

Figure (2) Filter Media (A) Sand (B) Gravel (C) Broken Bricks (D) Charcoal (E) Rice Husk Char and (F) Straw

Secondary Filtration

After treatment with the biofilter column, the filtrate was passed through secondary filter columns. Some colloidal matter was removed by filtration through the cylindrical-shaped sand filter column filled with 12 cm height sand medium [Figure (4)]. It was then passed through rice husk char column to again filtered. After filtering the water through the two columns, the filtered water was collected in sterilized plastic bottle, screw capped, placed in an ice box and taken to the laboratory within 3 hrs for analyzing the quality of treated water.

Figure (3) Primary Filtration Column

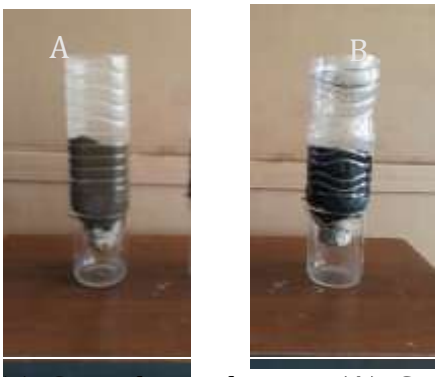

Figure (4) Secondary Filtration (A) Sand Filter (B) Rice Husk Char Filter Columns

Results and Discussion

Myanmar has an abundance of natural water resources, which are distributed unevenly spatially and temporally. Myitnge river rises on the Ayeyrawaddy-Salween watershed, flows westwards through northern Shan Plateau and eventually enters into the Ayeyarwady near

Mandalay (https://en.wikipedia.org/wiki/myitnge_River). **In most of the parts of Upper Myanmar,** groundwater as well as surface water quality varies seasonally according to the result of climate change and global warming. Due to the northern margins of dry zone, most of the dwellers in poor peri-urban area of Mandalay experienced water crisis problems during warm season.

Some of anthropogenic pressures such as river infrastructure and significant issues of water contamination as a result of urbanization, population expansion, the creation of special economic zones, extension of industrial sectors, agriculture and power generation are currently challenging the health of surface water bodies. The surface water quality in a particular region has varies from one season to another according to the result of rainfall, the source and the conditions of flow regimes, ecosystem and environment from which it is drawn (Bheemappa, 2015).

The water resource availability is strongly influence by climate change and the need to implement the demand of enough water is vital to the rural communities of Upper tropical region. One of the surface water resources like Myitnge river water near Mandalay city was necessary to analyzed for the purpose of potable as well as domestic uses of dwellers. For this purpose, physicochemical and biological characteristics of Myitnge river water were analyzed quarterly during the years of 2019 and 2020. According to the results shown in Tables (1) to (4), it is obvious that pH values were around 7 from the four sampling points during the sampling times. The turbidity value of river water at points A and B are within the desirable value of 5 NTU (**WHO, 2013**) except in July 2019 and 2020 because the rough flow of lower water level in river, mixed with a large proportional amount of erosive soil during the rainy season. Points C and D was above the desirable values all sampling times due to residue of some contaminants from the downstream of sewage Ditch [Figure (5)].

The reported values of one of the important chemical characteristics of thermal conductivity from all sampling locations were significantly higher than desirable values of 50 μS/cm ((https://en.wikipedia.org/wiki/Conductivity_(electrolytic)). The values of thermal conductivity not only related to the concentration of dissolved ions in water but the ions electrically remain neutral in water. **The conductivity value is more significant in rainy season of July 2019 and 2020.** Another characteristic likes total alkalinity value of river water in points A and B were lower than the maximum allowable values but points C and D were slightly higher than the desirable values in all investigating time as shown in Tables (1) to (4).

The total alkalinity of water is usually caused by the presence of carbonates, bicarbonates, hydroxides and less frequency by borates, silicates, and phosphates ions. Because of all the dissolved ions in the flow of river water were not only basic, but also some are acidic due to the pesticide residues from the growing areas and effluents near the sewage Ditch from northern part of Mandalay region. The wastes from other urban sewage materials can enter into streams and affect the alkalinity of natural water bodies.

The other important characteristic such as total hardness and calcium hardness were significantly higher than the desirable values in all locations but magnesium hardness was considerably lower than the desirable values**.** Weathering of limestone, erosion of sediment and calcium bearing minerals, percolation of domestic wastes and high rate of evaporation may enhance the total hardness value in the source of river water. Other factors such as geological formation, depth of water table, soil texture and filtration rate may be contributed to the high amount of hardness in this water sample.

Although the resultant values of some ions, namely chloride, iron, manganese, sulphate, copper were lower than the desirable values and some heavy metal ions such as arsenic, lead and cyanide concentrations in river water were not detected in all sampling points during the sampling periods.

For evaluation of biological characteristics, standard plate count, Probable Coliform count and *Esherichia coli* count were detected by agar plat method and it was observed that these values were unsatisfactory for drinking purposes throughout the sampling time during 2019 and 2020 [Tables (1) to (4)]. Therefore, some contaminated dissolving substances in river water must be removed by using appropriate treatment methods to access clean and safe water quality.

The technology for the removal of contaminated from river water has directed attention to the use of simple filtration in a carried out in a specially designed glass column using with readily available low-cost biomass owing to its great opportunity for the removal efficiency.

After analyzing the physicochemical and biological characteristics of Dokhtawaddy river water, the sequence of available water treatments such as plain sedimentation, aeration under sunlight and filtration were carried out to reduce the contaminants level in river water. Due to the comparison of contaminants level in selected four sampling points as reported in Tables (1) to (4), the river water sample from point D collected in October, 2020 was chosen to treat by the above series of treatments. Primary filtration was operated in glass column constructed with filter media of rice straw, sand, gravel, charcoal and broken brick. Each filtering media were separated by cotton fiber. Then, the secondary filtration was performed in a filter column consisting of 6 inches sand and rice husk char columns for the purpose of reducing the contaminants level in river water.

From the results reported in Table (5), the turbidity level was reduced to desirable limit (i.e., 2.2 NTU) and that of conductivity was also reduced. The total alkalinity value of treated water was decreased significantly in the sequence of treatments and found to reduce the removal efficiency of 52.5%. The values of total hardness, calcium hardness and magnesium hardness of treated water were significantly reduced and fall within the limit of desirable range. The amount of some contaminated ions like chloride, iron and manganese were more pronouncedly decreased after the series of treatment steps and the removal efficiency of above 80% was found to observed in treated water. The values of biochemical oxygen demand (BOD) decreased below the allowable values and that of chemical oxygen demand (COD) decreased slightly and the removal efficiency of above 50% was found to obtained after secondary filtration [Table (5)].

By using the treatments of river water in series of steps, the clear water with desirable level of water quality was found to obtain for clean and safe utilization of local communities in rural area near the river bank.

Table (1) Comparison of Characteristics of Selective Points of Dokhtawaddy (Myitnge) River Water Samples for January and April, 2019 (Before Treatment)

Sr No.	Characteristics	Sample Collection Time (January, 2019)				Sample Collection Time (April, 2019)				*Literature Value (WHO Standard)	
		Point (A)	Point (B)	Point (C)	Point (D)	Point (A)	Point (B)	Point (C)	Point (D)	Desirable	Imperative
1	pH	7.1	7.1	7.1	7.1	7.1	7.1	7.2	7.2	7-8.5	6.5-9.2
2	Colour (Units)	5	5	5	5	5	5	5	5	5	50
3	Turbidity (N.T.U)	4.02	4.08	5.38	5.50	4.12	4.18	5.89	5.90	5	25
4	Conductivity (micromohs/cm)	362	366	355	355	346	341	342	345	50	500
5	Total Alkalinity (mg/L)	184	189	200	210	183	192	220	220	200	500
6	Total Hardness (mg/L)	200	211	224	228	210	210	253	260	100	500
7	Calcium Hardness(mg/L)	172	200	180	200	172	200	180	200	75	200
8	Magnesium Hardness (mg/L)	20	20	24	27	20	22	29	29	30	150
9	Chloride (mg/L)	5	8	10	10	6	8	12	18	200	600
10	Iron (mg/L)	0.01	0.01	0.02	0.02	0.01	0.01	0.02	0.03	0.1	1.0
11	Manganese (mg/L)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.5
12	Sulphate (mg/L)	<200	<200	<200	<200	<200	<200	<200	<200	<200	200
13	Arsenic (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	-	0.01
14	Copper (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	1.0	2.0
15	Cyanide (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	-	0.07
16	Lead (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	-	0.01
17	BOD (mg/L)	5.2	5.3	6.6	6.5	5.5	5.1	7.2	6.9	5.99	12
18	COD (mg/L)	11.9	14.1	18.9	22.3	12.4	13.1	21.2	23.4	6.99	30
19	Probable Coliform Counts	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	0	50
20	*Escherichia Coli Count*	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	0	0

***Source:** World Health Organization (2013), Standards of Potable Water Quality and Water-Borne Diseases, Geneva
World Health Organization (2015), Drinking Water Quality Standards, Geneva.

Table (2) Comparison of Characteristics of Selective Point of Dokhtawaddy (Myitnge) River Water Samples for July and October, 2019 (Before Treatment)

Sr No.	Characteristics	Sample Collection Time (July, 2019)				Sample Collection Time (October, 2019)				*Literature Value (WHO Standard)	
		Point (A)	Point (B)	Point (C)	Point (D)	Point (A)	Point (B)	Point (C)	Point (D)	Desirable	Imperative
1	pH	7.1	7.1	7.2	7.2	7.1	7.1	7.2	7.2	7-8.5	6.5-9.2
2	Colour (Units)	5	5	5	5	5	5	5	5	5	50
3	Turbidity (N.T.U)	5.02	5.08	6.50	6.88	4.10	4.18	4.81	5.30	5	25
4	Conductivity (micromohs/cm)	363	366	376	385	326	320	340	351	50	500
5	Total Alkalinity (mg/L)	185	192	218	210	183	198	220	235	200	500
6	Total Hardness (mg/L)	165	160	184	173	210	210	253	260	100	500
7	Calcium Hardness(mg/L)	132	140	150	155	172	174	195	210	75	200
8	Magnesium Hardness (mg/L)	17	17	19	19	22	22	25	29	30	150
9	Chloride (mg/L)	5	8	10	10	12	12	25	29	200	600
10	Iron (mg/L)	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.1	1.0
11	Manganese (mg/L)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.5
12	Sulphate (mg/L)	<200	<200	<200	<200	<200	<200	<200	<200	<200	200
13	Arsenic (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	-	0.01
14	Copper (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	1.0	2.0
15	Cyanide (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	-	0.07
16	Lead (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	-	0.01
17	BOD (mg/L)	5.3	6.2	6.2	7.3	6.2	6.3	6.8	7.6	5.99	12
18	COD (mg/L)	12.5	12.3	13.8	18.4	13.3	13.1	17.6	19.2	6.99	30
19	Probable Coliform Counts	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	0	50
20	*Escherichia Coli Count*	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	0	0

Source: World Health Organization (2013), Standards of Potable Water Quality and Water-Borne Diseases, Geneva
World Health Organization (2015), Drinking Water Quality Standards, Geneva.

Table (3) Comparison of Characteristics of Selective Point of Dokhtawaddy (Myitnge) River Water Samples for January and April, 2020

Sr No.	Characteristics	Sample Collection Time (January, 2020)				Sample Collection Time (April, 2020)				*Literature Value (WHO Standard)	
		Point (A)	Point (B)	Point (C)	Point (D)	Point (A)	Point (B)	Point (C)	Point (D)	Desirable	Imperative
1	pH	7.1	7.1	7.1	7.1	7.2	7.2	7.4	74	7-8.5	6.5-9.2
2	Colour (Units)	5	5	5	5	5	5	5	5	5	50
3	Turbidity (N.T.U)	4.07	4.12	5.10	5.10	4.12	4.18	5.90	6.15	5	25
4	Conductivity (micromohs/cm)	365	363	358	365	343	351	372	350	50	500
5	Total Alkalinity (mg/L)	180	185	220	220	190	195	225	238	200	500
6	Total Hardness (mg/L)	200	211	224	228	210	210	253	260	100	500
7	Calcium Hardness(mg/L)	35	32	38	38	32	30	45	52	75	200
8	Magnesium Hardness (mg/L)	18	13	25	27	18	18	25	31	30	150
9	Chloride (mg/L)	5	5	10	10	8	8	16	18	200	600
10	Iron (mg/L)	0.01	0.01	0.02	0.02	0.01	0.01	0.02	0.03	0.1	1.0
11	Manganese (mg/L)	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.05	0.5
12	Sulphate (mg/L)	<200	<200	<200	<200	<200	<200	<200	<200	<200	200
13	Arsenic (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	-	0.01
14	Copper (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	1.0	2.0
15	Cyanide (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	-	0.07
16	Lead (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	-	0.01
17	BOD (mg/L)	4.3	4.6	5.9	6.2	5.2	5.9	6.7	7.2	5.99	12
18	COD (mg/L)	13.6	12.5	14.3	21.3	12.5	12.3	15.5	18.3	6.99	30
19	Probable Coliform Counts	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	0	50
20	*Escherichia Coli Count*	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	0	0

Source: World Health Organization (2013), Standards of Potable Water Quality and Water-Borne Diseases, Geneva
World Health Organization (2015), Drinking Water Quality Standards, Geneva.

Table (4) Comparison of Characteristics of Selective Point of Dokhtawaddy (Myitnge) River Water Samples for July and October, 2020 (Before Treatment)

Sr No.	Characteristics	Sample Collection Time (July, 2020)				Sample Collection Time (October, 2020)				*Literature Value (WHO Standard)	
		Point (A)	Point (B)	Point (C)	Point (D)	Point (A)	Point (B)	Point (C)	Point (D)	Desirable	Imperative
1	pH	7.1	7.1	7.2	7.2	7.2	7.2	7.5	7.5	7-8.5	6.5-9.2
2	Colour (Units)	5	5	5	5	5	5	5	5	5	50
3	Turbidity (N.T.U)	5.02	5.08	6.50	6.88	4.13	4.28	5.71	7.20	5	25
4	Conductivity (micromohs/cm)	372	372	384	389	343	340	350	372	50	500
5	Total Alkalinity (mg/L)	179	175	195	200	185	185	225	246	200	500
6	Total Hardness (mg/L)	165	160	184	173	210	210	253	260	100	500
7	Calcium Hardness(mg/L)	32	32	40	45	32	42	75	89	75	200
8	Magnesium Hardness (mg/L)	17	17	19	19	22	22	25	31	30	150
9	Chloride (mg/L)	5	5	12	12	10	10	25	28	200	600
10	Iron (mg/L)	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.05	0.1	1.0
11	Manganese (mg/L)	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.05	0.05	0.5
12	Sulphate (mg/L)	<200	<200	<200	<200	<200	<200	<200	<200	<200	200
13	Arsenic (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	-	0.01
14	Copper (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	1.0	2.0
15	Cyanide (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	-	0.07
16	Lead (mg/L)	ND	ND	ND	ND	ND	ND	ND	ND	-	0.01
17	BOD ((mg/L)	5.4	5.7	6.2	6.9	5.4	5.8	7.1	7.4	5.99	12
18	COD (mg/L)	12.3	13.5	18.9	24.5	13.4	15.6	18.9	27.8	6.99	30
19	Probable Coliform Counts	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	0	50
20	*Escherichia Coli Count*	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	0	0

***Source:** World Health Organization (2013), Standards of Potable Water Quality and Water-Borne Diseases, Geneva
World Health Organization (2015), Drinking Water Quality Standards, Geneva.

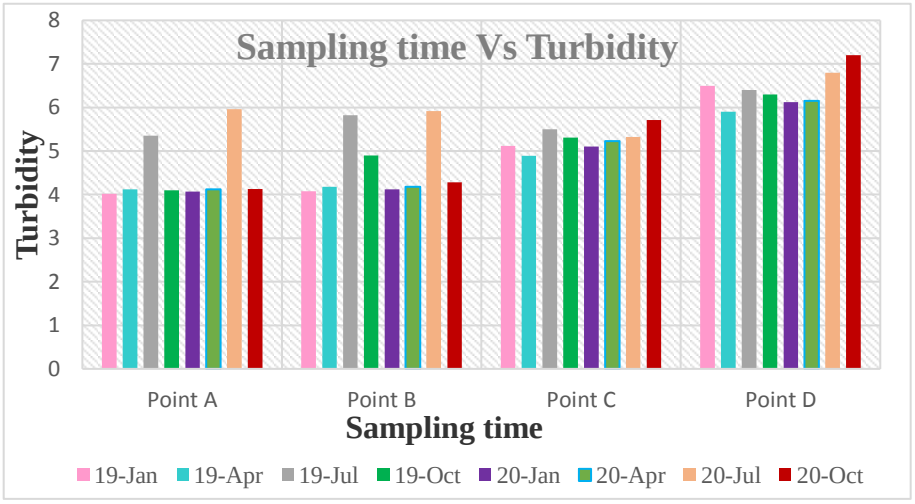

Figure (5) Comparison of Turbidity Values of Myitnge River Water Selected During 2019-2020

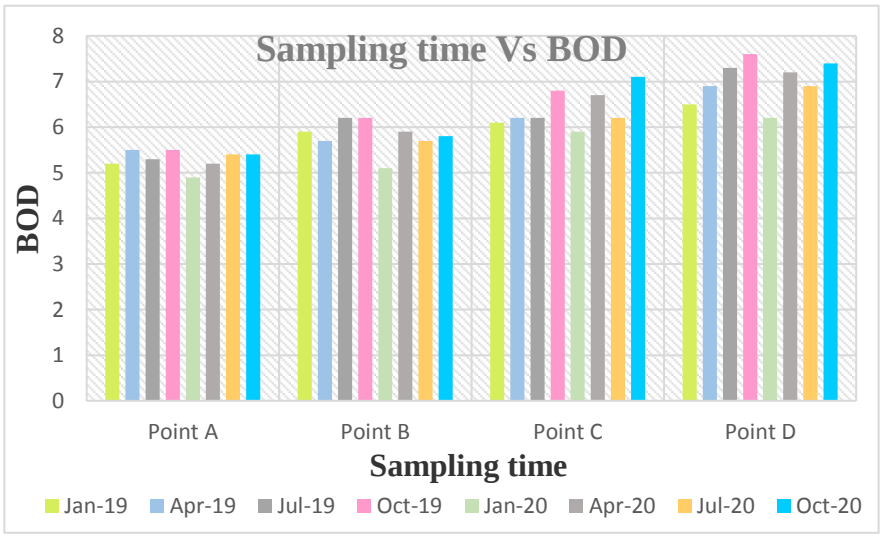

Figure (6) Comparison of BOD Values of Myitnga River Water During 2019-2020

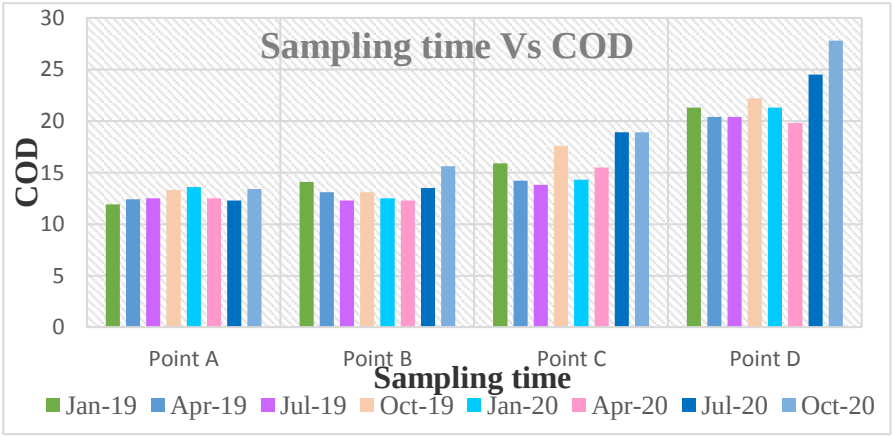

Figure (7) Comparison of COD Values of Myitnga River Water During 2019-2020

Table (5) Comparison of Characteristics of Dokhtawaddy (Myitnge) River Water Before and After Treatment from Selected Point (D) During October, 2020

Sr. No.	Characteristics	Before Treatment	After Treatment	Removal Efficiency (% w/v)	*Literature Value (WHO Standard)	
					Desirable	Imperative
1	pH	7.5	7.1	-	7-8.5	6.5-9.2
2	Colour (Units)	5	3	40	5	50
3	Turbidity (N.T.U)	7.20	2.2	69.4	5	25
4	Conductivity (ms/cm)	372	356	-	50	500
5	Total Alkalinity (mg/L)	240	122	52.5	200	500
6	Total Hardness (mg/L)	260	134	48.5	100	500
7	Calcium Hardness(mg/L)	89	35	60.6	75	200
8	Magnesium Hardness (mg/L)	31	21	32.3	30	150
9	Chloride (mg/L)	28	5	82.1	200	600
10	Iron (mg/L)	0.05	0.01	80	0.1	1.0
11	Manganese (mg/L)	0.05	0.01	80	0.05	0.5
12	Sulphate (mg/L)	<200	<200	-	<200	200
13	Arsenic (mg/L)	ND	ND	-	-	0.01
14	Copper (mg/L)	ND	ND	-	1.0	2.0
15	Cyanide (mg/L)	ND	ND	-	-	0.07
16	Lead (mg/L)	ND	ND	-	-	0.01
17	BOD(mg/L)	7.4	3.2	56.7	5.99	12
18	COD (mg/L)	27.8	12.5	55.0	6.99	30
19	Probable Coliform Counts	5/5	5/5	5/5	0	50
20	*Escherichia Coli Count*	Isolated	Isolated	Isolated	-	-

***Source:** World Health Organization (2013), Standards of Potable Water Quality and Water-Borne Diseases, Geneva

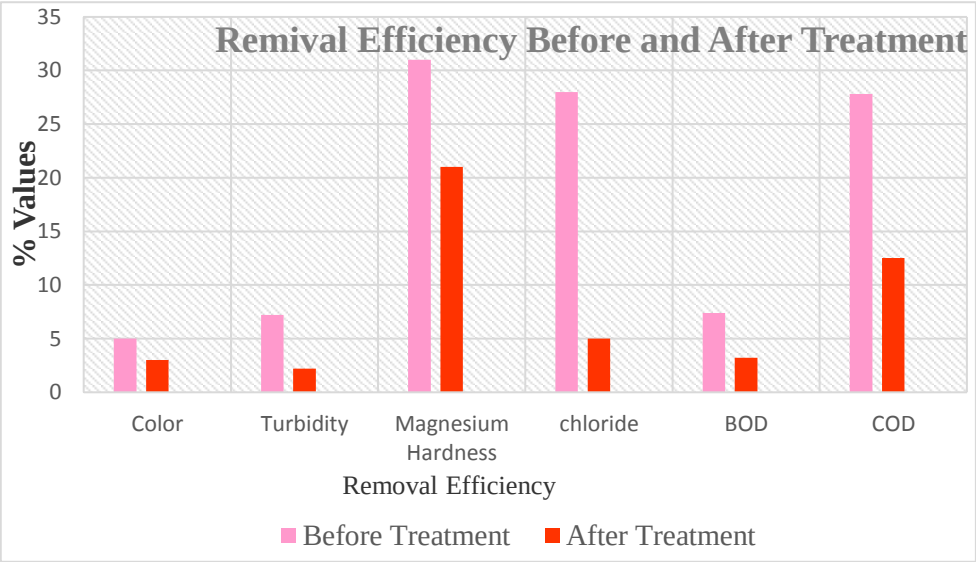

Figure (8) Comparison of Characteristics of Dokhtawaddy (Myitnge) River Water Before and After Treatment

Conclusion

Myanmar is heavily dependent on inland surface waterbodies for domestic use, agricultural irrigation and industrial production. Water quality monitoring data of Myitnge river revealed that water quality conditions in rivers are generally good, however need to treat for clean and safe uses.

In this research, the water samples were collected quarterly from upstream and downstream Mandalay Industrial Sewage Ditch of Dokhtawaddy River during 2019 and 2020. The characteristics of seasonally collected water samples are shown in Tables (1) to (4). Based on collected area as shown in tables, it must be concluded that the present investigation of site (C) and (D) is most polluted than others. The contamination degree of all collected water samples from the downstream side of Mandalay Industrial Sewage Ditch at points (C) and (D) were not suitable for potable purposes due to the presence of color, odor, suspended solids, several different minerals and bacteria for causing human intestinal diseases. And so, the water samples must be definitely treated by appropriate methods. Moreover, hydropower projects along Myitnge river can disrupt the natural flows river and freshwater ecosystem. These energy generation operations can destruct flow regime and influence the water quality parameters.

The effort of this research work contributes to the information of easier and inexpensive water treatments technology readily available for which rural people were early carried out. It is concluded that by contributing this low cost technology, the poor people could get the knowledge for the better clean water. From the results of this research work, this present study will be implemented the sustainable water resource development and support the downstream riverine ecosystem by controlling the impact risk on the flow regime. In addition, this work would confer a low cost approach to water treatment for poor peri-urban communities to get safe water and to maintain the native biodiversity and ecosystem integrity in the Dokhtawaddy river basin.

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BREWERY WASTEWATER TREATMENT WITH WATER HYACINTHS

Shwe Sin Oo[1], Wint Thuzar Than[2]

Abstract

Wastewater treatment technologies are essential, as water pollution is a major environmental concern. A natural treatment system is one of the most suitable treatment technologies, and it is a process of purifying contaminated water by growing aquatic plants that have the ability to absorb pollutants. This paper aims at investigating the removal efficiency (%) of brewery effluents (distillery effluents) using water hyacinth (*Eichhornia crassipes*). The treatment process in the present study was operated at the specified time duration, i.e., two hours, four hours, eight hours, one day, two days, four days and eight days. The physical and chemical characteristics, namely color, total dissolved solids (TDS), total suspended solids (TSS), conductivity, pH, biological oxygen demand (BOD_5), and chemical oxygen demand (COD) of the effluent samples before and after water hyacinth treatment were analyzed at the ISO TECH laboratory in Insein Township, Yangon. The physical and chemical parameters of the wastewater from the brewery are color (132 ± 2 TCU), total dissolved solids (1280 ± 4 mg/L), total suspended solids (633 ± 4.04 mg/L), pH (7.97 ± 0.19), conductivity (1806 ± 10.15µS/cm), biological oxygen demand (127 ± 8.62 mg/L), and chemical oxygen demand (196 ± 8.08 mg/L). The findings showed that color, TSS, and BOD_5 before treatment were higher than the guideline values for effluent. After eight days of treatment with natural plants, specifically water hyacinths, all analytical parameters were found to be well below the guidelines.

Keywords: wastewater treatment, water hyacinth, removal efficiency, physical and chemical characteristics, time duration.

Introduction

In recent years, the rapid growth of urbanization and the high standard of living have resulted in the rapid development of the industrial sector, leading to the generation of huge amounts of waste and wastewater (Ani Khare and Eugenia P.Lal, 2017). Large amounts of agricultural run-off and industrial effluents are increasing the pollution and contamination of freshwater resources and natural watercourses. Domestic waste, rubbish, and potentially toxic elements are also discharged into the environment by most people. Therefore, wastewater needs to be treated before it is released into the environment. This will reduce the contamination of the water resources around us. From the study of many research papers on water quality and its environment, many water bodies in Yangon are still suffering from sewage pollution. The most dangerous form of pollutants can be found in the wastewater that is discharged by many of the factories and industries in the industrial sector. Various factories, production companies, and manufacturing companies discharge different types of polluted wastewater. These wastewaters contain chemical pollutants and high concentrations of biodegradable organic compounds. These pollutants and organic compounds have an impact on water quality and make the water unsuitable for aquatic ecology (Zahra Mohebi, and Maryam Nazari 2021).

Water hyacinth, scientifically known as *Eichhornia crassipes*, is a perennial, free-floating aquatic plant that has been identified as capable of removing pollutants from industrial waste effluents (Jasmin Lad and Arti Pamnani, 2018). Water hyacinths, known for their capacity to absorb both organic and inorganic pollutants and their ease of management, play a crucial role in natural treatment systems. The utilization of water hyacinths in wastewater treatment has proven

[1] Department of Environment and Water Studies, University of Yangon, Myanmar
[2] Department of Environment and Water Studies, University of Yangon, Myanm

to be highly effective and economical (Ko Win and Aye Nyunt Kyi, 2020). These plants have demonstrated their efficiency in eliminating suspended solids, total dissolved solids,conductivity, organic matter, and various other dissolved pollutants from wastewater, resulting in the production of high-quality effluent. This paper explores the use of water hyacinth to remediate wastewater pollution from breweries.

Aim and Objectives

The aim of the present study is to evaluate the removal efficiencies (%) of physical and chemical characteristics for the treatment of distillery wastewater with water hyacinth. There are three objectives in this study, and they are as follows:

(1) to analyse physical characteristics, namely total dissolved solids (TDS), total suspended solids (TSS), colour and conductivity of the wastewater before and after treatment;
(2) to examine chemical characteristics such as pH, BOD_5 and COD of the effluent before and after treatment; and
(3) to calculate the removal efficiencies (%) of the physical and chemical characteristics of the effluent sample from the brewery followed by water hyacinth.

Materials and Method

The current research utilized water hyacinths to treat distillery wastewater. Two samples were collected for the study: water hyacinth (*Eichhornia crassipes*) and wastewater (distillery spent wash) obtained from a brewery. These samples were used as raw materials for experimental analysis.

Sample Collection

The plants used in this study were collected from upstream of the Aung Takhun Creek, which flows into Barla Creek and then into the Ngemoeyeik Creek, also known as Pazundaung Creek. After collecting the water hyacinth, rinse all samples thoroughly with tap water to remove impurities such as mud, microalgae, and insect larvae. Also, remove any unwanted parts of the plants, such as brown leaves and rotten roots. After cleaning, the sample plants were acclimatized for one week in tap water without any additional media or materials to allow them to adapt to their new environment (Mahesh W. and Jayaweera, 2008). Plants in good condition were then selected for experimental analysis.

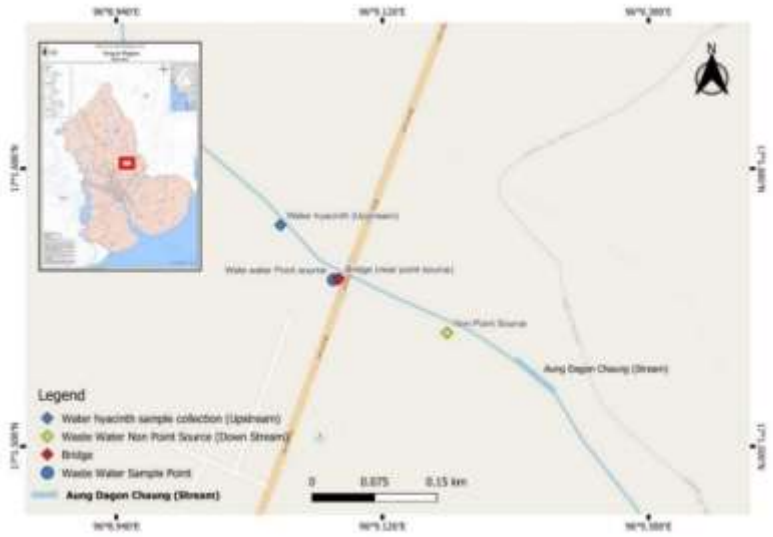

Figure 1 Location Map of Wastewater Sampling Site

Wastewater from the brewery is being discharged directly into Aung Takhun Creek and then flowing into Barla Creek. Wastewater sample were collected from the outlet of brewery directly and that is near No (3) Main Road at Pyinmabin Industrial Complex, Mingalardon Township, Yangon. Five 20-litre plastic bottles of wastewater samples were collected from the effluent (outlet) of the brewery's drainage system for the entire analysis.

Physico-chemical Analysis of Wastewater Sample

Wastewater sample before and after treatment were analysed for pH, colour, conductivity, total dissolved solid (TDS), total suspended solid (TSS), 5-day biological oxygen demand (BOD_5), and chemical oxygen demand (COD). For the analysis of wastewater sample before treatment, pH, conductivity and TDS of wastewater discharged from the brewery were measured on site using eXact Master Kit with pH & ORP, Part No. 486303. In addition, the pH, conductivity, and TDS of treated wastewater were measured in the laboratory at the Department of Environment and Water Studies, University of Yangon, after every treatment. Furthermore, after each treatment, the treated wastewater was sent to the ISO TECH laboratory in Insein Township at Yangon to test other parameters. The examination of wastewater characteristics before and after treatment, specifically BOD_5 and COD, was conducted following the UAS Standard Method 22nd Edition. The colour of water samples was tested using the Lovibond Spectro Direct Method No. 203. TSS was determined using the DR 3900 Spectrophotometer (HACH) with the Photometric Method.

Wastewater Treatment Process

Fifteen litres of collected wastewater were filtered and poured into a plastic bucket of 35 cm in diameter and 25 cm deep. Water hyacinth plants of the same size, with a total weight of about 1 kg, were evenly distributed in the wastewater sample contained in the plastic bucket. After the specified hours, four litres of treated wastewater were taken from each bucket and then sent to the laboratory. In this study, the duration of the experiments was set at two hours, four hours, eight hours, one day, two days, four days, and eight days.

Determination of Removal Efficiency (%)

After the analytical results had been obtained, removal efficiencies (%) of the characteristics of wastewater were calculated by the following equation (Rajnikant Prasad et al., 2021).

$$\text{Removal efficiency (\%)} = \frac{\text{Concentration before treatment} - \text{Concentration after treatment}}{\text{Concentration before treatment}} * 100\%$$

Results and Discussions

The ISO TECH laboratory in Insein Township, Yangon, analysed the TSS, BOD_5, and COD water quality characteristics of distillery wastewater before and after treatment, following the 'Standard Methods for the Examination of Water and Wastewater'. Table 1 compares the measured water quality parameters with the wastewater quality guidelines (Myanmar Emission Guidelines, 2015). Table 1 displays the minimum, maximum, and average values of the untreated wastewater characteristics. The characteristics of the treated wastewater are presented in Table 2. Table 3 shows the percentage of removal efficiency of water hyacinth.

Table 1. Characteristics of Untreated Wastewater

Sr No	Parameters	Unit	Min-Max	Average ± SD	Myanmar Emission Guidelines (2015)
1	Colour	TCU	129 - 135	132 ± 2	15
2	Total dissolved solids (TDS)	mg/L	1276 - 1284	1280 ± 4	1500
3	Total suspended solids (TSS)	mg/L	629 - 637	633 ± 4.04	50
4	pH	-	7.78 - 8.16	7.97 ± 0.19	6–9
5	Conductivity	µS/cm	1795 - 1815	1806 ± 10.15	2000
6	Biological oxygen demand (BOD_5)	mg/L	119 - 136	127 ± 8.62	50
7	Chemical oxygen demand (COD)	mg/L	189 - 205	196 ± 8.08	250

Table 2 shows the variations in process parameters (colour, TDS, TSS, BOD_5, and COD) in the wastewater after treatment during the study period.

Table 2. Characteristics of Treated Wastewater

Parameters	After Treatment						
	2 hours	**4 hours**	**8 hours**	**1 day**	**2 days**	**4 days**	**8 days**
Colour	121	98	75	49	25	17	15
Total dissolved solids (TDS)	1165	1150	1100	907	755	615	598
Total suspended solids (TSS)	563	490	411	253	137	49	45
pH	7.82	7.75	7.62	7.45	7.29	7.01	7.00
Conductivity	1631	1615	1551	1160	1025	867	421
Biological oxygen demand (BOD_5)	109	91	78	58	43	13	10
Chemical oxygen demand (COD)	180	164	131	108	82	33	28

The removal efficiencies of water hyacinth in the treatment of brewery effluent and the corresponding time periods are shown in Table 3.

Table 3. Removal Efficiency % of Water Hyacinth

	Removal Efficiency %				
Time Duration	**Colour**	**TDS**	**TSS**	**BOD_5**	**COD**
2 hours	12.88	8.98	11.06	14.17	8.16
4 hours	25.76	10.96	22.59	28.35	16.33
8 hours	43.18	14.06	35.07	38.58	33.16
1 day	62.88	29.14	60.03	54.33	44.90
2 days	81.06	41.02	78.36	66.14	58.16
4 days	89.39	51.95	92.26	89.76	83.16
8 days	91.67	55.23	94.00	92.13	85.71

Figure 2 shows the colour removal efficiencies (%) in the treatment of distillery wastewater with water hyacinth. From the graph, it can be seen that the minimum removal efficiency is 12.88 % after two hours of treatment, and the maximum removal efficiency is

91.67% over the eight-day treatment period. After four hours, 25.76% was removed, and after eight hours, 43.18% was removed. In addition, it was found that the removal efficiency was 62.88% after one day of treatment and 81.06% after two days of treatment.

Figure 3 shows the efficiency (%) of total dissolved solids (TDS) removal when treating distillery waste with water hyacinth. The results indicate that after two hours of treatment, the minimum removal efficiency is 8.98%, while the maximum removal efficiency is 55.23% after eight days of treatment. After four hours of treatment, the removal efficiency is 10.16%, and after eight hours, it is 14.106%. Additionally, the removal efficiency is 29.14% after one day and 42.10% after two days.

Figure 4 shows the percentage of total suspended solids (TSS) removed during the treatment of distillery wastewater using water hyacinth. The results indicate that the minimum TSS removal efficiency is 11.06% after two hours of treatment, while the maximum TSS removal efficiency is 94.00% after eight days of treatment. The removal efficiency for four hours of treatment is 22.59%, and for eight hours of treatment, it is 35.07%. Additionally, TSS removal is 60.03% after one day and 78.36% after two days.

In this study, the minimum removal efficiency of water hyacinth for 5-day biological oxygen demand (BOD_5) was observed to be 14.17% after two hours of treatment. The removal efficiency increased to 28.35% after four hours, 38.58% after eight hours, 54.33% after one day, and 66.4% after two days. At the end of the four-day treatment period, 89.76% of the BOD_5 was removed from the untreated distillery wastewater. After eight-day treatment, maximum removal efficiency was 92.13%. Figure 5 shows the recorded results.

During the two-hour treatment period, the COD removal efficiency was found to be a minimum of 8.16%. The removal efficiency increased to 16.33% after four hours, 33.16% after eight hours, 44.90% after one day, 58.16% after two days, 83.16% after four days and reached a maximum of 85.71% after eight-day treatment. The analytical results are presented in Figure 6.

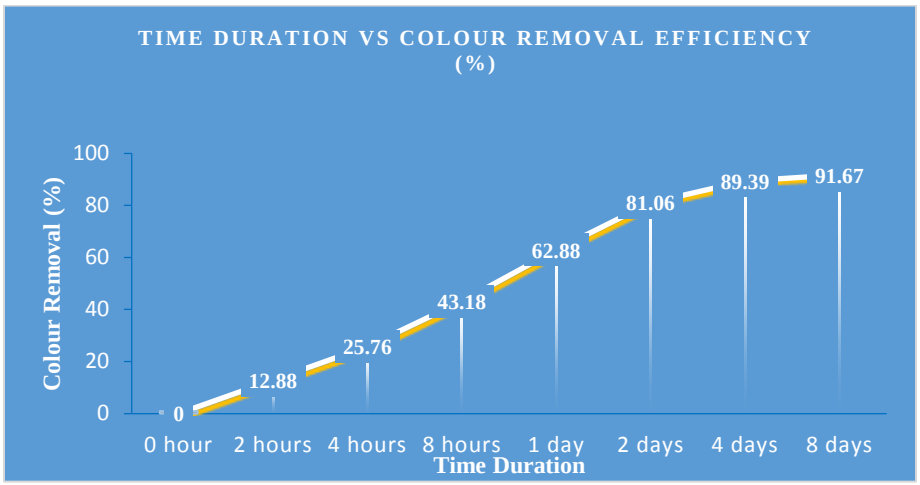

Figure 2. Colour Removal Efficiency (%) of Water Hyacinth

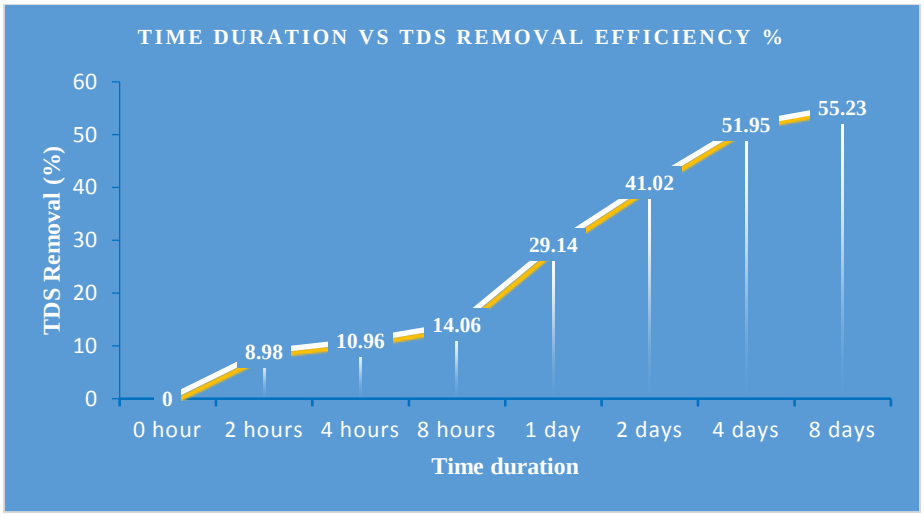

Figure 3. Removal Efficiency (%) of Water hyacinth for Total Dissolved Solids (TDS)

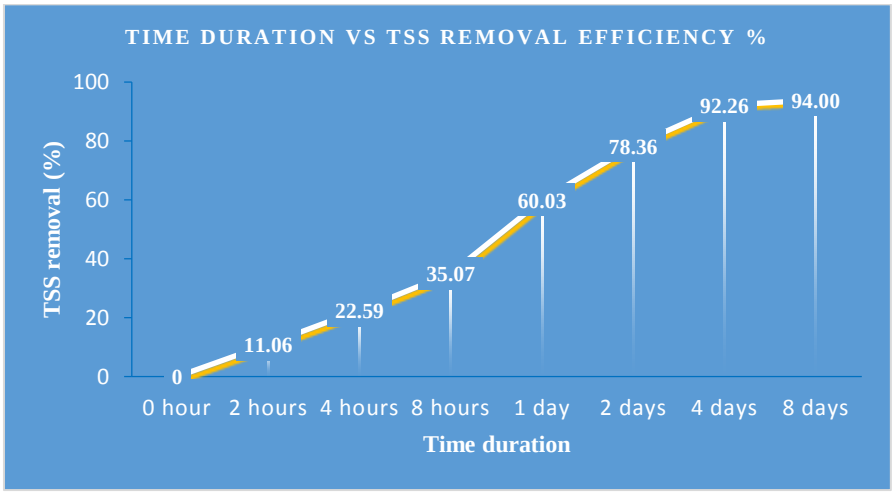

Figure 4. Removal Efficiency (%) of Water Hyacinth for Total Suspended Solids (TSS)

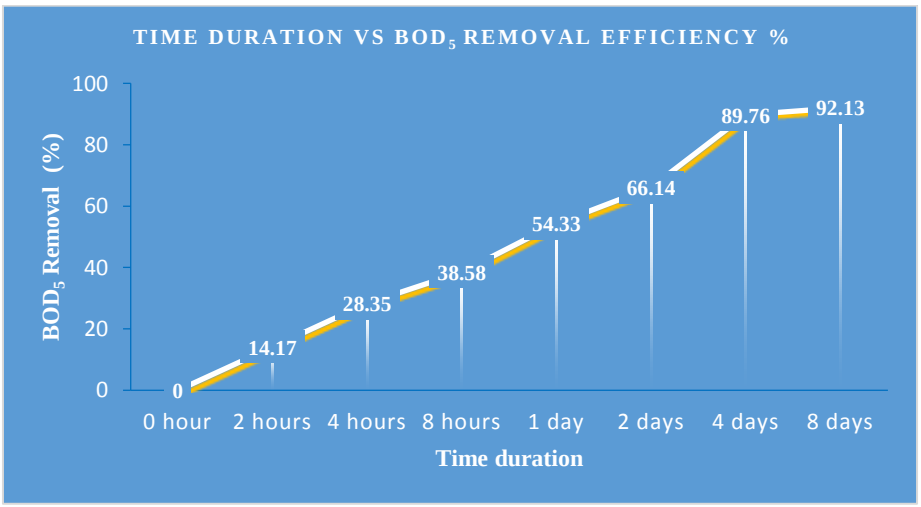

Figure 5. Removal Efficiency (%) of Water Hyacinth for Biological Oxygen Demand (BOD_5)

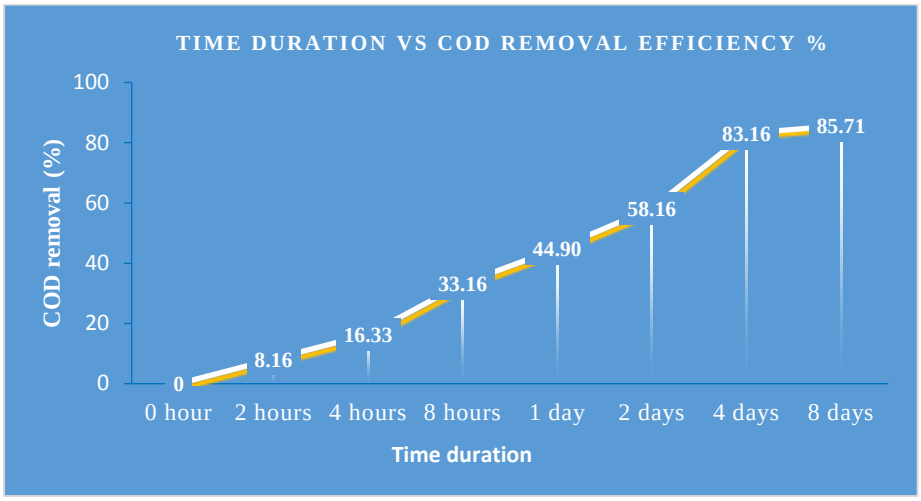

Figure 6. Removal Efficiency (%) of Water Hyacinth for Chemical Oxygen Demand (COD)

All the graphs in Figure 2 to Figure 6 were obtained using the analytical results, it can be seen clearly that the percentage removal of five parameters: colour, TDS, TSS, BOD_5, and COD are increasing with the increasing of time duration for the treatment. At the end of the eight days, physical and chemical parameters of treated wastewater are well below the guideline values limited by Myanmar Emission Guidelines (2015).

Conclusion

The longer the time duration, the higher the removal of color, TDS, TSS, BOD_5, and COD from the experimental results. Additionally, the removal percentage of TSS is superior to that of TDS, and the removal efficiency of 5-day biological oxygen demand (BOD_5) is greater than that of chemical oxygen demand (COD). For the specified experimental durations of four days and eight days, the rate of removal is lower than in other experimental periods. After eight days of treatment, all parameters of treated wastewater are well below the Myanmar National Environmental Quality (Emission) Guidelines (2015). This study concludes that the water hyacinth system is effective for treating brewery wastewater, particularly in Myanmar due to its tropical and subtropical climate, making the water hyacinth plant highly suitable for natural wastewater treatment technology.

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