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Contents

Zoology

Monogenean parasite in goldfish, *Carassius auratus* (Linnaeus, 1758)

Kyawt Kyawt Shinn[1] and Kay Lwin Tun[2]

Abstract

Dactylogyrus spp. are monogenean parasites that infect the gills of host species. Although, fish appear to co-exist with their specific monogeneans as natural habitats, they become pathogenic to their host fish in intensive culture conditions. In the present study, goldfish, *Carassius auratus*, from Hlaing Thar Yar ornamental fish farms were sampled and examined for monogenean parasitic infection. Fish were infected with *Dactylogyrus* sp. and prevalence of infection was very high (100%). According to the shape and size of hapter and copulatory complex, *Dactylogyrus* sp. was identified as *Dactylogyrus intermedius*. Thirty eight *C. auratus* specimens were investigated for spatial distribution of *D. intermedius. Dactylogyrus intermedius* showed the preference for distal part of the gills of host fish.

Key words: Monogenea, goldfish, *Dactylogyrus intermedius*, *Carassius auratus* prevalence of infection

Introduction

Fish parasites and their effects have become increasingly visible during the latest decades inconnection with the development of freshwater ornamental fish industries throughout the world (Alvarez-Pellitero, 2004). Diseases caused by parasites are widespread and cause loses of fish in intensively stocked pond and aquarium. Ectoparasites of freshwater ornamental fish come in all sizes and shapes and include single-celled protozoan, and multicellular trematodes (flatworms), crustaceans and arthropods (Roberts, 2010). Ectoparasites causing in ornamental fish only kills the fish but also reduces the market value of fish (Mousavi 2003, Koyuncu 2009).

Among harmful parasites, gill ectoparasite monogenean represent one of the most important group which facilitate in spreading of infection since their life history is direct and thus enabling the transmitting from one host to

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another. Monogeneans are flatworms (Platyhelminthes), ectoparasite and attached by special posteriorly positioned attachment organs to their host's skin on gills. Parasite populations can impact negatively through loss of fish growth, decreased market value due to parasite - induced damage on fish (Abowei and Ezekiel, 2011). Young fishes are subjected to the risk of infection with these parasites which might cause diseases and mortalities among fry in hatcheries, and among larger fishes (Amlacher, 1970). For example, *D. vastator* caused great damage to the gill filaments of carps and goldfishes in California hatcheries (Hoffman, 1998).

The present study was undertaken to investigate the monogenean infection in goldfish farm. Prevalence and intensity of infections of monogenea in ornamental fish farm were also reported.

Materials and methods

Sample collection and preparation of aquaria

Thirty eight *Carassius auratus* from Hlaing Thar Yar ornamental fish farm was sampled in November, 2010. Fish samples were taken by plastic bags filled with oxygen to the Laboratory of Aquatic Bioscience, Department of Zoology, University of Yangon. They were kept in the small aquaria in the laboratory and aeration was given. Before keeping the fish, aquaria were thoroughly cleaned, filled with tap water and given by aeration one day ahead. Some fishes were immediately dissected out for examining parasites and other were kept in the aquarium for about two days for later studies. Diagnostic symptoms were carefully recorded from individual fish.

Examination on spatial distribution of monogenean parasite

Fish were killed and the gills were excised and each arch placed in a separate Petri dish containing aquarium water and observed under a stereo microscope. Eight gill arches from each side of the fish were divided into 2 sections, distal and proximal, approximately equal in surface area. The number of worms on each section was recorded and their position plotted on a gill map as show in Figure 1.

Species identification

To verify the specific identity, the monogeneans were detached from the gill using strong water current. The worms were then transferred

individually into a drop of ammonium picrate-glycerine on a slide with a mounted needle. The preparation was then covered with cover slip. From these preparations, drawings were made of the sclerotised pieces of the haptor and of the copulatory complex using light microscope. The descriptive terminology and measured techniques were according to Gussev (1976). Parasites were identified following the description and figures of Yamaguti (1963).

Data analyses

Total number of parasites was determined by counting. Prevalence, abundance and mean intensity were calculated for each parasite species from their hosts in accordance with the following method by Margolis *et al*. (1982).

1. Prevalence (%)

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

2. Abundance

$$= \frac{\text{Number of parasites}}{\text{Total number of host examined}}$$

3. Mean intensity

$$= \frac{\text{Number of parasites}}{\text{Total number of infected host}}$$

Student t test were used to determine whether there were significant differences of distribution of monogenea between the left and right sides of each gill arch.

Results

Monogenean infection in *Carassius auratus*

Fish were infected with *Dactylogyrus intermedius* (Fig. 2). The following is an account on description and measurements of this parasite**:**

Host	-	*Carassius auratus*
Site of infection	-	gill filaments
Host locality	-	Hlaing Thar Yar ornamental fish farm, Yangon Division
Number of specimen measured	-	50

Body – Plump with blunt anterior end. Total length 2.68 mm ± 0.55 mm and greatest width 0.53mm ± 0.07mm. Posterior pair of eyes port larger and slightly overlapping with oval pharynx with its size. Copulatory complex is composed of a curved tube-like structure which is connected by a small tube.

Haptor – Anchor deeply forked and as broad as body width its size of 72 µm ± 0.25 µm. One pair of anchor joined by supporting bar. Single supporting bar saddle-shaped with two knobbed. Seven pairs of marginal hooklets with a dilated sickle and small thin handle. The distal end swollen and with the shape of a small bulb. Detail descriptions of parasite were described in Figure 3.

Distribution of *Dactylogyrus intermedius*

Thirty eight goldfish specimens (length range: 5.02 ± 1.60cm) collected from Hlaing Thar yar ornamental fish farm were found to be infected with *Dactylogyrus intermedius* (prevalence 100%). During the study period appromixly 14470 specimens of *D intermedius* were examined. Mean number of parasites recorded in each branchial arches are described in Table 1.

The mean intensity of infection in left and right gills was detected as 90 and 75, respectively. Mean abundance of infection in left and right gills was recorded as 76 and 70, respectively (Table.2). The data analysis did not

show any statistically significant difference in the number of *D. intermedius* between the right and left set of gill arches of goldfish (P>0.05).

There were a significantly greater mean intensity and abundance of *D. intermedius* on the hemibranch (P< 0.05). The mean intensity and abundance of *D. intermedius* on the distal and proximal hemibranch is given in Table 3. There were significant differences in the number of *D. intermedius* on the different gill areas (P<0.05). Except on abundance of parasite on right gills, a greater number of *D. intermedius* occurred on the distal than on the proximal segments of the gills.

Discussion

In the present study, *Carassius auratus* was infected with *Dactylogyrus intermedius* and prevalence of infection was very high (100%). *Dactylogyrus* spp. is common ectoparasite living on the gills in freshwater fish (Woo, 2006). This genus includes numerous species, while the pathologic significance is very dependent on the species and intensity of infection (Alvarez-Pellitero, 2004). *Dactylogyrus intermedius* is one of the important veterinary ectoparasites in Asia, Central Europe, Middle East, and North America (Paperna, 1963). *Dactylogyrus intermedius* may cause gill inflammation, excessive mucous secretions, accelerated respiration (Reed *et al*., 2009), and mixed infections with other parasites and secondary bacterial infections (Woo, 2006). The infection of *D. intermedius* in ornamental fish farm may lead to high mortality and serious economic damage.

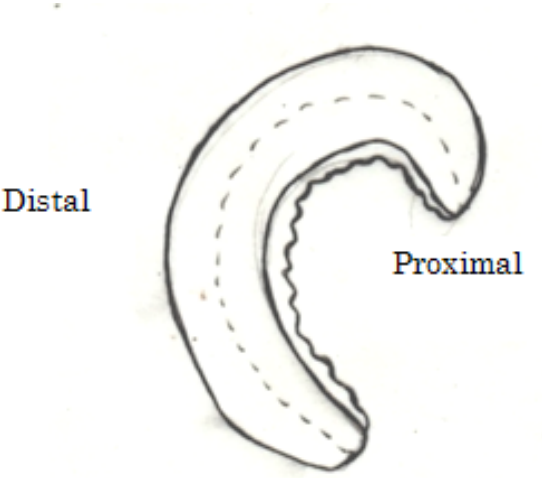
There are many studies on the microhabitat distribution of monogeneans on the gill of their host. (Dzika and Szymanski,1989; EI Hafidi *et al*.,1998; Simkova *et al*.,2000; Chapman *et al*.,2000; Lo and Morand,2000; Simkova *et al*., 2000, 2002; Matejusova *et al*.,2002; Kadlec *et al*.,2003). In the present study, the *D. intermedius* showed any significant differences in distribution between the right and left sets of gills. However, preferences the right side was recorded by *D. amphibothrium* (Wootten, 1974) and *Microcotyle mugilis* and also preferences for the left side was reported by *Metamicrocotyle cephalus* (EI Hafidi *et al*., 1998).

There was however, a significant preference for particular hemibranchs. Greater number of *D. intermedius* attached to the distal part of the gill filament, with fewer worm attached to the proximal segment of the hemibranch. Schaperclaus (1991) also found that *D.* extensus was mostly

located on the distal part of the gill filaments. *D. vastator* prefers to attach to the terminal edge of the gill filaments (Dzika & Szymalski, 1989). The specific preference might be effected by the interaction of the several factors such as differences in the hydrostatic pressure of the branchial pump, coughing action (Bijtel, 1949), water current over the gill surface during the respiratory cycle (Paling, 1968; Wooten, 1974).

Summary

Goldfish, *Carassius auratus*, from Hlaing Thar Yar ornamental fish farm were investigated for monogenean parasitic infection. Thirty eight goldfish specimens were infected with *Dactylogyrus intermedius* and the prevalence of infection was 100%. No significant preferences were found in the distribution of *D. intermedius* species on the gill arches between the left and right sides of its host. There was however, a significant preference for specific gill arches or for particular faces of the hemibranchs.



. Fig.1. Illustration of gill arch showing its division into two areas: 1. Distal, 2. Proximal

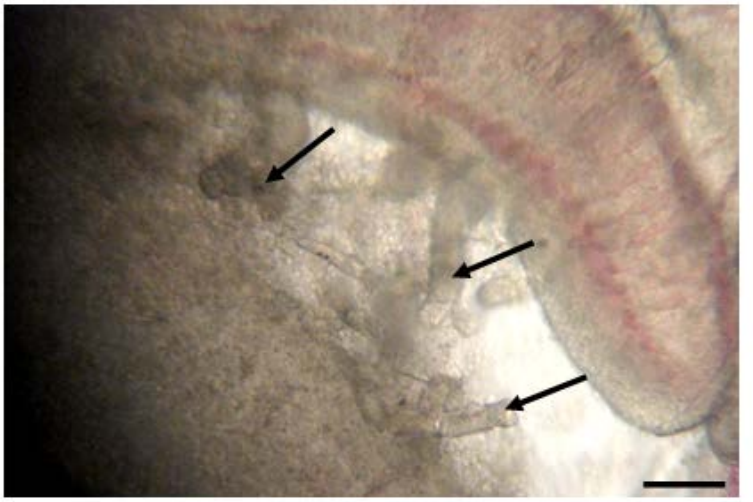


Fig. 2.Gills of *Carassius auratus* infected with *Dactylogyrus intermedius* (Scale bar = 2mm)

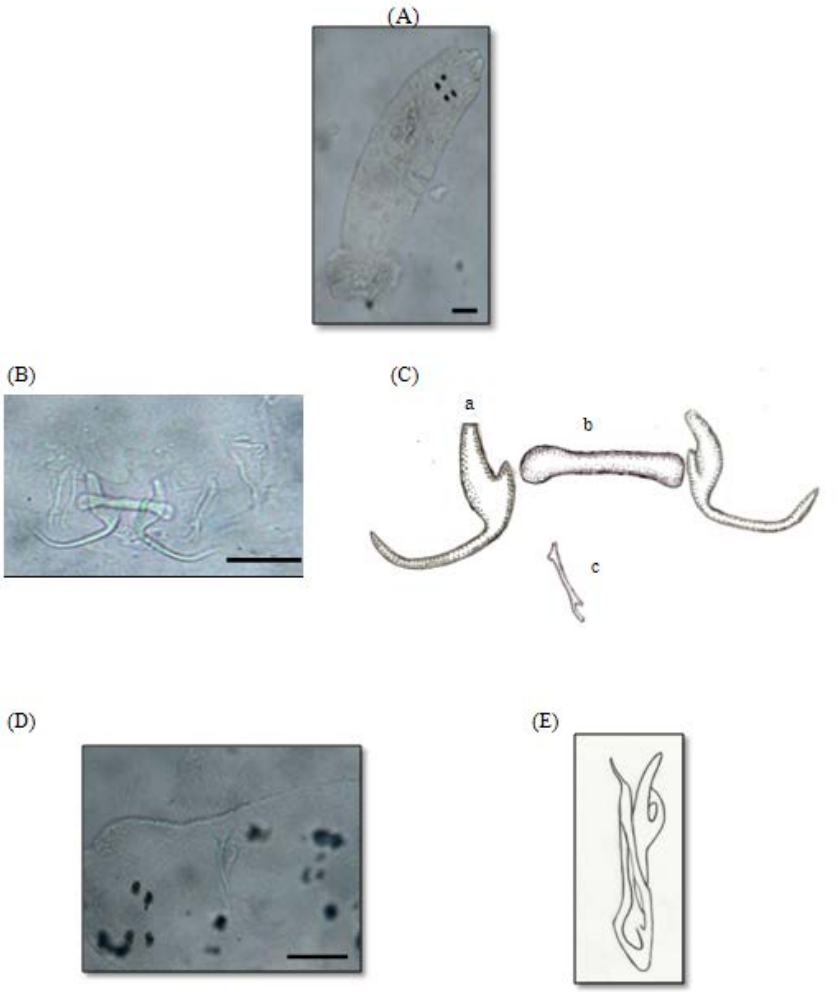


Fig.3. *Dactylogyrus intermedius* in *Carassius auratus* (A) Whole specimen of *D. intermedius* (B) Haptor (C) Line drawing of haptor; (a) anchor, (b) supporting bar, (c) marginal hook (D) copulatory complex (E) Line drawing of copulatory complex (Scale bar= 50µm

Table (1) Dactylogyrus intermedius in sectors distinguished in four branchial arch of gold fish

Arch	Side	Sector of Branchial arch		Total
		Distal	Proximal	
I	R	22.2 ±16.4	13±9.6	35.2±13
	L	27.6±15.9	17.8±9	45.4±12.4
II	R	34.2±27	14.8±11.7	49.0±19.35
	L	29.8±13.3	18±8.9	47.8±11.1
III	R	27.6±8	17.2±7.9	44.8±7.8
	L	33.4±91	26.8±29	60.2±60
IV	R	29.4±14.8	17.6±11.1	47±12.9
	L	29±21	15.4 ±12.1	44.4±16.5

Table (2). Prevalence, mean intensity and mean abundance of *Dactylogyrus intermedius* in *Carassius auratus*

Gill set	Left	Right

Prevalence	100%	100%
Mean intensity of infection	90	75
Mean abundance of parasite	76	70

Table (3). The spatial distribution of *Dactylogyrus intermedius* over the gill apparatus of *Carassius auratus*

Gill set	Left		Right	
Halves of primary lamella	Distal	Proximal	Distal	Proximal
Prevalence	100%	100%	60%	100%
Mean intensity of infection	62	30(*)	47	26(*)
Abundance of parasite	47	29(*)	38	29

(*) Mean intensity and abundance of parasite in proximal was significantly different from that of distal ($P<0.05$).

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References

Abowei and E.N. Ezekiel (2011) A Review of Myxosporea, Microspora and Monogenea Infections in African Fish. *British Journal of Pharmacology and Toxicology* 2(5): 236-250

Alvarez- Pellitero, P. (2004). Report about fish parasitic diseases. CIHEAM-IAMZ, Zaragoza, p 123

Amlacher E. (1970). Textbook of fish diseases. T.F.H. Publ., Jersey City: 302pp.

Bijtel, J.H. (1949).The structure and the mechanism of movements of the gill filaments in Teleostei. Archives Netherlandais Zoologie, 8: 267 – 288

Chapman, L.J., C.A Lanciani and C.A Chapman, (2000). Ecology of a diplozoon parasite on the gill of the

African Cyprinid *Barbus neumayeri*. *African Journal of Ecology*, 38: 312 – 320

Dzika, E. and S.Symanski, (1989). Co- occurrence and distribution of Monogenea of the genus *Dactylogyrus* on the gill of the bream,*Abramis brama* L. Acta Parasitologica Polonica, 34: 1-14.

EL Hafidi, F., O. Berrada- Rkhami, T. Benazzou and C. Gabrion, (1998). Microhabitat distribution and coexistence of Microcotylidae (Monogenea) on the gills of the striped mullet *Mugil cephalus*: change or competition? Parasitological Research, 84: 315 -320

Gussev, A. V., (1976). Freshwater Indian Monogenoidea: Principles of systematic analysis of the world faunas and their evolution. *Indian journal of Helminthology*.

Hoffman G.L., (1998) . Parasites of North American freshwater fishes. Cornell Univ. Press, London: 539pp.

Kadlec, D., A. Simkova, and M. Gaelnr, (2003). The microhabitat distribution of two *Dactylogyrus* species paracitizing the gill of the barbell, *Barbus barbus. Journal Helminthology*, 77 (4) : 317- 325.

Koyuncu, E. (2009). Parasites of ornamental fish in Turkey: A case study. *Bull. Eur. Ass. Fish Pathol.* 29 (1), 25-27.

Lo, C.M. and S. Morand, (2000).Spatial distribution and coexistence of monogenean gill parasites inhabiting two damselfishes from Moorea island in French polynesia. *Journal of Helminthology*, 74 94): 329-36.

Margolis, L., G. W. Esch, J., C. Homes, A. M. Kuris and G. A. Schad, (1982). The use ecological terms in parasitology (Report of an ad hoc committee of the American Society of Proctologists). *Journal of Parasitology*, 68, 131- 133.

Matejusova, I., A. Simkova, P. Sasal, and M. Gelnar, (2002). Anguillae and P.bini among and within gill arches of the European eel (*Anguilla Anguilla* L.). Parasitology Research, 89: 290- 296.

Mousavi H. A. E. (2003). Parasites of Ornamental fish in Iran: A case study. *Bulletin of the European Association of Fish Pathologists* 23(6), 297-300.

Paling, J.E., (1968). A method of estimating the relative volumes of water flowing over the different gill of a fresher water fish. *Journal of Experimental Biology*, 48: 533-544.

Paperna, I., (1963).Some observations on the biology of *Dactylogyrus* vastator in Israel. Bamidgeh (Bull, Fish Cult Israel).15:8-28.

Reed, P.A., R. Francis-Floyd and R.C. Klinger. (2009) FA28/FA033. Monogenean parasites of fish. EDIS-Electronic Data Information Source-UF/IFAS Extension.

University of Florida. Available at http://edis.ifas.ufl.edu/FA033. Accessed 17 May 2009

Roberts, H.E. (2010). Fundamentals of Ornamental Fish Health, 1. Edition ed. 28-108pp), USA, NY: Blackwell Publ.

Schaperclaus, W. 1991. Fish Diseases. Volume 2. Oxonian Press Pvt. Ltd., New Delhi. pp-1397.

Simkova, A., Y. Desdevises, M. Gelnar, and S. Morand, (2000). Co – existence of nine gill ectoparasites (*Dactylogyrus*: Monogenea) parasitizing the roach (Rutilus rutilus L.): history and present ecology. *International Journal of Parasitology*, 30 (10): 1077-1088.

Simkova, A., M. Ondrackova, M. Gelnar, and S. Morand, (2002).Morphology and coexistence of cogeneric ectoparasite species: reinforcement of reproductive isolation? *Biological Journal of the Linneans Society*, 76: 125-135.

Woo, P.T.K., (2006). Fish diseases and disorders: protozoan and metazoan infections, 2nd edn. CAB International, London, p 791.

Wootten, R., (1974). The spatial distribution of *Dactylogyrus amphibothrium* on the gill of ruff *Gymnocephalus cernua* and its relationton the relative amounts of water passing over the parts of the gills. *Journal of Helminthology*, 48: 167-174.

Yamaguti, S., (1963). Systema Helminthum- IV: Monogenea and Aspidocotylea. Interscience Pablication, N.Y, p-699

Occurrence of *Ichthyophthirius multifiliis* in some ornamental fish

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Abstract

Ichthyophthirius multifiliis can infect almost all freshwater fish causing devastating losses in susceptible fish. This study was conducted to determine prevalence and infection intensity of *I. multifiliis* in seven ornamental fish species. Ten specimen of each species, balashark (*Balantiocheilos melanopterus*), goldfish (*Carassius auratus*), blackmoor (*Carassius auratus*), guppies (*Lebistes reticulates*), rainbow tetra (*Paracheirodon innesi*), discus (*Synphesodon discus*) and swordtail (*Xiphophorus helleri*) and platy (*Xiphophorus maculates*) were collected from ornamental fish farm. Their gills and skin were checked under light microscope for *I. multifiliis* infection. The protozoan *I. multifiliis* was recorded in the five ornamental fish species examined. High prevalence and mean intensity of infections were observed in blackmoor and guppies. Balashark, Discuss and Rainbow tetra were not infected with *I. multifiliis*

Key words: *Ichthyophthirius multifiliis*, ornamental fish, white sport, protozoan parasite

Introduction

The ecto-parasitic ciliate *Ichthyophthirius multifiliis* is a common protozoan pathogen of freshwater fishes and etiological agent of Ich or white spot disease (Ventura and Paperna, 1985). The parasite is commonly distributed, occurring in tropical, subtropical and temperate regions, and extending north to the Arctic Circle (Matthews, 1994). It causes severe epizootics among different fish species in aquaria, hatcheries, and ponds, as well as in wild fish populations (Kim *et al*., 2002). In intensive aquaculture systems, Ich epizootics are more common due to the confinement of fish under stressful condition and the exponential increase in parasite numbers (Hines, 1973)

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The life cycle consists of 4 developmental stages: parasitic trophonts growing in size without cell division within the epithelial layer of the skin and fins of the host fish, protomonts leaving hosts and being encysted in water, encysted tomonts in which repeated cell fissions occur, and theronts released from tomonts into water (Colomi 1985, Colorni and Burgess 1997, Dickerson 2006). The life cycle takes 10 -18 days to complete, depending on temperature. Since the life cycle of *I. multifiliis* is a direct and requires no intermediate host (Ezz El-Dien et al., 1998), it can easily infect from one fish to another.

Recently, white spot disease in ornamental fish farms in Myanmar was reported (U Sai, personal communication). However, only limited research has been carried out regarding infection rates of *I. multifiliis* among ornamental fish (Thi Thi Thaw, 2007). The present study was undertaken to investigate the prevalence and intensity of *Ichthyophthirius multifiliis* infection in seven ornamental fish species that were collected from ornamental fish farm. Investigational approaches might provide a potential clue for management of Ich infection.

Materials and Methods

Sample collection

Ten specimen of each species, balashark (*Balantiocheilos melanopterus*), goldfish (*Carassius auratus*), blackmoor (*Carassius auratus*), guppies (*Lebistes reticulates*), rainbow tetra (*Paracheirodon innesi*), discus (*Synphesodon discus*), swordtail (*Xiphophorus helleri*) and platy (*Xiphophorus maculates*) were purchased from ornamaental fish farm located at South Okkalapa Township, Yangon Region in December 2010 (Fig. 1). Live ornamental fish were taken to the laboratory of aquatic bioscience, Department of Zoology, University of Yangon, in polythene bags filled with oxygenated pond water.

Examination of parasite

At the laboratory, fish were initially examined for the presence of any parasites or lesions visible to the naked eye. Wet mounts of scrapings (of body surface mucus from behind the pectoral fin adjacent to the dorsal fin and operculum,) and gills of freshly killed fish were examined separately for

parasites. Tissues were placed on the glass slide, added with physiological saline solution (0.7% Na sl Solution), covered with cover slip and examined under light microscope. Number of tomonts was counted.

For species identification, when parasite were detected, new smears were air dried and stained with May-Grunwald solutions (Sigma) for 5 min, washed with PBS briefly, then stained with Giemsa solution (BDH Chemicals, 1:20 diluted with distilled water) for 20 min and washed with distilled water. Coverslips were mounted on stained specimens with a small drop of Canada balsam. Species identification was carried out according to Lom and Dykova (1992) .

Prevalence, abundance and mean intensity were calculated in accordance with the following method by Margolis *et al*. (1982).

Prevalence (%)

$$= \frac{\text{Numbers of infected host}}{\text{Total number of fish}} \times 100$$

Abundance

$$= \frac{\text{Number of parasite}}{\text{Total number of host examined}}$$

Mean intensity

$$= \frac{\text{Number of parasite}}{\text{Total number of infected host}}$$

Experimental infection of *I. multifiliis*

I. multifiliis was harvested from the skin of infected goldfish purchased from ornamental fished farm located at South Okkalapa Township. Ten specimen of each species, goldfish (*Carassius auratus*), blackmoor (*Carassius auratus*), guppies (*Lebistes reticulates*), swordtail (*Xiphophorus helleri*) and platy (*Xiphophorus maculates*) reared under pathogen free fish farm were transferred to 50 L aquarium introduced with approximately 2490

individual of parasites. In control group, 20ml of distilled water was added instead of parasite. Fish were maintained for three weeks. One fish from each species were killed weekly to examine the number of parasite in each species. Water temperature during the study period was 25 to 28º C.

Prevalence, intensity and abundance among the fish species were compared using ANOVA and Tukey-Kramer test was employed as multiple comparison tests in order to pinpoint statistical differences. In all comparisons, significance level was set at 0.05.

Result

Ichthyopthirius multifiliis infection in ornamental fish

Ichthyopthirius multifiliis were detected in the gills of swordtail, black moor, goldfish, platy and guppies. The size of parasite was 380±30.8 µm (Fig. 2). It has an oval to round shape with uniformly ciliated in surface. A horseshoe-shaped nucleus can be seen in the center of parasite. Discuss, balashark and rainbow tetra were not infected with *I. multifiliis.*

Infection rate of *Ichthyopthirius multifiliis* in different ornamental fish

Ichthyophthirius multifiliis was found in five fish species. Black moor and guppy has low susceptibility to white spot disease when compared with other species as they showed the highest prevalence with the value of 100%. In goldfish and swordtail, prevalence of infection was more than 40% while platy was 30%. No infection was recorded in discuss, balashark and rainbow tetra (Fig. 3).

Number of parasite in infected fish was counted to calculate the abundance and intensity of infection. High abundance was recorded in skin of black moor and guppy with the value of 56.7 and 52, respectively. Abundance of parasite in goldfish, swordtail and platy were 20.3, 25 and 20, respectively. Abundance of parasite in goldfish and guppy were significantly higher than that of other infected fish (Fig. 4).

Similar results were obtained when calculated the mean intensity of infection. The highest mean intensity (58.9) was recorded in guppy while the lowest one (11) was found in platy. Intensity of parasite in goldfish, swordtail and blackmoore were 19.6, 28 and 52, respectively.

Intensity of parasite in Blackmoore and guppy were significantly higher than that of platy and goldfish (Fig. 5). Abundance and mean intensity of parasites in skin were higher than gill in all infected fish ($P<0.05$) (Fig.5).

Experimental infection of *I. multifiliis*

Number of parasites in experimental infection groups were described in Table (1). Parasites were abundantly occurred in goldfish and black moore while low infection was found in platy and swordtail. After 2nd week, number of parasite in swordtail decreased to zero while that of goldfish and black moore gradually increased. No parasite was recorded in control group.

Discussion

According to resent study, it was found that black moor and guppy has low susceptibility to white spot disease when compared with other species. Clayton and Price (1992) found significant interspecific differences in the susceptibility of freshwater fish to *I. multifilils*. They attributed this to the fact that the fish species *Ameca splendens* and *Ilyodon xantusi* were derived from wild population, and were more susceptible to *I. multifiliis* than the poecilid species which have a much longer history of domestication. Although different species of fish showed different susceptibility to *I. multifilils* infections, this parasite is not very fastidious in its host selection (Nlgrelli and Ruggieri 1966, Wilkie and Gordin 1969).

Discuss, balashark and rainbow tetra were not infected with *I. multifiliis.* It may be due to a number of reasons. They were held in a high water column to ensure low concentration of the infective agents. Natural lectins have been reported to occur in the skin mucus of different species of fish (Kamiya & Shimizu 1980, Kamiya *et al*., 1988). On the other hand, carbohydrates have been reported in the surface coat of parasites, such as *Cryptobia* spp. (Vommaro et at., 1997, Feng & Woo 1998) where they are thought to be involved in recognition of the specific host and attachment to host cells.

Infection rate in guppies was higher than all other fish species, which is a statistically significant result ($p < 0.01$) and indicates that this parasite is more common in guppies. This observation may also be explained by the lack of disease resistance in guppy varieties due to development of newer varieties for improved appearance and color, with little emphasis on other factors. Breeding for new varieties is commonly practiced in guppies, which constitute

the major share of Myanmar market.

Summary

High prevalence of *Ichthyopthirius multifiliis* were detected in the gills of swordtail, black moor, goldfish, platy and guppies. Parasites were more prevalent in guppies and black moor suggesting that they were specific for such fish species. Histological sections of the skin of guppy revealed large lesion in the epidermal layers.

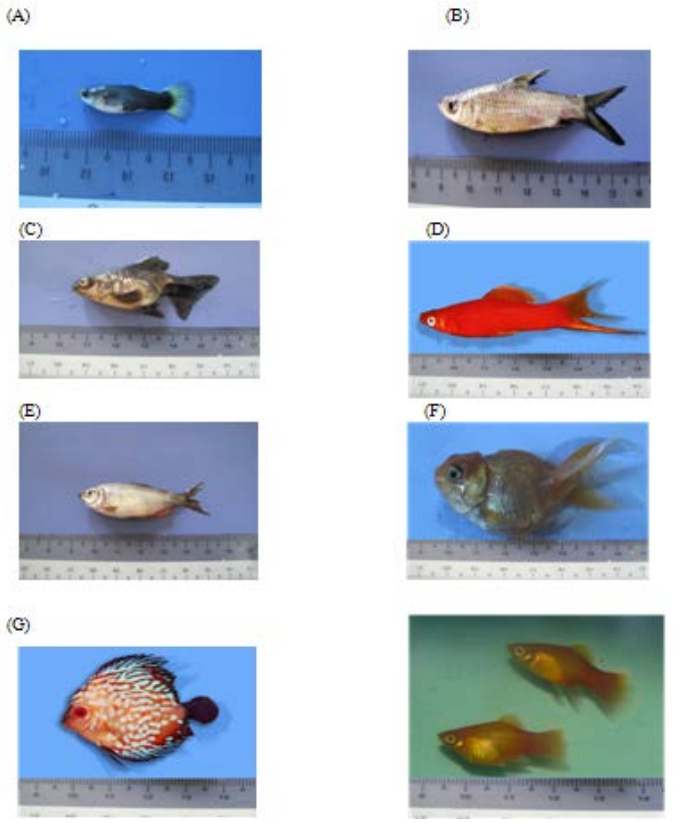

Fig.1. Fish species examined (A) Guppies (*Lebistes reticulates*), (B) Balashark (*Balantiocheilosmelanopterus)*, (C) Black moor (*Carassius auratus*), (D) Swordtail (*Xiphophorus helleri*) , (E) Rainvow tetra (*Paracheirodon innesi*) (F) Gold fish (*Carassius auratus*) and (G) Discus (*Synphesodon discus*) (H) platy (*Xiphophorus maculates*)

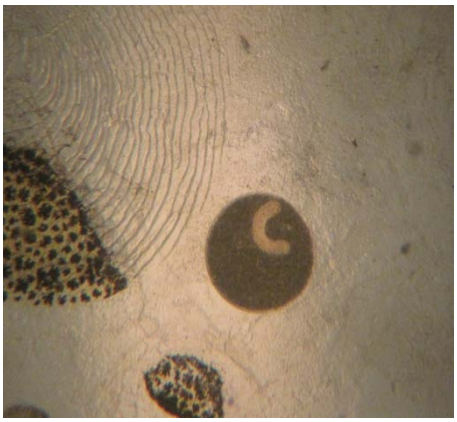

Fig 2. *Ichthyophthirius multifiliis* (A) Wet mount of the *Carassius auratus* skin during infection with *I. multifiliis* . Note the horseshoe-shape nucleus of the *I. multifiliis* (bar= 300 µm).

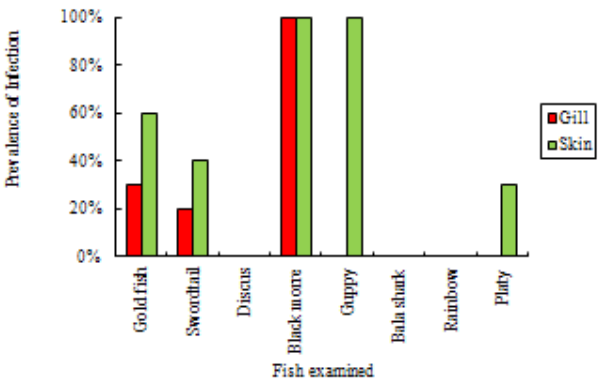

Fig. 3. Prevalence of with *Ichthyophthirius multifiliis* occurred in the gilla

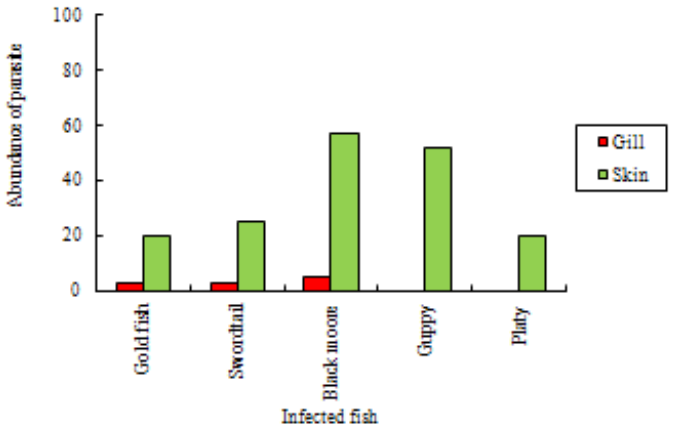

Fig. 4. Abundance of *Ichthyophthirius multifiliis* occurred on the gills and skin of fish

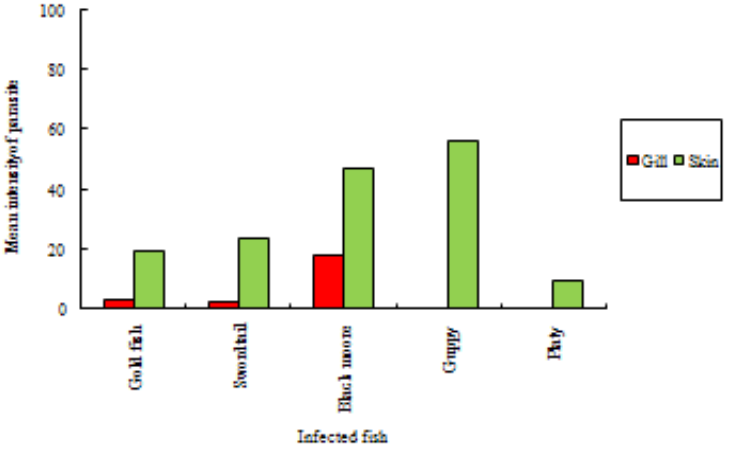

Fig. 5. Mean intensity of with *Ichthyophthirius multifiliis* occurred in the gill and skin of fish

Table 1. Number of recorded parasite from experimental tank

Fish species	Mean number of parasites		
	1st week	2nd week	3rd week
Gold fish	51	62	134
Black moore	40	49	96
Swordtail	3	0	0
Guppy	21	35	49
Platy	0	7	8

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Reference

Clayton GM and Price DJ (1992). Interspecific and intraspecific viriation in resistance to *Ichthyophthirius multifiliis* among poecilliid goodeid fishes, Journal to fish biology 40:445-453

Colorni A (1985) Aspects of the biology of *Cryptocaryon irritans* and hyposalinity as a control measure in cultured gilthead sea bream Sparas aurata. Disease Aquatic Organism 1:19-27

Colorni A, Burgess PI(1997) Vryptocaryon irritons Brown, 1951, the cause of 'white spot disease' in marine fish: on update. Aquartic Science Conservation 1:217-238

Dickerson HW (2006) *Ichthyophthirius multifiliis* and *cryptocaryon irritans* (Phyium Ciliophora). In: Woo PKT (ed) Fish diseases and disorders, vol 1. Protozoon and metazoan infections, 2nd edn. CAB international, Wallingford, P 116-153

Ezz El-Dien N M, Aly SM & Elsayed EE (1998). Outbreak of *Ichthyophithirius multifiliis* in ornamental goldfish (*Carassius auratus*) in Egypt. Egypytian Journal of Comarative Pathology and Clinical Pathology 2, 235-244.

Feng S, Woo PTK (1998) Identification of carbohydrates on the surface membrane of pathogenic and nonpathogenic

Hines, R.S., Spira, D.T., 1973. *Ichthyophthirius multifiliis* (Fouquet) in the mirror carp, *Cyprinus carpio* L. I. Course of infection. J. Fish. Biol. 5, 385–392.

Kim Jeong-Ho, Hayward CJ, Joh Seong-Joh & Heo Gang-Joon (2002). Parasitic infections in live freshwater tropical fishes imported to Korea. Diseases of Aquatic Organisms 52,169-173.

Kamiya H, Shimizu Y (1980) Marine biopolymers with cell specificity II. Purification and characterization of agglutinins from mucus of windowpane flounder *Lophopsetta maculata*. Biochemistry Biophysics Acta 622:171–178

Kamiya H, Muramoto K, Goto R (1988) Purification and properties of agglutinins from conger eel, *Conger myriaster* (Brevoort), skin mucus. Development of Comp Immunology 12:309–331

Nigrelli, R. F., Ruggieri, G. D. (1966). Enzootics in the New York Aquarium caused by *Cryptocqon initans* Brown, 1951 (= *Ichthyophthirius marinus* Sikama, 1961), a histo- phagous ciliate in the skin, eyes and gills of marine fishes. Zoologica, New York 51 (9): 97-102

Margolis, L., G. W. Esch, J., C. Homes, A. M. Kuris and G. A. Schad, (1982). The use ecological terms in parasitology (Report of an ad hoc committee of the American Society of Proctologists). Journal of Parasitology, 68, 131- 133.

Matthews RA (1994). *Ichthyophthirius multifiliis* Fouquet, 1876: Infection and protective response within the fish host. In (Pike AW & Lewis JW Eds.), pp.17-42. Parasitic Disease of Fish. Samara Publishing, Tresaith, UK.

Valtonen ET & Keränen AL (1981). Ichthyophthiriasis of Atlantic salmon, Salmo salar L. at the Montta hatchery in northern Finland in 1978-1979. Journal of Fish Diseases 4, 405-411.

Ventura MT & Paperna I (1985). Histopathology of Ichthyophthirius multifiliis infections in fishes. Journal of Fish Biology 27, 185-203.

Lom J, Dykova I (1992) Protozoan parasites of fishes. Developments in Aquaculture and Fisheries Science 26. Elsevier,

Amsterdam, p 253 Vommaro RC, Attias M, Silva Filho FC, Woo PTK, De Souza W (1997) Surface charge and surface carbohydrates of *Cryptobia salmositica* virulent and avirulent forms and of *C. bullocki* (Kinetoplastida: Cryptobiidae). Parasitol Res 76: 294–300

Wilkie, D. W., Gordin, H. (1969). Outbreak of cryptocary- oniasis in marine aquaria at Scripps Institute of Oceano- graphy. Calif. Fish Game 55 (3): 227-236

Small scale integrated mud crab farming in Mangrove Area, Kyein-ta-li Sub township, Southern Rakhine Coastal Region

Thet Su Mar

Abstract

The present study on small scale integrated mud crab (*Scylla serrata*) farming in related mangrove area of Kyein-ta-li Sub Township, Southern Rakhine Coastal region was carried out from July to December 2011. One crab farming experiment was conducted in friendly mangrove forests that are situated in lower zone. Mud pond was used in 0.35 ha at low inter tidal region. Small young crabs of under commercial size (80 g) were collected from local crab traders and cultured in this pond. 1621 crabs were stocked on pond and all were harvested after growth out period of 180 days. In the present study, 65 % survival rate of crabs were harvested from small scale farming in mangrove related area that were population of male crabs 43% and female crabs 22% in farming periods while 35 % motility were lost in respectively Total weight increased from 114.4 kg to 215.0 kg. Clam meat, trash fish and domestic waste products were used as feed. Number of trees with species and environmental factors of soil salinity, pH, water content and temperature were also investigated in pond. The present study showed mangrove related mud crab farming is less investment and quick return of profit and used for future management of mud crab resources. This result will support to develop mangrove friendly small scale aquaculture for mud crab farming as one of income generation and poverty alleviation for local peoples.

Key Words: Mangrove, Kyein-ta-li, Farming, Poverty alleviation

Introduction

The mud crab *Scylla serrata* is widely distributed in the Indo pacific region. It is a member of a group of swimming crab. Mud crab are common in the mud flats of the littoral, parts of the supra littoral and the intertidal zones of Bay of Bangal. Mud crabs are more predominantly in estuarine and mainly inhabit mangrove, but move into offshore areas to spawn (Macnae,1968). Early larval stages are marine and they being their mangrove life only at the final larval stage, magalopa, which is a benthic form. Small crab those measuring about 2- 7 cm carapace width (CW), inhabit sea grass bed and the root props of mangrove (Keenan,1998). They usually stay hidden in sheltered areas in the sub tidal zone of the mangrove. It is distributed over a wide range

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of salinity .from 2 ppt to 35 ppt oceanic waters, from the coast to the interior brackish waters. Crab live in mud burrows, which occur densely in intertidal mangrove swamps, a little above the low tide mark (Macintosh,1984).

The largest male crab reaches 2.5kg, due to eaten a good size and tasty meat, mud crabs harvested and consumed by human beings at nearly everywhere. However as demand on mud crabs as seafood materials increased. International crab trade has been developed. In this context, the mud crab has become a golden crab which earns foreign currencies for exporting as well as precious cash income for local fisherman in rural mangrove territories. The estimated of total catch in the Bay of Bangal region is between 10000 t / year. Mud crab culture and farming operations depend solely on seed collected from wild. The lack of straightly management controls on the indiscriminate collection of natural seed has led to a decline in mud crab landings in most of the regions. There had also been a gradual reduction in the maximum landing size and another indicator of overexploitation. Science 1980, overexploitation of mud crab has been faced in Southeast Asia countries. In the economic decreasing in both in number and size of mud crab has been reported (Angell,1992). The supplement the decreased natural stock, aquaculture has been attempted and some methods has been developed in some countries. According to the market sources, mud crab is a better price and greater demand for crab in China but price is low in the domestic market. Moreover, undersize crabs were rejected at export oriented markets which are now sold at very low price (Kosuge,2001). These crabs stocked for responsible aquaculture in this mangrove without disturbing with natural ecosystem. The objective of this study was to determine the growth, harvesting and production of the mud crabs stocked in mangrove related earthen pond,to estimate costs and income (Profit) in the related earthen pond crab farming and to assess the ecofriendly small scale aquaculture benefits for income generation and poverty alleviation among mangrove caretaker families. The present study intends to develop mangrove friendly small scale aquaculture for mangrove caretakers as one of income generation and poverty alleviation for their household family.

Materials and methods

Study area

Kyein-ta-li is a Sub-township of Gwa Township and about 43 miles far form Gwa Town, in the southern part of Rakhine State adjoining with Ayeyarwaddy Division. Rakhine State is situated in the western part of the nation, bordering Chin State in the north and Magway Division, Bago Division and Ayeyawady Division in the east and facing Bay of Bengal in the west. It falls between latitudes 17°30' and 21°30' north and longitudes 92°10' and 94°20' east with the total extent of 36,352 km2(Fig.1).

Fig 1. Study area of Kyain ta li Sub township, Gwa Township.

Site selection

Mud crabs grow best in brackish water such as tidal flat, estuarine areas, bays and lagoons. For pond culture, coastal low-lying areas which are intended to brackish water flushing are preferred. The site selected the oldest protected site likes secondary forest with degradation and well protected tidal swamp and abundant tree diversity .

Pond construction

0.35 ha area were selected for farming. Pond was fenced with bamboo stakes, equipped with two water gales, inlet and outlet canal by using old hollow trees and shelters made of covered with bam bamboo baskets for water exchange. Sometimes, hollow blocks or old cans were placed at the pond bottom to serve as hiding areas for crabs. This mud pond was covered with wire mesh to prevent crab escape. Diggers which is minimal, provides soil for band construction. A central mound of soil is usually provided for crab to burrow during molting and to shelter at the of poor water quality.

Water exchange

The water level was maintained at about 60 cm. Water was discharged at least three times weekly about 20-25 % water was being exchanged each time.

Stocking

Stock crabs were bought from fishermen who had stocking collected them by trapping baiting and commercials trader. The body weight of the crablings ranged from 50 g to 90 g each with the average weighing about 70 g. The cost of each crab was 400 k/kg. Mud crab1621 were sampled randomly to determine size, carapace width and fresh bodyweight(Table.1).

Cultural period

As stocking and harvesting are continuous operations, and the culture period was mostly 1to 6 months. The period is dependent on the initial size of the crab stocked and the size derived at harvest. Partial harvesting is carried out from the to time watch the crabs of various size are stocked in the size pond.

Table 1. Stocking the crabs in small scale integrated farm

Stock size	Male		Female		Total	
	No. of crabs	Avg: Weight (kg)	No. of crabs	Avg: Weight (kg)	No. of crabs	Avg: Weight (kg)
50g	71	3.5	150	7.1	221	10.6
60g	100	5.9	150	8.5	250	14.4
70g	200	14.0	200	11.5	400	25.5
80g	200	16.3	100	6.8	300	23.1
90g	200	18.0	100	8.3	300	26.3
100g	150	14.5	0	0	150	14.5
Total	921	72.2	700	42.2	1621	114.4

Harvesting

Partial harvesting 5 times were carried out in neap tide period after 3 months farming by using box trap and hook

Feeding

Feeding was done daily by fish, mollusks, clam and head of crustacean. The total quantity of feed in the pond varied according to changes in the number of crab.

Data analysis

Average daily weight gain = final weight (g) – initial weight (g) /Time (days) (ADG) (Nguyen *et al*., 2003)

Weight gain =final weight (g) – initial weight (g)

Survival rate (%) = 100 x (no. of stock at the end / no. of stock at start)

Catching rate (%) = 100 x (no. of caught / no. of stock)

Production rate (%) = 100 x (weight harvested / weight stocked)

(Zaki *et al*., 2004)

Result

Harvesting on stock

65 % survival rate of crabs were harvested from small scale farming in mangrove related area that were population of male crabs 43% survival rate and female crabs 22% survival rate in farming periods while 35 % motility were lost in respectively (Fig 1).

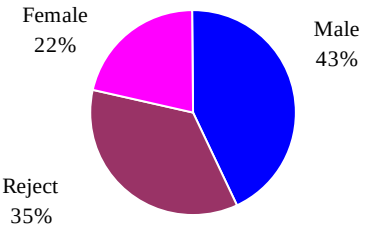

Fig 1. Survival rate of total harvested crabs

Total of harvesting weight (215.0) kg was recorded in this pond. 145.5 kg weight in male and 69.5 kg in female were harvested in this pond during the culture period. Harvesting was made (5) times in each pond. In the first time of harvesting, 13 kg were harvested during farming periods and successfully followed by 40.20kg, 43.5 kg and 47.5 kg in each harvesting times. The last time of harvesting was recorded 71.0kg. The highest harvesting weight (71.0) kg in last time of harvest and the second highest harvesting was recorded weight (47.5) kg in fourth time and followed by 43.6 kg and 40.2 kg weights were harvested in each harvesting times. Lowest harvesting weight 13.0 kg was recorded in first time of harvesting during farming periods (Fig 2).

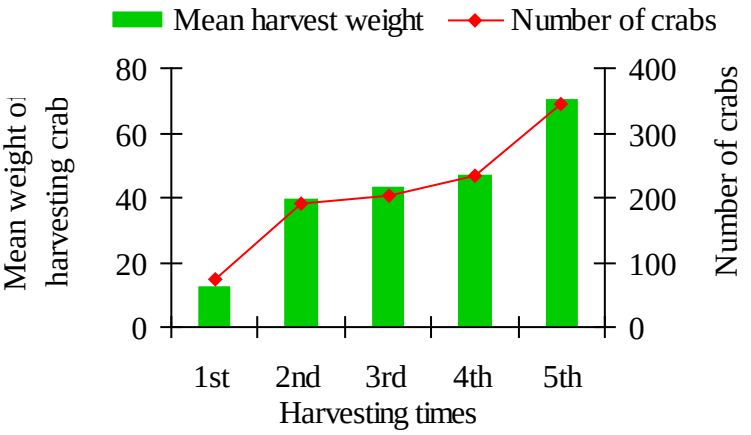

Fig 2. Partial harvestings in crab farming.

In male crabs initial stage of total stock weight was 72.2 kg and the final production weight was 145.5 kg. The weight gain was recorded 73.1 kg. Average weight gain of male was (225) g. In female crabs initial stage of total stock weight was 42 kg and the final production weight was 69.5 kg. The weight gain was recorded 27.5 kg. Average weight gain of female was (166) g.Average daily weight gain (ADG) was (1.08) g.

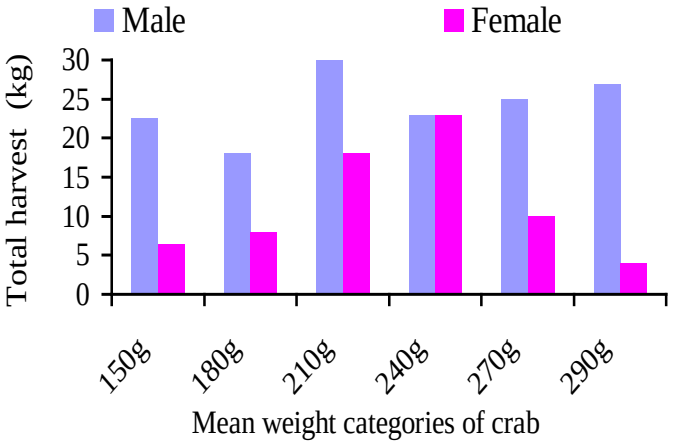

Fig 3. Harvested weight of various categories

In male crabs (145.5kg) were harvested with average carapace width (12.0) cm with (250) g of weight in this study period. Maximum length and weight was recorded in 13.5 cm and 300 g and minimum was 10.0cm with 150 g in respectively. In female crabs (72.2kg) were harvested with average

carapace width (11.0) cm with (170)g weight. Maximum length (12.0 cm) and weight 250g were investigated and minimum in 9.5cm length and 140g. Harvested crabs were categorized by sizes of catching crabs as large, medium and small group. Large sizes were harvested 64kg from farming followed by small sizes in 55kg and the medium was recorded 95.8kg in respectively (Fig 3).

Catching rate

1050 marketable crabs were caught after retaining in farm at mangrove related area. The average catching rate of mud crab maintained in the earthen ponds of farming was 64.8% while that of the production rate was 87.9%.

The highest catching rate 21.3% with (346) crabs were recorded in last time of catching. The second highest catching rate 14.5% with (235) crabs were recorded in fourth time followed by catching rate 12.5% with 202 crabs in third time and 11.9% with (192) crabs in second time were in each periods and while the lowest catching rate 4.66% with (75) crabs were collected in farming of crabs after (140) days of first time (Fig 4).

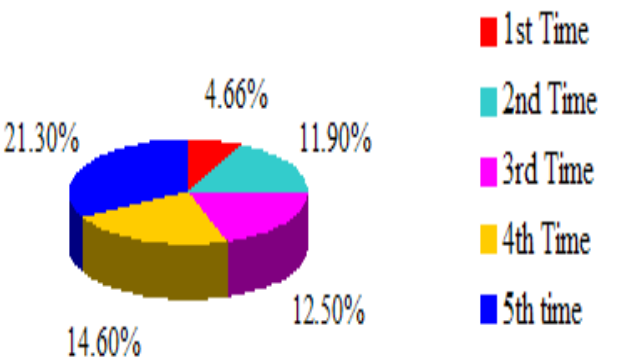

Fig 4 Catching rates of crab stock in different harvesting times

Growth performance

The carapace width of the inter moulted crab increased from 6.6.cm to 9.2 cm in the third months of farming and to 12.72 cm at harvest in the last months. The bodyweight increased from any harvest 70 g to 140 g in the middle month and to 250 g at harvest (Fig 5).

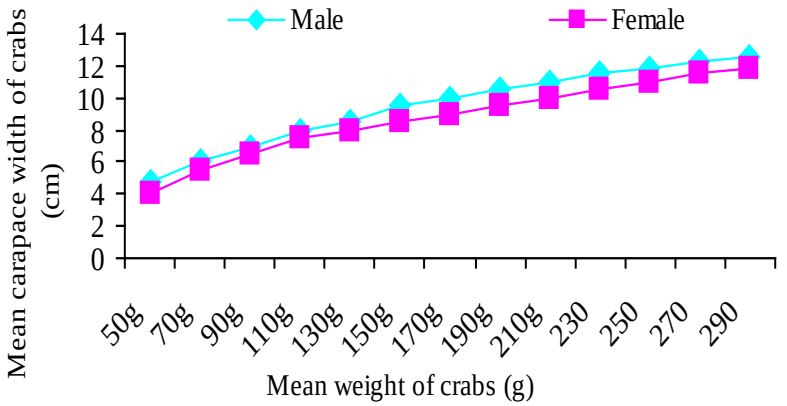

Fig 5. Growth performance of stcking crabs

Marketing categories

The growth rates of harvested crab were graded according to size into three categories after catching. The first group I of small size (150g-200g) were collected 250 male and 100 female and successfully followed by included the second group IIof medium size (200g -250g) was harvested (250) male, (200) female while that of the last group III in large size (250g-300g) was caught (200) male, (50) female in respectively(Fig 6).

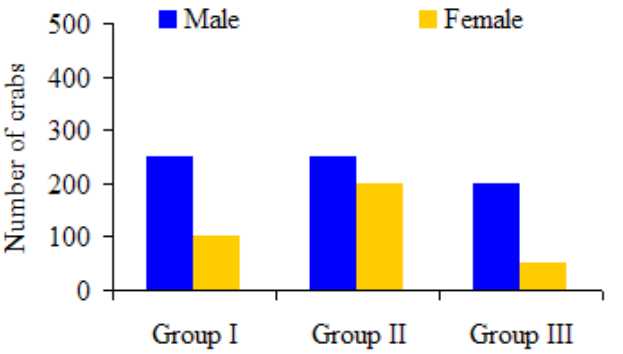

Fig 6. Sex composition of marketing categories of harvestedcrabs

Environmental factors

Total numbers of 42 trees belonging to 10 mangroves and associated shrubs were recorded in this farm. The average high and diameter of tree was 7.2 m and 10.2cm respectively. This region vegetation stand showed *Avicennia officinalis ,Sonneracia apeculata, Bruguiera gymnorhiza,* and *Excoecaria agallocha* tree species in above 10m height. It was included the mangrove associated shrubs and herbs Nipa *frutican, Aegicerus cariculatum ,Acrostichum-aurium, Derris indica and Phonix pludosa.*

The average soil salinity 20.0 ppt with a range 8.7ppt to 29.8 ppt , temperature 30.7 °C with a range 27.0°C to 35.0 °C and pH 6.5 with a range 5.65 to 7.9 were recorded in respectively (Table 2).

Table 2. Environmental factors of mud crab farming pond

Environ mental factors			Range	Average
Pond area	0.35 ha	Temperature(°C)	27.0- 35.0	30.7
No. of tree	42 trees	pH	5.65 – 7.9	6.5
No. of species	6	Salinity (ppt)	8.7 – 29.8	20

Feeding

Feedings are wet food, namely fish. Feeding was done daily by 3.5 kg either fish, mollusks, clam and head of crustacean. The total quantity of feed in the pond varied according to changes in the number of crab. Feed was provided daily.It daily avg food as follows fresh of shrimps head 0.5 kg, fish 2 kg, snail mollusks 0.5 kg and clam 0.5 kg per day. Normally, Feed was consisted of domestic utilized, like rice, vegetables, fish and animal entrails. The total feed cost of crabs was 30000k per month. Shrimps head price was 250 k/kg, Trash fish for 375 kyats/kg and snail molluscs (or) clean for 250 k/kg in respectively price (Table 3) .

Table 3. Feed consumption of mud crab farming in daily

Type of food	Weight(kg)	Price(Kyat)	Cost (Kyat)
Fresh shrimps head	0.5 kg	250/kg	125 k
Trash fish	2 kg	375/kg	750 k
Snail & molluscs	0.5 kg	250/kg	125 k
Waste material (Domestic utilized)	0.5 kg	-	-
Total	3.5 kg		1000 k

Production rate

The average production rate was 87.9%. Total production stock weight 100.6 kg were increased from 114.4kg to 215kg. The highest rate of production was recorded 62.1 % and followed by 41.5% in fouth time catching while 38.1 % were recorded in third time .The lowest production rate 11.4% was recorded in first time of harvesting(Fig 7).

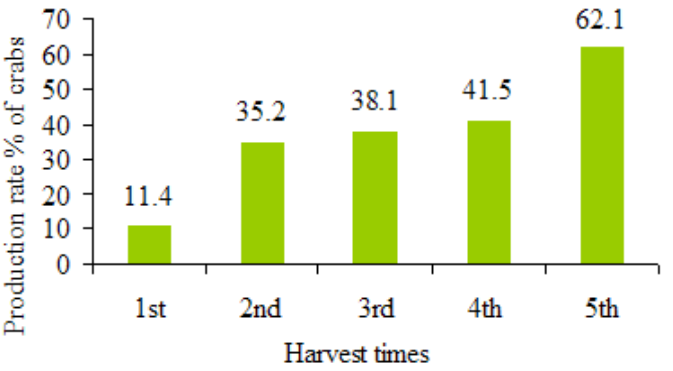

Fig 7. Production rate of small scale mud crab farming.

Table 4. Variation of cost between stocking and harvesting of mud crab farming

Group	Harvested weight (g)		Selling price
	Male	Female	Price / kg
Small	(150g- 230g)	(150g- 190g)	3000 kyats
Mid size	(230g- 270g)	(190g- 240g)	3500 / 4000 kyats
Large	(270g above)	(250g above)	4000 kyats

Cost and profit

Production Rate of the harvest crabs were graded according to size into three categories after catching. The smaller size (150 g-230g) was get 3000k price and middle size (included 190 g-270g) was paid 3500k price while larger size were consisted (250 g above) above price earned 4000k in respectively Table 4. According to this experiment, the total investment cost of small scale mud crab farming was recorded (325760 kyat) in culturing periods which were included investment of cost of stock, cost of pond construction (100000 kyat), cost of stock (45760 kyat) and the feeding cost (180000 kyat) for farming period during 6 months.

In the stock investment cost were recorded the price of 400k/kg for seed stock as immature crab of under marketable sizes. Total feeding cost was also recorded by 1000 k/kg in daily (Table 5).

Table 5. Stocking, harvesting and selling price of mud crab farming

Stocking				Harvesting		
Number	Stock crabs	Weight	Cost (Kyat)	Harvest crabs	Weight	Sale (Kyat)
Male	921	72.2 kg	28960	700	145.5kg	549000
Female	700	42.0 kg	16800	350	69.5kg	197000
Total	1621	114.4 kg	45760	1050	215.0kg	746000

Now, there was a total profit of 746,000 kyat from harvested crabs from small scale mud crab farming which were earned with 549,000 kyat for male and 197,000k for female in respectively. Therefore, a net profit was 420240 kyat from mud crab farming periods (Table 1.6).

Table 6. Cost and return between stocking and harvesting of mud crab farming

Investment (Kyat)		Production(Kyat)	
Cost of stock	45760	Cost of harvest	746000
Cost of pond/3 years	100000		
Cost of feed /6 months	180000		
Total investment cost	325760	Total investment cost	325760
		Net profit	420240

Discussion

Mud crab in Gwa were consumed at a local scale price of crabs were very cheap since they could be able to capture early and everywhere. Thus, mud crab was very abundant in the past. The catch amount of crab had been decreasing during the periods. Therefore, it is evidence that mud crab stock has badly decreased of mud crab population size, over fishing could be pointed out. Also, area of mangrove forest has also decreased rapid many rove forest depletion was limited impact on the population size of the crab. Annual number of crabs captured by one fisherman is calculated for GWa. It is assumed that the fisherman would go to fishing during spring tide days or 12 days per month. Average number of catch of crabs per day was recorded 30 crabs based on average of 30 crab catcher men. Thus, the number of crab captured appeared to be much numerous than crabs occurred in field. It is stated that crab recruitment from outside areas was plenty and the larval supply may manage to sustain the crab population. Crab culture is profitable. Restricted movement promotes rapid growth and minimizes cannibalism and squabbles which result in the loss of appendages. A more efficient system of sampling is required. The system of harvesting was needed to be updated.

The large extend of tidal flats from mangrove areas and lagoons provide tremendous potential for crab culture. It is assumed that the present study 1050 marketable crabs (65% stock) were produced after retaining in farm at mangrove related area. Small scale mud crab farming would provide additional income for the coastal fishing folk and general self employment. If the insufficient supply of the seedling mud crab for culture in this area, aquaculture activities will be affected. This indicated that the natural stock of mud crab is depleted perhaps due of these causes the high demand of gravid female for consumption over fishing habitat destruction and weak on culture techniques. Natural mud crab stock in Kyein ta li sub township is depleted through over fishing. If this continues at current rate, the stock will shrink where both the number of total catch and size of crabs will decrease. A low proportion of mortality was (35%) which were sold at a very low price and do not contribute very much in terms of weight to the total production. The maximum carapace width of *Scylla serrata* ranged from 19.0cm to 28.0 cm and the average was 22 cm (Angell,1992) .In this study, the average carapace width ranged 9.5cm to 13.0 cm and average was 11.0 cm. In the present study, more crabs of 60g-90g bodyweight were stocked, over 250g crabs could be harvested. It indicated the initial size of mud crab to be stocked should be between 120g and 200g as they can gain substantial weight within 2 months.

In the present survey, 64% of stocked crabs were harvested as marketable products during 6 months of farming. Average bodyweight increased from 70 g to 140 g in months and to 270 g at 6 months. Science 1980, overexploitation of mud crab has been increasing in Southeast Asia countries. Trade in mud crabs decreasing both in number and size has been reported Angell, 1992. Crab price is very low in local trade but the price was determined by the buyers. Cross border and local Chinese traders certainly profit from the cheaper prices of crabs from local fishermen.

Conclusion

Mud crab has become a golden crab which earns foreign currencies for exporting as well as precious cash income for local fisherman in rural mangrove territories. Feeding with cheap raw materials of good nutritive value should be tested in different combination to reach the optimum nutrient requirement of mud crab. An acceptable mortality rate would make small scale mud crab farming a more profitable and stable business. Fishing at exploitation levels should be controlled to some degree and crabs smaller than 8.5cm in carapace should be release and not permitted to be sold. Total number of catch per day per fisherman should limited. Mangrove related mud crab farming is less investment and quick return of profit.The small scale mud crab farming will support income generation and poverty alleviation for their household family. Simples but modern methods of aquaculture should be introduced to small scale mud crab farming in mangrove forest. The lack of controls on the indiscriminate collection of natural seed has led to, decline in mud crab landings in most of the regions. Local people should be educated for awareness of mud crab fisheries crisis and its relationship to mangrove conservation.

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References

Alcock ,A. (1895). Materials for a Carcinological Fauna of India. *Journal of Asiastic Society Bangal*. Vol

Angell, C.A. (1992). The mud crab. Report of the seminar of the mud crab culture anf trade held at Surat Thani, Thailand, November 5-8, 1991, Bay of Bangle Programme, Madras, India.

Keenan,C., Davie,P.J.F. &Mann, D.L.(1998). A revision of the genus Scylla De Haan,1883 (Crustacea: Decapoda:Brachyura: Potunidae). Raffles Bull.Zoo.46(1).

Kosuge Takeharu & Than, (2001). Status of mud crab fishery in Ayeyawady mangroves, Myanmar.

Macnae, W., (1968). A general account of the flora and fauna of mangrove swamps and forests on the Indo-West-Pacific Region. *Advances in Marine Biology*. 6: 73-270.

Macintosh, D. J., (1984). Ecology and productivity of Malaysian mangrove crab populations (Decapoda: Brachyura), pp. 354-377 *Proceedings of the Asian Symposium on Mangrove Environment-Research.* University of Malaya and UNESCO.

Efficencies of Seven Types of Fishing Gears in Ayeyarwady Region

Nant Thin Thin Kywe

Abstract

Efficiency of seven types of fishing gears in Ayeyarwady region was studied during a period of October 2005 to December 2007. Seven types of fishing gears belonging to three groups were recorded from the study area. The greatest fishing duration was recorded in bottom set gill net (Sein-pike), followed by net fence (Pike- Bawoun), drift gill net (Nga-Moke-pike), beach seine net (Thaun-swe-pike), bush park seine net (Kalar-pike), trammel net (Thone-htat-pike) and bamboo fish filter trap (Myin-wonn-sae). The greatest catch per unit effort (CPUE) was recorded in bamboo fish filter trap, followed by bush park seine net, beach seine net, drift gill net, bottom set gill net, trammel net and net fence. Based on the number of utilized gears, bush park seine net and bottom set gill net caught annual catch quantities over 10000 tonns.

Key words : *efficiency, fishing duration, CPUE, catch quantity,*

Introduction

Fishing is a very ancient practice that dates back at least to the Paleolithic period which began about 40,000 years ago. The Neolithic culture and technology spread worldwide between 4,000 and 8,000 years ago. With the new technologies of farming and pottery came basic forms of all the main fishing methods that are still used today (Wikipedia,2007).Technological development of fishing gear and methods in the past was aimed to increase production, the present situation with many over fished stock, limited possibilities to expand fishing on underexploited resources and concerns about the environmental impact of fishing operation, gear development focused on selective fishing and gears with less impact on the environment (Hovgard and Lassen, 2000).

The efficiency of fishing gear is dependent upon several different parameters. It is influenced especially by the shape and behavior of a particular fish species, and the seasons. Knowledge on the efficiency of a particular type of gear is essential for determining the intensity of fishing to be undertaken on

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a given water body at a given time of year(Prado, 1990). The effectiveness of nets is reduced if they are left in the water for long periods without periodic removal of the catch; if they are made of easily visible material; if water transparency rises; if water temperature falls; if atmospheric pressure falls; and if northerly winds blow(Reali, 1991).

The last three factors reduce the swimming activity of the fish. Some species have a regular diurnal movement between in the water column, but this may depend upon the time of year, and different species may move in opposite direction at a given season affecting efficiency of gears (Berka, 1990).

Any decisions of prohibition of gears should be based on sound biological, social and economic advice, taking into account on local views, rather than a reaction to hearsay (FAO, 1997).

The effectiveness and seasonality of each gear type could pose relative threats to the sustainability of the fishery. Finally variations in the seasonal effectiveness of gears are highly dependent on local ecological and social conditions. This dependence emphasises the need for local understanding and involvement in the management of fisheries (Min Thu Aung, 2006).

Thus, present research is carried out with the following objectives:

-To observed the fish species caught and quantity

-To assess the efficiency of fishing gear based on the catches.

-To determine the most effective fishing gear.

Material and methods

The Ayeyarwady Region lies between 15°40' N to18°31' N and 91°11' E to 96°06' E. It has an area of approximately 35,034.93 sq km, embracing 26 townships and 2,129 village tracts. Field surveys and interviews with local fishermen were conducted from June 2005 to May 2007. Types of fishing gears, fishing frequency, fishing duration, fishing time, number of fisherman and catch quantity of fish were recorded. Fish specimens were collected from the fishermen in the study area(Fig 1). Scaled photographs of specimens were taken immediately after the collection and preserved in 10% formalin for reference. The preserved fish specimens were washed thoroughly with tap water and identified according to Day (1978), Talwar and Jhingran (1991) and Rainboth (1996). Fishing gears were classified according to Nedelece and

Prado(1990). Catch Per Unit Effort (CPUE) and fishing effort were calculated to evaluate the effect of fishing gears.

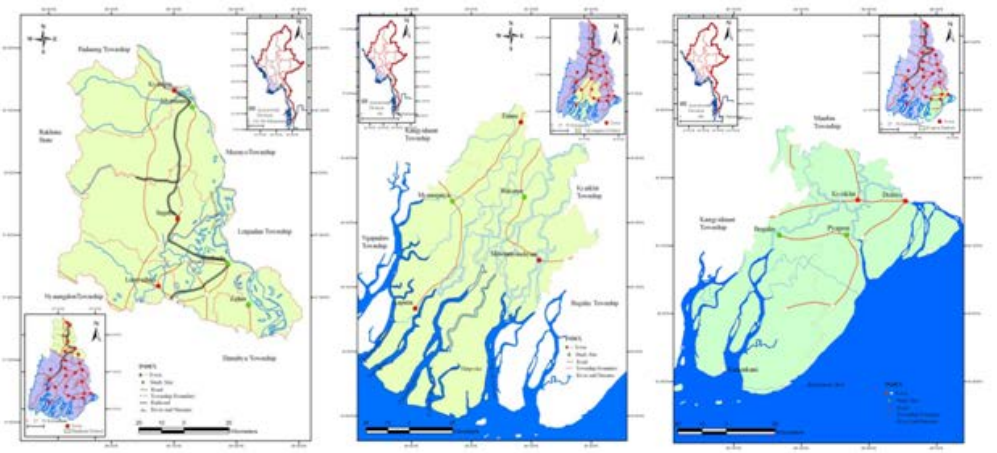

Fig. 1. Map of the Studies Area

Source: Department of Geography,Yangon University

Results

A total of seven types of fishing gears belonging to three groups were recorded in study area. Among the recorded gears, only one type was found as trap gears, three types were also found not only in gill net but also in surrounding net(Table 1).

Highest number of fish species (42) was found to be caught by bush park seine net followed by beach seine net (35), bamboo fish filter trap (34) and the least number of fish species(4) were caught by bottom set gill net (Fig.2, Table 2).

Bottom set gill net (Seine-pike) was recorded to be utilized to catch fish in the whole year round. Fishing activity of bamboo fish filter trap was the lowest (three months) (Fig.2,Table 2).The largest number of fisherman (25) applied with bamboo fish filter trap was recorded in study area followed by beach seine net (17) and bush park seine net (16), while the smallest number in net fence (2)(Fig.3, Table 2)

The whole day fishing was observed in bamboo fish filter trap and bottom set gill net in study area although fishing frequency of beach seine net was 10 times per day and those of trammel net, net fence and bush park seine net were 2 times per day each, respectively (Table 2). Monthly utilized number of fishing gears was found vary in the study area. The number of trammel net (2470) was the greatest followed by net fence (1832), bottom set gill net (857) while the lowest in drift gill net (78) (Fig.4, Table 2).

Catch per unit effort (CPUE) was the highest in bamboo fish filter trap (181 kg/day) followed by bush park seine net (163 kg/day) and beach seine net (82 kg/day) although that of the lowest was recorded in net fence (7 kg/day) (Fig. 4 and Table 2).

Estimated annual catch quantity of seven types of fishing gears varied from 931 tonns to 18037 tonns. The highest quantity was observed in bush park seine net (18037) followed by bottom set gill net(10197) and trammel net(9221) while that of the lowest in drift gill net(931) (Fig.5, Table 2).

Table 1. Different fishing gears used in Ayeyarwady region

Sr. No	Groups of Gears	Common Name	Local Name
1	Trap	(G1) Bamboo fish filter trap	Myin-wonn-sae
2	Gill net	(G2) Drift gill net	Nga- moke-pike
		(G3) Bottom set gill net	Seine-pike
		(G4) Trammel net	Thone-htat-pike
3	Surrounding net	(G5) Net fence	Pike-bawoun
		(G6) Beach seine net	Thaun-swe pike
		(G7) Bush park seine net	Kalar-pike

Table 2. The fishing activities and efficiencies of recorded fishing gears

Sr. No	Performance of gear	G1	G2	G3	G4	G5	G6	G7
1	fishing duration	3	8	12	6	9	6	6
2	fishing time	day+ night	day	day+ night	day	day+ night	day+ night	day
3	fishing frequency (time/day)	whole day	2	whole day	2	2	10	1
4	fisherman	25	6	14	2	2	17	16
5	no.of catch species	34	7	4	7	21	35	42
6	no. of utilized gears	203	78	857	2,470	1,832	146	608
7	CPUE(kg)	181	49	33	21	7	82	163
8	estimated annual catch quantity (tonns)	3,307	931	10,197	9,221	3,261	2,178	18,037

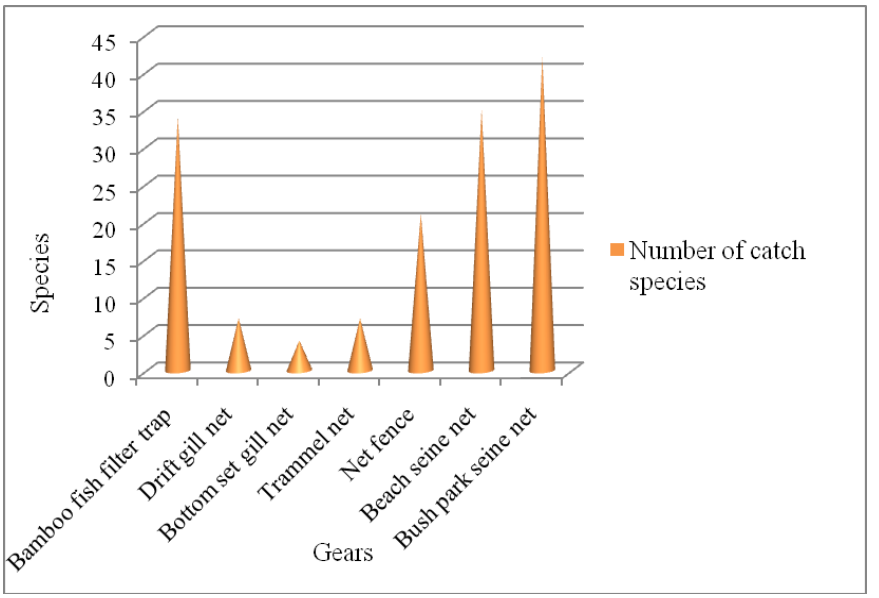

Fig.2 Number of catch species by gears

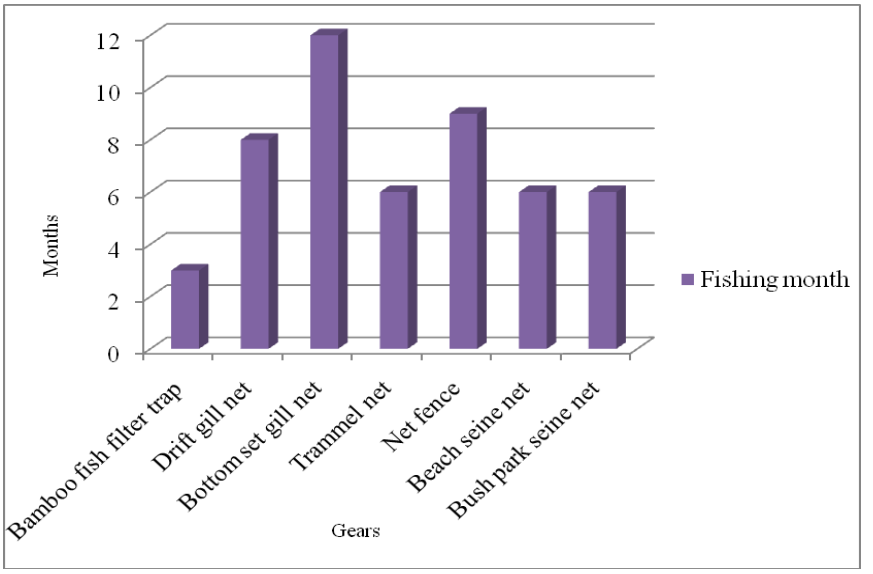

Fig. 3 Active fishing month of recorded fishing gear

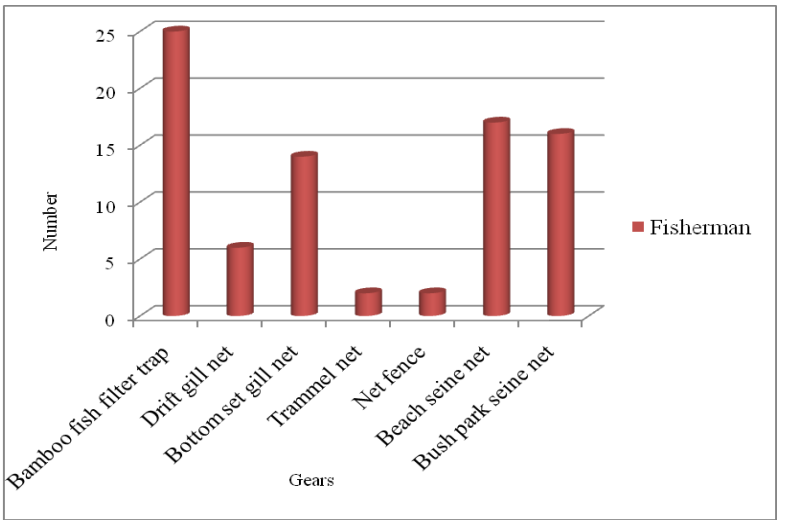

Fig.4 Operated fisherman in recorded fishing gears

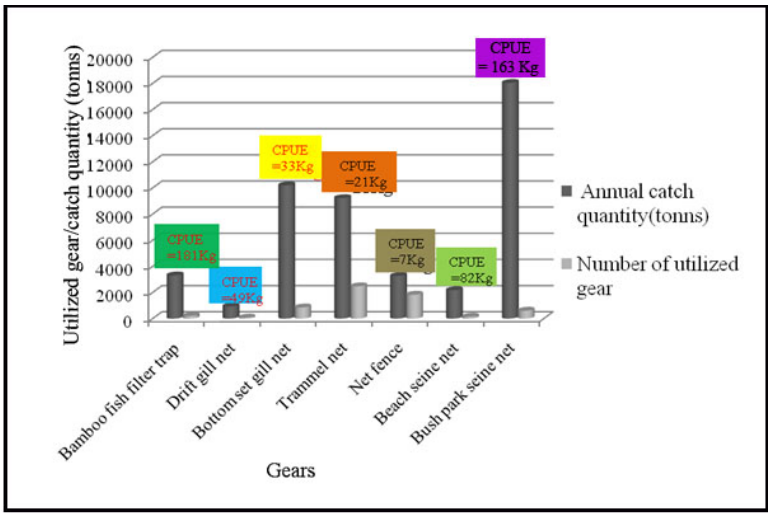

Fig. 5 Recorded fishing effect of gears in study area (*Number of CPUE per gear was described on its respective bar)

Bamboo fish filter trap

Drift gillnet

Bottom set gill net

Trammel net

Net fence

Beach seine net

Bush park seine net

Discussion

A total of seven types of fishing gears belonging to three groups were recorded. Only bamboo fish filter trap was included in trap gear group. All seven types of gears were non selective fishing gear.

All of the gill nets were caught in brackish water that found in Pyapon District. This finding indicated that demersal species could lead to exploitation by gill net. Bottom set gill net(sein-pike) is operated the whole year either at day or night. It may be assumed that the life of respective target fish species may be disturbed by this gear.

Bamboo fish filter trap (myin-woun-sae) is found to be the least used for only three months although preferred fishermen and CPUE are greater in this trap. Hence this type of gear have more effect on fishing operations.

Regarding the number of species caught by gear categories, 42 species were found to be caught by bush park seine net (Kalar-pike), 35 species by beach seine net (Thaun-swe-pike) and 34 species by bamboo fish filter trap (Myin-wonn-sae). Depending on these findings, it may be concluded that a wide range of species were vulnerable to the mentioned fishing gears.

More fish species were vulnerable to the trammel net (Thone-htat-pike) because this type of net has three layers which provide to catch more fish with no chance of escaping. However, the CPUE of this gear was calculated to be the lowest among other gillnets.

Among the three groups of fishing gears, although the number of catch species by gill net were lesser, their estimated annual catch quantity was greater than the other groups of gears. Hence, these results revealed that the group of gill net to be the most efficient fishing gears in the study areas.

Although the number of catch species and CPUE of bottom set gill net and trammel net were found to be lesser, the estimated annual catch quantity was greater. These results may be the catch of lager fish by mentioned gears. The number of catch species were greater in net fence (Pike-bawoun) but CPUE was lesser. These results may be the cause of catching smaller fish by mentioned gears. In addition, the number of catch species, CPUE and annual catch quantity of bush park sein net were higher. It is assumed that the above mentioned gear is the most effective fishing gear in the study area. On the aspect of number of catch species, CPUE and annual catch quantity, the efficiency drift gill net was the lowest.

The estimated annual catch quantity of gear types showed that the highest level was observed to be achieved by the bush park seine net followed by bottom set gill net, trammel net, bamboo fish filter trap, net fence, beach seine net and drift gill net. Based on these findings, the efficiency level regarding amount is assumed to be highest in the above mentioned the first two fishing gears in the study periods. Regarding the other four gears were recorded to achieve fairly high catch quantities while the remaining drift gill net had low levels of annual catch.

The present finding indicated that the three different categories of fishing gears used in three districts of Ayeyarwady floodplain fisheries and describes several examples of each type. The effectiveness and seasonality of each gear type was showing their relative threats to the sustainability of the fishery. The seasonality of gear used also influences the potential technical interactions between different gears. Various fish species may be over-exploited by the effective fishing gears. Thus, heavy exploitation of fishes may affect the sustainable yield. Some guidelines for conservation of fish resources should therefore be considered. Finally, variations in the seasonal effectiveness of gears are shown to be strong and highly dependent on local ecological and social conditions.

Summary

1. Efficiencies of seven types of fishing gears in Ayeyarwady Regoin was studied within the period of June 2005 to May 2007. Hinthada, Myaungmya and Pyapon districts were designated as the study sites.

2 A total of 7 types of fishing gears categorized as three groups were recorded, among which 3 types in Hinthada, 1 types in Myaungmya and 3 types in Pyapon were included.

3 A total of 42 species were found to be caught by bush park seine net (Kalar-pike), 35 species by beach seine net (Thaun-swe-pike) and 34 by bamboo fish filter trap(Myin-wonn-sae).

4 According to the estimated annual catch quantity of gear types, the highest level was observed in bush park seine net (Kalar-pike) which was followed by bottom set gill net (Sein-pike), trammel net (Thone-htat-pike), bamboo fish filter trap (Myin-wonn sae) and net fence (Pike bawonn).

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References

Berka, R. (1990). Inland capture fisheries of the USSR. *FAO Fisheries Technical Paper* 311, FAO, Rome, 1990.

Carpenten, K.E and Myint Pe, (1996). *Identification guide to the proposed marine fishery statistical units of Myanmar.* (FAO abbreviated species identification field guide for fishery purposes.) Food and Agriculture Organization of the United Nations, Rome.

FAO, (1997). *Inland Fisheries*. Food and Agriculture Organization Technical Guidelines for Responsible Fisheries . No. 6. Rome, 36 P.

Hovgard, H and Lassen, H., (2000). Manual on estimation of selectivity for gillnet and long line gears in abundance surveys. *FAO Fisheries Technical Paper* 397. FAO, Rome, 2000.

Min Thu Aung, (2006). Population dynamics and the effect of fishing gears in inshore fishing grounds of Kawthoung District, Tanintharyi Division. *PhD Thesis*, Department of Zoology, University of Yangon, Myanmar.

Nedelec, C and Prado, J., (1990). Definition and classification of fishing gear categories. *FAO Fisheries Technical Paper* 222. FAO, Rome.

Prado. J., 1990. *Fisherman's workbook,* Fishery industries Division, Food and Agriculture Organization of the United Nations, Oxford.

Reali, P., (1991). *Fish for food and development*. Food and Agriculture Organization of the United Nations, Rome.

Talwer, P. K. and Jhingran, A. G., (1991). *Inland fishes of India and adjacent Countries*, Vol. I and II. India.

Wikipedia, the free encyclopedia (internet): Fishing; (cited 2007 Aug). Available from : http://en. Wikipedia. org/wiki/ Fishing.

Factors Influencing respective Elevational Landsnail Fauna (Mollusca: Gastropoda) in Different parts of Myanmar

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Abstract

The present study was to investigate the influencing factors that determined the diversity of landsnail species from three elevational different study sites. The land snail fauna in Taunggyi limestone hill(Southern Shan Plateau), non-limestone substrate, Hlawga National Park(Yangon Region) and Dawei township, Tanintharyi Region were investigated. A total of 28 mollucks species were recorded in relation to physicochemical factors. Field surveys were from April, 2011 to February, 2012. The fauna was sampled in 12 plots, four of which were in lime stone hill ,four in non-lime stone substrates, and four in Dawei region. In the taxomony, some small microsnail species, *Pupisoma* spp. and *Kaliella* sp. were identified with the help of SEM and others were visually searched. Of them, *Allopeas grancile* was the highest distributing species. The species diversity of limestone area was the highest which was alkaline rich soil and the lowest diversity of landsnail on non-calciferous soil were found in Dawei township. The highest value of similarity index used for the species comunities from different study sites was found in Yangon region and Dawei ,and the lowest value of similarity index was found in Taunggyi and Dawei region.

Key word: land snail and microsnails, elevational limestone hill and non-limestone substrate, soil calcium (%), water calcium carbonate, and water p^H, species diversity, similarity index

Introduction

The land snail species richness in some tropical rainfall has been assessed recently by several author ((Emberton , 1995; Tattersfield,1996; and Schuilthuizen & Ratjis, 2001). Solem(1984) revealed that mollusk are richly represented in natural vegetationThe number of species in individual localities is also largely influenced by the diversity of the micro- habitats e.g. the range of fallen leaves and branches , rocks, human rubbish, felled timber and the presence of trees and herbaceous plants. In addition, the high calcium carbonate content and consequently alkaline p^H of the limestone provide suitable conditions for organisms with high calcium requirements or low

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acidity tolerance. Among animals, landsnails are one of the most distinctive groups of karst inhabitants. These organisms have high calcium requirements for their shells and reproduction (Graveland *et al*. 1994), and their abundance is usually strongly positively correlated with both calcium carbonate ($CaCO_3$) concentration and p^H (Walden, 1995). Graveland *et al*, 1994 also said that land-snails have high calcium requirements for the production of both their eggs and their shells; hence, calcium poor substrates generally support fewer individuals and species land snails when compared to calcium rich ones. Highly acidic habitats such as bog, pine forest have been regarded as unfavourable condition for landsnail diversity (Tweedie, M.1961). Although altitude, canopy density, and soil p^H explained significant variation in species composition independent of the effect of habitat type, these three environmental variables were closely correlated with variation in species composition between gardens and forest (Raheem *et a.l*, 2008). Thus, the present study was to investigate the influencing factors that determined the diversity of landsnail species from three elevational different study sites.

Materials and Methods

The field surveys were conducted from April 2010 to March 2011. Sampling was carried out in April 4-15 2010, September 19, October 15, November 17, January 1st 2011, March 30. The fauna was sampled in 12 plots, four of which were on lime stone hill, Southen Shan State, (20º 47.47' N, 97' 03.12' E, elevation – 1436 m) , four on non- lime stone substrate, designed. Hlawga National Park (17º 02. 58' N, 96º 06.36' E , elevation- 14 m)and Dawei township, Tanintharyi Region(14 09'N, 9820'E , elevation >10m). Plots of 1 ×10 m^2 were searched for both dead and living land snail. 4 plots of 5x5 m each were selected and searching was done during two person-hours.

Specimen were collected from the natural vegetation, leaf litter, rock crevices, ground patches of forest and around lime stone crops and caves. Soil samples and water samples collected from studied sites were measured for the content of soil calcium (%), water P^H, and water calcium carbonate (mg/l). Soil calcium (%) were examined with EDXRF.

Species were identified from the taxonomic criteria given by Blandford and Godwin- Austen (1908); Vaught (1989); Naggs and Raheem

(2000) and Panha and Burch (2005). Some were identified with the help of Scaning Electron Microscope (SEM).

The measure of similarity Index (I = 2 Z / X +Y) , used for the species comunites from different study sites which was followed after Colwell,.2000. The index ranges from 0 (no similarity) to 1 (complete similarity). In two communities, one with x number of species, and the other with y number of species , and with z species occurring in both communities. Moreover, the measures of diversity used in this study were overall species richness(S) and Whitakkers's index I, which is the total number of species recorded (S) divided by the mean number of species per site (α), providing a measure of diversity difference among sites. If I equals 1, sites have identical faunas and higher values indicate increasing differentiation (Whittaker, 1975).

Results

Twenty One species under (10) families belonging to (12) genera were recorded from limestone hill, 15 species under (15) families belonging to (17) genera from non- limestone area(Yangon Region), six species under 5 families belonging to 6 genera from Dawei township (Table. 1, 2, and 3). Of them, some microsnail species, *Pupisoma* spp and *Kaliella* sp, were identified with SEM.

Table 1. Systematic position of land snails from Taunggyi limestone hill,Southen Shan State

Phylum – Mollusca

Class – Gastropoda

Subclass	Order	Family	Sub family	Genus	Species
Pulmon-ata Cuvier	Stylomma-tophora Schmidt	Zonitidae	Sophimniae	*Hemiplecta* Albers, Heliceen, 1850	*Hemiplecta Humphreysiana*
		Ariophanti-dae	Macrochlamyi-nae	*Sayama* Godwin-Austen	*Sayama primiscua* Godwin-Austen

Subclass	Order	Family	Sub family	Genus	Species
			Ariophantinae	*Euplecta* Godwin-Austen (1899)	*Euplecta hyphasma* Pfeiffer 1854
					Euplecta sp.
		Pupillidae	Nesopupinae	*Pupisoma* Stokick, 1873	*Pupisoma* sp TG-1
		Glessulidae	Glessulinae	*Glessula* Von Martens	*Glessula taprobanica* Pilsbry, 1908
					G.pochycheila Benson, 1853
					G.p. taprobanica Pilsbry, 1908
		Achatinidae	Stenogyrinae	*Opeas* Albers	*Opeas filiforme*
		Helicarioni-dae	Sesarinae	*Kaliella* Blandford, 1863	*Kaliella barrakporensis* Pferiffer, 1852
					Kaliella sp.
			Durgellinae	*Sitala* Adams,1885	*Sitala attegia*
		Subulinidae	Subulininae	*Allopeas* (Benson,1863)	*Allopeas grancile*
					Allopeas sp1
					Allopeas sp2
	Stylomm-atophora	Endodonti-dae		*Philalanka* Godwin-Austen,1898	*Philalanka* sp a
					P.sp b

Subclass	Order	Family	Sub family	Genus	Species
					*P.*sp c
		Helicidae	Corillina	*Plectophylis* Benson, 1860	*Plectopylis perorcta* Blanford, 1865
					*P.*sp 1
Prosobranchia		Helicinidae		*Alcadia* Baker, 1927	*Alcadia* sp TG

Table 2. Systematic position of land snails from Hlawga National Park , Yangon Region (Non - lime stone substrate)

Phylum – Mollusca

Class – Gastropoda

Subclass	Order	Family	Sub family	Genus	Species
Pulmonata	Stylommato-phora	Helicarioni-dae	Sesarinae	*Kaliella* Blandford, 1863	*Kaliella barrakporensis* Pferiffer, 1852
	Stylommatoph-ora (Schmidt)	Endodonti-dae		*Philalanka* Godwin-Austen 1898	*Philalanka* sp YG 2.
		Achatinidae	Stenogyrinae	*Opeas* Albers	*Opeas gracile*
		Subulinidae	Subulininae	*Allopeas*	*Allopeas gracile* Hutton 1834
				Paropeas	*Paropea achatinaceum* (Pferffer 1846)
				Subulina	*Subulina octona* Bruguiera, 1789
	Stylommato-phora	Helicarionidae	Macrochlamy i-nae	*Macrochyla-mys* Benson 1832	*Macrochylamys prava*
			Durgellinae	*Sitala* Godwin-Austen 1823	*Sitala* sp 4.
	Stylommato-phora	Pupillidae	Nesopupinae	*Pupisoma* Stoliczka, 1873	*Pupisoma lignicola,* var.unidentata
	Heterurethara	Succineidae	Succineinae	*Succinea* Draparnaud,	*Succinea raoi* (Subba Raot &

Subclass	Order	Family	Sub family	Genus	Species
				1801	mitra, 1976)
	Mesogastropoda	Streptaxidae	Enneinae	*Gulella* Pfeiffer, 1856	*Gulella.bicolar* Hutton, 1834
		Diplomma-tinidae	Diplophorinae	*Diplommati-na* Benson	*Diplommatina* sp 5.
Pulmonata	Stylommat-ophora	Achatinidae		*Achatina*	*Achatina fulica* Bowdch,1822
	Pectinibran-chiata	Tiaridae	Tiarinae	*Tiara*	*Tiara jugicostis*
Prosobran-chia		Cyclophoridae		*Cyclophorus* Benson	*Cyclophorus mencheanus*

Table 3.Systematic position of land snails from Dawei township. Tanintharyi Region

Subclass	Order	Family	Sub family	Genus	Species
Prosobr-anchia		Cyclophoridae		*Cyclophorus Benson*	*Cyclophorus mencheanus*
	Heterurethara	Succineidae	Succineinae	*Succinea Draparnaud, 1801*	*Sucinnea sp.*
		Subulinidae	Subulininae	*Allopeas*	*Allopeas gracile* Hutton 1834
				Subulina	*Subulina octona* Bruguiera , 1789
Pulmonata	Stylommat-ophora	Achatinidae		*Achatina*	*Achatina fulica* Bowdch,1822
	Stylommat-ophora (Schmidt)	Endodontidae		*Philalanka* Godwin-Austen 1898	*Philalanka* sp

In limestone area, Southern Shan plateau, the average alkaline P^H value in water found in the four plots were 8.1. In Hlawgar National park in Yangon Region, the four non-limestone plots had an average P^H of 7.0. The calcium carbonate ($CaCO_3$) content of water sample was also much higher in limestone area. In Southern Shan Plateau, the average $CaCO_3$ content in water

was 175 mg/L^3 , Ca% in soil was 64% whereas the average value for non-limestone, Hlawgar National Parks, the average calcium carbonate ($CaCO_3$) content in water was 27 mg/L and Ca% in soil was 6.8%. 15 species were found in Hlawgar national park (non-limestone area) and 21 species were found in Southern Shan State plateau(limestone hill). Six species were found in Dawei Towship. In Dawei, the average alkaline P^H value in water found in the four plots were 5.5. Calcium carbonate content and calcium % in soil were not found in Dawei township.

All comparisons of chemical factors in each study sites were examined. Mann- Whitney W test showed that the value of Ca% in soil, $CaCO_3$ in water, and p^H in water found in Taunggyi was significantly higher than the value of Ca% found in Yangon ($P< 0.05$, $W= 10$), $CaCO_3$in Yangon($P < 0.05$, $W= 10$) and p^H in Yangon ($P< 0.05$, $W=10$) . Hence, the value of Ca%, $CaCO_3$ and p^H found in Yangon was also higher than the value of Ca% in Dawei $P< 0.05$, $W=26$) and p^H ($P< 0.05$, $W= 20$).

Table 4. Index of species diversity from different study sites

Plot	Index of species diversity
Yangon	3.00
Taunggyi	4.00
Dawei	1.88

I= 1 identical fauna

I>1= increasing differentiation

In each studied site, 21species under (10) families belonging to (12) genera were recorded from limestone, 15 species under (15) families belonging to (17) genera from non- limestone area(Yangon Region), six species under 5 families belonging to 6 genera from Dawei township. The critical analysis of the distribution of species in these communities was calculated in this study. The highest species diversity was found in Southern Shan Plateu, Taunggyi. (Whitaker's index of species differentiation, I = 4) and the lowest was found in Tanintharyi Region, Dawei. (Whitaker's index of species differentiation, I = 1.66) (Table 4). The value of species number in limestone hill was significantly higher than that in non-limestone area, Hlawgar National Park (Yangon region) and Dawei township.

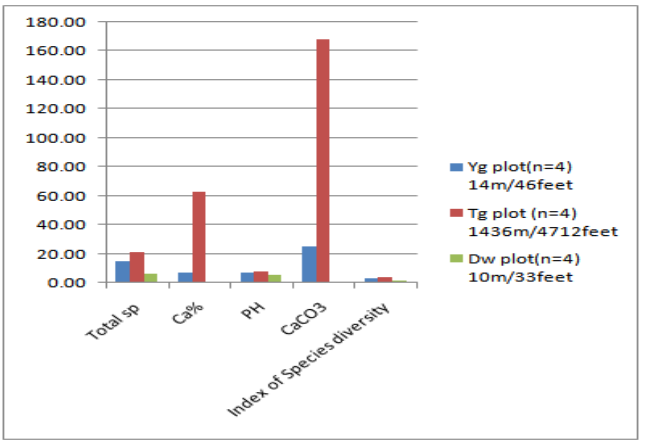

Fig 1. Total number of species, p^H and $CaCO_3$ in water, Ca% in soil from different study sites

The given line graph illustrated that the highest number of species, water p^H and $CaCo_3$ in water ,Ca% in soil was found in Taunggyi (Altitude 1436 m) whereas the lowest number of species and water p^H was found in Dawei (Altitude > 10 m) Fig.1 .

Table.5. Showed the relationship between total species number and water p^H, $CaCO_3$ in water,& Ca% soil , index of species diversity ;and between index of species diversity and water p^H,$CaCO_3$ in water , Ca% in soil

Variable	Water p^H		Index of species diversity		$CaCO_3$ mg/L3 in water		Ca% in soil	
Total species number	p	R^2	P	R^2	P	R^2	P	R^2
	0.01	0.99	0.01	0.99	0.31	0.77	0.34	0.73
Index of species diversity	0.01	0.99	-	-	0.29	0.59	0.30	0.52

In all these malacofauna, a total number of species in each studied sites was highly significantly correlated with P^H in water (P=0.01, R^2= 0.99) and index of species diversity (P= 0.02, R^2= 0.99). More than that, the index of species differentiation was also significantly correlated with water P^H (P < 0.01, R^2 = 0.99). However, no significant differences were found in $CaCO_3$ in water and Ca% in soil. Index of species diversity (I) was not correlated with $CaCO_3$ in water and Ca% in soil. (Table.5).

Table 6. Similarity of Index (I) from different study site

Study sites	Index of similarity
Yg and Tg	0.44
Yg and Dw	0.57
Tg and Dw	0.22

I=1 (complete similarity)

I = 0 (no similarity)

More than that, the highest value of similarity of index (I) was 0.57 found in Yangon and Dawei whereas the lowest value of similarity of index (I) found in Dawei and Taunggyi township was 0.22 . (Table 6, Fig .2).

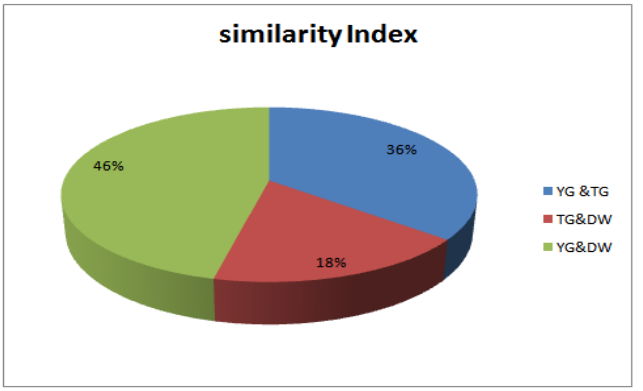

Fig.2 . Index of similarity shown in three different study sites

Discussion

Twenty eight species of the different elevational malacofauna in limestone and non-limestone area from Myanmar were recorded in relation to physicochemical factors in this study . 21species were recorded from highly elevational limestone area (Shan Plateau) , 15 species from low land, non-limestone area ,Yangon Region, and six species from Dawei township, Tanintharyi Region. Some microsnails, *Pupisoma* spp. were too small to notice with naked eyes and were identified with SEM. The lists of species showed indeed that some species are restricted to limestone and non-limestone area. Of them, *Allopeas* spp. were the most diverse species in all area.

Raheem *et al.* (2008) noted that the correlation between land snail composition and altitude was strong. The observed effects of altitude may also reflect altitudinal differences in the abundances of species, which was also reported by Nekola, 2011. Based on this, in the present study, the value of limestone land snail species number found in high altitude area was higher than that of non- limestone substrate type found in low altitude. Of the 15 species studied, *Sayama* sp. was more abundant at high altitude area found in Taunggyi whereas they were not found in Dawei where the altitude is low. Of them, *Cyclophrous mencheanus* , was prosobranchids so that these species were restricted to limestone area (Solem ,1984).

Schilthuizen *et al.*, 2003 reported that the chemical analyses of soils indicated that abundances correlate positively with p^H and calcium carbonate content. In the present study, the number of species in limestone area and non-limstone area were highly correlated with pH in water . However , no

significant differences were found in $CaCO_3$ in water and Ca% in soil. More than that, Whitaker's index of species diversity (I) was also correlated with p^H in water, but did not correlated with $CaCO_3$ in water and Ca% in soil.

Tattersfield, 1996 stated that in east Africa forest may provide tentative evidence for a maximum level of landsnail richness at intermediate elevations of about 1000- 1500m . Based on this, in the present study, the diversity of molluscan fauna found in high altitude area, Taunggyi, was the highest which was alkaline rich soil. The lowest diversity of landsnail was found in low land substrate, Dawei, which was acidic soil. Only six species were found in Dawei environs. Prosobranchid species, *Cyclophorous mencheanus* species was well adapted in acidic soil. Whitaker's index of species diversity (I) took the highest value in Taunggyi (I=4) and the lowest value in Dawei (I=1.6) .

The value of high similarity Index of species was found in Yangon and Dawei (Index of similarity) I= 0.57 whereras the low value of similarity of Index was found in Taunggyi and Dawei region, I=0.22 which was agreed with Nekola,2011. Nekola, 2011stated that the presence of base–rich limestone habitats, geographic factors appear important as the frequency of unique species within the kempsey non-limestone fauna remains significantly higher than that observed from other sites. Of the six species studied, molluscan fauna found in Dawei was similar to that reported for other tropical rainforests species which was agreed with Schiltuizen *et al*., 2001.

It was thus concluded that limestone land snail species number found in Taunggyi, highly elevational limestone area (Southern Shan Plateau) was higher than that of non- limestone substrate type found in low altitude area. It may be due to obligate calcicoles. Limestone hill may be considered important reservoirs for regional malacofauna. Moreover, the indications of lowest number of species were found in Dawei township that would be adapted for acidic soil.

Acknowledgements

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References

Blandford. W.T. and Godwin-Austin, H.H. (1908). *Fauna of British India*. Mollusca Vol.1 Testacellidace and Zonitidae. Talyor and Francis, London.

Colwell, R. K(2000). Estimate S: *Statistical estimation of species richness and shared species from samples*.Version 6.

Emberton, K.C.(1995). Land snail community morphologies of the highest diversity sites of Madagascar, North America, and New Zealand, with recommended alternatives to height-diameter plots. *Malacologia. 36*: 43-66

Graveland,J.,R.van der wal,J.H.Van Balen&A.J.,Van Noordwijk,(1994).Poor reproduction in forest passerines from decline of snail abundance on acidified soils.*Nature*,368: 446-448.

Naggs, F. and D. Raheem(2000). Land Snails diversity in Srilanka. *The Natural History Museum London*:214pp

Nekola, J.C. (2011). Acidophilic terrestrial gastropod communities in North America. *Journal of Molluscan Studies* (in press).

Panha, S. and J.B Burch (2005) An introduction to the microsnails of Thailand. *Malacological review*. Vol.37/98:1-1

Raheem, D.C., Naggs, F., Preece, R.C., Maptuna, Y., Kariyawasam, L. and Eggleton, P. (2008). Structure and conservation of Sri Lankan land-snail assemblages in fragmented lowland rainforest and village home gardens. Journal of Applied Ecology, 45: 1019-1028. doi: 10.1111/j.1365-2664.2008.01470.x

Schilthuizen, M & Rajes, H. (2001). Land snail diversity irnal of Molluscan a square kilometer of tropical rainfoest in Sabah, Malaysian Boneo. *Journal of Molluscan Studies, 67*: 417-423

Schilthuizen,M.,M.H.Chai.,T.E.Kimsin and J.J.Vermeulen (2003). Abundance and diversity of land snails (Mollusca:Gastropoda) on the limestone hills in Borneo. *Bulletin of the Reffels Museum, Singapore*, 51 (1) : 35 – 42

Solem,A.,(1984). A world model for land snail diversity abundance.In: A.Solem, and A.C. van Bruggen, eds.*Worldwide snails: biogeographical studies on non marine Mollusca*, 6-12.E.J.Brill/W, Backhuys , Leiden.

Tattersfield, P.(1996). Local patterns of landsnail diversity in a Kenyan rainforest. *Malacologia,38*: 161-180

Tweedie, M.(1961). On certain Mollusca of the Malayan limestone hills. *Bulletin of the Raffles Museum,26*: 49-65

Vaught, K,C. (1989). *A classification of the living Mollusca*. (Eds. R.T. Abott and K.J. Boss). American malcogists, Melbourne, Florida 32902, USA

Walden, H.W (1995). *Norway as an environment for terrestrial moullus, with viewpoints on threats against speicies and diversity*. Pages 111-editors. Biodiversity and conservation of the mollouscs. Backhuys, Oegstgeest, the Netherlands

Whittaker,R.H. (1975). *Community and Ecosystem*.Macmillan, New York.

The Effect of Industrial and Urban Effluenton the Water Quality of Taungthaman Lake

Khin Than Htway

Abstract

Water quality degradation of Taungthaman Lake transformed it into a eutrophic water body. Caused particularly by domestic sewage, agricultural runoff and industrial pollution. The changes in lake water quality caused by eutrophication. Water quality is typically assessed by chemical, physical and microbiological parameters. Each parameter includes a comprehensive list of water quality measures. Among these, some of the most common measured are nitrate, phosphorus, dissolved oxygen and sedimentation. Wastewater is often one of the largest single sources of pollution into freshwater systems, where it has dramatic effect on water quality.

Key words: eutrophication, assessed, microbiological parameters, dramatic effect

Introduction

Taungthaman Lake is situated in the AmarapuraTownship, positing at 21˚54”N, 96˚ 03″ E, and the water body of the Lake is approximately 600ha. Dokhtawady River flows to the south of it and Ayeyarwaddy River flows to the west. Taungthaman Lake is a large floodplain transformed into a permanent Lake by the construction of water control atTadarphyu sluice gate in the year 1993 disrupts the natural flow of the Lake.Urban and industrial development around Myothit area is the point source of pollution since the waste discharges directly through Payandaw Chaung into the Lake.(Figure 1)

The demand for surface water for many purpose is increasing globally mainly due to population growth and irrigation. Owing to the fact that people have not realized the frailty of the Lake ecosystem and lack of environmental awareness, human activities such as land reclamations, and destruction of plants around Lakes, discharge of large quantities of industrial and agricultural waste water into Lakes and so on have greatly affected. Many Lakes throughout the country are commonly undergoing the process of eutrophication. As a result, the cycling of the ecosystem of many Lakes is

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demaged, causing great losses to production and people's life in the Lake region (Xiangcan, 2003).The United Nations Environment Programme (UNEP) reported that it is possible to identify six major environmental problems for fresh water Lakes. Eutrophication process can be subdivided into Oligotrophic, Mesotrophic, Eutrophic and Hypertrophic (Vollenweider, 1968). Oligotrophic Lakes are low primary productivity and low biomass associated with low concentrations of nutrients (nitrogen and phosphorus). Mesotrophic Lakes are less well defined than either oligotrophic or eutrophic lakes and are generally through to be lakes in transition between the two conditions. Eutrophic Lakes are high concentration of nutrients and associated high biomass production, usually with a low transparency. Oxygen concentrations can get very low. Hypertrophic Lakes are at the extreme end of the eutrophic range with exceedingly high nutrient concentrations and associated biomass production. Anoxia or complete loss of oxygen often occurs in the hypolimnion UNDP (2002).

There are two general sources of water pollution; point and non point sources. Point sources are industrial discharge pipes and municipal sewer outlets that discharge pollutants directly into an aquatic ecosystem. Non point sources are indirect sources of pollution such as runoff from agriculture, forestry, urban and industrial activities as well as land fill leaches and air-borne matter. Water pollution from human activities includes nutrients, heavy metals, persistent pesticides and other toxins (Painchaud and Jean, 1997).

The aim of the study is to assess the impact of urban and industrial wastes on the Taungthaman Lake ecosystem and to explore the possibility of stopping and hopefully reversing the process of eutrophication.

Fig.1. Map of Taungthaman Lake 2012 Google image

Fig.2.Statelline image of Taungthaman Lake showing the inflow of wastewater

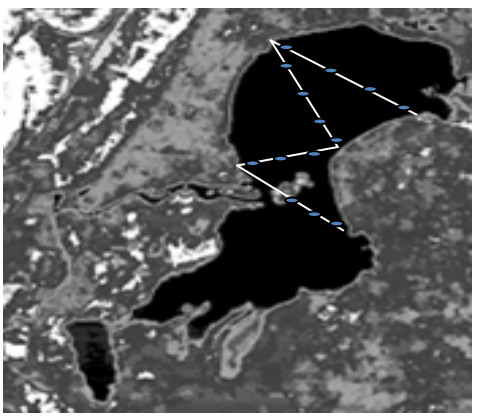

Fig.3.Satellite image of Taungthaman Lake showing line transect of sampling stations

Materials and Methods

Water samples for the measurement of Dissolved Oxygen (DO) and bottom sediments were collected along four transects as follows

(1) Htandaw to Bu Pagoda

(2) Bu Pagoda to Kyauk Taw Gyi

(3) From Kyauk Taw Gyi to GandaryoneBaikman and

(4) From Baikman to Duck hut.

Total of 14 stations were marked on the transect at approximately regular distance sample at two week interval from April 2006 to March 2007.

Water for BOD measurement was collected from 1.67 m below the surface. DO was measured with DO meter DO-24P, DKK TOA CORPORATION. DO was measured initially and after incubation. The BOD was computed from the difference between initial and final DO.

BOD measurement was conducted according to the method of standard examination of water and waste water (APHA, 1979).

pH measurement Q/GHSC 1544-1999 universal indicator paper Shanghai SSS Reagent Co. Ltd was used to take the readings.

Estimation of Biochemical Oxygen Demand

The method involves measuring the dissolved oxygen of the sample before and after incubating for 5 days at room temperature, the difference between two values being the biochemical oxygen demand (APHA, 1979).

$$BOD_5 = DO_I - DO_F$$

In addition, the biochemical oxygen demand test is used to determine the relative oxygen requirements of treated effluents and polluted water.

Calculation

$$BOD_5 \text{ (mg/L)} = \frac{DO_I - DO_F}{V_S/V_B}$$

where

DO_I = initial dissolved oxygen (in mg/l)

DO_F = final dissolved oxygen (in mg/L)

V_S = Volume (in ml) of water sample

V_B = Volume (in ml) of the bottle

Reagent Used

(a) 0.05% Urea Solution.

About 0.05 g of urea was dissolved in distilled water and the volume made up to 100ml in a volumetric flask.

(b) Phosphate Buffer Solution

0.85 g of dipotassium hydrogen phosphate (K_2HPO_4), 2.18 g of potassium dihydrogen phosphate (KH_2PO_4) and 0.17 g of ammonium chloride (NH_4CL) were dissolved in 80ml of distilled water and the volume made up to 100 ml. The pH of the solution was adjusted to 7.2 with approximately 0.1 M hydrochloric acid solution.

Procedure

Water sample was filled into glass bottle taking care so that bubbling did not occur and the dissolved oxygen content was initially determined with DO meter. After adding 1 ml solution of 0.05% urea and 1 ml of phosphate buffer solution, the bottles were incubated at room temperature for 5 days. After incubation, oxygen concentration was measured with DO meter again. Finally, the 5 days biochemical oxygen demand was obtained from the difference between the initial DO content and after 5 days incubation.

Results and Observations

Surface water for DO of 14 stations is well oxygenated than the bottom water of the Taungthaman Lake. For the bottom water DO is low. The lowest DO levels usually occur just before drawn. Oxygen production or photosynthesis is normally varies because it is light dependent. The sun rises, aquatic plants and algae or photosynthetic organism begin to produce oxygen which increases in concentration throughout the day. Production of oxygen is greater than consumption. Around sunset, photosynthesis essentially ceases and “DO” levels begin to drop due to respiration and consumption by fish and other aquatic organisms is greater than productively.

The pH of water sample in hot season of Taungthaman Lake lie between 6.4 to7.3, the rainy season and cold season is between 6.3 to 7.3. The pH of the Taungthaman Lake is normal.

The temperature of Taungthaman Lake in hot season is high over 30ºC. The rainy season is over 29˚C. And the cold season of the water temperature less than 27˚C. Elevated temperature reduce solubility of DO and

decrease the amount of O_2 in the water body. High temperature increase metabolism, respiration and the demand for oxygen by fish and other aquatic organisms. From April 2006 to 2007 March, the surface, bottom, and final of DO level is highest in the rainy season but BOD value is lowest in cold season. DO(final) is lowest in hot season.The surface DO level is 7.30 mg/l in December is the lowest throughout the year. BOD value 3.42 mg/l in October, it is the highest of all months of the year. In March, DO(final) is less than1mg/l is the lowest of all the months.

Based on oxygen measurements, Taungthaman Lake can be classified as a highly eutrophic lake possibly leading to a hypertrophic stage.

Table (1) Distribution of DOi (Top), (Bottom), DO_f(final), BOD, pH, and Temperature of October 2006

Stations	**DO_i(Top) (mg/l)**	**DO_i(Bottom) (mg/l)**	**DO_f (Final) (mg/l)**	**BOD (mg/l)**	**pH**	**Temperature (^oC)**
1	10.42	5.66	2.4	3.26	6.7	29.2
2	9.78	2.53	1.67	0.86	6.9	29.3
3	8.89	3.55	1.27	2.28	6.5	29.7
4	9.63	1.46	0.98	0.48	6.6	29.9
5	9.32	2.78	1.69	1.09	6.8	30.1
6	9.03	5.07	1.36	3.71	6.9	29.9
7	12.37	5.46	2.35	3.11	7.2	30
8	11.14	6.12	2.48	3.64	6.8	30.2
9	12.9	6.22	2.86	3.36	6.7	30.4
10	12.42	6.21	2.44	3.77	6.6	30.6
11	11.84	8.33	2.61	5.72	6.8	30.4
12	13.83	7.14	2.48	4.66	6.9	30.2
13	10.99	8.35	2.53	5.82	6.8	30.1
14	12.85	9.49	3.34	6.15	7.2	30.2

Table (2) Distribution of DO_i (Top),(Bottom), DO_f(final), BOD, pH, and Temperature of December 2006

Stations	DO_i (Top) (mg/l)	DO_i (bottom) (mg/l)	DO_f (Final) (mg/l)	BOD (mg/l)	pH	Temperature (ºC)
1	6.35	0.76	0.38	0.38	6.9	23.2
2	6.28	0.62	0.2	0.42	6.7	23.6
3	6.65	0.59	0.19	0.4	6.8	23.3
4	6.73	0.89	0.35	0.54	7.2	23.6
5	5.86	0.53	0.3	0.23	6.7	23.8
6	6.11	1.83	0.64	1.19	6.5	24
7	6.89	2.55	1.29	1.26	6.8	24.1
8	7.14	3.03	1.88	1.15	6.6	24.3
9	6.94	2.52	1.43	1.09	6.7	24.2
10	6.5	2.67	0.98	1.69	6.9	24.4
11	9.7	3.38	1.48	1.9	7.3	24.5
12	9.77	4.09	1.92	2.17	6.8	24.9
13	8.23	4.43	1.86	2.57	6.7	24.7
14	9.02	5.21	2.36	2.85	6.6	25.1

Table (3) Distribution of DOi (Top), (Bottom), DO_f (final), BOD, pH, andTemperature of March 2007

Stations	DO_i (Top) (mg/l)	DO_i (bottom) (mg/l)	DO_f(Final) (mg/l)	BOD (mg/l)	pH	Temperature (ºC)
1	10.34	0.9	0.31	0.59	6.5	30
2	10.11	0.56	0.18	0.38	6.4	30.1

Stations	**DO_i (Top)**	**DO_i (bottom)**	**DO_f(Final)**	**BOD**	**pH**	**Temperature**
	(mg/l)	**(mg/l)**	**(mg/l)**	**(mg/l)**		**(ºC)**
3	9.88	0.89	0.52	0.31	6.7	30.4
4	8.99	0.76	0.24	0.58	6.5	30.6
5	9.02	0.95	0.28	0.87	6.6	30.5
6	8.21	1.91	0.81	0.67	6.8	30.6
7	9.33	2.24	0.96	0.47	6.9	30.6
8	9.51	0.88	0.48	0.4	6.8	30.8
9	10.26	2.57	1.1	1.09	7.2	30.6
10	10.24	0.8	0.33	1.48	7.2	30.7
11	9.14	1.33	0.46	1.28	6.9	30.8
12	8.68	4.28	1.79	2.49	7.3	30.6
13	9.59	3.15	1.1	2.05	6.9	30.8
14	10.96	2.49	0.98	1.51	6.9	31.2

Table (4) Distribution of DO_i (Top), (Bottom),DO_f (Final),pH,Tempertaure of Hot season,Rainy season and Cold season of Taungthaman Lake.

Months	DO_i (Top) (mg/l)	DO_i(Bottom) (mg/l)	DO_f(Final) (mg/l)	BOD (mg/l)	pH	Temperature (°C)
March	9.59	1.69	0.68	1.01	6.8	30.6
April	9.3	2.32	1.19	1.13	6.8	31.97
May	10.51	5.24	2.02	3.18	6.8	31.21
Hot season	9.8	3.08	1.3	1.77	6.8	31.26
June	10.77	4.91	1.74	3.17	6.9	30.51
July	11.27	3.15	1.99	1.16	6.8	29.83
August	10.28	4.97	3.08	1.99	6.8	30.64

Months	DO_i (Top) (mg/l)	DO_i(Bottom) (mg/l)	DO_f(Final) (mg/l)	BOD (mg/l)	pH	Temperature (°C)
September	10.01	3.95	2.24	1.71	6.7	31.08
October	11.1	5.6	2.18	3.42	6.8	30.01
Rainy season	10.69	4.52	2.25	2.29	6.8	30.41
November	10.06	6.32	4.18	2.14	6.7	27.75
December	7.3	2.36	1.09	1.27	6.8	24.12
January	11.85	2.44	1.68	0.78	6.7	24.24
February	11	1.82	1.2	0.62	6.6	23.58
Cold season	10.05	3.24	2.04	1.2	6.7	24.92

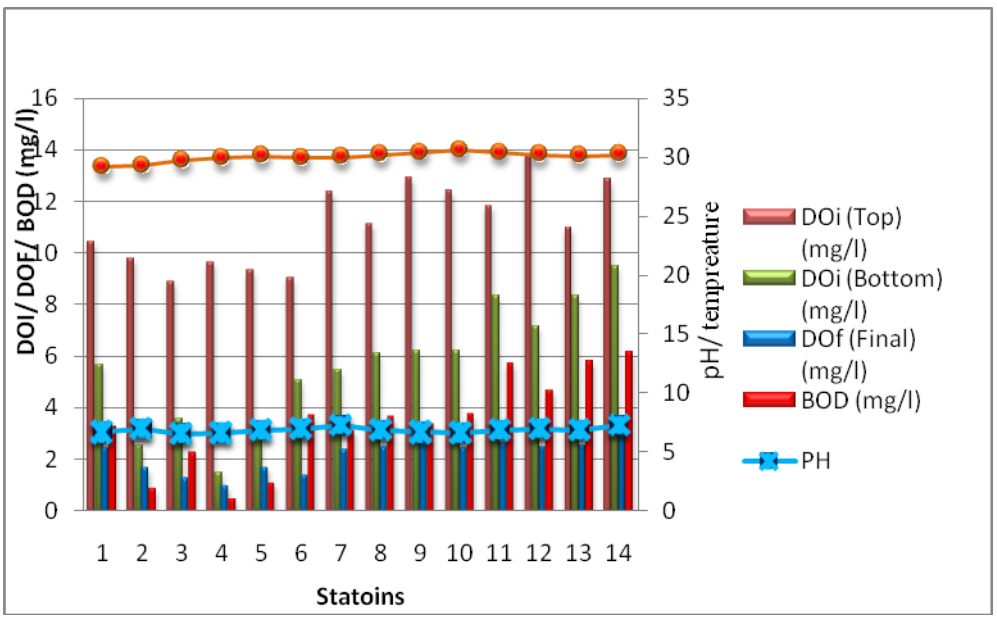

Fig.(4)Distribution of DOi (Top), (Bottom), DO_f (final), BOD, pH, and Temperature of October 2006

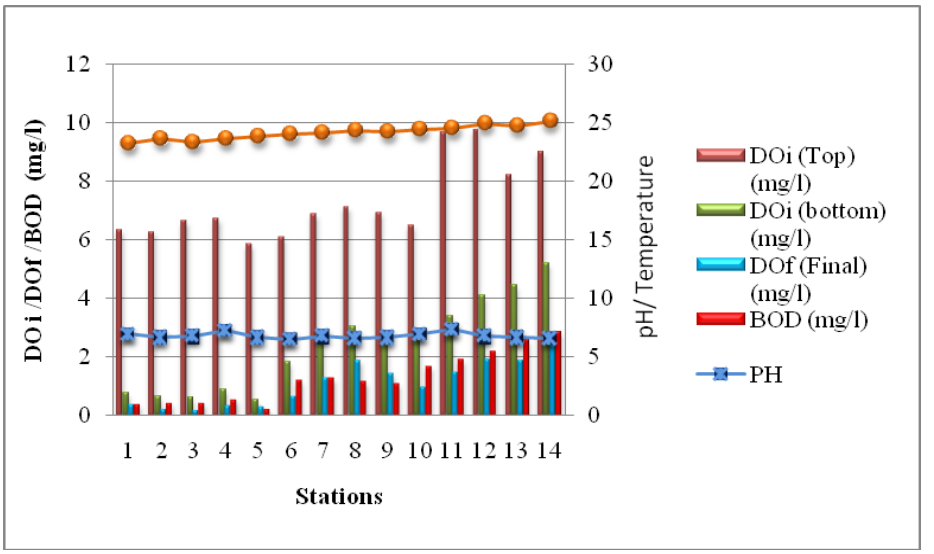

Fig.(5) Distribution of DOi (Top), (Bottom), DO_f (final), BOD, pH, and Temperature of December 2006

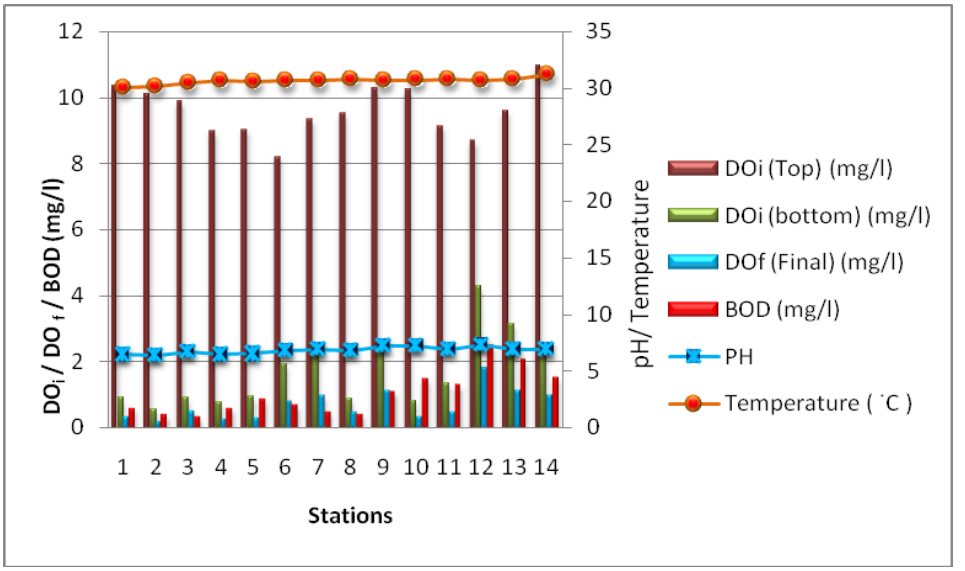

Fig.(6) Distribution of DOi (Top), (Bottom), DO_f (final), BOD, pH, and Temperature of March 2007

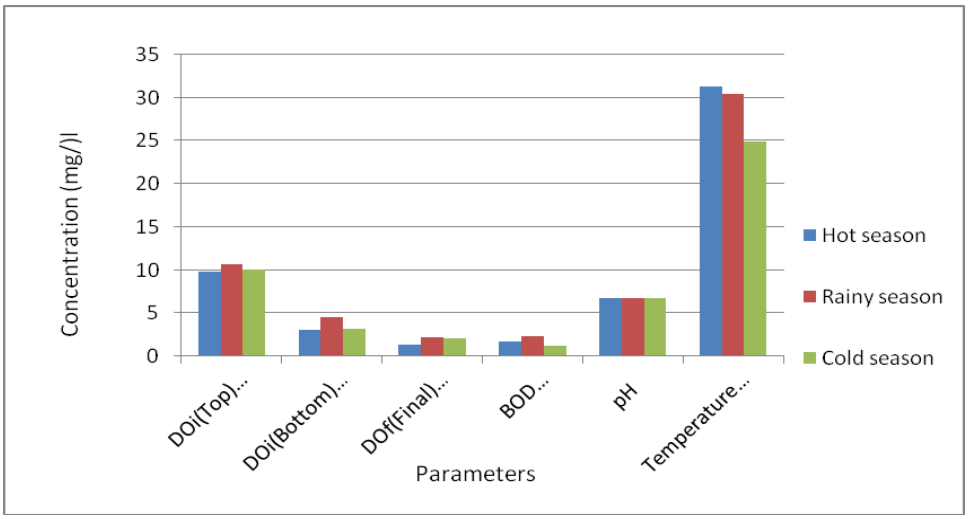

Fig.(7) Distribution of DO_i,(Top), (Bottom),DO_f (Final), pH ,Tempertaure of Hot season, Rainy season and Cold season of Taungthaman Lake

Fig 8. Membrane type DO meter

Fig 9. Tadarphyu sluice gate

Fig 10. Water sample for BOD measurement

Fig 11.Waste water from Myothit area

Fig 12. Typical debris input of Payandaw Chaung

Fig 13. Domestic waste dumped under U Bein Bridge

Fig 14. Bloated carcass on the lake

Fig 15. Polluted water in Taungthaman lake

Fig 16. Floating mass of blue green algae

Fig 17. Bed of cyanobacteria on north east foreshore of the lake

Fig 18. Mass of cyanobacteria

Fig 19. Fishkill in Taungthaman Lake

Fig 20. Duck Farm, solid organics waste

Fig 21. Diffused source o**f** organic pollution

Discussion

DO value indicate that the water is fresh or not. Heavy algal growth increase DO during the day but also causes the oxygen to drop at night. The reduction of DO is commonly associated with excessive nutrients and oxygen demanding waste. Low DO levels result when the balance is disrupted between oxygen production and it physical, chemical and biological processes. Over a 24 hour period, DO levels fluctuate naturally in most water bodies.

The fluctuation of dissolved oxygen in Taungthaman Lake is in (Fig. 4 to 6) There is an indication of low oxygen content of the bottom water of the lake throughout the year. The oxygen depletion is well marked in stations in hot season and in the cold seasons when the water level is low, due to lack of inflow and low precipitation. High temperature during hotseason also reduces the ability of the water to absorb oxygen from the atmosphere.

In Taungthaman Lake major sources of nutrients include fertilizers and manure from agricultural activities, Urban runoff contain human, animal, domestic and industrials wastewater. Plant nutrients that were essential to plant growth, can have a negative impact on water quality and the aquatic environment. Accumulation of solid organic waste, carried by rain water and by the inflow of the Chaungs flowing into the Taungthaman Lake may also cause the low oxygen in the bottom water. The decomposition of such waste put a high demand of dissolved oxygen increasing BOD.

Conclusion

Taungthaman Lake is a historically important site and a major tourist attraction for Mandalay City. Because of the changes in the water quality due to eutrophication, Taungthaman Lake has lost its scenic beauty. Discharge of polluted water from the Lake is already effecting the water quality of the Ayeyarwaddy River immediate efforts should be undertaken to stop the discharge of waste into the Lake. Environmental awareness and envionmental education of public should be strengthened.

Acknowledgement

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References

APHA (1979). *Standard Methods for the Examination of water and wastewater*.Ed. Greenberg, A.J. Connors and D.Jerkins., American public Health Association, Washington DC. ISBN 0-87553-091-5.PP.1-1136.

Painchaud and Jean (1997). "Substantial Progress Has Been Made in Quebec's Water Quality." *Environmental Science and Engineering* (March) : 34-37.

UNDP (2002). Proceeding of the workshop on lakeManagement and Eutrophication control for DonghuLake; *Newsletter and technical publication fresh water Management Series* No.8.124pp.

UNEP (2000). How bad is Eutrophication at present? International Environmental Technology Center.*Newsletter and Technical Publication Lakes and Reservoir* 3**:**1-6.

Vollenweider, R.A.(1979). Fundamentals of the Eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in Eutrophication *OECD.. TechnicalRport DA 5/SCI /*68**:**27-250.

Xiangcan, J. (2003). Analysis of eutrophication state and trend for lakes in china. *Journal of Limnology* (2)**:**60-66.

The Aggressive Behaviour of *Betta Splendens* and their Social Hierarchy

San San Myint[1], Omar Myint[2] and Maung Maung Gyi [3]

Abstract

The Siamese fighting fish, *Betta splendens*, is very well known for its aggressive behaviour. In this species, although resident males are solitary and kept aloof from one another, females can discriminate male social hierarchy, especially for their potential mate. Present study examined the correlation of male aggressive behaviour and its social hierarchy to hypothesize that male in higher social rank will be more aggressive than that of lower social rank male. It was found that male of *B. splendens* showed aggressive behavior to its own mirror image. The level of individual aggressiveness was not related to the male social status. *B. splendens* female prefers dominant males. Thus, the present study suggested that male social status might be positively correlated with their reproductive fitness. It was also suggested that individual aggressive behaviour in *B. splendens* evolves among males in a population in order to increase their reproductive fitness.

Key words: aggressive behaviour, reproductive success, social hierarchy

Introduction

Aggressive behavior is exhibited by most of the species of animals on this world. The purpose of aggressive behavior is to increase the chance of survival. In aggressive interactions, male competitors exhibit a ritualized sequence of signal behaviors, also called displays, during which they exchange information about their readiness and ability to fight (Baerends and Baerends-van-Roon,1950; Enquist *et al*., 1990: Huntingford *et al*., 2000; Parker, 1974). Aggressive behavior also occurs in the establishment and maintenance of social hierarchies. For example, adult male baboon competes violently for social dominance (Drews, 1996) because female prefers to mate with dominant male (Frank *et al*., 1995; Frank, 1997). Thus, the aggression of males may also have access to resources (e.g., food, mate and nesting place). Like many other animals, aggressive behavior can also be seen in Siamese fighting fish, *Betta splendens,* especially between males. They aggressively defend their territory against intruding male conspecific during which they use multiple highly stereotyped and conspicuous visual displays (Simpson, 1968). This agonistic behaviour allows male exclusive access to resources (food,

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mates and nesting sites). Aggressive pattern of *B. splendens* includes frontal displays (erecting the operculae, fins and tail), lateral or broadside displays (swimming with erection of fins and tail), and physically attacking and biting the intruder. *B. splendens* are colorful and sexually dimorphic, males are brighter than the females. The Siamese fighting fish, *Betta splendens*, is very well known for their aggressive behaviour. *B. splendens* live and breed in rice paddies fields, shallow ponds and small streams. Males built a bubble nest on the surface of the water and females laid the eggs inside the male's bubble nest. Males guard the nest until the young reach to the safety state. Adult individuals reach a total length of about 7cm, although females are quite smaller. The present study was carried out to observe the relationship between the level of individual aggressiveness and their dominance hierarchy, and to examine whether the dominance hierarchy influence on female mate choice or not.

Materials and methods

Fish used in this experiment were obtained from local aquarium shop during the study period, from March 2012 to May 2012. Fish were kept in separate plastic bottles (1Litre) and fed food pallet twice a day and kept under a 12:12 light-dark cycle. Water in the plastic bottles was refreshed twice a week. The total length (TL), the distance between tip of the snout and tip of the caudal fin, of males (5.35$\pm$ 0.26) and females (4.74$\pm$ 0.26) were used. All females used in the experiment were gravid and ready to spawn. Males with similar maturity and red colour morph were used. Behavioural analysis were done by the video images recorded with a video camera (Panasonic HDC-TM 45).

Observation on unilateral attack (experiment 1)

A mirror test was carried out to examine the level of individual aggressiveness of a male *B. splendens* in a small aquarium (17x30x22cm) (Fig.1a) by placing a 14x14cm glass mirror inside the aquarium for 5minutes and observed the frequency of male unilateral attack. Then, the fishes were returned to their plastic bottles and kept for further experiments.

Observation on mutual attack (experiment 2)

To observe the male social rank, an experimental tank was equally divided into two compartments with a transparent plastic plate (Fig. 1b). Two males were placed into each male compartment, and were observed the frequency of mutual attack between them for 30 minutes (if one individual

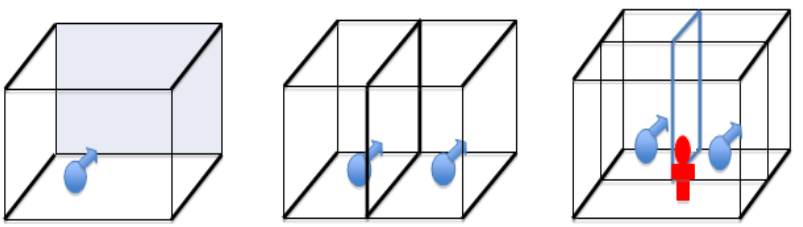
defeats the other within the observation period, the experiment was finished and recorded the time).

Female choice on dominance hierarchy (experiment 3)

To examine the female choice on dominance hierarchy of the male, an experimental tank with three compartments was set up as shown in Fig (Fig. 1c). A single female was kept in a compartment, partitioned with a transparent plastic plate and face to face with the male compartments, which was separated by an opaque plastic partition. Hence, female fish could see both of the males and vice versa, but the males could not see each other. Then, the two males, winner and loser from experiment 2, were placed into each male compartment for 15 minutes. The time spent by the female in the active region, 5cm from the male compartment, was observed. If males did not perform courtship actively, we cancelled the experiment.

Statistical analysis

All data were checked for normality, using the Kolmogorov-Smirnov test. We therefore used non-parametric Mann-Whitney *U* test to analyse whether the level of male individual aggressiveness influences on dominant status or not. It was also used Mann-Whitney *U* test to test the female mate choice on different male social status. Spearman correlation test was used to know the relationship between the frequency of male aggressiveness and female mate choice. All the statistical tests were performed with statistical software (STATISTICA).



(a) Experiment 1 (b) Experiment 2 (c) Experiment 3

Fig. 1. Experimental design: (a) mirror test, (b) mutual attack and (c) female choice on dominance hierarchy

Results

In the mirror test, many males showed aggressive behaviour to their own image (34.35±9.21, N=20). This behavior indicated that the common habit of individual aggressiveness in this fish. However, this level of aggressiveness was not related to their dominance status for winner or loser in the subsequent mutual attack (Winner: 38.20±5.59, N=10; Loser: 30.70±10.79, N=10; Mann-Whitney *U* test; p=0.12, U=29.5, Fig.2). This result also revealed that the aggressive behavior of *B. splendens* was evolved not only for their social status but also for their survivorship. Thus, the level of male aggressiveness towards their own image was not related to their future winning possibility.

In female mate choice experiment, females usually chose winner male from the mutual attack indicating that female preference on dominant male might be indirectly related with the male aggressiveness. The time spent by female in front of the winner male compartment was significantly higher than that of loser male (p=0.02, U=20.0, N=10, Fig.3). Thus, *B. splendens* females prefer dominant male or more aggressive males like in many other fishes.

There was highly positive correlation between female mate choice and male aggressiveness (Spearman correlation test: p=0.002, t (N-2)=3.74, N=20, Fig.4). Thus, female can judge the level of male aggressiveness or boldness and their social hierarchy.

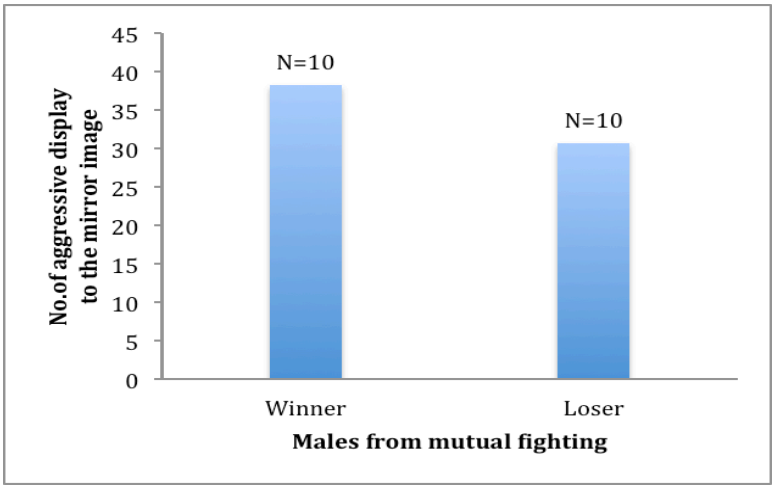


Fig. 2. Agggressive behavior of males from the mirror test

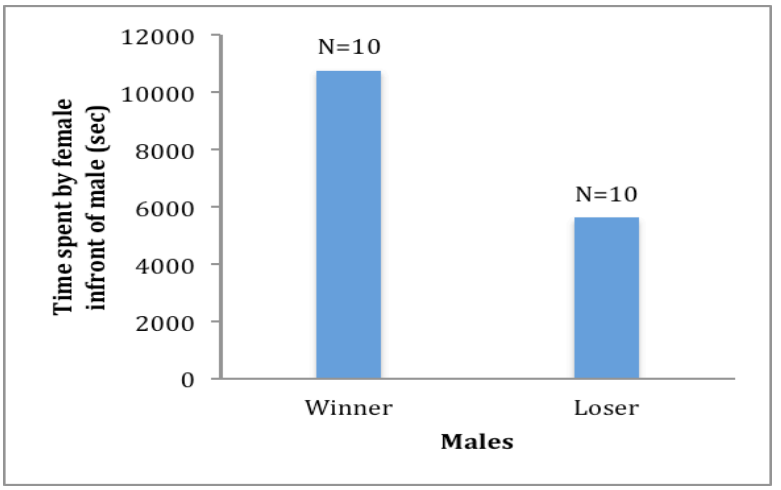


Fig.3. Female choice on male social status

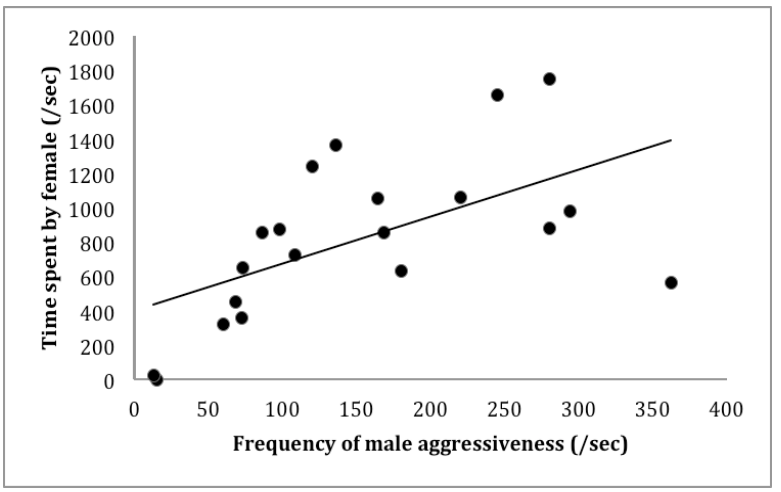


Fig. 4. Female preference on male aggressiveness

Discussion

In the first experiment, *B. splendens* males showed intensive aggressiveness to their own image indicating this behaviour is very common in this species. In addition, females were found to prefer more aggressive males, which also explains why males are so aggressive in this species. The

present study showed that the intensity of male aggressiveness in the mirror test was not related to the possibility of future winning. This result is consistent with the previous empirical research of Simpson (1968). However, Beaugrand et al., (1991) reported that in swordtail fish future winners were more aggressive to their own image than that of future losers before they encounter with other individuals. In addition, several studies reported that aggressive displays of males to the mirror image seem to have reinforcing properties for the subsequent attack (Hogan, 1967; Bols, 1977; Hogan and Roper, 1978; Ginsburg and Allee,1942; Scott, 1946). If this hypothesis is true, aggressive males from the mirror test should always win in the subsequent mutual attack. However, in the present study many males showed agonistic behavior to their own mirror image indicating that the competitions between males are very intense in nature and this aggressive behavior might come innately.

In general, dominant males usually monopolize the females in order to maximize their reproductive success. Thus the battles among males are very common in life on earth. One prominent manifestation of conflict among males is the dominance hierarchy and many studies had been reported that there is a relationship between dominance and reproductive success (Drews, 1996; Widdig *et al*., 2004). Although fighting between males are not seriously damage to the contestants, in this fish this conflict can lead to an unpleasant death (Castro., *et al.,* 2006). During the battle, *B. splendens* males attack aggressively towards each other until the rank appears between them. Once one knows his place, the battle is finished. The winner learns to attack or chase the specific rival, whilst the loser learns to avoid or flee away from his rival. In the present study, it was found that aggressive males in the mirror test was not always win in the subsequent mutual fighting indicating that the dominance hierarchy in *B. splendens* might not base on the individual aggressive level because all individuals are aggressive to their own mirror image. Thus, from our results we suggested that not only individual aggressive level but also dominance hierarchy is important in *B. splendens*, and it might be strongly related with their survivorship.

In addition, in the present study it was found that females prefer dominant males. In many animal species, females usually choose potential mate and male usually compete for access to mate (Anderson, 1994). Female choice bases on male characteristic or resources provided by male. Female usually prefers dominant male because they can provide resources than other males thus the rank in a dominant hierarchy correlates with male mating

success (McCann, 1981; Hoelzel *et al*., 1999). It was suggested that female preference on high rank male in this study might be correlated with female reproductive success. By choosing a dominant male or territory male, female can produce many offspring because dominant male will defend their territory successfully from other conspecific male. Hence, female may gain direct benefits from its mate choice.

Conclusion

It was concluded that aggressive behavior is a common habit in *B. splendens*. In addition, female mate choice plays an important role in male aggressiveness and the dominance hierarchy, since it is strongly related with male reproductive success. Further studies concerned with innate mechanism of male aggressiveness in *B. splenden* should be encouraged.

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References

Anderson, M., (1994) "*Sexual Selection."* Princeton University Press, Princeton.

Baerends, G. P., J. M. Baerends-van-Roon, (1950). "An introduction to the study of the ethology of cichlid fishes." *Behaviour*, vol. 1, S1-S242.

Beaugrand, U.P., C., Goulet, & D. Payette, (1991). "Outcome of dyadic conflict in male green swordtail fish, *Xiphophorus helleri*: effects of body size and prior dominance." *Animal Behaviour*, vol. 41, pp. 417-424.

Bols, R.J., (1977). "Display reinforcement in the Siamese fighting fish, *Betta splendens:* aggressive motivation or curiosity?" *Journal of Comparative Physiology and Psycology*. Vol. 91, pp. 233-244.

Castro, N., A. F. H, Ross., K Becker., and R. F. Oliveira., (2006). Metabolic cost of aggressive behavior in the Siamese Fighting Fish, *Betta splendens* "*Aggressive behavior*", vol. 32, pp. 474-480.

Drews, C., (1996). "Contests and patterns of injuries in free-ranging male baboons (*Papio cynocephalus*)." *Behaviour*, vol. 133, pp. 443-474.

Enquist, M., O. Leimar, T. Ljungberg, Y. Mallner, & N. Segerdahl, (1990). "A test of the sequential assessment game: Fighting in the cichlid fish, Nannacara anomala." *Animal Behaviour*, vol. 47, pp.387-410.

Frank, L. G., H. E. Holekamp, & L. Smale., (1995). "Dominance, demographics and reproductive success in female spotted hyeanas: A long-term study." *In: Serengeti II: Research, Management, and Conservation of an Ecosystem*, A. R. E. Sinclair and P. Arcese (edis.). University of Chicago Press, Chicago.

Frank, L. G., (1997). "Evolution of genital masculinization: Why do female hyeanas have such a large penis?" *Trends in Ecology and Evolution*, vol.12, pp. 58-62.

Ginsburg, B., & W.C. Allee., (1942). "Some effects of conditioning on social dominance and subordination in inbred strains of mice." *Physiology and* Zoology., 15, 485-506.

Hoelzel, A. R., B. J. Le Boeuf, J. Reiter, & C. Campagna., (1999). "Alpha-male paternity in elephant seals." *Behavioral Ecology and Sociobiology*, vol. 46, pp. 298-306.

Hogan, J. A., (1967). "Fighting and reinforcement in Siamese fighting fish *Betta splendens." Journal of Comparative Physiology and Psycology.* Vol. 64, pp. 356-359.

Hogan, J. A., & Roper., T. J., (1978). "*A comparison of the properties of different reinforcers*." In: Rosenblatt, J. S., Hinde, R. A., Beer, C., and Busnel, M. C. (Eds.), Advances in the study of Behaviour, 8. Academic Press, pp. 155-255.

Huntingford, F. A., A. C. Taylor, L. U. Sneddon, & F. C. Neat, (2000). "Prowess and the resolution of animal fights. In: Esmark Y, Amundsen T, Roseqvist G (eds): "*Animal Signals: Signalling and Signal Design in Animal Communication*," Trondheim, Norway: Tapir Academic Press, pp. 259-276.

Kraaijeveld, K., F. J. L. Kraaijeveld-Smit, & J. Komdeur, (2007). "The evolution of mutual ornamentation." *Animal Behaviour*, vol. 74, pp. 657–677.

McCann, T. S., (1981). "Aggression and sexual activity of male southern elephant seals, *Mirounga leonina*." *Journal of Zoology*, vol. 195, pp. 295-310.

Parker, G. A., (1974). "Assessment strategy and the evolution of fighting behaviour." *Journal of Theoretical Biology*, vol. 47, pp. 223-243.

Scott, J. P., (1946). "Incomplete adjustment caused by frustration of untrained fighting mice." *Journal of Comparative Physiology and Psycology.* Vol. 39, pp. 379-390.

Simpson, M. J. A., (1968). "The display of the Siamese fighting fish *Betta splendens*." *Animal Behavior.* Monog., 1, 1-73.

Widdig, A., F. B. Bercovitch, W. J. Streich, U. Sauermann, P. Numberg, & M. krawczak., (2004). "A longitudinal analysis of reproductive skew in male rhesus macaques." *Proceedings of the Royal Society of London B*, vol. 271, pp. 819-826.

Motility and fertilization capacity of cryopreserved spermatozoa of *Pangasius hypophthalmus* (Sauvage, 1878) in different conditions

Kyaw Naing Oo

Abstract

In cryopreservation, the highest post-freezing motility was recorded for effective equilibration period (15 minutes).To determine appropriate extender and proper type of cryoprotective agents with their concentrations, the motility of spermatozoa was studied up to 365 days after freezing. The spermatozoa motility in the two extenders; (HBSS Vs CFHBSS) was not significantly different (P>0.05).Successfully cryopreserved fish spermatozoa were able to fertilize ova of the same species. The developmental stages of embryos produced from fresh ova inseminated with cryopreserved semen were compared with those of control in which freshly collected semen was used. Although very low percent of eggs fertilized with cryopreserved spermatozoa had arrested mitosis, no malformed larvae were observed, and the zygotes took 20-24 hrs or longer to hatch. The fitness of cryopreserved spermatozoa was confirmed by comparing the hatching rate after 365 days of freezing with control. Hatching percents of ova fertilized with frozen thawed semen and freshly collected semen were not significantly different when 9 % DMSO had used with each of the two extenders (HBSS and CFHBSS).The highest fertilization capacity (hatching percent) was 65.39 ± 1.22 in 9 % DMSO added with CFHBSS at 365 days of freezing.

Key words ; cryopreserved, spermatozoa, freezing, thawing, extender , ova, thawed, fertilization, hatching.

Introduction

Cryobiology comes from the Greek word “Kryos” which means “cold”. Cryobiology is the study of living system at any temperature below the standard physiological range (Fashy, 2007).

Habitat lost has been occurred due to human exploitation such as urbanization, industrialization, deforestation and global warming leading to natural disasters. World-wide 11% birds, 25 % mammals and 34 % of fish species are threatened (Vemuganti and Balasubramanian, 2002) and aquatic ecosystems are becoming more vulnerable to disasters. In current the cyclone,

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Nargis (2008, May) made the lower Myanmar fresh water ecosystem upset. Salt water flooded over fresh water bodies during Nargis that had destroyed the breeding sites, spawning ground and habitats of some fresh water species. The conservation of the whole organism in nature becomes rather difficult and hence the conservation of the genetic resources has to be urgently needed and essential issue for sustainable ecosystem. Actually, cryopreservation is the best way to keep the genetic resources or biodiversity of rare and endangered species (Bart, 2000). Many fish species are in decline and some have become endangered due to a combination of over-exploitation of pesticide and aquatic pollution, spread of disease, uncontrolled introduction of exotic fishes, and habitat modification (Mijkherjee *et al.*, 2002).

In order to sustain food production, research and its applications will have to use all available technologies, especially the rapidly developing modern biotechnologies (Mehra, 2001). Of these crypreservation is one of the effective technologies for keeping the genetic resources of biodiversity of rare and endangered species (Li *et al*., 2004).

Spermatozoa can be stored for a few hours to several days at 0º C depending on the species while cryopreserved gametes can be theoretically stored between 200 and 32,000 years without deleterious effect (Ashwood-Smith, 1980).

Since Polge *et al*., (1949) first discovered the protective effect of glycerol on the preservation of fowl spermatozoa. Cryopreservation technology began in 1950 and spermatozoa of 200 fish species had been cryopreserved (Rana and Gilmour, 1996).Optimum conditions for cryopreservation of fish spermatozoa are highly variable for each fish species (Leung and Jamieson, 1991). Blesbois and Labbe (2003) also stated that each fish species had high variability of cryopreservation success between male and between ejaculates within a species. Successful cryopreservation depends not only on the right choice of cryoprotective agents and extender, but also on the freezing protocol used (Mazur, 1970).

In the family Pangasiidae, 45 species under three genera; *Helicophagus, Pangasianodon* and *Pangasius* are globally recorded. Among them, 21 species are endemic to Asia. Of three species in Myanmar; *Pangasius pangasius, P. myanmar* and *P. hypophthalmus, P. myanmar* is newly described as endemic and very few individuals were caught in the wild and data on this species is very rare (Roberts and Vidthayanon, 1991).

Pangasius hypophthalmus (Sauvage, 1878) is native to India and Burma but was presumably introduced to Indonesia, Thailand and Malaysia.

Because of population decline in nature, international market demand and its rapid growth rate, culture of *P. hypophthalmus* is being attempted in captive condition (San Aung, 2009). The amount of *P.hypophthalmus* seed in the wild was only a tenth of what was caught ten years ago (Khanh *et al.*,1999). Other species of Pangasiidae are also similarly affected so there is an urgent need to preserve genetic material as some of these species are facing extinction. New and innovative tool, such as cryopreservation can assist in the preservation of genetic diversity and assist in hatchery production of fries and fingerlings (Kwantong, 2000). This catfish, Nga dan is one of the most popular fresh water fish in Myanmar because its meat is used in many ways to traditional dishes. In addition, *P.hypophthalmus* has now become an important export item after processing. Hence, the present study aimed

1. To examine the life span of the cryopreserved spermatozoa of the catfish based on different freezing and storage times.
2. To evaluate the effects of freezing shock upon the sperm viability and motility (fitness) of the spermatozoa cryopreserved by using extenders and three cryoprotective agents (CPAs).
3. To determine the most suitable combination of cryoprotective agents and extenders by comparing motility and hatching rates of embryos from cryopreserved sperm and fresh ova.

Materials and methods

Collection site of fish germ cells and experimentation

Ripe males and females were taken from the stock and were acclimated in concrete tanks at Hlawga Fishery Department. The experiments which evaluate the fitness (motility %) of the spermatozoa were carried out in Livestock Breeding Improvement Section (LVBD), Ministry of Fishery and Livestock, Mingaladon and that of fertilization capacity (hatching %) was done at Hlawga Fishery Department.

Collection of Semen

The males were injected with 15μg/kg leuteinizing hormone-releasing hormone, ($LHRH_a$), (Suprefact) and 5 mg / kg of domperidone (Motilium) for stimulation of semen release (Tiersch,1994).After six hours of hormonal

stimulation semen was collected by stripping method and care was taken to avoid contamination by urine, faeces and blood (Tiersch *et al*., 1998).

Collection of eggs

After 6 hours of injection of females with Suprefact (30 μg / kg), leuteinizing hormone – releasing hormone ($LHRH_a$) and 10 mg / kg of domperidone (Motilium) to stimulate egg production (Tiersch *et al*., 1995) the eggs were collected by abdominal stripping method and weighed. Counting of the individual egg number was done to evaluate the percentage of fertilization capacity after inseminated and fertilized by thawed cryopreserved semen. Freshly collected eggs were inseminated with cryopreserved semen while the same batch of eggs was fertilized with fresh semen in control.

Laboratory Facilities

Different glass wares, stryofoam, thermometer, ice pieces, warm water, refrigerator, digital camera and compound microscope with monitor, liquid nitrogen, 0.5 ml straws, sealing powder, thermostat (temperature controller),cold cabinet, freezing tanks, sperm counter, cryobox, cryocan, and hatching aquaria were used to conduct cryopreservation and fertilization experiments.

Cryoprotective agents (CPAs) and extenders

Three CPAs used are Dimethysulfoxide (DMSO), Glycerol (Gly) and Methylhydroxide (MeoH) while two types of extenders Hank's Balanced Salt Solution (HBSS) and Calcium Free Hank's Balanced Salt Solution (CF-HBSS) are use for freezing and storage of semen.

Statistical Analysis

Data were subjected by analysis of variance (ANOVA). The Tukey's HSD (multiple comparison tests) was used to compare the treatments. Walcoxin Stratified Test was also used whenever necessary to compare different strata. These statistical analyses were conducted with Statistical Package for Social Science (SPSS-Version16).

Experimental design

Three experiments were conducted in present work:

(1) the evaluation of the motility of the spermatozoa in short term storage

(2) the same evaluation was done on spermatozoa in long term storage (cryopreservation) and

(3) artificial insemination or fertilization experiment in which the fresh ova were inseminated with cryopreserved thawed semen and fresh semen was used as control.

Motility analysis of semen of Striped catfish

Motility and movement for at least 200 spermatozoa were evaluated based on the following criteria:

1. Mass progressive motility when most of the spermatozoa were actively swimming with progressive movement and
2. Total movement duration in seconds recorded until most spermatozoa stopped swimming. All samples were examined in replicates (Akeay *et al*. 2004).

Long term storage (cryopreservation) of semen of Striped catfish

Semen collected from 6 male *P.hypophthalmus* was used for freezing process for evaluation of the post - freezing fitness (motility %). Two different experiments were used for long term storage as follows:

(i) Effect of freezing shock on viability (survival) of spermatozoa and effect of different CPAs on post - thaw motility % of spermatozoa.

(ii) Post - thaw motility % of cryopreserved spermatozoa with different storage time at different concentrations of CPAs with two extenders.

The semen samples of adult males were mixed with Negrosin-eosine staining thoroughly. Semen and stain mixture were smeared on the slides and examined with microscope for live and dead sperm count percent of 200 individual spermatozoa (Moss *et al.*, 1979) and then compared and assessed viability (live and dead percent) before and after freezing. All straws were freezed in liquid nitrogen for 2 days by using two steps freezing method (Tiersch 1994). After keeping in liquid nitrogen for 2 days, straws were thawed at 40ºC in water bath and the motility of spermatozoa was examined. Each examination was replicated two times for spermatozoa from each male.

Fertilization capacity (hatching) % of ova inseminated with frozen thawed (cryopreserved) spermatozoa of *P.hypophthalmus*

The straws cryopreserved after one year were thawed and used to fertilize fresh ova while the same amount of fresh semen was used as control. The number of hatched fries was counted after 24 hours after insemination. The hatching % was evaluated based on the following:

(i) Developmental capacity of embryos from ova fertilized with spermatozoa cryopreserved

(ii) Spermatozoa cryopreserved in different concentrations of CPAs for one year was used for fertilization.

After hormonal stimulation of two female eggs were collected by abdominal stripping and fertilized with cryopreserved semen while the same batch of eggs were fertilized with the same amount of fresh semen as a control. The series of developmental stages of embryos with duration of seven stages of embryo development was recorded. Methods were after Kimmel *et al*. (1995) and Hill(2007).

Sperm density was estimated by using a micrometer (sperm counter). The sperm: egg ratio (4.2×10^6:1) was used for the fertilization experiments (Tiersch, 1994). After the eggs and sperm were mixed thoroughly, rinsed in water and incubated at aquaria($170 \times 72 \times 30$) cm^3 with continuous aeration of 27 ^oC. Photographs of the stages were prepared by a digital camera and phase contrast microscope (Olympus×10).The percentage of hatched fries related to egg numbers after 24 hours of insemination was calculated. The fertilization capacity of the spermatozoa after one year of storage could be assessed and compared with the results of control.

Results

Motility Rate of The Spermatozoa of The Raw Semen at Different Extenders

After storage of 3 successive days, spermatozoa motility was a little decreased and the most favorable in fructose compared with that of in HBSS and in CFHBSS.Motility declined from 74 % to the range of 55%-60.2% but it was active (Fig 1).

Effects of Freezing Shock on Post Thaw Viability and Motility of Spermatozoa in Two Extenders

Some of spermatozoa got cryoinjuries which could be revealed with microscopic assessment of live and dead sperm. Before freezing 84 ± 4 % of live sperm occurred while after freezing 61 ± 5 % of live sperm occurred. Dead sperm were pink due to Negrosin-eosin staining and live spermatozoa were unstained (Plate1.a).

DMSO 9 % in HBSS was the best CPA for spermatozoa motility, 42.54 (± 1.27)% in freezing while motility, 41.50 (± 1.21)% in MeOH 9% with CFHBSS and 41.20 (± 1.54)% in DMSO 12 % with HBSS, 40.78(± 1.19)% in MeOH 12% with CFHBSS were also recommended a moderate efficacy. All concentrations of Glycerol (GLY) in both extenders resulted in poor post thaw motility percent. Post thaw motility of spermatozoa 37.50(±1.24)% in DMSO 9% in CFHBSS was active for future use (Table 1) and (Fig 2).

Motility rate (%) of long term cryopreserved spermatozoa in different concentrations of DMSO and two extenders

DMSO 9 % in CFHBSS gave the highest motility of 45.89 ± 2.65 % while second highest motility was 42.60 ± 1.30 % in DMSO 9 % with HBSS. These motility rate were not significantly different from motility at 46.36 ± 1.25 % and 45.00 ± 1.57 % found at 2d of cryopreservation in the same two combinations of CPA and extender. Moderate spermatozoa motility at 39.23 ± 1.28 % in 12 % DMSO with CFHBSS was higher than that fond in DMSO 6 % with HBSS at 38.00 ± 1.75 % after one year of freezing. In addition, spermatozoa motility in DMSO with extenders of other combinations were also higher than the recommended level (30 %) after one year of freezing .Spermatozoa motility was significantly different at($p < 0.01$) level among three different concentrations with two extenders at 365 ds of freezing (Fig 3).

Developmental Capacity of Embryos and Hatching % of *P.hypophthalmus* with Cryopreserved Spermatozoa

Normal development of embryos of *P.hypophthalmus* in which frozen thawed semen fertilized with fresh ova occurred from 0 minute to 24 hours and later. All internal developments observed from outside. Although a few fertilized eggs did not hatch to hatchling, no malformed larvae occurred in fertilization experiment in which frozen thawed semen and freshly collected ova. Seven developmental stages of embryo with a time table was shown (Plate 1. d, e, f, g, h, i).

Fertilization Capacity (Hatching %) of Ova Inseminated with Cryopreserved Spermatozoa in Three Different Concentrations of DMSO

The combination of extender and cryoprotective agents (DMSO 9 % and CF HBSS) was the most favorable for hatching percent 65.43(± 122) while 63.43(± 1.41) % in DMSO 9% with HBSS was the moderate one compared with that of others 6 % and 12 % DMSO with both extender. It was not significantly differently different between test combinations and that of control (Fig 4).

Discussion and Conclusion

Cryopreservation technique can also increase economic utilization of males and is a prerequisite for the establishment of gene (sperm) bank concerning all endangered and endemic species of vertebrates because many species are facing extinction on a world wide scale with rapidly declining and disappearing habitats. The genetic conservation of fish gametes by cryopreservation technique has not yet been done in Myanmar.

Short term storage (chilling at 4 -5 ºC) of striped catfish semen Motility analysis

Motility of semen is one of the simplest characteristics to determine for viability of spermatozoa because motility is largely related to viability (Stoss,1983). Billard *et al.,* 1981 reported that in salmonid fishes, duration of motility after dilution with water was short for about 15 seconds. This short duration is related with the consumption of ATP during the movement (Billard and Cosson, 1992). Sperm motility in raw semen of the catfish was found to decrease at 10 sec and later. Hence, raw sperm motility was very short in this studied species. The motility 74.00 % of spermatozoa of *P. hypophthalmus* was higher at the condition of 4 – 5 ºC than that of 16.89 (± 0.93)% under room temperature at storage of 70 second. At the room temperature, the motility rate declined within 20sec (Kyaw Naing Oo, 2010).

Lahnsteiner *et al.,* (2003) reported that sperm motility rate was much higher in the saline solution than in water. The findings of the present study are in agreement with that of the above authors. Spermatozoa were relatively active and (54 % - 60 %) lasted for more than three days in the three extenders. However, motility was not different markedly among the three extenders. The spermatozoa motility was quite stable (71 % - 74 %) up to six

hs or later in the extenders while the semen of control experiment in water changed into colloidal form after 6 hs (Kyaw Naing Oo,2010).

Long term storage (cryopreservation) of striped catfish semen

Cryopreservation is a process where cells or whole tissues are preserved by cooling to low sub-zero temperature such as (typically) – 80 ºC or -196 ºC (the boiling point of liquid nitrogen). At the low temperature any biological reaction that would lead to stop death of cells effectively (Mazur, 1970).

The type of CPAs used varied widely between species and sometimes within one species: a CPA successful used in one study was unsuitable in another study within the same species (Viveiros *et al.,* 2000). The present findings were agreed with the above statements because different effective of different CPA_s on spermatozoa motility of *P.hypophthalmus*. During freezing fifty percent of spermatozoa were reported to get cryo-injuries (Moss *et al.,* 1979).DMSO was found to be more effective than MeOH and GLY as a cryoprotective agent having maximum mean values of motility % in this experiment. In each type of CPAs, tested 9 % concentration had the best post freezing motility compared to those in 6 % and respectively. This may be due to the relative cellular toxicity or the differing permeability of different CPAs for the spermatozoa of the studied species. GLY and DMSO have been used for decades as CPAs to reduce ice formation in sperm and embryo that are cold preserved in liquid nitrogen (Wikipedia, 2007). But the present findings revealed that MeOH was recommended for high post - freezing motility of spermatozoa. Suquet *et al.* (2000) however reported that DMSO generally gave the best results in cryopreservation of semen of marine fish species. The present findings of 9 % DMSO as the most suitable CPAs in either HBSS or CFHBSS extender were similar to with the statement of above authors although striped catfish is a freshwater fish. Suquet *et al.,* (2000) also reported that penetration of DMSO is faster into spermatozoa and interacted with the phospholipids of the sperm membrane compared to other CPAs in marine fish species. Glycerol (GLY) provided no protection in black grouper (Gwo, 1993) and low protection in the turbot (Dreanno *et al.,* 1997).

Although methanol (MeOH) is a good CPA for spermatozoa of some species of fresh water fish (Harvey *et al.,* 1982), but it has low cryoprotective efficiency in marine fish species such as barramundi (Leung and Jamieson, 1991). The present findings of suitability of MeOH as CPA in the studied freshwater species confirmed the statements of the above authors and the

present results also showed species specific efficiency of different CPAs. Thirumala *et al.* (2005) also reported that the spermatozoa cryopreservation of live bearing fish (*Xiphophorus hellen),* extender solutions of HBSS with 4 % (v/v) GLY and HBSS with 10 % (v/v) DMSO gave satisfactory results. The present finding of good results in HBSS with 9 % DMSO was agreement with the above statement regarding good spermatozoa motility. GLY has heavier molecular weight (92.09) whilst DMSO and MeOH have low molecular weight of 78.13 and 32.04 (Kasai,1998).The lower post thaw motility could be due to the large molecular weight of GLY which could have slower permeation into spermatozoa. Freezing process resulted in damage to an average of 40 – 50 percent of motile sperm (Perry, 1968). In the present study, good motility was maintained above recommended 30 % at 365 ds storage of spermatozoa. After six months of frozen storage, the spermatozoa motility became mostly stabilized compared with that of initial months. This may be due to the freezing shock that influenced spermatozoa motility more in early periods of freezing process while in later periods the rest of spermatozoa got cryoresistance and maintained motility for long period. Up to 365 ds of storage duration spermatozoa motility was significantly higher in 9 % DMSO than that in 6% and 12%. This might probable be due to more osmotic balance of 9 % DMSO. Spermatozoa can be stored for a few hours to several days at 0º C depending on the species while cryopreserved gametes can be theoretically stored between 200 and 32,000 years without deleterious effect (Ashwood-Smith, 1980).

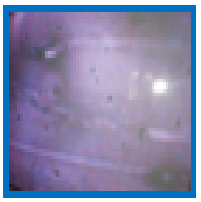
Fertilization capacity (hatching %) of cryopreserved spermatozoa of *P. hypophthalmus*

Horvath and Urbanyi (2000) found that the highest fertilization and hatching rates were achieved with DMSO and dimethyl acetamide (DMA) while ethylene glycol, glycerol, methanol and propylene glycol yielded poor results in this species. In the present findings, good hatching percent were obtained with DMSO 9% compared with that of 6% and 12% which gave lower hatching percent for the studied species after long term (365ds) of cryopreservation. The fertilization capacity(63%-65%) of frozen-thawed spermatozoa was lower than that of fresh sperm (75 %).The present findings agreed with the statement of Munkittrick and Moccia (1984) who said that the fertilization capacity of cryopreserved spermatozoa was lower than that of fresh sperm.

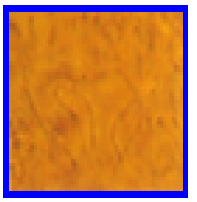
Development of embryo and fertilization capacity

Timing of the developmental stages of zygotes up to hatching was not different between the ova from the same female fertilized with cryopreserved sperm and fresh sperm. In the present study, eggs fertilized with cryopreserved sperm had nearly similar hatching % to eggs inseminated with fresh sperm. According to Mongkonpunya *et al.* (1995) the fertilization percent of cryopreserved spermatozoa of *P. gigas,* at 66.0 ± 6.5 % was nearly the same with the present work of 63.39 ± 1.72 % in 9 % DMSO plus CFHBSS after one year of cryopreservation. Mongkonpunya *et al*., (1992) also found that the ranges of fertilization were 8 % – 14% when sperm cryopreserved in glass ampoules were used and 22 % – 77 % for AI catheters compare to 42 % – 95% in control.Hence, the result of present work on fertilization capacity was in accordance with those of Mongkonpunya *et al*., (1992). However, hatching capacity of the range was not significantly different between two extenders (HBSS and CFHBSS) with 9 % DMSO. The present findings revealed that 9% DMSO in either HBSS or CFHBSS extender is the most favorable for high fertilization capacity.

As a conclusion the motility and the life span of spermatozoa could be extended at state of 4 – 5 ºC. Three extenders tested were reliable for short term storage of the spermatozoa of *P.hypophthalmus*. Spermatozoa motility was favorable in DMSO and MeOH compared with that in GLY after freezing (cryopreservation) up to 365 ds. CPA, 9 % DMSO with CFHBSS was the highest hatching percentage compared with that of 6% and 12% for both extenders. After 365 ds of cryopreservation hatching percents were high but not significantly different in 9 % DMSO for both extenders that indicated the fitness of cryopreserved spermatozoa of *P. hypopthalmus*. The fitness of spermatozoa at 365 ds of cryopreservation was revealed by their good motility percent and fertilization compared with that of control in which fresh semen was used.



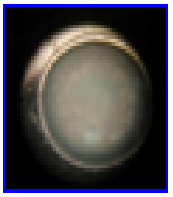
(a)Live and dead estimation of spermatozoa (staining) (× 400)



(b)Spermatozoa in dried smear (× 600)

(c)egg

(d) Zygote Stage



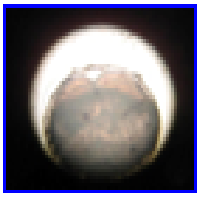
Clear (fertilized) and

opaque (unfertilized) eggs

One cell(Chorion swelling), (0-40 mins)
(Red = Animal pole, Green = vegetative pole)

(e) Cleavage Stage

Two cell stage (30 – 50 mins)



Four cell stage (40 – 60 mins)

Eight cell stage(60 –70 mins)
(Arrow head - Cleavage lines)

16 cell stage (60 – 90 mins)

Cleavage Stage

32 cell stage (1½ - 1¾ hours)

64 cell stage (1¾ - 2 hours)

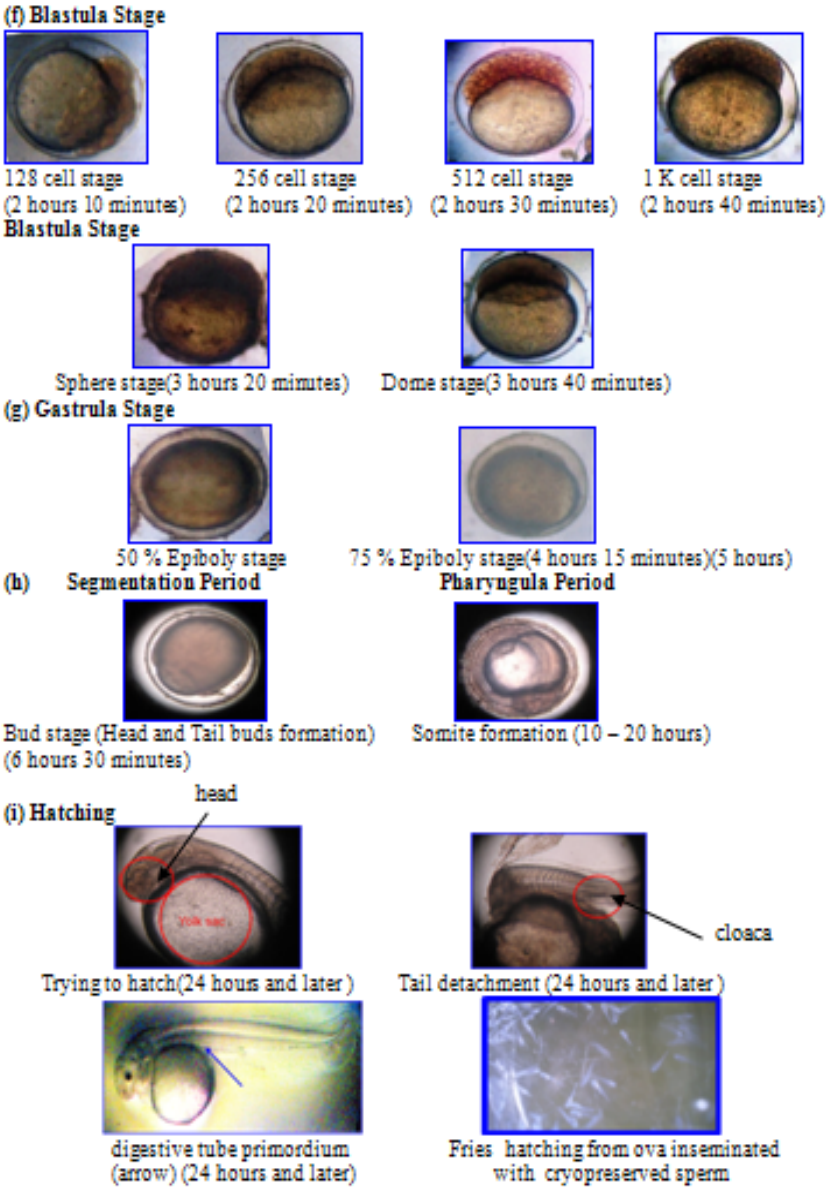


Plate 1. Gametes and Developmental stages of embryos from ova fertilized with cryopreserved spermatozoa

Table 1. Effects of different cryoprotective agents (CPAs) on post-thaw motility % of *P.hypophthalmus* semen in two extenders (HBBS and CFHBSS) after two days of freezing

Extenders	CPAs (Conc:)	Motility % of spermatozoa			
		0 %	6 %	9 %	12 %
HBSS	GLY	0.00	30.06 ± 1.45[a]	32.50 ± 1.32[c]	31.50 ± 1.32[b]
	DMSO	0.00	32.00 ± 1.29[a]	42.54 ± 1.27*[c]	41.20 ± 1.54[b]
	MeOH	0.00	27.50 ± 1.33[a]	35.00 ± 1.42[c]	33.89 ± 1.52[b]
CFHBSS	GLY	0.00	31.49 ± 1.21[b]	34.44 ± 1.26[c]	30.64 ± 1.85[a]
	DMSO	0.00	28.59 ± 1.25[a]	37.50 ± 1.24*[c]	36.38 ± 1.23[b]
	MeOH	0.00	32.00 ± 1.24[a]	41.50 ± 1.21[c]	40.78 ± 1.19[b]

N = 3 * maximum

a, b, c - Different superscripts in the same row differ significantly ($p < 0.05$)

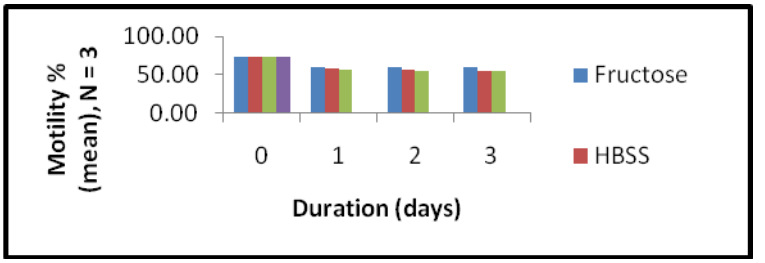
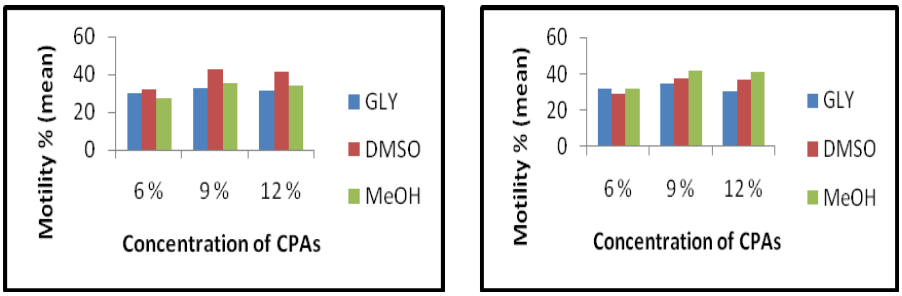
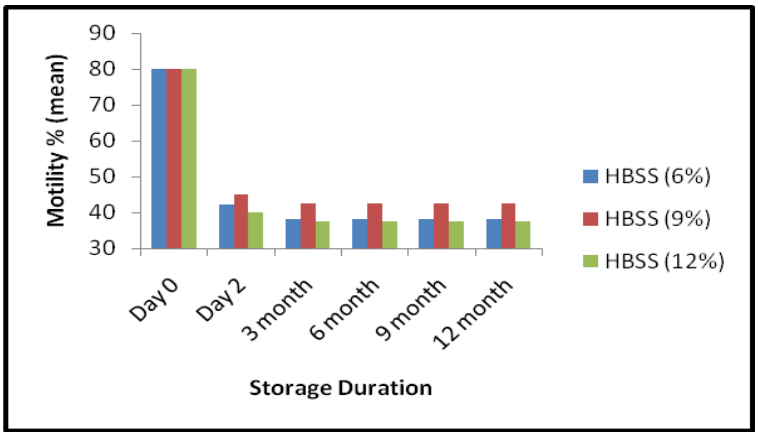


Fig 1. Motility % of raw semen in different extenders at 4–5ºC and 72 h storage

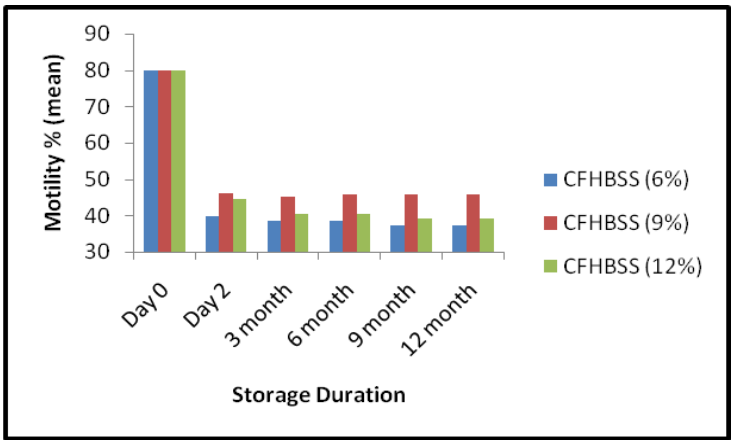


A. Spermatozoa in different CPAs with HBSS B. Spermatozoa in different CPAs with CFHBSS

Fig 2. Motility rate (%) of spermatozoa cryopreserved in different concentrations of CPAs and two extenders



A. Motility rate (%) in different concentrations of DMSO added with HBSS



B. Motility rate (%) in different concentrations of DMSO added with CFHBSS

Fig 3. Motility rate (%) of long term cryopreserved spermatozoa in different concentrations of DMSO added with two extenders

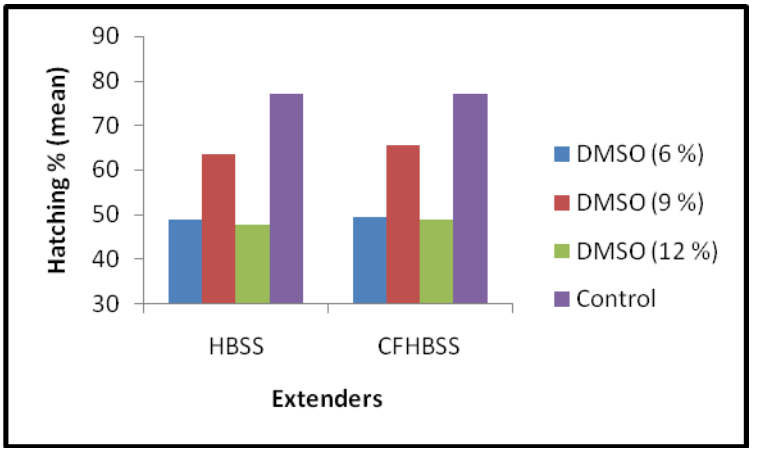


Fig 4. Fertilization capacity (hatching %) of ova inseminated with cryopreserved spermatozoa in three different DMSO concentrations at 365 ds after freezing

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References

Akeay, E., Bozkurt, Y. Secer, S.and Tikin, N. (2004). Cryopreservation of Mirror Carp Semen. Turk.*J.Vet.Anim.Sci*. 28:837-843

Ashwood-Smith, M.J. (1980): Low temperature preservation of cells, tissues and organs. *In: Low temperature preservation in medicine and biology* (M.J.Ashowood-Smith ed.), Pitman Medical Ltd., Turnbridge Wells. 19-44

Bart, A.N, Wolfe, D.F, and Dunham, R.A., (2000). Effects of cryoprotectant, sperm density and straw size on cryopreservation of blue catfish, *Ictalurus furcatus, sperm Transactions of the American Fisheries Society.* V. 127 No. 5: 819-824.

Billard ,R. and M.P. Cosson (1992): Some problems related to the assessment of sperm motility in fresh water fish. *J. Exp. Zool* 261: 122 – 131

Blesbois, E., and Labbe C. (2003): Main improvements in semen and embryo cryopreservation for fish and fowl. In: *Workshop on Cryopreservation of Animal Genetic Resources in Europe. Paris 2003.* (D. Planchenault ed.) ISBN 2-908447-25-8, 55-65

Dreanno, C., Suquet, M., Quemener, L., Cosson, J., Fierville, F., Normant, Y. and Billard, R. (1997). Cryopreservation of turbot (*Scophthalmus maximus*) spermatozoa, *Theriogenology* 48, 495-498

Fashy, G.M. (2007). Cryobiology: The Study of Life and Death at Low Temperatures. http://www.21cm.com/articles/cryobiology.html

Gwo, J.C., (1993). Cryopreservation of black grouper (Epinephelus malabaricus) spermatozoa. *Theriogenology* 39: 1331 – 1342.

Gwo, H.H.T.S., Weng, L.SI Fan and Y.H.Lee (2005). Development of cryopreservation procedures for semen of Pacific bluefin tuna *Thunus orietalis. Aquaculture* 249 (1 – 4): 205-221

Harvey, B. (1982). Cryobiology and storage of teleost gametes. In: Proceedings of the International Symposium on the Reproductive Physiology of Fish. (Richer C.J. and H.J. Goos. Eds.). Pudoc, Wageningen. 123-127

Hill, M., (2007). UNSW *Embryology: Zebrafish development and other embryos.* The University of New South Wales. Sydney. Australia.

Horvath, A. and Urbanyi B. 2000. The effect of cryoprotectants on the motility and fertilizing capacity of cropreserved African catfish *Clarias gariepinus* (Burchell 1822) sperm. *Aquaculture Research* 31(3):317

Kasai, M, (1998). Principles of the cryopreservation of mammalian embryos by vitrification. In: *Reproductive Biology Update*. Shoukadou Bookseller Co., Kyoto, Pp. 415

Khanh, P.V, Tuan, N, Hao, N.V, Jeney, Z, Trong, T.Q, and Thanh, N.M. (1999). *In : Review of Biology and Breeding of some Indigenous Fish Species in the Mekong Delta of Vietnam*. Cai Be. Tien Giang. Vietnam. Pp-32

Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., and Schilling, T.F., (1995). Stages of embryonic development of the zebrafish. *Developmenalt Dynamics.* 203: 253 - 310

Kwantong, S., (2000). Cryopreservation of striped catfish, Pangasius hypophthalmus sperm. Aquaculture and Aquatic Resources Management. AIT Serl; Planting seeds in mind, water, and earths. *AIT Dissertation* No, AQ-03-1. 2044 http;//www.aqua.ait.ac.th/ modules/library/singlefile.php? 389 (20-10-2006)

Kyaw Naing Oo, (2011). Fitness and Fertilization Capacity of Spermatozoa of *Pangasius hypophthalmus*. (Sauvage, 1878) in Cryopreservation, Thesis.

Lahnsteiner, F. Berger, B. and Weismann, T. 2003. Effects of media, fertilization technique, extender, straw volume, and sperm to egg ratio on hatchability of cyprinid embryos, using cryopreserved semen. *Theriogenology* 60(5): 829-841

Leung, K.P. and. Jamieson G.M. (1991). Live preservation of gametes. In: *Fish Evolution and Systmatics: Evidence from Spermatozoa.* (Jamieson, G.M. ed.) Cambridge University Press, Cambridge. Pp.245-269

Li, X. Gao. X.and Ji. W. (2004). Cryopreservation of Sperm of an Endangered Species; Assamese Macaque. *Cell Preservation Technology*, 2 (1): 29-33

Mazur, P. (1970). Cryobiology: the freezing of biological system: The response of cells to ice formation

Mehra, K.L., (2001). *Animal Biotechnologies; Benefits and Concerns.* Proceedings of National Workshop on Conservation and Management of Genetic Resources of Livestock. Natl. Acad. Agric. Sci., New Delhi-110067 India http://www.biotech-info.net/animal ag.html (13-4-2007)

Mijkherjee, M., A., Praharaj and D. Shamik (2002). Conservation of endangered fish stocks through artificial propagation and larval rearing technique in West Bengal, India. *Aquaculture Asia* 7(2): 1- 4.

Mongkonpunya, K, Pupipat, T, Pholprasith, S, Chantasut, M, Rittaporn, R.,Pimolboot, S, Wiwatcharakoses, S, and Chaengkij, m. (1992). Cryopreservation of sperm of the Mekong giant catfish, Pangasinodon gigas Chevey. Aquaculture and Schistosomiasis. Proceedings of a network meeting held in manila, phillipines August 6 -10. PP. 56 – 60.

Mongkonpunya, K, Chairak. N, Pupipat. T and Tiersch-T.R. (1995). Cryopreservation of Mekong Giant catfish sperm

Moss, J.A., Melrose,D.R. Reed, H.C.B. and Vandeplassche, M.(1979). Spermatozoa, semen and artificialinsemination. In: Fertility and *Iinfertility in Domestic Animals.* 3rded. 9Ed. Laing, J.A. Bailliere Tindall-London.

Munkittrick, K.R., and Moccia, R.D: (1984). Advances in the cryopreservation of salmonid semen and suitability for a production scale artificial fertilization program. *Theriogorology* 1984; 21: 645-659.

Perry, E.J. (1968). *Artificial Insemination in Farm Animals.* 4th Revised ed., Oxford and IBH Publishing Co., Calcutta.

Polge. C., A. Smith and Parkes. A.S. (1949). Revival of spermatozoa after vitrification and degydratin at low temperature. *Nature* 164:666.

Rana, K.J., and Gilmour, A. (1996). *Cryopreservation of fish spermatozoa*, Refrigeration and aquaculture conference, Bordeax, 20-22/03/96, 3-12

Roberts, T.R. and C. Vidthayanon (1991). Systematic revision of the Asian catfish family Pangasiidae. With biological observations and descriptions of three new species. *Proc. Acad. Nat. Sci Phil.* 143: 97-144

San Aung, (2009). Commercial River Fishes of Myanmar P.108-109

Sauvage, H.E. 1878. Notes sur quelques poisons d'especes nouvelles provenant des ea douces de I;Indochine. *Bulletin de la souete' hilomatheque de Paris* (7) 2: 223-242

Stoss, J. (1983): Fish gamete preservation and spermatozoan physiology. In: *Fish Physiology*. Hora, W.S., D.J. Rindall and E.M. Donaldson (eds.) Vol. IX. B. Academic Press. 305-350

Suquet, M. Dreanno, C. Fauvel, C Cosson. J.and Billard R. (2000). Cryopreservation of sperm in marine fish. *Aquaculture Research* 31 (3): 231-243.

Thirumala, A.. Huang. C. Dong, Q. Tiersch TR. and Devireddy R.V. (2005). A theoretically estimated optimal cooling rate for the cryopreservation of sperm cells from a live-bearing fish, the green swordtail Xiphophorus helleri. *Theriogenology*. 63(9): 2395-415

Tiersch, T.R. (1994). Cryopreservation of channel catfish sperm, *Transactions of American Fisheries society*, 123:580-586

Tiersch, T.R, Mongkonpunya, K and Chairak,N, and Pupipat, T . (1995). Cryopreservation of Mekong giant Catfish sperm.Asian Fishery Society,Manila, Phillipine 211-221

Tiersch, T.R., Figiel C.R., Wayman W.R., Williamson J.H., Carmichael G.J. and Gorman O.T. (1998). Cryopreservation of sperm of the endangered razorback sucker. *Transactions of the American Fisheries Society* 127, 95-104

Vemuganti, G.S and Balasubramanian, D., (2002). Heralding the dawn of ultured adult stem cell transplantation. *Indian J. Biotechnol*. 1 - 39

Viveiros, A.T. So. .N. and komen. J.(2000). Sperm cryopreservation of kAfrican catifish. Clarias gariepinus: cryoprotectants, freezing rates and sperm: egg dilution ratio. *Theriogenology* 54: 1359-1408

Wikipedia (2007). *Fish.* Media Wiki, Wikipedia Foundation. Inc.http:// embryology.med.unsw.edu.ar / OtherEmb/EmbHome.htm (22-6-2007).

Species Composition of Some Nocturnal Insects from Meiktila University Campus, Meiktila and Relation between Abundance of Insects with Environmental Factors

Hla Swe[1] and Khin Maung Oo[2]

Abstract

In the present research, a total number of 2,258 representing 45 species of insects confined to 37 genera and distributed under 24 families in eight orders were collected from Meiktila University Campus, Meiktila during 2006 to 2008. In 2006, 2007 and 2008, a total of 28, 24 and 26 species of insects were recorded respectively. In 2006, species composition in plantation site was the highest 88.97%, followed by 6.57% in aquatic site and 4.46% in grass-lawn site. In 2007, species composition in aquatic site was the highest 48.67%, followed by 41.74% in plantation site, 7.82% in grass-lawn site and 1.77% in scattered tree site. In 2008, composition of species in plantation site was the highest 42.98%, followed by 24.52% scattered tree site, 16.90% in grass-lawn site and 15.60% in aquatic site. During 2006 the numbers of insects were low in February, June and July and sharp and very sharp seasonal peaks occurred at the onset of the rain in March and April with a little peak in September. During 2007 the numbers of insects were low in February, April and June and very sharp peak happened at the onset of the rain in March and May with a second very sharp peak in August. In 2008 population was low in January and February, and climbed to a peak in August with a little fall in May and June, and then, a sudden fall in September, October and November. The number of specimens (species) in plantation, grass-lawn, aquatic and scattered tree sites were 1158 (33) 267 (24), 538 (27) and 295 (16) respectively. The most common species were found to be ranked in different positions over the three year periods. True bug species *Cydnus indicus* accounting for 62.13% of the total individuals collected, was the most dominant species in all study sites. *Macrotermes* sp. and *Phyllophaga* sp. were the second and third most abundant species collected.

Key words: insect, species composition, abundance, environmental factors, Meiktila University campus

Introduction

Several studies have provided evidences showing that tropical insects undergo seasonal changes in abundance (Davis, 1945). It seems likely that the seasonal presence of insect species is synchronized with a seasonal presence of

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its food, if food availability varies seasonally. The apparent abundance of foliage in a forest does not necessarily imply an abundance of food for herbivores (Voute; 1957, cited in Wolda, 1978). Peaks of insect abundance can roughly be correlated with certain flowering, fruiting or leaf flush peaks (Wolda in press, Fozden 1972, Smythe 1974b, Burkish 1976, cited in Lubin 1978).

Rainfall is the most important climatic factor affecting plant phenology in the tropics, and Janzen (1973) has shown that insect abundance in and over vegetation is directly related to seasonal patterns of flowering, fruiting and leaf flush in that vegetation.

Fluctuation of climatic conditions can affect appreciable changes in abundance of arthropod population, interaction, and community structure. Population of insects is highly sensitive to changes in abiotic condition, such as temperature and water availability, which could affect insect growth and survival.

Seasonal variability of arthropods can be extremely high, reflecting pediodic food supplies or environmental changes such as rainfall (Denlinger, 1980) or temperature (Mani, 1968, cited by Lowman, 2006). Seasonality is a common phenomenon among tropical insects. Since the classic studies by Dobzhansky and Paran (1950) and Bigger (1976), cited by Wolda, O'Brien and Stockwell (1998) a fair number of papers have been published showing that tropical insect species range from a seasonal to sharply seasonal even in relatively aseasonal climates, a variation much larger than that found in the temperate zone. For some species there is information on between year similarities in seasonal patterns (Patil and Thontadarya, 1983; Wolda, 1982, 1983c, 1989) suggesting that between-year differences in seasonal abundance patterns do exist, but they are small. Almost all species investigated show seasonal peaks of abundance that in one way or another are associated with the alternation of wet and dry seasons. Indeed, it appears that most biological events in the tropics are seasonal and are dependent on the distribution and amount of rainfall (Owen, 1966) (cited in Owen and Chanter, 1972).

Few studies exist, however, that have examined seasonal changes in insect populations of rain forests and rare study in Myanmar.

The present research aimed:

- to determine the species composition of insects among four habitat types of Meiktila University campus and

- to establish the relationship between abundance of insects and environmental factors (temperature, rainfall and humidity) in the study area.

Materials and Methods

Study Area

Meiktila University campus is situated between 20° 52' North and 95° 50' East and located in Meiktila Township, Mandalay Region.

Study Period

The study was conducted from January 2006 to December 2008.

Insect Sampling

The specimen collection was made by light trap (constructed according to Sutherland, 1997). The light trap uses an eight watts fluorescence light powered by a 12 volt battery at the centre of four aluminium baffles placed at right angle to one another. It was supported by four wooden legs each having one metre height. A bucket is placed in between and insects attracted to the light were fell through a funnel below and were collected in the bucket. Four sampling sites were allocated in the study area, namely, plantation, grass-lawn and aquatic and scattered tree sites. One light trap was placed at the centre of each site. Samples were taken from each study site during 19: 00 hr to 01: 00 hr of every fortnight during studied periods. The insects trapped in the bucket were collected in the next morning and preserved in 70% alcohol except moths.

Weather Record

Meteorological data during sampling times of January 2006 to December 2008 were obtained from Air Force Station, Meiktila.

Results

In the present research, a total number of 2,258 representing 45 species of insects confined to 37 genera and distributed under 24 families in eight orders were collected from Meiktila University campus, Meiktila during 2006 to 2008.

In plantation site, a total of 1,158 (51.28%) insects confined to eight orders , in grass-lawn, 267 (11.82%) insects confined to eight orders, in

aquatic site, 538 (23.83%) insects confined to eight orders and in scattered tree site, 295 (13.06%) insects confined to six orders were collected (Table 1).

The eight orders of insects recorded from the plantation, grass-lawn and aquatic sites were namely, Odonata, Orthoptera, Isoptera, Dermaptera, Hemiptera, Homoptera, Coleoptera and Lepidoptera, while there confined to six orders were identified from the scattered tree site. Out of the recorded eight orders, six were found to be common to all areas of study. Insects of order Odonata were not encountered and insects of order Lepidoptera were missed for identification at the scattered tree site. In 2006, a total of 426 (18.87%) insects confined to eight orders were collected, in 2007, 678 (30.03%) insects confined to seven orders and in 2008, 1154 (51.11%) insects confined to seven orders were collected. Out of the eight orders, six were found to be common to three years of study. Insects of order Dermaptera which were encountered in 2006 and 2008 was not collected in 2007, similarly, insects of the order Lepidoptera recorded in 2006 and 2007 were missed for identification in 2008 (Table 1).

When the number of insects confined to the respective orders was taken into consideration, it was revealed that Hemiptera (677, 58.46%) peaked among the eight orders of insects collected from plantation site, followed by Isoptera (263, 22.71%) and Coleoptera (194, 16.75%). In grass-lawn, Hemiptera (194, 72.66%) was predominant, followed by Coleoptera (41, 15.36%) and Isoptera (18, 6.74%). In aquatic site, Hemiptera (300, 55.76%) also peaked in the position, followed by Isoptera (156, 28.99%) and Coleoptera (32, 5.95%). In scattered tree site, Hemiptera (248, 84.07%) also peaked in the rank, followed by Coleoptera (31, 10.51%) (Table 1 & Fig. 1).

In 2006, Isoptera (243, 57.04%) was predominant, followed by Coleoptera (148, 34.74%). In 2007, Hemiptera (402, 59.29%) was predominant followed by Isoptera (193, 28.47%) and Coleoptera (53, 7.82%). In 2008, Hemiptera (1014, 87.87%) was also predominant, followed by Coleoptera (97, 8.41 %) (Table 1 & Fig. 2).

From 2006 to 2008, the insects in plantation (1158, 51.28%) was dominated, followed by aquatic site (538, 23.83%), scattered tree site (295, 13.06%) and grass-lawn site (267, 11.82%). In the study period, Hemiptera (62.84%) peaked in the order, followed by Isoptera (19.53%) and Coleoptera (13.20%)

A total of 2,258 specimens were collected from the plantation site, grass-lawn site, aquatic site and scattered tree site (Table 2). Approximately twice as many individuals were collected in the plantation site as in the aquatic site, with fewer still collected from the grass-lawn site and scattered tree site respectively.

The catch data of insects from four sampling sites during 2006 to 2008 is shown in Table 2. During 2006, the highest percentage of abundance was found in plantation site (88.97%, 379 individuals), followed by in aquatic site (6.57%, 28 individuals) and in grass-lawn (4.46%, 19 individuals) (Table 2 & Fig. 4).

During 2007, the highest percentage of abundance was observed in aquatic site (48.67%), followed by in plantation site (41.74%), in grass-lawn site (7.82%) and in scattered site (1.77%) (Table 2 & Fig. 4).

During 2008, the highest percentage of abundance was noted in plantation site (42.98%), followed by in scattered tree site (24.52%), in grass-lawn (16.90%) and in aquatic site (15.60%) (Table 2 & Fig. 4).

Abiotic factors examined in this study include temperature, rainfall and humidity during the study period. The total annual rainfalls for 2006, 2007 and 2008 were 985 mm, 973 mm and 852 mm respectively. Temperature had an effect on the abundance of insects collected each month in 2006 (Table 3). None of the years showed a correlation between abundance of insects with rainfall and humidity.

The seasonal distribution based on rainfall, also had the seasonal patterns in terms of insect abundance as determined by using the light traps. During 2006, the numbers of insects were low in February, June and July and sharp and very sharp seasonal peaks occurred at the onset of rain in March and April with a second little peak in September. The number of insects fell greatly in October, November and December. During 2007, the number of individual insects were low in February, April and June and very sharp peaks happened at the onset of rain in March and May with a second very sharp peak in August. The numbers then fell and fluctuated a little, with a fall in September and October. The numbers of insects greatly declined in November and December. In 2008, the population was low in January and February, and climbed to a peak in August with a little fall in May and June, and then, a sudden fall in September, October and November. The numbers of insects greatly also drop in December (Fig. 5 & 6).

The peak of insect abundance was observed at the onset of the rain in 2006, and it is assumed to be in some way associated with rain, and it is a seasonal effect, as it was repeated in 2007 and in 2008 (Fig. 5).

Table 1. Percentage species composition of insect orders in respective community of insects in different capture sites of Meiktila University campus from 2006 to 2008

Sr. No.	Order	Plantation		Grass-lawn		Aquatic		Scattered tree		2006		2007		2008		3-year average	
		Total No. of Individual	(%) Composition	Total No. of Individual	(%) Composition	Total No. of Individual	(%) Composition	Total No. of Individual	(%) Composition	Total No. of Individual	(%) Composition	Total No. of Individual	(%) Composition	Total No. of Individual	(%) Composition	Total No. of Individual	(%) Composition
1	Odonata	10	0.86	3	1.12	5	0.93	0	0	12	2.82	4	0.59	2	0.17	6	0.80
2	Orthoptera	4	0.35	2	0.75	13	2.42	1	0.34	2	0.47	17	2.51	1	0.09	6.67	0.89
3	Isoptera	263	22.71	18	6.74	156	28.99	4	1.36	243	57.04	193	28.47	5	0.43	147	19.53
4	Dermaptera	7	0.60	5	1.87	10	1.86	7	2.37	1	0.23	0	0	28	2.43	9.67	1.28
5	Hemiptera	677	58.46	194	72.66	300	55.76	248	84.07	3	0.70	402	59.29	1014	87.87	473	62.84
6	Homoptera	2	0.17	2	0.75	21	3.90	4	1.36	15	3.52	7	1.03	7	0.61	9.67	1.28
7	Coleoptera	194	16.75	41	15.36	32	5.95	31	10.51	148	34.74	53	7.82	97	8.41	99.33	13.20
8	Lepidoptera	1	0.09	2	0.75	1	0.19	0	0	2	0.47	2	0.29	0	0	1.33	0.18
	Total	1158		267		538		295		426		678		1154		752.67	

Table 2. Summary of catch data from January 2006 to December 2008

Sr. No.	Sites	No. of Order			No. of Family			No. of Species			No. of Individual			Composition (%)		
		2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008
1.	Plantation	7	6	6	11	9	14	21	13	21	379	283	496	88.97	41.74	42.98
			8			22			33			1158			51.28	
2	Grass-lawn	5	7	3	8	10	6	13	11	8	19	53	195	4.46	7.82	16.90
			8			13			24			267			11.82	
3	Aquatic	7	6	4	10	10	7	11	15	11	28	330	180	6.57	48.67	15.60
			8			16			27			538			23.83	
4	Scattered tree	0	4	5	0	7	12	0	7	15	0	12	283	0	1.77	24.52
			6			12			16			295			13.06	
	Total	8	7	7	17	13	18	28	24	26	426	678	1154			
	Grand Total		8			24			45			2258				

Table 3. Correlation coefficients of the total monthly abundance of insects collected in each year, with temperature, rainfall and humidity

	Abundance		
	2006	2007	2008
Temperature	0.4843	0.2845	0.2668
Rainfall	0.1103	0.0786	-0.1844
Humidity	-0.3951	-0.0280	0.0834

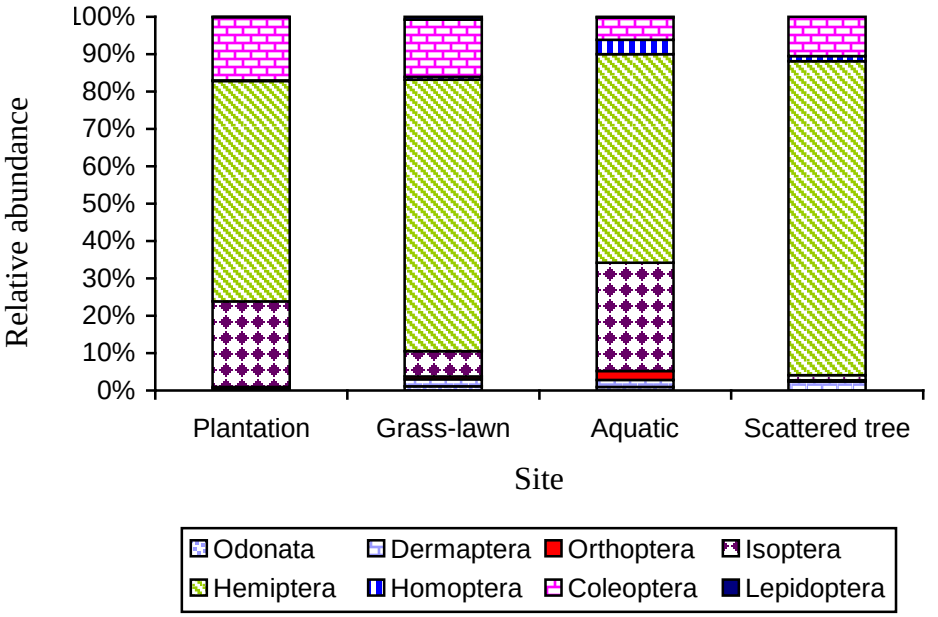

Fig. 1 Relative abundance of insect orders at various sites from 2006 to 2008

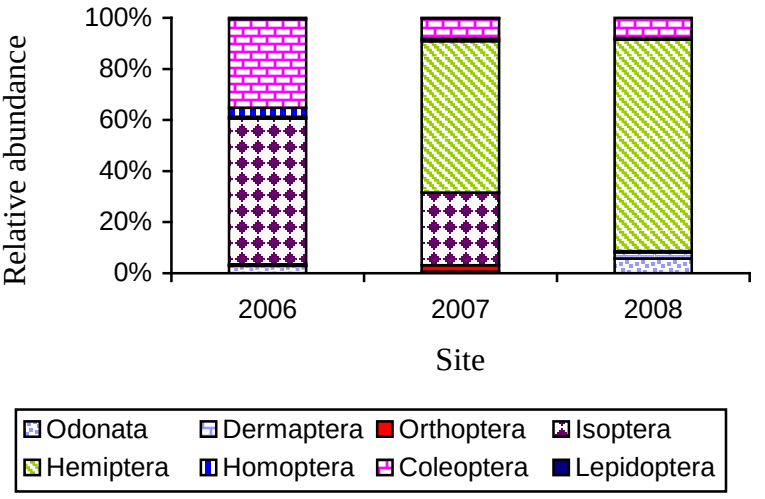

Fig.2 Relative abundance of insect orders from 2006 to 2008

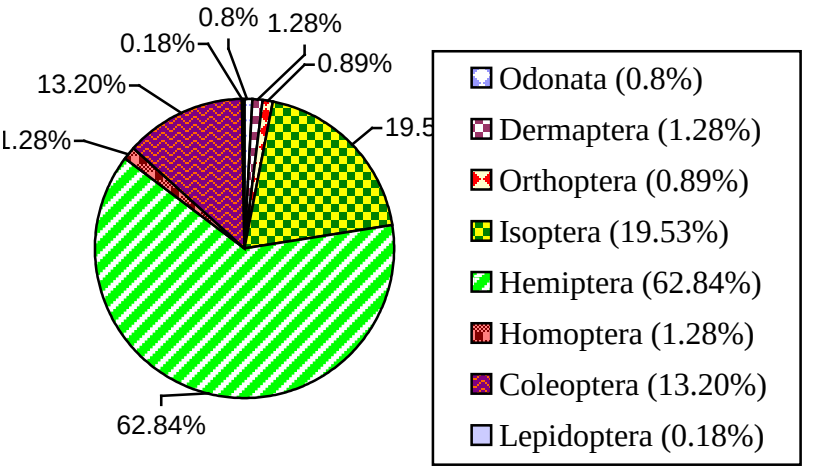

Fig. 3 Relative abundance of insect orders of 3-year average (from 2006 to 2008)

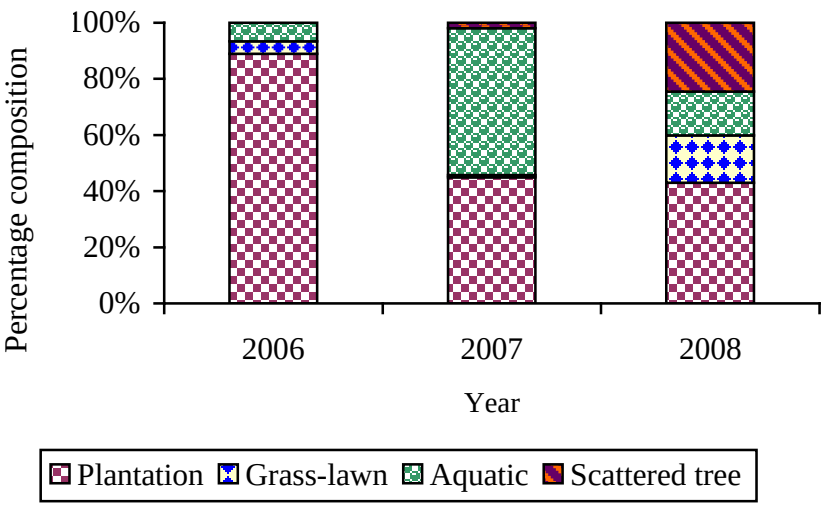

Fig. 4. Comparison of percentage composition of insects in four habitat types from 2006 to 2008

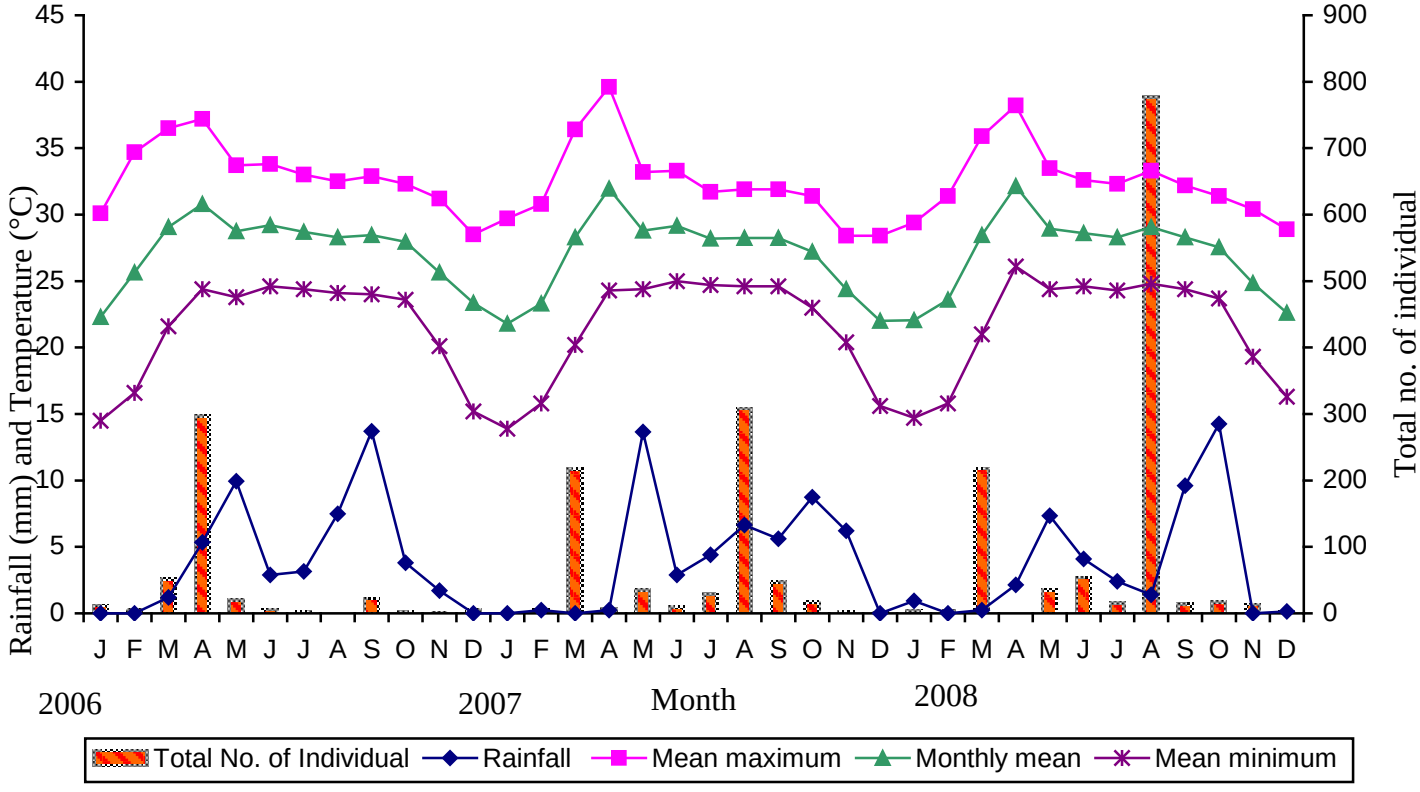

Fig. 5. Relationship between monthly abundance of insects and weather conditions from January 2006 to December 2008

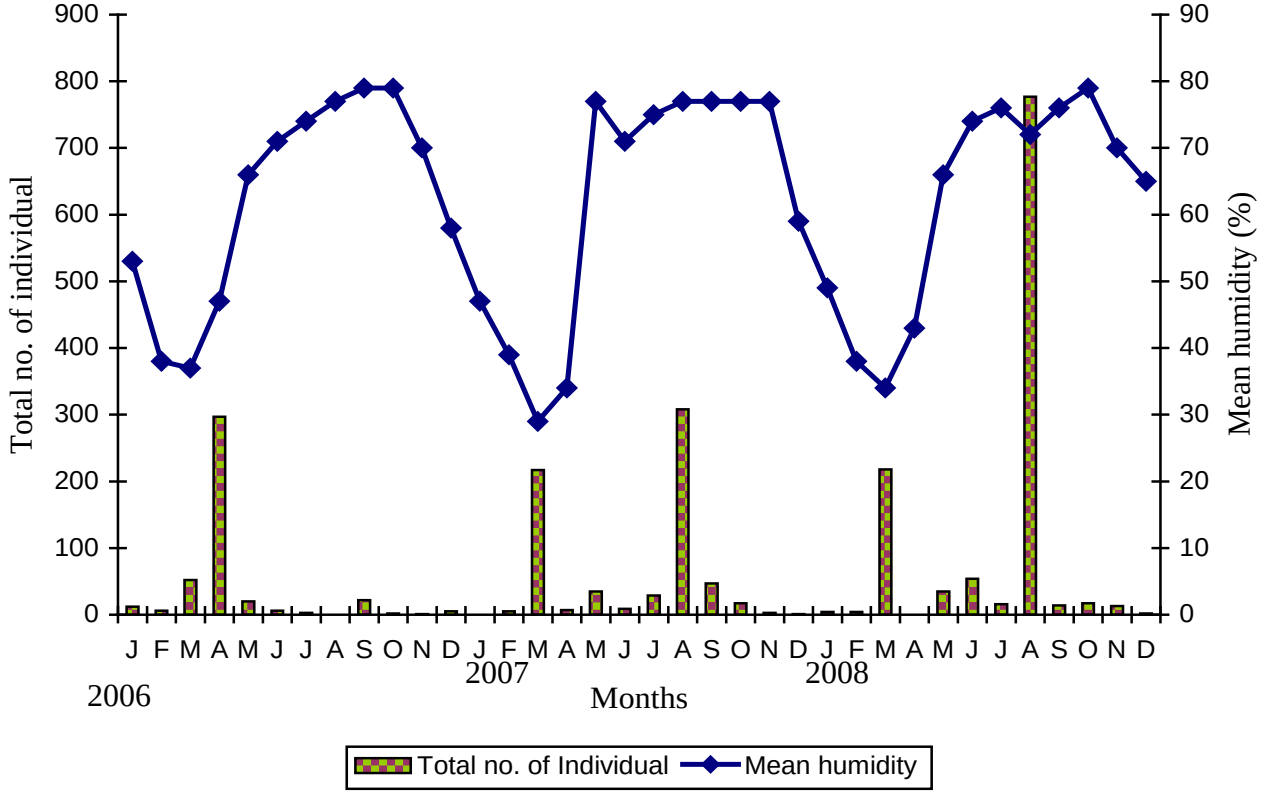

Fig. 6. Relationship between monthly abundance of insects and mean humidity from January 2006 to December 2008

Discussion

In the present research, a total of 2,258 specimens representing 45 species from the plantation, grass-lawn, and aquatic and scattered tree sites distributed under 24 families in eight orders were collected.

In comparing the four habitat types, the more uncommon species were found in aquatic site than the other three sites. Eleven species were represented by one individual only in plantation and grass-lawn sites, 12 species in aquatic site and five in scattered tree site respectively. In all sites, *Cydnus indicus* was most common having more than 50% of total individuals. On the other hand, *Ictinus rapax, Forcipula quadrispinosa, Labidura riparia, Dysdercus singulatus, Copris sinicus, Megalodacne promensis, Ancycloprotus bigibbosus, Batocera titana, Deiopia pulchella, Spilosoma* sp, *Nyctipao glaucopis* and *Nyctipao* sp were the most uncommon.

The relation between weather condition in the area during the period of sampling and the total numbers of insects taken in each month was assessed. In Myanmar, it is clearly defined that hot season from February to May, rainy season from June to September and cold season from October to January. There was no rain in January and February 2006, in March 2007 and February 2008, whereas in May 2006, 2007 and 2008 rainfall is exceptionally heavy and continuous. The temperature does not vary markedly with the season of the year although ranges of usually 8-10°C being slightly and lower in cold than in hot season. The seasonal distribution based on rainfall, also had the seasonal patterns in terms of insect abundance as determined by the light traps.

During 2006, the numbers of insects were low in February, June and July and sharp and very sharp seasonal peaks occurred at the onset of the rain in March and April with a second little peak in September. The numbers fell greatly in October, November and December. During 2007, the numbers of insects were low in February, April and June and the very sharp peaks happened at the onset of the rain in March and May with a second very sharp peak in August. The numbers then fell and fluctuated a little, with a fall in September and October. The numbers of insects greatly dropped in November and December. In 2008, the population was low in January and February, and climbed to a peak with the onset of the rain in March, with a second peak in August with a little fall in May and June, and then, a sudden fall in September, October and November. The numbers of insects also greatly dropped in December.

The peak number of insect species was observed at the onset of the rains in 2006, and it is assumed to be in some way associated with the rain, and it is a seasonal effect, as it was repeated in 2007 and in 2008.

During 2006, the peak occurred at the plantation and grass-lawn in April, but it occurred at the aquatic site in September. In all habitat types, number of insect species climbed to a peak in March and April perhaps due to the vigorous vegetation growth. During 2007, the peak occurred at the plantation site in August, at the grass-lawn in March and at the aquatic site it also occurred in March and August and collection of insects was not complete and it was missed for identification at the scattered tree site. In three habitat types, the number of insect species climbed to a peak in March and August. During 2008, the peak occurred at the plantation and grass-lawn sites in March, and August, at the aquatic site and scattered tree sites it also occurred in March and August. In all four habitat types, the numbers of insect species also climbed to a peak in March and August.

Several factors that restrict the beetles to a certain habitat include moisture content and exposure to deer dung, latitude, thermal factors, wind and vagility (Godon, 1983) (cited in Price, 2004). Though Landin (1961) argued that fluctuations in natural populations of dung–beetles depend on the abiotic factors rather than on competition, competition may also be a factor when food resources are limited, which is often the case with deer dung (Gordon, 1983) (cited in Price, 2004).

Temperature appears to have had a moderate effect on the abundance of insects collected each month in 2006. None of the years showed a correlation between abundance of insects with rainfall and humidity.

Price (2004) stated that the majority of the beetles were collected during the warmer months (May to September), with general peaks appearing to be correlated with temperature. The present finding is agreeable with his statement since there was a considerable drop in species abundance during January, February, November and December.

The finding of the present study is similar to the observation of Lowman (2006) who studied the insects of Australian forest communities. In his study, there was considerable seasonal variation in the numbers of insects, particularly those active at night. Nocturnal samples exhibited a marked seasonal variation in numbers, perhaps due to the stronger influence of

temperature at night, and to the fact that night samples included a high proportion of adult Lepidoptera, which were very seasonal.

Owen (1969) reported that the tropical insects exhibit two general features not normally encountered in temperate regions. Within a taxon there are generally many more species than in a comparable temperate group, and many tropical species occur as adults all the year round, but with conspicuous peaks of abundance that are often associated with the alternation of wet and dry seasons.

Dudgeon and Corlett (1994) described the annual pattern of insect activity in Hong Kong as a rapid rise in total numbers in April with a peak in May followed by another in late July; a gradual decline follows with numbers remaining low from November throughout the dry season(cited in Ades and Dudgeon 1999).

Insects were present during all months of the year, but distinct seasonal changes in trap catches were evident, with increases in insect abundance and morphospecies richness when temperatures rose at the onset of the wet season (March through May) and annual lows during the cooler, dry season (especially January and February).

Periodic fluctuations in insect abundance are typical of many tropical and subtropical environments, and are usually associated with the alternation of wet and dry seasons (Owen and Chanter, 1970; Owen, *et al.*, 1972; Denlinger, 1980, Young 1982, cited in Ades and Dudgeon 1999).

Ades and Dudgeon (1999) showed that insect seasonality in Hong Kong can be related to temperature. Similar results were obtained by So and Dudgeon (1990) in a seasonal study of dipteran diversity and abundance, e.g., a rise in the abundance and diversity of insects coincided with the onset of the monsoon when both temperature and relative humidly increase and rainfall becomes more frequent and intense. The onset of rain can as a direct trigger for seasonal activity in some insects (Wolda, 1978), as appeared to be the case for termites (Denlinger, 1980). Insect activity is probably governed by a combination of factors such as temperature, rainfall, humidity and atmospheric pressure (Wolda, 1988; 1992). Moreover, seasonal changes in the weather may be direct effects on insects through an influence on plant growth and hence food availability for phytophagous insects. Photoperiod may determine the timing of insect activity through an effect on the host plant (Wolda, 1989), and

Boinski and Fowler (1989) have invoked plant phenology to explain seasonal changes in insect abundance.

During 2006, in plantation site, species of insect possess 88.97% of total insects while those of aquatic site (6.57%) and grass-lawn site (4.46%). During 2007, in aquatic site, species of insect possess (48.67%) of total insects while those of plantation site (41.74%), grass-lawn site (7.82%) and scattered tree site (1.77%). During 2008, in plantation site, species of insect possess 42.98% of total insects while those of scattered tree site (24.52%), grass-lawn (16.90%) and aquatic site (15.60%).

In comparing mean percent composition among plantation site, grass-lawn site, aquatic site and scattered tree site, it was revealed that the insect species were more abundant in plantation site which is more shaded area and more food resources. This finding was in congruous with the findings of Lowman (2006) who studied in both nocturnal and diurnal samples; the insect numbers were greatest during times of vigorous vegetation growth.

Conclusion

The insect species composition was highest in plantation site. In all sites, order Hemiptera was most common having more than 50% of total individuals. The peak number of insect species was observed at the onset of the rains in 2006, and it is assumed to be in some way associated with the rain, and it is a seasonal effect, as it was repeated in 2007 and in 2008. Temperature appears to have had a moderate effect on the abundance of insects collected each month in 2006.

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References

Ades, G.W.J. and Dudgeon, D., (1999). Insect Seasonality in Hong Kong: *A Monsoonal Environment in the Northern Tropics,* 18: 81 – 98.

Denlinger, D.L, (1980). Seasonal and Annual Variation of Insect Abundance in the Nairobi National Park, Kenya. *Biotropica*, 12: 100-6.

Janzen, D.H., (1973). Sweep Samples of Tropical Foliage Insects: Descriptions of Study Sites with Data on Species Abundances and Size Distributions. *Ecology*, 54: 650 – 86.

Landin, J., (1961). Ecological studies on dung beetles (Col. Scarbaeidae). *Opuscula. Entomologica Supplement*, 19: 1-228.

Lowman, M.D., (2006). Seasonal Variation in Insect Abundance among Three Australian Rain Forest, with Particular Reference to Phytophagous types. *Australian Journal of Ecology*, 7: 325- 361.

Lubin, Y.D., (1978). Seasonal Abundance and Diversity of Web-building species in Relation to Habitat Structure on Barre Colorado Island, Panama. *J. Arachnal*, 6: 31-51.

Owen D.F. and Chanter, D.O., (1972). Species Diversity and Seasonal Abundance in Charaxes butterflies (Nympalidae). *J. Entomology* (A), 46: 135 - 143.

Price, D.L., (2004). Species Diversity and Seasonal Abundance of Scarabaeoid Dung Beetles (Coleoptera: Scarabaeidae, Geotrupidae and Trogidae) Attracted to Cow Dung in Central New Jersey. *J. New York Entomol. Soc.*, 112(4): 334-347.

Sutherland, W.J., (1997). *Ecological Census Techniques.* Low Price Ed. Cambridge University Press. 336 pp.

Wolda, H., O' Brien, C.W. and Stockwell, H.P., (1998). Weevil Diversity and Seasonality in Tropical Panama as Deduced from Light-Trap Catches (Coleoptera: Curculionoidea). Smithsonian Institution Press; Washington, D.C. 79 pp.

Wolda. H., (1978). Seasonal Fluctuations in Rainfall, Food and Abundance of Tropical Insects. *J. Anim. Ecol.*, 47: 369-381.

Varietal Resistance and Management of Rice Root Nematode *Hirschmanniella oryzae* (Luc and Goodey, 1964) in Rice Variety

Hla Hla Maw

Abstract

Hirschmanniella oryzae species is known as rice root nematode. This species is endo-parasitic and causes rice root rot disease. The *H. oryzae* nematodes were extracted from the roots of infected rice plants. Among the nematode inoculated treatments of five rice varieties, the number of nematodes per plant of Manwathukha was significantly higher than others and followed by that of Sinthwelatt, and Shwewartun. The numbers were significantly lower both in Yezin 3 and Shweyinaye than others, although there were no significances between the two varieties. It was observed that Manawthukha was likely to be the most susceptible variety, where as Sinethwelatt and Shwewartun were moderately susceptible and other two varieties Yezin and Shweyinaye were moderately resistant. Investigation of treatments for control of *H. oryzae* on Manawthukha was made in rice field of Plant Protection Division (PPD) campus. Chemicals; carbofuran 3 G, diazinon 10 G, phenthoate 50 EC were applied and jatropha as biopesticide. Application rates were carbonfuran 3 G 11.4g/plot (i.e, 49kg/ha), diazinon 10 G 5.7g/plot (i.e, 24kg/ha), phenthoate 50 EC 0.5 ml/plot (i.e, 4942ml/ha), and jatropha 522.1 g/plot (i.e, 1976lb/ha) and those were applied as basal. The numbers of nematode recovered from carbonfuran 3 G treated plot was significantly lower than the rest of plots. The numbers of nematode from Diazinon 10 G was significantly lower than jatropha, phenthoate 50 EC, and control. There was no significant difference between jatropha and phenthoate 50 EC. The highest numbers of nematode were obtained from control plots.

keywords: *Hirschmanniella oryzae*, rice, resistant varieties, manangement

Introduction

Rice is the dominant staple food crop in the developing countries. Almost 90 percent of rice is produce and consumed in Asia, and 96 percent in developing countries (FAO, 2004). In Myanmar, rice is the national food crop. Rice production needed for local consumption as well as for export. Among the rice diseases, nematode infestation can result in yield losses of up to 30 percent in general (Doberman and Fairhurst, 2000). More than one hundred species of plant parasitic nematodes have been found associated with

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cultivated rice. Four major species occur in the rice growing areas of Myanmar. They are *Ditylenchus angustus, Aphelenchchoides besseyi, Meloidogyne graminicola* and *Hirschmanniellia oryzae* is found mainly in low land areas of Myanmar (Mya Mya, 1983). *Hirschmanniella* spp are long, slender nematodes, which enter the roots to feed and reproduce. Plants infested heavily with *H. oryzae* started to show symptoms after about 4-8 weeks, plant stopped growing and remained small, the grains produced were fewer and their roots were much shorter in length and were thin (Mathur and Prasad, 1972; Babatola and Bridge, 1979). They survive longer in roots than in soil but survival of root populations is shorter in flooded soil due to the more rapid decay of roots (Fortuner and Merny, 1979). Yield losses due to the root of disease caused by *H. oryzae* may be much as 50-60% (Mian and Mondal, 1988). Although there has been an increasing appreciation of the damage caused by plant parasitic nematodes in recent years, control of *H. oryzae* has been achieved by various practices; in particular fallow, weed control, use of resistance cultivars, rotation with non-host plant, chemical soil treatments of nurseries and fields, and chemical root dipping and seed coating (Bridge *et al*, 1990). Nematicidal treatments have been successfully used but nematicides are usually expensive and create problem of environmental pollution and accumulation of toxic residue in edible plant products (Lamberti, 1997). However cultural and non-chemical practices that can be used by farmers can control nematodes and many of these are sustainable in the long term (Luc, *et al*.; 1990). Used of resistant cultivars can be considered the best choice for reducing yields loss (James, 2004). Thus a comprehensive research programmed for *H. oryzae*, including chemical application and resistant varietal screening trials are initiated for long term increase in rice productivity.

Materials and Methods

Study site and period

The investigation was conducted in Plant Protection Division (PPD), West Gyogone, Insein Township, Yangon Region. Study period lasted from December 2006 to January 2008.

Collection of rice plants and soils

Diseased plants with root and soil samples were collected from rice fields in Hlaingtharyar Township. Collected samples were placed in each plastic bag (15x30)cm, with attached labels containing sampling date, variety,

locality, date of sowing, date of transplanting and collector name. Samples are stored in a cool place of Nematology laboratory, PPD.

Extraction nematode from rice soils and roots

The *H. oryzae* nematode was identified according to Hunt (Hunt, 2000). Nematodes of *H. oryzae* were extracted by using the Whitehead tray Method (Whitehead, 1965). Nematodes of soil and root were singly extracted from collected samples. Randomly collected soil samples were thoroughly mixed and 100 ml was taken out for nematode extraction. Infected rice root samples were washed with tap water, cut into small pieces about 1 cm long then mixed together. Then, 100 ml of mixed soil and 50 g of roots were separately spread in a thin layer over a muslin cloth in each plastic sieve (15x20) cm. The sieves were placed in each of plastic tray (20x25) cm. Amount of 200 ml tap water was carefully added down from the edge of each tray. After 24 h, the sieve was removed and the nematode suspension in the tray was poured into a glass beaker (300 ml Pyrex) and left for 2-3 h. After which upper portion about 170 ml of suspension was discarded. Remaining 30 ml of nematode suspension in the beaker was thoroughly shaken and 5 ml of the nematode suspension was pipette into a counting dish where *H. oryzae* was examined under dissecting microscope (Plate I).

Preparation of test plants in plastic bag

Five test rice varieties namely Manawthukha, Sinethwelatt, Shwewartun, Shweyinaye and Yezin3, obtained from Rice Division, Department of Agricultural Research (DAR), Yezin were used. Seeds of test varieties were washed with sterilized water for two times. The seeds were pre-germinated in the Petri dishes for one week. Hundred Plastic bags were filled with composite soil. One-week-old seedlings of each test rice varieties were transplanted individually in each plastic bag.

Inoculum preparation

Inoculums were prepared by extracting *H. oryzae* nematode from the soil and roots of rice. The extracted nematode suspension was collected in a beaker and nematodes containing in 1.0 ml of the suspension were counted in a counting dish for three times. The nematode suspension was adjusted to obtain required inoculums level.

Inoculation

Two-weeks after transplanting, inoculation was carried out immediately with nematodes. Inoculation was done in the plastic bags by pipetting the adjusted nematode suspension into three pencil-holes that were made around the roots at about 5 cm depth. One thousand nematodes were inoculated in each plant. The bags with inoculated plants were kept in a screen house and watered whenever necessary (Plate I).

Preparation of test plants

Forty clay pots were applied and each pot (50x37) cm was filled with 20 kg soil respectively. Urea at the rate of 1.1 g/pot, T. super 0.86 g/pot and Potash 0.43 g/pot were added and mixed with the potted soil. Two-weeks-old seedlings of test varieties were transplanted individually in each pot. Experiment was laid out in a Randomized Complete Block Design (RCBD) with four replications. The plants were harvested at 120 days after transplanting (Plate I). Numbers of extracted nematodes from soil and roots, root and seed weight were recorded.

Manangement of *H. oryzae* in field

This field experiment was conducted in the field of PPD campus. The field was worked out into 540 square feet and five treatments and three replications were used. The five treatments were T1, Carbofuran 3 G, at the rate of 49 kg/ha, T2, Diazinon 10 G at the rate of 24 kg/ha, T3 Phenthoate 50 EC, at the rate of 1976 ml/ac, T4 Biopesticide Jastropha at the rate of 4942lbs/ha and T5 control. Pesticides were applied as basal. Before the treatments, soil sampling was done in each plot and nematode extracted from soil to obtain the initial population of *H. oryzae*. One week after treatments, five rice plants, including roots and soil were taken out from each plot and nematodes extracted as above. The rice plant sampling from each plot was done weekly for ten times (Plate I). Data were analyzed by SPSS 11.5 programmed.


Nematology Laboratory


Infected rice plants

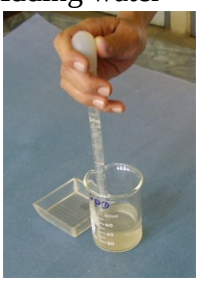

Washing rice roots


Sieve with tray

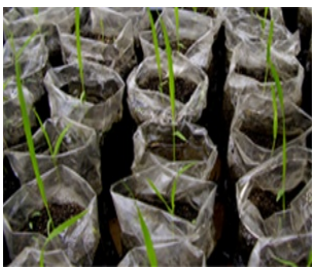

Adding water


Removing sieve


Pouring nematode suspension


Suspension placed in counting dish


Examinig nematode


Seedling of five test rice varieties


Inoculation


Varietal resistant experiment


Management of *H. oryzae*

Plate I. Extraction, experiment and management of nematode

Results

Systematic position of *Hirschmanniella oryzae*

Phylum - Nematoda

Class - Secernentea

Order - Tylenchida

Family - Pratylenchidae

Genus - *Hirschmanniella*

Species - *H. oryzae*

Morphological characters

Body elongated head hemispherical, stylet with well-developed basal knobs, median bulb ovoid, ventral overlapping oesophagus, tail elongate with mucron at tip, male with slightly arcuate spicules and terminal bursa (Plate II).

Multiplication number of nematodes in five rice varieties

The numbers of nematode was also observed, Manawthukha (3900/plant) was significantly higher than the rest of other varieties. Such as Sinethwelatt, Shwewarhtun, Yezin 3 and Shweyinaye. It was also found that there was no significant between Shwewarhtun (2600/plant) and Sinethwelatt (2400/plant). The numbers of nematode in Shweyinaye (1570/plant) was significantly lower than Yezin 3 (1757/plant) (Fig.1).

Effect of *H. oryzae* on the fresh root weights

It was found that total fresh root weight per plant of Manawthukha was significantly lower than other inoculated and non-inoculated of test varieties. There was no significant difference between Sinethwelatt and Shwewarhtun. However, Shweyinaye was significantly higher than the inoculated plants of test varieties but, no significant difference between non-inoculated plants (Fig. 2).

Effect of *H. oryzae* on total seed weights

The result of investigation showed that, total seed weights per plant, Yezin 3 and Shweyinaye were not significantly different between them, but significant than rest of other inoculated plants of test varieties. Manawthukha was significantly lower than in inoculated and non-inoculated plant of test

varieties. There was no significant difference between sinethwelatt and Shwewarhtun, but significant than rest of other test varieties (Fig. 3).

Management of rice root nematode *Hirschmanniella oryzae*

Control of *H. oryzae* was also observed that the least numbers of nematode obtained from carbofuran 3 G plot (6/5g of root) and highest numbers of nematode in control plot (141/5g of roots). Diazinon 10 G was (36/5g of roots) significantly lower than jatropha (69/5g of roots) and phenthoate 50 EC (74/5g of roots) and control. Carbofuran 3 G treated plot was significantly lower than the rest of plots (Fig. 4)

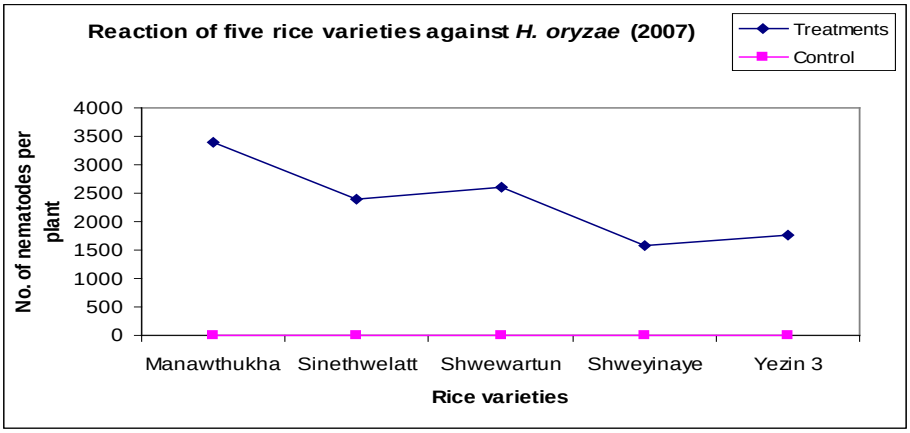


Fig. 1. Number of *H. oryzae* in five rice varieties

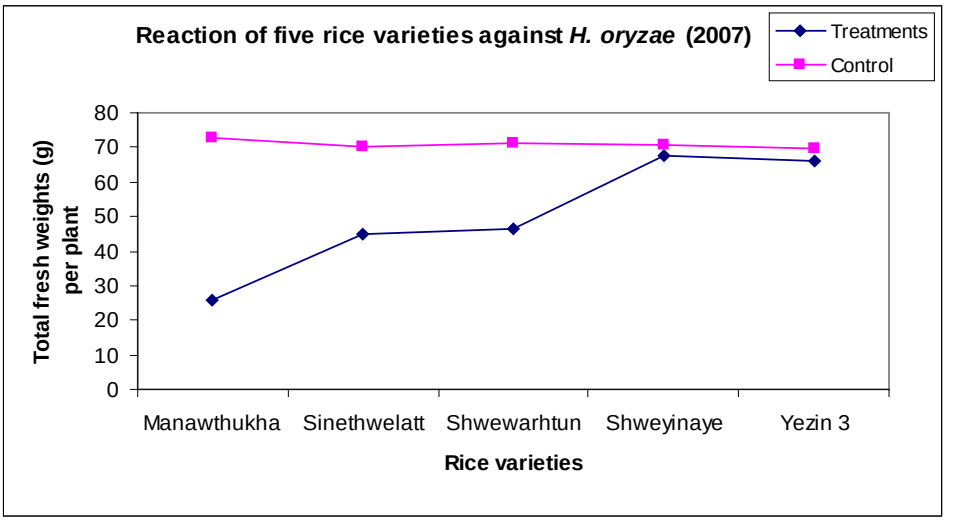


Fig. 2. Fresh roots weight (g/plant)

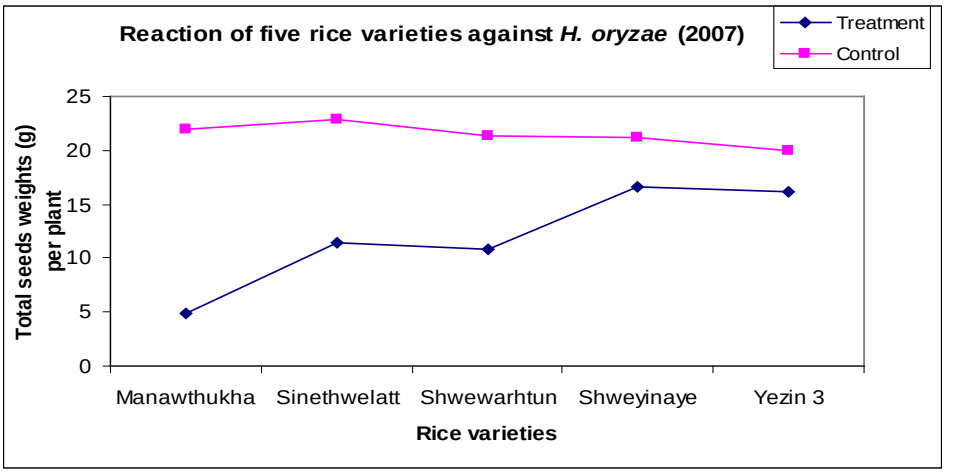


Fig. 3. Seed weight (g/1000seeds)

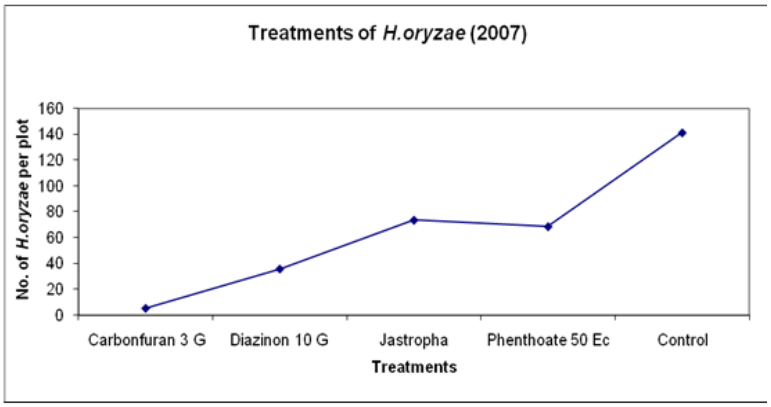
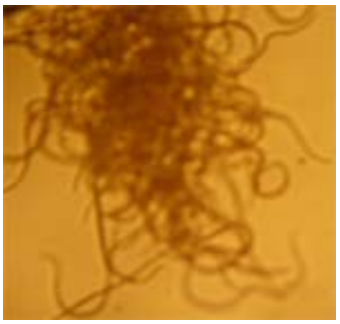
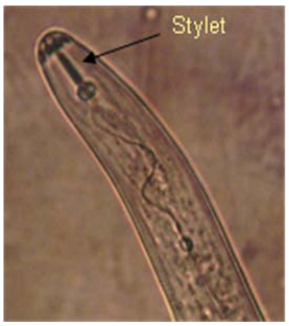


Fig 4. Management of rice root nematodes



Grouping nematodes

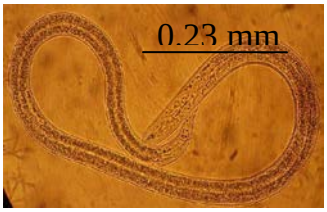


Female head

Vulva

Female

Juvenile



Male Male head Spicule

Plate II. Morphology of *Hirschmanniella oryzae* male

Discussion

Hirrschmanniella oryzae species is known as rice root nematode and endoparasite of rice plant. *H. oryzae* is distributed throughout the rice growing region of Myanmar and causes "root-rot" disease. Plants with the root system damaged by nematode showed retarded growth, chlorosis and reduced yield. Solving nematode problems plays an important role in improving crop yield. For managing nematode population to reduce crop loss, it is important to know the life cycle and population changes of *H. oryzae*.

In my study, five rice varieties namely Manawthukha, Sinethwelatt, Shwewarhtun, Shweyinaye and Yezin 3 received the same inoculum of 1000 nematode per plant, in the present study on screening of five rice varieties for resistance to *H. oryzae* showed that the highest population growth of nematode was observed in Manawthukha, followed by Sinethwelatt and Shwewarhtun; it was low in Yezin 3 and Shweyinaye. This study showed that Manawthukha was susceptible, whereas Sinethwelatt and Shwewarhtun were moderately susceptible and other two varieties such as Yezin 3 and Shweyinaye were moderately resistant. This finding was similar to previous report by Po Po Than (2003). Starr and Cook (2002) stated that resistance of plants to nematode has become the most attractive control method and seemed to be confirmed by present study.

Other investigators also showed that population level of 100 to 1000 nematodes per plant could already cause significant reduction in growth and yield of rice plants (Babatola and Bridge, 1979). Much higher population peak was observed in varieties which may indicate the variety was more susceptible to nematode (Walawala and Davide, 1984).

The result obtained from the effect of different chemical treatments showed there was no significantly difference in nematode numbers between Jatropha, and phenthoate 50 EC plots. However, the numbers of nematode in carbofuran 3 G treated plots was significantly lower than untreated plot. It was also observed that the number of nematodes in carbofuran 3 G was significantly lower than rest of other treatments such as diazinon 10 G, Phanthoate 50 EC and jatropha .

In the present experiment, carbonfuran 3 G effectively controlled of *H. oryzae*. This finding was similar to previous record by Plowright *et al* (1990) who noted that cabofuran 3 G effectively controlled the nematode. Ying and Li

(1984) also mentioned that carbofuran 3 G was the most effective to control *H. oryzae*.

Number of nematodes in Diazinon treated plot was significantly lower than that in Jatropha, Phanthoate 50 EC treated plots. Ohnmar Thein (1997) found that the least number of nematode was recovered from carbonfuran 3 G followed by diazinon and seemed to confirm the present finding. Jatropha and Phenthoate 50 EC could not reduce the number of nematodes than control because the numbers of nematodes from those plots were not significantly different from control untreated plot. Ichinohe (1988) had also pointed out that chemicals with nematicidal action have been applied and found that the chemicals had varying success against *H. oryzae*. Lamberti (1997) observed nematicidal treatments have been successfully used but create problem of environmental pollution. FAO conference (2004) mentioned that the best solution is the development of resistant varieties, the program should continue with screening of available rice varieties for nematode resistance. James (2004) had also indicated use of resistant cultivars can be considered the best choice for reducing yields losses.

All the above studies gave basic information about associated with resistant of five rice varieties on root-rot disease in Myanmar. Moreover, the present finding on the effect of chemical treatment on root-rot disease should be confirmed under naturally infested rice growing areas.

Conclusion

Using resistant variety can not harm human and plant health, and environmental pollution. Using chemicals very often can occur nematode species that can resist to chemicals. Chemicals make risks to human and plant health and environment although it can control nematode effectively. While there is still lack of resistant varieties., chemical can be used with 'the least is the best'.

Acknowledgements

I am greatly indebted to Rector Dr. Khaing Myae West Yangon University for his encouragement. I sincerely thank to Professor Dr. Thida Lay Thawe, Head of Zoology Department, West Yangon University, for her kind permission to conduct this research. I also thanks Dr. Thet Thet Win, Professor, Department of Zoology, West Yangon University for her advice and encouragement. I warmly thank to Dr. Pyone Pyone Kyi, Assistant Manager, Plant Protection Division, Myanma Agriculture Service, for her permission to use laboratory, suggestion and encouragement my research work.

References

Babatola, J.O., Bridge, J., (1979). Pathogenicity of *Hirschmanniella oryzae*, *H.spinicaudata*, *H.mamuri* on rice. J.Nematol. 11:128-132.

Bridge, J., Luc, M., Plowright, R.A., (1990). Nematode Parasite of rice. Pp. 69-70.I*n plant parasitic Nematodes in subtropical and Tropical Agriculture* M.Luc, R.A. Sikora, and J.Bridge(Eds).CAB International, UK.

Dobermann, A., Fairhurst, T., (2000). Rice: *Nutrient disorder and Nutrient Management.* 1st ed., pp. 9. Oxford Graphic Printers Pte. Ltd., Philippine.

Fortuner, T. and Merny, G., (1979). Root-parasitic nematodes of rice. *Rev. Nematol.* 2(i):79-102.

Hunt, (2002). General morphology. Myanmar Training Course on Plant Nematodes. In: *Training Course on Plant Parasitic Nematodes of Economic Important*. Hunt, D.J. (Ed.), Bonn University, Bonn, Germany CABI Bioscience, Egham, UK. February-March, 2002. pp.20-31.

Ichinohe, M., (1988). Current research on the major nematode problems in japan. *Journal of Nematology*, 20:184-190.

James, P., Noe., (2004). Plant Parasitic Nematodes. In (Eds. Robert, N. T., Mark, T. W and Alan S, W.) Plant Pathology Concept and Laboratory Exercise.

Lamberti, F., (1997). Plant nematology in developing countries; problems and progess. Pp 13-27 *In Proc. Plant nematode problems and their control in the Near East Region*, 1992, FAO.

Luc, M., Sikora, R.A., Bridge, J., (1990). Reflection on Nematology in subtropical and Tropical Agriculture. Pp. xi- xvii. In *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. M.Luc, R.A.

Mathur, V.K., Prasad, S.K., (1972). Role of the rice root nematode, *Hirschmanniella oryzae* in rice culture. *Indian Journal of Nematology* 2, 158-168.

Miah, S.A., Mondal, A.H., 1988. Nematodes in deepwater rice. Pp. 575-581. In proc. Int. int. *Deepwater Rice Workshop, IRRI*. Manila, Philippines.

Mya Mya. (1983). Initial Survy of soil and Plant Parasitic Nematodes in Burma. M.Sc. Thesis. YAU, Myanmar.

Plowright, R.A., Matias, D., Aung, T. Mew, T.W., (1990). The effect of Pratylenchus zeae on upland rice. Revue de Nematologie (inpress).

Po Po Than, (2003). Survey of Plant-Parasitic Nematodes on some economic crops and study on rice root-rot disease caused by *Hirschmanniella oryzae*. M.Sc Thesis. YAU Myanmar.

Starr J.L., Cook R., Bridge J., 2002. Plant resistance to parasitic nematodes. CAB International, Wallingford (UK), 107-139.

Whitehead, A.G., Hemming, J.R.., 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of applied Biology* 55:25-3

Multiple cues used by female in an ornamental fish *Poecilia latipinna*

Omar Myint[1], San San Myint[2] and Maung Maung Gyi[3]

Abstract

Female mate preferences are based on more than one cue. However, in many empirical studies female mate choice cues are treated separately ignoring their possible interactions. In this study, we will examine how do male territory and its body size affect female mate choice, by using the ornamental fish *Poecilia latipinna*. In this study, females are given a binary choice between males that differ in body size or social status. Females prefer large body size male when males body size are different. Females prefer dominant male when males body size are quite similar. Specifically, large males occupy the territory and show intensive courtship, an important cue for female mate choice. It was suggested that neither male body size nor social status alone will affect female mate choice, but the two cues will work together in order to maximise female reproductive fitness. Since there is underestimating or ignoring the importance of various cues in sexual selection, the present work clearly explains that females use multiple cues in female mate choice.

Key words: mate choice, multiple cues, sexual selection, territory

Introduction

In many species, males compete for access to mate and females usually choose. Female mate choice decision is very important because female may suffer lower reproductive success if they choose a wrong mate. Female preference on particular traits or cues arises because it may increase (1) direct fitness benefits of the female such as improved parental care which enhances offspring survival (Hoelzer, 1989), (2) indirect genetic benefits in the form of the inheritance of genes for viability (Zahavi, 1975) and (3) attractiveness, i.e., the Fisherian runaway process (Fisher, 1930), and exploitation of pre-existing sensory biases in the receiver (Ryan & Rand, 1993; Endler & Basolo, 1998). Female preferences on male quality and/or resources provided by the male before or during reproduction had been reported in several empirical studies. For example, in fishes especially with paternal care, females gain benefits by choosing large males because large males defend their territory successfully (Cote and Hunte, 1989; Dowhower and Brown, 1980). In addition, different

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signals give information about different mate qualities (Moller and Pomiankowski, 1993; Johnstone, 1997). To investigate information on the overall quality of a potential mate, an individual may pay attention to several traits that reflect different qualities. Thus, in mate choice female may use one or several cues to assess the male quality. For example, sand goby females do not necessarily base their mate choice on male body size or nest size, but they choose rather on a combination of these two cues (Lehtonen *et al*., 2007). The use of multiple cues in mate choice has been regarded as an adaptive behaviour because it may increase female fitness by reducing mate choice errors or costs of choice (Pominankowski and Iwasa, 1993). In addition, the use of multiple cues indicates the quality of the receiver and also explains the existence of their life in the evolution.

However, female mate choice based on several cues has received little attention and the generality still remains unknown. The present study was carried out to examine how do *P. latipinna* females choose a male, and to investigate whether females use multiple cues in mate choice or not.

The subject fish, *P. latipinna*, in common name sailfin molly, is a live bearing fish without parental care. They live in a mixed sex shoal and reproduce the whole round year (Schlupp and Ryan, 1996a). Females exhibit a preference for large males, and sometimes they copy the mate choice of other individuals (Schlupp and Ryan, 1996b; Witte and Ueding, 2002). Adult individual can reach up to 7cm in total length.

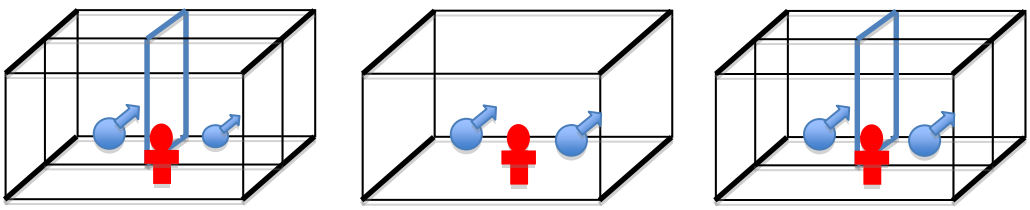
Materials and methods

The present study was conducted from March to May 2012. All fish used in this experiments were collected from local aquarium shop, and the same sex were maintained in separate stock tanks. Fish were fed food pallet 3 times a day. The room temperature and light were followed the natural environment. To test female preference on male body size, the experimental tank (30cm x 60cm x 45cm), consisting three compartments, were set up as shown in figure (Fig.1a). Male compartments were divided with an opaque plastic board, thus males cannot see each other. However, the male and female compartment was divided with a thin transparent plastic sheet, thus female can see the two males and vice versa. Total length (TL) of males and females were recorded. Before starting the experiment, female (mean$\pm$SD: 4.29$\pm$0.29cm (TL)) and males (TL_{large}=5.12$\pm$0.32cm, TL_{small}=3.58$\pm$0.29cm) were placed into the experimental tank for 10 minutes to acclimate with the experimental tank. During acclimatization period, male and female compartment were

covered with a removable opaque plastic board, and it was removed when the experiment starts. The first experiment lasted for 30 minutes. At the half of the experiment the two males were shifted from one side to another to prevent side bias of the female. If female spent 80% of the time on one side of the experimental tank, we ignored the data and cancelled that trial. The time spent by the female in the active zone, 5cm from the male compartment, was recorded. Male and female courtship behaviour was also recorded with a video camera (Panasonic HDC-TM 45).

The male chosen by the female from the 1st experiment and another match-sized male were placed into the tank. Then the choice female was introduced into the experimental tank (Fig.1b). The interaction between the two males was observed10 minutes to know the social status of the males (Fig. 1b).

After learning the male social status, males and female were placed into the experimental tank (Fig.1c) to observe female choice on male social status. This experiment lasted 30 minutes. The experimental procedure was the same with the above experiment (experiment. 1). Fishes were returned into the stock tank, after finishing all the experiment.



(a) Experiment (1) (b) Probation period (c) Experiment (2)

Fig. 1.Experimental design of the female mate choice: (a) female choice on male body size, (b) probation period for dominance hierarchy, and (c) female choice on dominance hierarchy

Statistical analysis

Data distribution for normality was checked with Kolmogorov Smirnov test. Mann-Whitney *U* test was used, to analyse the female mate choice on male body size and male social status. Courtship intensity between males were also analysed with Mann-Whitney *U* test. All data analysis were two tailed and conducted using STATISTICA (statistical software).

Results

Sailfin female preferred larger male when males were different in size (Time spent by female in front of male compartment: T_{large}=660.67±253.79sec, N=18; T_{small}=192.28±125.35sec, N=18; Mann-Whitney *U* test: p=0.0001, U=19.0, Fig. 2). The time that female spent in front of large male compartment was significantly higher than that of the smaller male indicating that sailfin female preferred large body size male as in other Poecillids fish. Large male intensively courted towards female but the small male did not (Large=984.50±127.29 sec, N=18, Small=229.80±86.199 sec, N=18; p=0.001, U=0.00, Fig. 3).

Dominant males usually attacked subordinate males. The male chosen by female in the first experiment was not always be the dominant male 38.9% (7/18) when they met with another match-sized male.

When male sizes were not different, females usually chose dominant males (Time spent by female in front of male: $T_{dominant}$= 510.56±163.86 sec, $T_{subordinate}$= 206.94±132.03 sec; p=0.0002, U=26.50, N=18, Fig. 4) indicating that female choice not only base on male body size but also on the male social status. In addition, courtship frequencies of males were not statistically different ($T_{dominant}$= 1350.06±84.52 sec, $T_{subordinate}$= 1296.44±69.34 sec; p=0.10, U=110.50, N=18, Fig. 5) indicating that female choice shift to male social status.

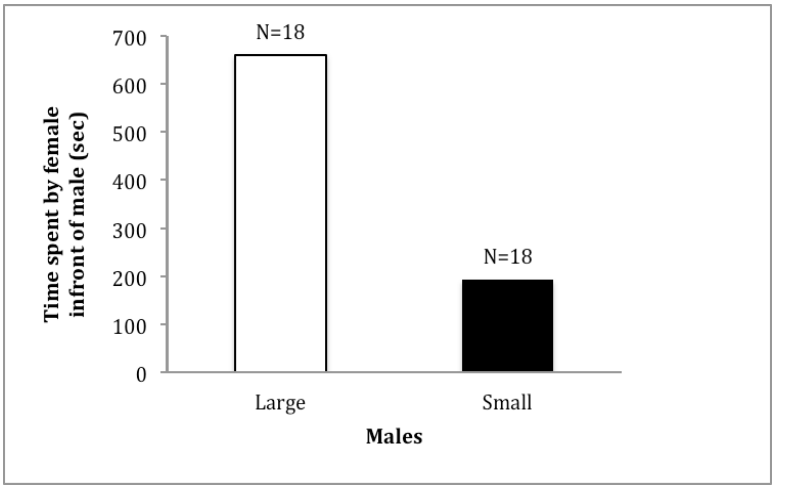


Fig. 2. Female choice on male body size

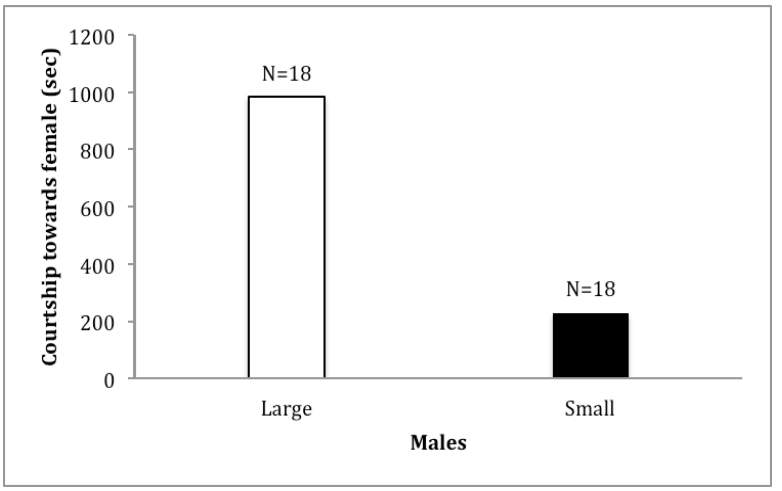


Fig. 3. Males courtship towards female

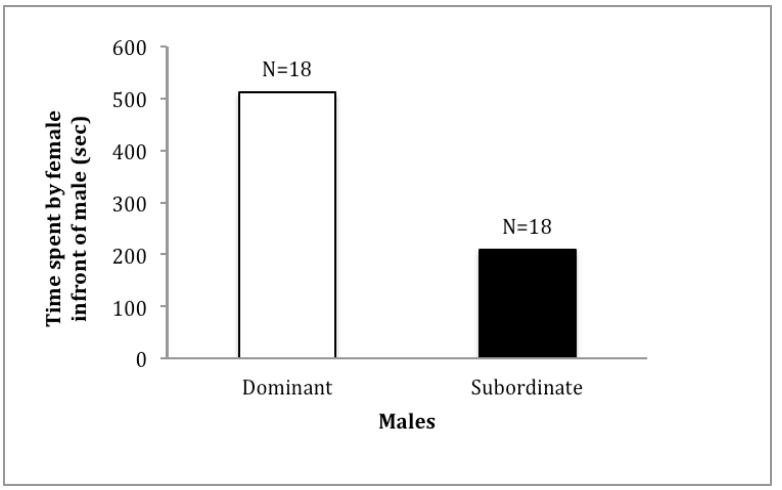


Fig.4. Female choice on male social status

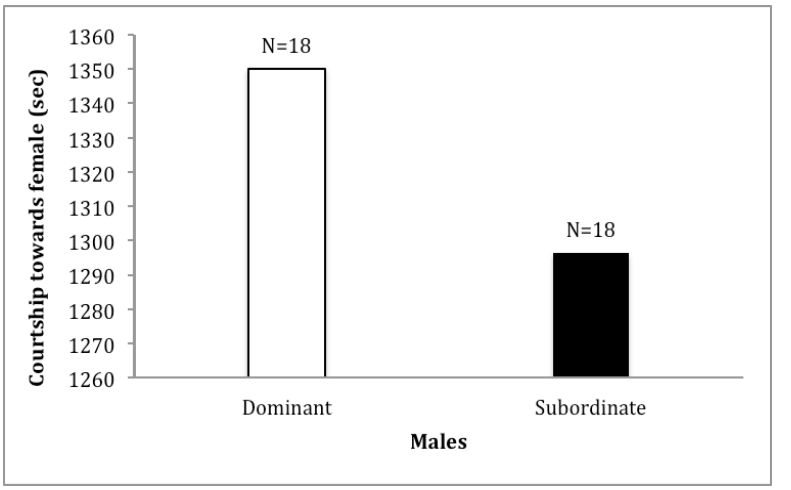


Fig. 5. Males courtship towards female

Discussion

Our results clearly show that sailfin molly female use multiple cues in choosing a potential mate. Like many other fish species, sailfin molly females showed a preference for large body size male. In some fish species, females prefer large males because large male defend their brood successfully and/or guard the brood for a long period (Cote and Hunte, 1989; Downhower and Brown, 1980; Bisazza and Marconato, 1988). This female preference on larger males enhances female reproductive success and gains direct benefits from it. However, in some fish species females prefer large male although males do not provide any parental care, i.e., Fisherian runaway process, model of good genes, or pleiotropic effects resulting in sensory bias for larger size (Ryan, 1997). Meanwhile, in this study we found that sailfin molly females preferred large body size males (1^{st} experiment) and this result is consistent with the previous studies (Witte and Ryan, 1998; Schlupp *et al*., 2001). We suggested that this female preference on larger males might be resulted from different male reproductive tactics. In this fish, large males usually show courtship display and defend the female successfully from another males (Schlupp *et al*., 2001). However, small males never court females and exclusively rely on forced copulation, a sneaky mating strategy (Schlupp *et al*., 2001). Thus, the interruption of small males reduces female foraging time, which indirectly affects the female reproductive success (Schlupp *et al*., 2001). This male different reproductive tactics may strongly influence on female mate choice in

Poecillids fish. Thus, in this fish choosing a larger male may enhance female fitness although males do not provide any parental care or resources.

In addition, Anderson (1994) reported that females prefer males with an elaborate secondary sexual character, such as conspicuous plumage, nuptial coloration or courtship display. Females usually choose males with intensive courtship because courtship is the honest signal for female in choosing mate, especially in paternal care fish (Myint *et al.*, 2011). In this study we found that sailfin molly female preferred large male, which display courtship intensively. However, when males were not different in body size, females chose dominant males although courtship frequencies of males were not different. We suggested that this female preference on dominant male may came from the conflict between sexes. Choosing dominant male is to avoid the interruption of sneaker males because dominant males can defend the females successfully from sneaker males by chasing them. In addition, the operational sex ratio (OSR) of Poecillid fish is extremely male bias because of intensive male sexual harassment (Parzefall, 1973). Female receptivity is usually lesser than that of male in these fishes. Thus according to their general breeding ecology, female Poecillids may have some control over mating access by males. Schlupp *et al*., 2001) reported that male attention reduces feeding time for females and induces a shift in female time allocation. If female choose a dominant male, she may ensure to live safely in that male territory. Thus, this female preference on dominant male might be an adaptive female mate choice because female may enjoy direct benefits from its choice.

Conclusion

It was concluded that sailfin molly females use multiple cues in mate choice. Firstly, females choose large body size male, then they swift their choice towards dominant male after learning the social status of the males. Thus our results strongly reveal that the use of multiple cues in female mate choice is an adaptive behavior and it may also reflect the quality of the receiver.

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References

Anderson, M., (1994). *exual Selection.* Princeton University Press, Princeton.

Bisazza, A., & M. Marconato, (1988). Female mate choice, male- male competition and parental care in the river bullhead, *Cottus gobio L.* (Pisces, Cottidae) *Animal Behaviour*, vol. 36, pp. 1352 - 1360.

Cote, I. M. & W. Hunte, (1989). Male and female mate choice in the redlip blenny why bigger is better. *Animal Behaviour*, vol.38, pp. 78 – 88.

Downhower, J. F., & L. Brown, (1980). Mate preferences of female mottled sculpins, *Cottus bairdi*. *Animal Behaviour*, vol. 28, pp. 728 – 734.

Endler, J. A & A. L. Basolo, (1998). Sensory ecology, receiver biases and selection. *Trends In Ecology and Evolution*, vol. 13, pp. 415 - 420.

Fisher, R. A., (1930). *The Genetical Theory of Natural Selection.* Clarendon Press, Oxford.

Hoelzer, G. A., (1989). The good parent process of sexual selection. *Animal Behaviour*, vol. 38, pp. 1067 -1078.

Johnstone, R. A., (1997). The evolution of animal signals. In: *Behaviour Ecology. An Evolutionary Approach* (ed. J. R. Krebs and N. B. Davies), pp. 155 - 178. Blackwell Science, Oxford.

Lehtonen, T. K., S. Rintakoski, & K. Lindstrom, (2007). Mate preference for multiple cues: interplay between male and nest size in the sand goby, *Pomatoschistus minutus*. *Behavioural Ecology*, vol. Ecol. 2007

Moller, A. P. & A. Pomiankowski, (1993). Why have birds got multiple sexual ornaments? *Behavioural Ecology and Sociobiology*, vol. 32, pp. 167 - 176.

Myint, O., H. Tsujimoto, N. Ohnishi, T. Takeyama & M. Kohda, (2011). Mate availability affects female choice in a fish with paternal care: female counterstrategies against filial cannibalism. *Journal of Ethology*, vol. 29, pp. 153-159.

Parzefall, J., (1973). *Attraction and sexual cycle of poecillids. In: Genetics and mutagenesis of fish*. (ed. J. H. Schroder), Springer Verlag, Berlin, p. 177 -183.

Pomiankowski, A. & Y. Iwasa, (1993). Evolution of multiple sexual preferences by Fisher's runaway process of sexual seletction. *Proceeding of the Royal Society of London Series B*, vol. 268, pp. 557 -563.

Ryan, M. J. & A. S. Rand, (1993b). Species recognition and sexual selection as a unitary problem in animal communication. *Evolution*, vol. 47, pp. 647 - 657.

Ryan, M. J., (1997). *Sexual selection and mate choice. In: Behavioural ecology: an evolutionary approach.* 4th ed. (Eds. Kerbs, J. B., & Davies, N. B.,). Oxford, Blackwell. pp. 179-202.

Schlupp, I., & M. Ryan, (1996a). Mixed species shoals and the maintenance of a sexual-asexual mating system in mollies. *Animal Behaviour*, vol. 52, p. 885 - 890.

Schlupp, I., & M. Ryan, (1996b). Male sailfin mollies (*Poecilia latipinna*) copy the mate choice of other males. *Behavioral Ecology*, vol. 8, p. 104 -107.

Schlupp, I., R. M. Knab & M. J. Ryan, (2001). Sexual harassment as a cost for molly females: bigger males cost less. *Behaviour*, vol. 138, pp. 277-286.

Witte, K. &U. Kirsten, (2002). Sailfin molly females (*Poecilia latipinna*) copy the rejection of a male. *Behavioral Ecology*, vol. 14, No. 3, pp. 389-395.

Witte, K. & M. J. Ryan, (1998). Male body length influence copying in the sailfin molly (*Poecilia latipinna*) in the wild. *Animal Behaviour.* vol. 63, pp. 943-949.

Zahavi, A., (1975). Mate selection a selection for a handicap. *Journal of Theoretical Biology*, vol. 53, pp. 205 - 214.

Rodent Outbreaks on Upland Rice and Maize Cultivation in Hakha, Htantalang and Phalum Townships in Chin State

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Abstract

Rodent outbreaks on upland rice and maize cultivation in Chin State was studied in Hakha, Hyantalan and Phalum townships. Chin State is the poorest state of Myanmar and is not self-sufficient in rice production, most of its population is chronically food insecure. These outbreaks led to severe losses to crops and stored food, and resulted in severe food shortages. In the upland environment, rodents are considered one of the most important pests of upland rice and maize. Overall damage to upland rice was 67.7% in Hakha Township, 21.9% in Htantalang Township, and 38.9% in Phalum Township. Maize is the main staple food after rice for farmers in the regions. In Hakha and Phalum Township, 72.6% and 36.7% of maize were also attacked by rodents. In three townships, 487.63 ha of upland rice fields in 28 villages were destroyed by rodents. Aswarm rat attacked not only upland rice but also maize in these sites, 260.4 ha of maize in 16 villages were damage. *Rattus rattus* was the most common species in and around rice fields during the outbreaks.

Keywords: rodent outbreaks, Chin State, upland rice, maize

Introduction

Rodents are most important remarkable mammalian agricultural pests at the global level. Rats damage and destroy many crops prior to harvest and also are a major pest for grain stored post-harvest (Rennison and Buckle, 1987). Rice is the staple food in most Asian countries and more than 90% of the world's rice is produced in Asia as reported by Khush (1993). Agricultural is a major component of the Myanmar economy, contributing 42% to its GDP with 65% of the labor force involved in agriculture (Ministry of Agriculture and Irrigation, 2004). Rodent problems have a major impact in Myanmar during pre-harvest, where 75% of population residing in rural area and depend on agriculture for their livelihood. Rodents are major pests in agricultural production. In Asia, rodents cause, on average, annual preharvest losses of 5–10% in rice crops. A loss of 6% is substantial, as this is enough rice to feed 225 million people for a year. (Singleton, 2003)

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Trina Leah Mendoza reported that rat population outbreaks can cause severe crop losses, especially in the uplands, where such losses can lead to major food shortages. Since 2005, such cases have been reported in Mizoram (eastern India), Chittagong Hill Tracts (Bangladesh), Chin State (Myanmar), and the provinces of Oudomxay, Luang Namtha, Sayaboury, and Luang Prabang (Lao PDR). These infestations often happen after an expansive bamboo flowering takes place. Usually rats give birth twice a year but when the bamboo is flowering they can give birth dozen times in a year as the bamboo flowering give them good nutrition to support their reproduction.

The objectives of this study are to document the impact of rodents on smallholder village people and to quantify the extent of rodent damage to crop and whether there is different timing of rodent outbreaks in three townships in Chin State.

Materials and Methods

General description of study area and study period

Location

Chin state is located in the north-west part of Myanmar, bordering India and Bangladesh. Most of the state is hilly and mountainous having an average height of 4,000 ft. During the study period April 2008 to March 2009 in nine villages for three Townships. In Hakha Township; Loankywe, Lamtuk and Vantalan located within 22° 24' 48", 22° 23' 52", 22° 32' 16" N and 93° 52' 52", 93° 47' 56", 93° 49' 41" E. In Htantalang Township; Sopum, Htanzan and Sihhmuh located within 22° 46' 36", 22° 51' 15", 22° 48' 55" N and 93° 22' 45", 93° 21' 07", 93° 18' 30" E. In Phalum Township; Waibula, Zalang and Hmawlzauk located within 23° 00' 44", 23° 03' 25", 22° 59' 48" N and 93° 55' 14", 93° 53' 34", 93° 53' 52" E respectively. (Fig.1)

Climate

Chin State is dry zone and upland rainfed areas. The annual average temperature is maximum 29.8 ºC and minimum -5 ºC; annual rainfall is 168 mm (Department of Meteorology and Hydrology, Yangon).

Soil condition

Valley, bottom and plain in chin state are meadow and meadow alluvial soils with silty loam and clay loam. Which had the pH of 7.0 – 7.5, suitable to grow rice, maize and vegetable. Hill slopes and tops were red brown forest soils with 5.0 – 6.5 suitable for gardening steep slopes of mountain ranges are mostly sandy loam clay with gravel.

Land use pattern

In Chin state, out of 116952 ha of cultivated land, Hakha possessed 15289 ha, Htantalang and Phalum had 15043 ha and 16982 ha, respectively.

Data collection

We were selected the most affected villages of three townships. Trapping was conducted for per night at each village. 100 local kill traps were used. Captured rats collected were recorded and identified individually, sexed, weighed, measured and breeding condition determined. All males and females were necropsied. Females were dissected to determine the condition of the uterus, number of embryos, size of embryos and number of uterine scars. Representative specimens were preserved in 70% ethanol. Rats were identified to species with reference to a taxonomic key developed by Aplin *et al*., 2003.

Conduct household surveys to obtain the following information:

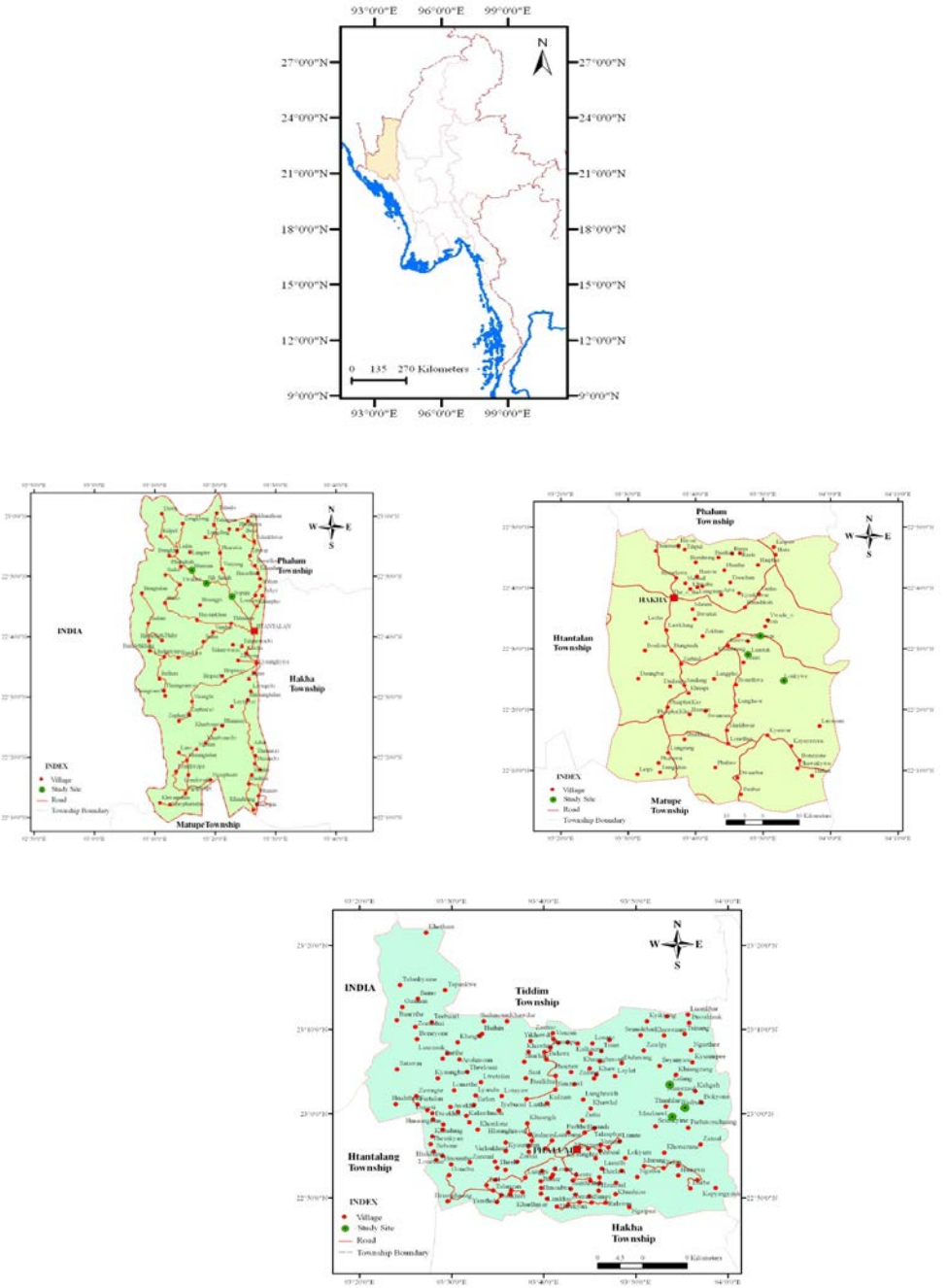
a) Cropping calendar

b) Timing and extent of rodent damage to crops (estimates of yield loss; estimates of area affected)

c) Agronomic data yield of crops in previous year to outbreak, % of crops sold for income; major constraints to crop production

Conduct interview of village head or of older people to capture information on:

a) When was the last outbreak of rats?
b) When did rats cause famine conditions from previous “rat floods”?



Source : Based on Topographic Map (1:63360) and Field Survey

Figure 1. Map of the study sites

Results and Discussion

Major crop cultivation

Upland rice and maize were the main crops in all study sites. The main livelihood activity is agriculture and shifting cultivation or slash and burn farming, is widely practiced. Terrace rice cultivation traditionally practice with shifting system alternated at every three years interval due to depletion of fertilized soil. Soil preparation for rice cultivation was done during March and April. Seeds were sown in May. Paddy grown in May was usually harvested in October and November according to cultivars. Paddy cultivation mainly depends on monsoon. (Table 1)

Table 1. Generalized crop calendar of the study areas

Particulars	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Site selection											
Slashing & burning											
Seeding											
Weeding											
Weeding, thinning, rodenticide use											
Crop Harvesting											
Maize harvesting											
Rice harvesting											

Rodent outbreaks on food security and damage to crops

Most of the upland rice and maize crops in these three townships were devastated by rodents in 2008. Overall damage to upland rice was 67.7% in Hakha Township, 21.9% in Htantalang Township, and 38.9% in Phalum Township. The total upland rice area in Htantalang was greater than in other townships, therefore, more people in Htantalang were affected by these rodent outbreaks. In 2008, 94 households in three villages were affected by a food shortage in Hakha and 97 households were affected by rodent outbreaks in three villages in Htantalang. In Phalum, 18 households faced a substantial food

deficit in three villages. Across the three study sites, we estimated that 31% of the population experienced food insecurity because of these rodent outbreaks on upland rice. (Table 2)

In Chin State maize is one of the main staple foods after rice in some regions. In Hakha and Phalum, the overall damage in maize fields was 72.6% and 36.7%. In Chin State, Hmawlzauk Village, Phalum had 100% of maize crops damaged and the next highest intensity of damage to maize was in Loankwe Village, Hakha (99%) in 2008. In 2008, of those households relying on maize as their staple crop, in three villages, 41 households in Hakha and 16 households in Phalum had a food deficit problem. At the three study sites, the rodent outbreaks lead to food shortages for 13% of the population. (Table 3) In both upland rice and maize cultivation, some farmers completely lost their crop whereas some farmers were able to harvest only 10% of their crop.

Table 2. Impact of rodent outbreaks on upland rice in three villages in Hakha, Htantalang, and Phalum townships in Chin State in 2008. Damage refers to a minimum of 20% but in most cases farmers reported damage greater than 50%.

Townships	Villages	Area of crop production (ha)	Area damaged (ha)	Percent of area damaged	Total households (no.)	Affected households (no.)	Affected households (%)
Hakha	Loankywe	64.63	62.10	96.06	61	50	82
	Lamtuk	41.67	16.67	40	52	13	25
	Vantalan	67.92	39.17	57.76	82	31	38
Total		174.22	117.94	67.70	194	94	48
Htantalang	Sopum	72.29	32.10	44.38	107	26	24
	Htanzan	420.83	84.17	20	145	67	46
	Sihhmuh	57.50	4.38	7.61	46	4	9
Total		550.62	120.65	21.90	298	97	33
Phalum	Waibula	36.67	0.54	1.48	102	0	0
	Zalang	0	0	0	41	0	0
	Hmawlzauk	22.50	22.50	100	40	18	45
Total		59.17	23.04	38.9	183	18	10

Source: Myanma Agriculture Service.

Table 3. Impact of rodent outbreaks on maize in three villages in Hakha, Htantalang, and Phalum townships in Chin State in 2008. Damage level was not defined.

Townships	Villages	Area of crop production (ha)	Area damaged (ha)	Percent of area damaged	Total house-holds (no.)	Affected house-holds (no.)	Affected house-holds (%)
Hakha	Loankywe	50.42	49.9	99.0	61	20	33
	Lamtuk	33.33	12.5	37.5	52	5	10
	Vantalan	56.67	39.58	69.9	82	16	20
Total		140.42	101.98	72.62	194	41	21
Htanta-lang	Sopum	0	0	0	107	0	0
	Htanzan	50.42	0	0	145	0	0
	Sihhmuh	0	0	0	46	0	0
Total		50.42	0	0	298	0	0
Phalum	Waibula	66.67	15.52	23.3	102	6	6
	Zalang	29.38	10.83	36.4	41	4	10
	Hmawlzauk	14.17	14.16	100.0	40	6	15
Total		110.22	40.51	36.75	183	16	9

Source: Myanma Agriculture Service.

In Chin State, upland rice 8.1% and maize 3.5% was destroyed by rats in nine villages of Hakha Township. Damage of 2.2% upland rice by rat was recorded from twelve villages of Htantalang Township. Seven villages from Phalum Township suffered 1.7% loss of upland rice and 7.1% loss of maize. (Table 4).

Table 4. Rodent outbreaks on upland rice and maize in three townships in Chin State

Crop	Townships	Villages	Total area (ha)	Area damaged (ha)	Percent of area damaged	Source
Upland rice	Htantalang	12	7,890.42	170	2.2	MAS
	Hakha	9	2,452.92	198.33	8.1	MAS
	Phalum	7	7,070.83	119.30	1.7	MAS
Total	3	28	17414.17	487.63	35.7	
Maize	Hakha	9	6,113.75	210.73	3.5	MAS
	Phalum	7	695.83	49.71	7.1	MAS
Total	2	16	6809.58	260.44	26.2	

Rodent outbreak and its damage Hakha, Htantalang and Phalum townships were observed in seedling, tillering and harvesting stage. Damage of rice in flowering stage was observed in Hakha and Htantalang. In Phalum rice damage due to rat occurred in booting and ripening stage (Table 5). Maize in Hakha Township was caused more damage by rats at seedling, germinating, flowering and ripening stages. In Phalum Township, rat damage to germinating, budding and harvesting stage of maize (Table 6).

Table 5. The timing of the highest intensity of damage to upland rice crops due to rodent outbreaks in three different townships in Chin State during 2008

Townships	Seedling	Tillering	Booting	Flowering	Ripening	Harvesting
Hakha	✓	✓	-	✓	-	✓
Htantalang	✓	✓	-	✓	-	✓
Phalum	✓	✓	✓	-	✓	✓

Source: Farmer Interview.

Table 6. The timing of the highest intensity of damage to maize crops due to rodent outbreak in three different townships in Chin State in 2008

Townships	Seeding	Germinating	Budding	Flowering	Ripening	Harvesting
Hakha	✓	✓	-	✓	✓	-
Htantalang	-	-	-	-	-	-
Phalum	-	✓	✓	-	-	✓

Source: Farmer Interview.

Rodent population outbreaks and control activities

Seven adults (three adult females and four adult males) and fifteen juveniles *R. rattus* were caught in one night. In Mizoram, there were also a high proportion of juveniles caught in the crops during the period of high losses (Aplin and Lalgiamliana, 2010). Only one adult female (n=3) had uterine scars. *R. rattus* is one and the only rat species that occur in that places giving serious damage to crops and human utensils. In Chin State, the main rodent species involved in the population outbreaks is *R. rattus*. *R. rattus* in its various forms is the dominant rat of agricultural environments as well as village habitats throughout much of Asia (Aplin *et al.* 2003).

R. rattus breed 4 times a year. Breeding occurred in fallow stage and harvesting stage. Pronounced damage occurs in bamboo fruiting season. As much as 30 rats per night were caught in bamboo blooming period. A maize plant in its budding time host 5-9 rats. Rodent damage sown seeds to harvest stage plant were foraged. Application of rodenticide three times in growing season. Rat population was mostly controllers by traps which accounted for 50% of control methods. Four types of kill traps were used by a single villager. Traps are local called as Pial, Mang Khawng, Luang Rap and Cep trap. Followed by the use of rodenticides with 35% catapults and crossed bow were also used to catch trap when there was high population of rats.

Rodent impact on livelihoods

The chin people always depend on their own crops. Maize and rice are their major food. Bamboo is the primary vegetation in much of southern Chin State and the areas along India-Burma borders. In Chin State, the economic activities are mostly agriculture activities (vegetable and fruit production), livestock, gardening, small trade and seasonal labor work. In 2007/2008 during

the outbreak season the farmers losing their harvest. Every stage of crop was destroyed by the rats. No crop yield was expected if seeding in the nurseries were devoured by rats. They became desperate after selling off all their livestock such as chicken, pigs and cows to buy food. The price of rice has 7,000 kyats for one basket (Table 7).

Few families have received some financial assistance from relatives living abroad. But the majority of people are finding themselves. Evident of starvation and outbreak of rat related diseases were not detected although a lot of damages occurred in house, churches and barns. As well as utilization such as blanket, pillow, bed sheet, etc. Villagers are not bitter but stored crop were damaged. Rats eat every kind of crops. They attack not only crops, also have been found to eat bamboo matted floors inside houses and have been destroyed home materials.

Table 7. Market price of main crops in study areas.

Crops	Count	Price (Kyat)
Paddy	0ne basket	7,000
Maize	0ne basket	6,000

Conclusion

Chin state is poorest State of Myanmar. Not self-sufficient in rice and maize. Rats have damaged 487.63 ha of upland rice in 28 villages and 260.4 ha of maize in 16 villages (Source: MAS in Chin State). Many farmers lost all of their harvest. Some farmers received 10 % of their normal harvest. Localized food insecurity in affected villages is expected. Most of its population is food insecure. Shorter cycle of shifting cultivation not adequate to rebuild the soil fertility. A serious rodent problem in monsoon crop. Outbreak of rodent population should be investigated based on different sources such as availability of food, bamboo blooming internal and damage. Now, highest % of expenditure on food (Household expenditure – UNDP 2007).

Rodent damage to upland rice field

Rodent damage to maize field

Rodent damage to stored grain (upland rice) (maize)

Rodent damage to stored grain

Rodent population (*Rattus rattus*)

Plate I

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References

Aplin, K.P., Brown P.R., Jacob J., Krebs C.J. and Singleton G.R. (2003). *Field methods for rodent studies in Asia and the Indo-Pacific*. ACIAR Monograph 100. Australian Centre for International Agricultural Research: Canberra. pp 223.

Aplin, K.P and J. lalsiamliana (2010). *Chronicle and impacts of the 2005-09 mautam in Mizoram*. In Rodent Outbreaks: Ecology and Impacts, (Ed. Singleton, G.R. ,S. R. Belmain, P. R. Brown and Bill Hardy. Los Banos (Philippines): International Rice Research Institute. 13pp.

Khush, G.S. (1993). *More food and a safe environment*. Food comes first for Asia. Crawford Fund of International Agricultural Research. pp. 31-33. Parkville, Australia.

M.O.A.I. (2004). *Rice varieties in Myanmar*, Ministry of Agriculture and Irrigation. 25 pp.

Rennison, B.D. and Buckle A.P. (1987). Methods for measuring and evaluating the losses caused in rice and other crops by rodents. In: *Control of Mammal Pests*. (Ed. Richards, C.G.J. and T.Y. Ku).CRC Press pp. 3-14.

Singleton, G.R. (2003). *Impacts of Rodents on Rice Production in Asia*. IRRI Discussion Paper Series No. 43. International Rice Research Institute, Los Baños, Philippines. 30 pp.

"When rats attack" Rice Today, Trina Leah Mendoza, October-December 2009**.** http://www.irri.org/publications/today/pdfs/44-45.pdf

Screening of Calorie Restriction-induced Genes in the Rotifer *Brachionus Plicatilis**

Aung Kyaw Swar Oo

Abstract

Rotifers play an important role in an aquatic ecosystem and are used as model organisms for various study areas. Although many studies have been conducted to identify environmental factors that influence rotifer populations, the molecular mechanisms involved still remain to be elucidated. The availability of food resource is also a one of the most important environmental factors that fluctuate rotifer populations over time. In this study, gene(s) differentially expressed by calorie restriction in the monogonont rotifers, *Brachionus plicatilis*, was analyzed, where a calorie-restricted group was fed 3 h/day and a well-fed group fed *ad libitum*. A subtracted cDNA library from the calorie-restricted rotifers was constructed using suppression subtractive hybridization (SSH). One hundred sixty three expressed sequence tags (ESTs) were identified, which included 109 putative genes with a high identity to known genes in the publicly available database as well as 54 unknown ESTs. A total of 38 different genes were obtained among 109 ESTs. Gene ontology study showed the differentially expressed genes related to cellular structure, transport, and division; DNA synthesis; metabolism; transcription; RNA biosynthesis; and other functions were 24, 11, 36, 5, 3, 18% respectively, whereas genes with functionally unclassified were 3%.

Key words: Monogonont rotifer, *Brachionus plicatilis*, calorie restriction, SSH, EST

Introduction

In an ecosystem all the organisms living together in a specific habitat rely on each other. The amount of energy within the ecosystem is always maintained at a constant level and is hardly created nor declined. The energy flow through a food web and a food chain is carried out by various trophic levels in a particular system. Therefore, individuals in every trophic level are important for the sustainable ecosystem in which they live and for their own survival. Biotic and/or abiotic factors also play key roles for the survival of animal species. It is postulated that a number of animals are influenced by both population density-dependent and -independent factors, and their impacts are

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relative and depend on the physiological status of the population itself (Mori, 1988). The population of any animal species fluctuates depending on the changes in their surrounding environments and within their own population. These fluctuations strongly influence humans in various ways such as food resources, public health, economy and environment.

Nowadays population dynamics studies have become popular and animals from diverse taxa with different economic values of importance are used as model organisms in these studies. The rotifer *Brachionus plicatilis* is a small zooplankton approximately 0.3 mm in length that inhabits brackish waters. It is an economically important species as a live food organism in aquaculture (Hagiwara *et al.*, 2001) because it has a rich nutritional profile and a suitable size for larval fish and shrimp. It reproduces either sexually or asexually with one of the highest population growth rate among metazons. Cyclically parthenogenetic organisms provide a valuable model for investigating the relationships between reproductive mode and population structure, and the maintenance of genetic variation in natural populations (Bell, 1982; Hebert, 1987). There are several reports on obligate parthenogenetic strains (reproduce only asexually) in *Brachionus* sp. (Bennett and Borass, 1989; Fussmann *et al.*, 2003) and Ishikawa strain of *B. plicatilis* used in the present study has been known to reproduce asexually: mictic females or males are not observed (Yoshinaga *et al.*, 2000). The parthenogenetic rotifer is now widely used as a model organism in population dynamics studies and in the laboratory it shows a typical sigmoid growth curve as observed with other laboratory-reared model organisms (Yoshinaga *et al.*, 2001a).

Environmental factors, either climatic or biological, cause an alternation in the individual life history parameters of the rotifer, such as reproductive pattern and life span, leading to the fluctuation of population (Yoshinaga *et al.*, 2003). The rotifer subjected to calorie restriction (CR) in a feeding schedule of 3 h/day showed its life span two times longer than that subjected to well fed, and offspring production concomitantly decreased about ten times under CR (Yoshinaga *et al.*, 2003). Thus, CR is one of the biological factors that shift a mode of reproduction and concomitantly a mode of life span in the rotifer. Such trade-off between lifetime fecundity and life span is proposed as an alternative life history strategy of the rotifer under starved conditions to maintain its population size stable (Yoshinaga *et al.*, 2000). It is also proposed that the effects of CR on the starvation tolerance are transmitted from parents to their offspring in the rotifer (Yoshinaga *et al.*, 2001b).

Molecular approaches to the effects of CR on life span have been conducted using rotifer as a model organism. It is widely accepted that life span is regulated by the interaction between oxidative stress and an enzymatic antioxidation. The major antioxidant enzyme, superoxide dismutase (SOD), catalyzes decomposition of reactive oxygen species (ROS), which provokes massive damages to DNA, proteins, and lipids (Finkel and Holbrook, 2000). The accumulated mRNA levels of manganese-SOD (Mn-SOD), which functions in mitochondria, were found to increase in calorie-restricted, long-lived rotifer (Kaneko *et al.*, 2005). It has been also claimed that the dietary restriction (another term of CR) retarded the rate of nuclear division in the gastric glands and vitellarium (yolk-secreting gland) of the rotifer *Asplanchna brightwelli* (Verdone-Smith and Enesco, 1982). Although it is generally accepted that an energy saving through the suppression of reproduction during the period of food shortage is a prerequisite to a longer life span and maintaining the population size, little is known at present about the genes expressed under CR.

Suppression subtractive hybridization (SSH) is a powerful, reliable technique to identify differentially expressed genes that are involved in physiological processes of both aquatic invertebrates (Brown *et al.*, 2006; Soetaert *et al.*, 2006) and vertebrates (Reynders *et al.*, 2006; Wang and Wu, 2007) responding to various environmental conditions. In this study, SSH was used to identify differentially expressed genes in calorie-restricted rotifer *B. plicatilis*.

Materials and Methods

Culture, feeding regimen, and sample collection

The parthenogenetic rotifer *B. plicatilis* (Ishikawa strain) was used in the present study. Rotifers were cultured using Brujewicz artificial seawater (BAS). The BSA, consisting of 454 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 27 mM $MgSO_4$, 26 mM $MgCl_2$, 2.4 mM $NaHCO_3$ and 0.8 mM NaBr, was sterilized and filtered by 0.45-μm filter. The half-diluted BAS culture media were used in the present study. The rotifers were precultured under a batch culture system at 25°C using a cool-incubator (HCRCS2V150W-A1202, Ikuta Industries, Tokyo, Japan) and subjected to a continuous feeding with commercially available concentrated algae *Nannochloropsis oculata* (Nikkai Center, Tokyo, Japan). Eggs deposited on the bottom of the precultures were

collected and hatched out. Neonates were cultured under the same conditions as mentioned above in total darkness except during observation and subsequently divided into two groups: one for well-feeding (WF) as the control and the other for CR. CR was imposed by periodical food limiting at a 3 h/day feeding regimen, while WF was instituted by feeding at *ad libitum*. Culture media were changed daily at the beginning of the CR period. The rotifers in the CR group were transferred using a plankton net (50 μM mesh size) into a fresh medium without algae, whereas those in the WF group were into a fresh medium previously suspended with food algae. Samples were collected on day 2, when the reproductive performance between the WF and CR groups was clearly distinct, using the plankton net and washed two times with fresh BAS.

Total RNA extraction

Total RNAs were extracted using Isogen (Nippon Gene, Toyama, Japan) according to the manufacturer's protocols with a little modification (Fig. 1). Harvested rotifers (about 40,000 ind/200ml) were homogenized with 1 ml of Isogen in 1.5 ml tubes. The tubes were then swirled using a vortex and stored at room temperature for 5 minutes. An aliquot of 0.2 ml of 99.5% chloroform was added into the tubes, which were subsequently shaken vigorously for 15 seconds and stored at room temperature for 2 to 3 minutes. After storage, the tubes were centrifuged at 16,000xg for 15 minutes at 4℃. The uppermost aqueous layers containing the extracted RNAs were transferred into new tubes, added with 0.5 ml of 99.5% isopropanol, and stored at −20℃ overnight to precipitate RNAs. The precipitated RNAs were collected by centrifugation at 16,000xg for 30 minutes at 4℃. All aqueous phase was discarded and the precipitated RNAs were washed with 1 ml of 70% ethanol. The tubes were again centrifuged at 4,600xg for 10 minutes at 4℃. Alcohol was discarded and the tubes were dried briefly till the alcohol residues were completely evaporated. Finally, the RNA pellets were re-suspended into sterile distilled water.

Poly $(A)^+$ RNA isolation

Poly $(A)^+$ RNAs were isolated from the total RNAs using Oligotex-dT30 (super) mRNA purification Kit (TaKaRa, Otsu, Japan) according to the manufacturer's instructions. RNA integrity was examined by using agarose gels containing 1% formaldehyde. The quantity and quality of RNA were determined by absorbance at A260 and at A260/280 using a DU® 530 Life Science UV/Vis spectrophotometer (Beckman Instruments, Inc., Fullerton, CA,

USA).

SSH library construction and plasmid isolation

cDNAs from the CR group were used as a tester, whereas cDNAs form the WF group were used as a driver, and the driver cDNAs were subtracted from the tester cDNAs (Fig. 2). SSH was carried out using PCR-Select^{TM} cDNA Subtraction Kit (TaKaRa) with minor modifications. PCR amplification was conducted using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The optimized cycles for the primary and secondary PCRs were 27 and 15, respectively. The resulting subtracted cDNAs were ligated to the pGEM-T vectors using pGEM-T Vector Systems (Promega, Madison, WI, USA) and transformed into *Escherichia coli* strain JM109. White colonies were randomly picked up from the subtracted cDNA library and the presence of inserts was checked by agarose gel electrophoresis. The clones with inserts were grown overnight in Luria-Bertani (LB) broth media containing ampicillin (200 μg/ml) at 37 °C under shaking. Plasmid isolation was accomplished using GenElute Plasmid Miniprep Kit (Sigma, St. Louis, MO, USA).

Sequencing and search for homologous sequences

The purified plasmid DNAs with inserts were subjected to PCR labeling using BigDye^{TM} Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and sequencing was performed using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Adaptor and vector sequences flanking either side of partial sequences were detached using SeqEd v1.0.3 software (Applied Biosystems). Finally, modified expressed sequence tags (ESTs) were submitted to the National Center for Biotechnology Information database (NCBI) using the blastx program in the Basic Local Alignment Search Tool (BLAST) to search any known gene counterparts with homologous sequences in the database.

Results

Reproductive performance under calorie restriction

The WF group on day 2 showed observable reproduction and most individuals carried at least two eggs. However, reproduction in the CR group was indistinct and very few individuals carried one egg. Thus, the numbers of eggs and individuals carrying eggs were remarkably different between the CR

and WF groups, which were regarded as a visual parameter for distinguishing the effects of CR.

SSH and sequence homology

After SSH, different band patterns were seen between the subtracted and unsubtracted PCR products. The bands with the molecular weights of 612, 495, 345, and 210 bp were predominantly observed in the subtracted PCR products (Fig. 3). Subtracted PCR products were subcloned into the pGEM-T vectors and inserted clones were subjected to sequencing.

Randomly collected 163 clones containing inserts were sequenced and submitted to the NCBI database using the blastx program in BLAST. Among 163 ESTs submitted, 109 ESTs (66.9%) retrieved their homologous sequences, whereas other 54 ESTs (33.1%) showed no significant similarity to known genes in the database. Sequence alignment of the ESTs with the same gene products was conducted using the ClustalW multiple sequence alignment program, yielding 38 different genes among 109 ESTs (Table 1).

Gene ontology study showed one group of gene having unknown function (3%) and 6 functional groups of gene related to cellular structure, transport, and division (24%), DNA synthesis (11%), metabolism (36%), other functions (18%), transcription (5%), and RNA biosynthesis (3%) were observed among the differentially expressed genes (Fig. 4).

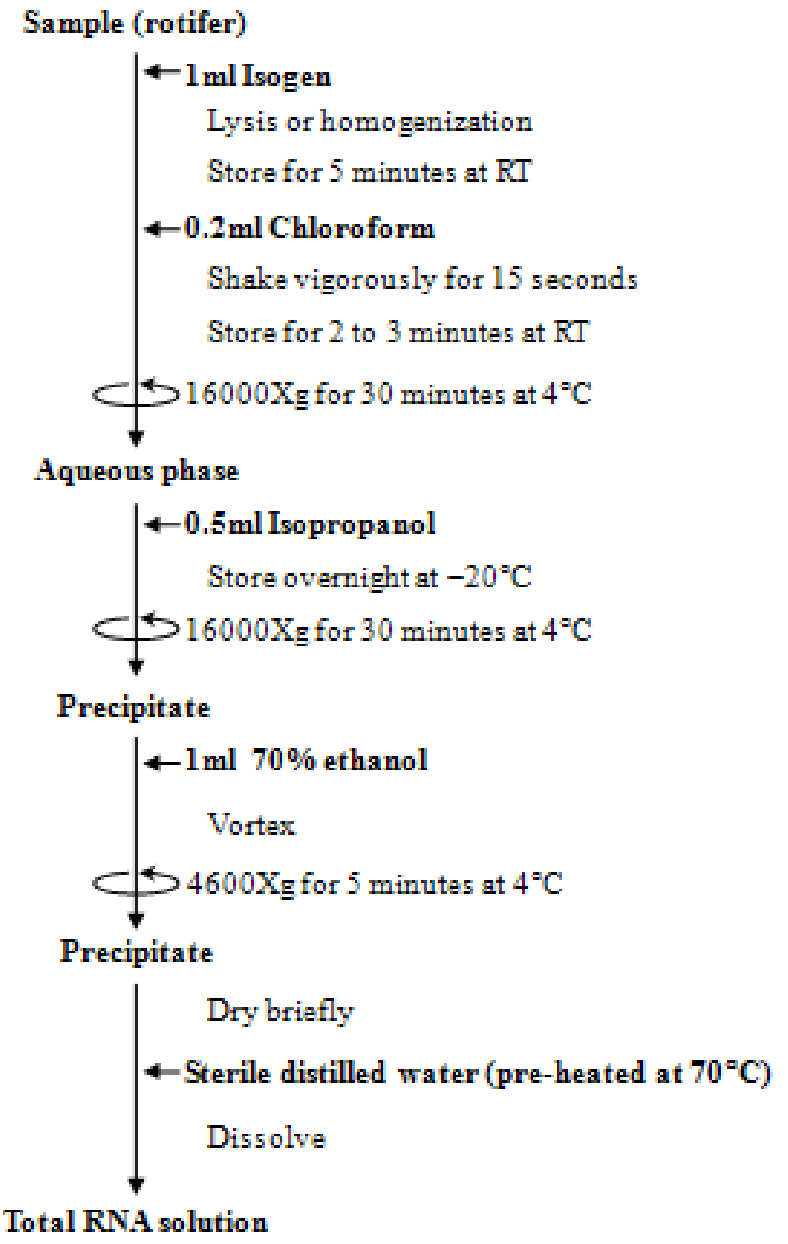

Fig. 1. General outline for total RNA extraction. RT, room temperature

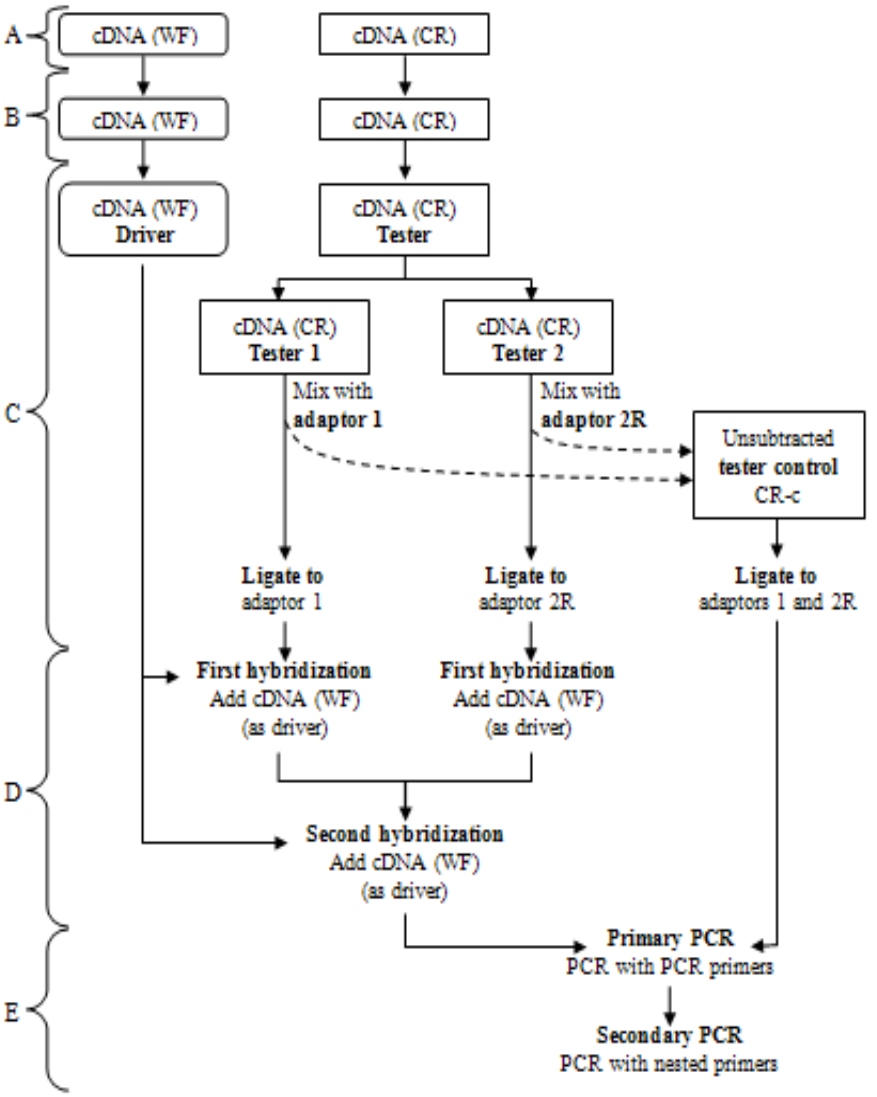

Fig. 2. Basic steps in SSH (A-E) and type of cDNA subtraction performed for the present study. SSH technique basically covers cDNA synthesis (A), enzyme digestion (B), adaptor ligation (C), hybridization (D), and PCR amplification (E). As the present study was focused on calorie restriction (CR) cDNA prepared from the CR samples were used as a tester, whereas those prepared from the control well-fed (WF) samples were used as a driver. The tester cDNAs were subtracted from the driver cDNAs. CR-c, CR unsubtracted tester control; PCR, polymeric chain reaction; cDNA, complementary DNA

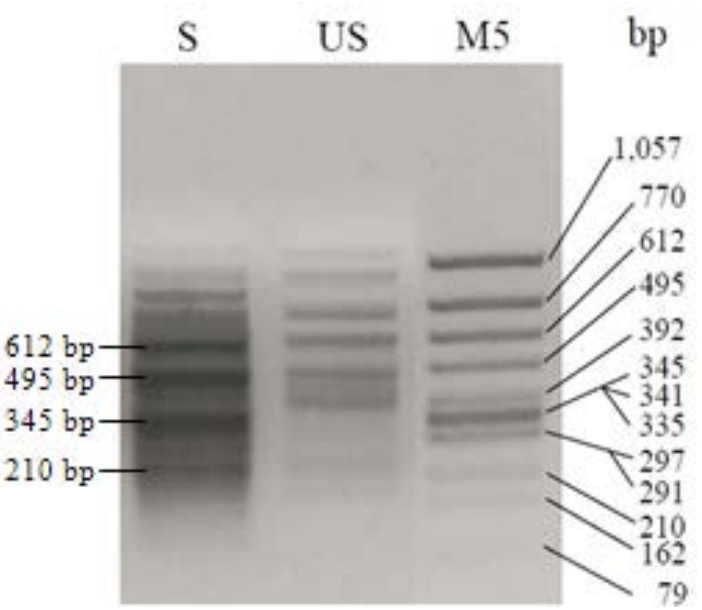

Fig. 3. PCR products resulted from SSH. PCR products were run on 2% agarose gel containing ethidium bromide. S and US stand respectively for the subtracted and unsubtracted PCR products; M5, molecular weight marker 5

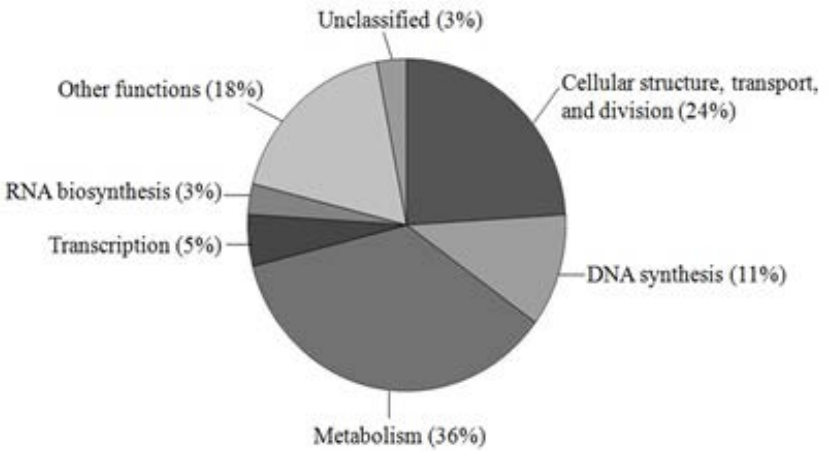

Fig. 4. Gene ontology analysis on 38 differentially expressed genes in calorie-restricted *Brachionus plicatilis* by functional classification for predicted proteins.

Table 1. Differentially expressed genes in calorie-restricted *Brachionus plicatilis* as revealed by SSH

Putative gene	Abbrevia-tion	Accession no.	E value[1]	Length of query sequence	Identity (%)
Genes related to cellular structure, transport, and division					
Lissencephaly-1	*Lis1*	XP_392399	4e-18	288	69
Dynein heavy chain domain 3	*Dnahc3*	XP_997725	1e-42	481	58
Beta 2 tubulin	*tub*	AAX09675	1e-37	301	97
Microtubule-associated protein EB 1	*MT-EB1*	BAC05521	4e-08	230	61
BRCA2 and CDKN1A-interact-ing protein, isoform BCCIPβ	*BCCIPβ*	XP_423888	3e-17	676	32
Calmodulin (synthetic construct)	*CaM64B*	AAD34268	1e-12	427	40
Genes related to DNA replication					
CDT1 protein	*CDT1*	AAH21126	0.031	303	31
Mismatch repair protein in mitosis and meiosis	*Msh6p*	NP_010382	8.6	424	38
DNA polymerase epsilon	*Ploe*	EAT45963	7.3	495	41
DNA polymerase sigma	*Pols*	NP_001012968	1e-26	239	73
Genes related to metabolism					
Stom protein	*stom*	AAH91908	9e-46	550	65
Galactose-4-epimerase, UDP	*Gale*	EAT40915	3e-21	432	60
Glycogen phosphorylase	*Glase*	NP_001001904	5e-12	158	67
β-galactosidase	*Glb*	XP_792349	2e-11	262	49

Putative gene	Abbrevia -tion	Accession no.	E value[1]	Length of query sequence	Identity (%)
2-isopropylmalate synthase	*IPS2*	YP_609867	0.76	343	78
Succinate dehydrogenase complex subunit D	*SDHD*	AAW70035	8.4	182	50
Iron regulatory protein	*Irp*	AAR15297	2e-09	428	51
Peptidylglycine-hydroxylating monooxygenase	*Phm*	NP_477225	9e-13	351	47
Genes related to other functions					
Tissue factor pathway inhibitor	*TFPI*	AAB26836	5e-14	384	38
Serine protease	*Ser*	EAT46744	3e-08	521	38
Multifunctional 14-3-3 family chaperone	*14-3-3*	ABF18291	1e-28	249	83
Serine/threonine phosphatase	*STPP*	AAD01260	2e-26	174	98
Serine/threonine protein kinase with TRP repeats	*PK-TRP*	YP_593054	8.6	200	38
Transposase	*Tsase*	NP_602772	1.3	382	54
Envelope glycoprotein	*gp*	ABA61554	5.0	459	45
Genes related to transcription					
Zinc finger protein	*Znf*	FAA00107	3e-05	307	48
EBF protein	*EBF*	XP_688771	5.4	304	42
Genes related to RNA biosynthesis					
NOL1/NOP2/Sun domain family 2 protein	*NSUN2*	XP_419023	2e-14	234	53
Predicted metal-dependent RNase	*COG1782*	ZP_00366321	5.6	494	42
Unknown					
Conserved hypothetical protein	*CHP*	EAT32996	0.26	369	27

[1]E value or expectation value — the number of the different alignments with scores equivalent to or better than raw score (S) that are expected to occur in the database search by chance.

Discussion

CR without essential nutrient deficiency is the only known experimental intervention that extends life span and retards age-related defects of various species across wide phylogenetic differences. In any organisms, adaptation to changes occurring in the environment is controlled by molecular-based mechanisms. Relevant expression or suppression of genes to time and conditions is a prerequisite to adjusting metabolism at the molecular level in all organisms. Proper gene expression confers energetically favorable mechanistic pathways in energy-demanding cells and hereby cells sustain their normal activities required for maintaining life.

SSH is a powerful technique to identify differentially expressed genes that involve in physiobiological processes of organisms under a particular condition. In the present study, SSH was used to identify genes induced by CR in the rotifer *B. plicatilis*. Annotated sequences were classified by Gene Ontology followed by manual adjustment, showing 6 functional groups among 38 differentially expressed genes (see Table 1).

Predicted metal-dependent RNase (*COG1782*) was most abundantly found (32 ESTs) among 109 ESTs encoding 38 different genes, but its putative function is largely unknown. Among genes related to cellular structure, transport and division, the number of EST encoding *Dnahc3* was 17, the second most abundant transcript. Dynein motor protein has several roles, in combination with other molecules, in cellular activities. In *Drosophila*, dynein is required during germline cell divisions and oocyte differentiation (McGrail and Hays, 1997). The dynein heavy chain gene is differentially expressed during development with the highest levels of transcripts in ovaries and embryos (Li *et al.*, 1994). Dynein localization along the oocyte cortex in wild-type *Drosophila* egg chambers is dependent on *Drosophila* Lis1, DLis1 (Swan *et al.*, 1999). It has been speculated that a membrane-associated protein, spectrin, is required for proper localization of DLis1 to the oocyte cortex in the *Drosophil*a ovary (Swan *et al.*, 1999). Lis1 interacts physically with β-spectrin *in vitro* (Wang *et al.*, 1995). In the present study, the gene encoding β-spectrin was also observed among 38 different genes. Based on these findings and the present results, expression of genes encoding Dnahc3, Lis1 and β-spectrin protein are probably attributable to reproductive suppression of the rotifer

under CR.

BCCIPβ is an isoform of BCCIP, a BRCA2 and CDKN1A (p21 or $p21^{Waf1/Cip1}$) interacting protein. BCCIPβ interacts with p21 *in vivo*, inhibits cell growth and delays progression of G1 to S phase (Meng *et al.*, 2004). It has been reported that CR decreases the rate of cell division as well as the total number of dividing cells in rat colonic mucosa (Albanes *et al.*, 1990). In the present study, *BCCIPβ* expression was observed by CR. Dietary restriction retards the rate of organ-specific nuclear division in the rotifer *A. brightwelli* (Verdone-Smith and Enesco, 1982). Cell division in the rotifer is known to occur only in their eggs and CR suppresses reproduction (Egami, 1972). Taken together, expression of BCCIPβ may regulate the metabolic shift of rotifers from reproduction to body maintenance under CR.

In this study, the genes encoding DNA polymerase epsilon (*Pole*), DNA polymerase sigma (*Pols*), and replication protein (*CDT1*) were expressed by CR. In *S. cerevisiae*, Pols is necessary to stimulate DNA polymerase activity of Pole holoenzyme (Edwards *et al.*, 2003) and this activity is also required for rapid and efficient chromosomal DNA replication in *Xenopus*, a higher eukaryote (Shikata *et al.*, 2006). Pole in *S. cerevisiae* localizes and functions at the replication forks (Hiraga *et al.*, 2005) and its expression peaks at G1/S (Sugino, 1995). CDT1 is also specifically recruited to chromatin during G1 and S phase (Bell and Dutta, 2002). Therefore, the expression of *Pole* and *CDT1* would affect cell cycle progression. Intracellular iron metabolism is important for cell-cycle progression and regulated by iron regulatory protein, Irp. *Irp* was expressed by CR in the present study. Expression of the genes that regulate cell-cycle progression (e.g., *GADD45* participating in growth arrest and DNA damage) are induced by iron chelators (Gao and Richardson, 2001). On the other hand, GADD45 arrests the cell cycle and is involved in DNA nucleotide excision repair (Kastan *et al.*, 1992; Levine, 1997). *GADD45B* expression is also inducible by tissue factor pathway inhibitor, TFPI (Shirotani-Ikejima *et al.*, 2002). *TFPI* was also expressed by CR in the present study and its expression might also related to cell cycle arrest at G1/S phase. NOL1/NOP2/Sun domain family 2 protein (NSUN2) is an RNA methyltransferase. NSUN2 has high sequence homology to mammalian Misu protein, which contains SUN domain. It has been reported that *Misu* expression is highest in S phase (Frye and Watt, 2006). Therefore, in the present study expression of *NSUN2* indicates that the cells in the rotifers were mostly in S phase of the cell cycle under CR.

In addition to its roles in cell cycle delays, BCCIP functions in genome stability through its direct interaction with homologous recombinational repair (HRR) protein, BRCA2 (Lu *et al.*, 2005), indicating BCCIP participates in DNA repair. Mismatches result from DNA replication errors and genetic recombination, and DNA damages are fixed in the genome if uncorrected (Crouse, 1996; Kolodner, 1996; Modrich and Lahue, 1996; Modrich, 1997). Mismatches are corrected by mismatch repair proteins, Msh2p and Msh6p (Alani, 1996; Iaccarino *et al.*, 1996). In this study *Msh6p* expression was observed under CR, suggesting that CR induces the expression of genes involved in DNA repairs and their expression is attributable to genome stability.

CR not only functions at cellular and molecular levels, but also has many effects on metabolism of various animals. Lowered plasma glucose content consequent to a variety of CR regimens has been demonstrated in mouse, rat, and non-human primates of different ages (Masoro *et al.*, 1992; Harris *et al.*, 1994; Kemnitz *et al.*, 1994; Cefalu *et al.*, 1995). The genes encoding glycogen phosphorylase (*Glase*) and β-galactosidase (*Glb*) were expressed by CR in this study. Glycogen is degraded for metabolic use by Glase, liberating glucose units from the liver cells into bloodstream. Glb (also called lactase) is commonly used to cleave lactose into galactose and glucose. Lactase activity is consistently higher in dietary restricted animals than their counterparts fed *ad libitum* (Maier *et al.*, 2007). The expression of these genes is likely to regulate the body glucose levels required for maintaining important metabolic processes under CR. Enzyme IPS2 in *S. cerevisiae* catalyzes leucine biosynthesis (Ryan *et al.*, 1973). Leucine is an essential amino acid and also a potent activator of serine/threonine kinase involved in many cellular processes, including protein synthesis, cell growth, and metabolism (Inoki *et al.*, 2005; Cota *et al.*, 2006). In this study, the gene encoding serine/threonine protein kinase with TRP repeats (*PK-TRP*) was also expressed together with *IPS2*.

The members of the 14-3-3 family mediate interactions between diverse components having different biological activities and 14-3-3 proteins have been implicated in the regulation of cell cycle (Stoica *et al.*, 2006). Because of its diverse biological functions, the expression of the gene would be important for various biological processes under CR.

The transposable element (TE) gene encodes transposase (Tsase), which confers translocation of TE in the genome. It has been observed that TEs are differentially expressed in black tiger shrimp *Penaeus monodon* exposed to

a range of environmental stressors (de la Vega *et al.*, 2007). In fish, the exprssion of *Tsase* is induced by external stimuli such as toxin, stress, and bacterial antigens (Krasnov *et al.*, 2005). In this study, *Tsase* and the gene encoding viral envelope glycoportein (*gp*) were also expressed by CR. Based on previous findings and the present results, *Tsase* expression seems necessary for responding to various environmental stressors.

Early B cell factor (EBF) is a transcription factor known to be responsible for the development of B lymphocytes. *Collier* (*col*, the *Dorsophila* ortholog of the vertebrate gene encoding EBF) has been implicated in developing lamellocytes, which function in cellular immune response to parasitization in *Drosophila* (Crozatier *et al.*, 2004). Therefore, the expression of *EBF* might protect the rotifer from various potential diseases under CR.

CR functions at physiological, cellular, and molecular levels. At cellular levels, CR mediates cell proliferation and inhibits the organ-specific cell proliferation (Lok *et al.*, 1990; Lu *et al.*, 1993). CR reduces the follicle size of reproductive females of tree lizard *Urosaurus ornatus* (French *et al.*, 2007). Verdone-Smith and Enesco (1982) reported that the rate of nuclear division in gastric glands and vitellarium (yolk-secreting gland) of the rotifer *A. brightwelli* was retarded by dietary restriction. A trade-off between life span and lifetime fecundity has been proposed as an alternative life-history strategy of *B. plicatilis* under starved conditions (Yoshinaga *et al.*, 2000).

Conclusion

The primary role of CR seems to postpone reproductive senescence upon the somatic maintenance, thereby animals gain an increased chance of survival with a reduced intrinsic rate of senescence. Based on previous studies and the present findings, we predict here that these aspects of CR may also be found in the calorie-restricted rotifer of *B. plicatilis* (Ishikawa strain). Most of the up-regulated genes in the present study may be important for the rotifer in maintaining their metabolic processes under CR. It is likely that there are other genes still remaining out of the present study due to differences in experimental procedures. However, the differentially expressed genes observed in the present study shed light into the molecular mechanisms that control the metabolic processes of a particular animal under CR.

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References

Alani, E (1996). The *Saccharomyces cerevisiae* Msh2 and Msh6 proteins form a complex that specifically binds to duplex oligonucleotides containing mismatched DNA base pairs. *Mol Cell Biol* vol.16, pp.5604–5615

Albanes, D, Salbe, A. D., Levander, O. A., Taylor, P. R., Nixon, D. W., Winick, M. (1990). The effect of early caloric restriction on colonic cellular growth in rats. *Nutr Cancer* vol.13, pp.73–80

Bell, G. (1982). *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*. Croom-Helm, London

Bell, S. P. and Dutta, A. (2002). DNA replication in eukaryotic cells. *Annu Rev Biochem* vol.71, pp.333–374

Bennett, W. N. and Boraas, M. E. (1989). A demographic profile of the fastest growing metazoan: a strain of *Brachionus calyciflorus* (Rotifera). *Oikos* vol.55, pp.365–369

Brown, M., Davies, I. M., Moffat, C. F., Craft, J. A. (2006). Application of SSH and a macroarray to investigate altered gene expression in *Mytilus edulis* in response to exposure to benzo[a]pyrene. *Mar Environ Res* vol.62, pp.S128–S135

Cefalu, W. T., Bell-Farrow, A. D., Wang, Z. Q., Sonntag, W. E., Fu, M. X., Baynes, J. W., Thorpe, S. R. (1995). Caloric restriction decreases age-dependent accumulation of the glycoxidation products, N epsilon-(carboxymethyl)lysine and pentosidine, in rat skin collagen. *J Gerontol A Biol Sci Med Sci* vol.50, pp.B337–B341

Cota, D., Proulx, K., Smith, K. A., Kozma, S. C., Thomas, G., Woods, S. C., Seeley, R. J. (2006). Hypothalamic mTOR signaling regulates food intake. *Science* vol.312, pp.927–930

Crouse, G. F. (1996). Mismatch repair systems in *Saccharomyces cerevisiae*. In: Nickoloff, J., Hoekstra, M. (ed) *DNA damage and repair—biochemistry, genetics and cell biology*. Humana Press, Clifton NJ, pp.411–448

Crozatier, M., Ubeda, J. M., Vincent, A., Meister, M. (2004). Cellular immune response to parasitization in *Drosophila* requires the EBF orthologue collier. *PLoS Biol*

vol.2, pp.1107–1113

de la Vega, E., Degnan, B. M., Hall, M. R., Wilson, K. J. (2007). Differential expression of immune- related genes and transposable elements in black tiger shrimp (*Penaeus monodon*) exposed to a range of environmental stressors. *Fish Shellfish Immunol* vol.23, pp.1072–1088

Edwards, S., Li, C. M., Levy, D. L., Brown, J., Snow, P. M., Campbell, J. L. (2003). *Saccharomyces cerevisiae* DNA polymerase epsilon and polymerase sigma interact physically and functionally, suggesting a role for polymerase epsilon in sister chromatid cohesion. *Mol Cell Biol* vol.23, pp.2733–2748

Egami, N. (1972). Tasaiboudoubutsu no Baai. In: *Aging no Seibutsugaku (Biology of Aging) in Japanese*. Iwanami Shoten, Tokyo, pp.99–121

Finkel, T., and Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature* vol.408, pp.239–47

French, S. S., DeNardo, D. F., Moore, M. C. (2007) Trade-offs between the reproductive and immune systems: facultative responses to resources or obligate responses to reproduction? *Am Nat* vol.170, pp.79–89

Frye, M., and Watt, F. M. (2006). The RNA methyltransferase Misu (NSun2) mediates Myc-induced proliferation and is upregulated in tumors. *Curr Biol* vol.16, pp.971–981

Fussmann, G. F., Ellner, S. P., Hairston, N. G. (2003). Evolution as a critical component of plankton dynamics. *Proc Biol Sci* vol.270, pp.1015–1022

Gao, J., and Richardson, D. R. (2001). The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents, IV: The mechanisms involved in inhibiting cell-cycle progression. *Blood* vol. 98, pp.842–850

Hagiwara, A., Gallardo, W. G., Assavaaree, M., Kotani, T., de Araujo, A. B. (2001). Live food production in Japan: recent progress and future aspects. *Aquaculture* vol. 200, pp.111–127

Harris, S. B., Gunion, M. W., Rosenthal, M. J., Walford, R. L. (1994). Serum glucose, glucose tolerance, corticosterone and free fatty acids during aging in energy restricted mice. *Mech Ageing Dev* vol.73, pp.209–221

Hebert, P. D. N. (1987). Genotypic characteristics of cyclical parthenogens and their obligately asexual derivatives. In: Stearns, S. C. (ed) *The Evolution of Sex and its Consequences*. Birkhäuser, Basel, pp.175–195

Hiraga, S., Hagihara-Hayashi, A., Ohya, T., Sugino, A. (2005). DNA polymerases alpha, delta, and epsilon localize and function together at replication forks in *Saccharomyces cerevisiae*. *Genes Cells* vol.10, pp.297–309

Iaccarino, I., Palombo, F., Drummond, J., Totty, N. F., Hsuan, J. J., Modrich, P., Jiricny, J. (1996). MSH6, a *Saccharomyces cerevisiae* protein that binds to mismatches as a heterodimer with MSH2. *Curr Biol* vol.6, pp.484–486

Inoki, K., Corradetti, M. N., Guan, K. L. (2005). Dysregulation of the TSC-mTOR pathway in human disease. *Nat Genet* vol.37, pp.19–24

Kaneko, G., Yoshinaga, T., Yanagawa, Y., Kinoshita, S., Tsukamoto, K., Watabe, S. (2005). Molecular Characterization of Mn-superoxide Dismutase and Gene Expression Studies in Dietary Restricted *Brachionus plicatilis* Rotifers. *Hydrobiologia* vol.546, pp.117–123

Kastan, M. B., Zhan, Q., el-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., Fornace, A. J., Jr (1992). A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. *Cell* vol.71, pp.587–597

Kemnitz, J. W., Roecker, E. B., Weindruch, R., Elson, D. F., Baum, S. T., Bergman, R. N. (1994). Dietary restriction increases insulin sensitivity and lowers blood glucose in rhesus monkeys. *Am J Physiol* vol. 266, pp.E540–E547

Kolodner, R. (1996). Biochemistry and genetics of eukaryotic mismatch repair. *Genes Dev* vol.10, pp.1433–1442

Krasnov, A., Koskinen, H., Afanasyev, S., Mölsä, H. (2005). Transcribed Tc1-like transposons in salmonid fish. *BMC Genomics* vol.6, pp.107

Levine, A. J. (1997). p53, the cellular gatekeeper for growth and division. *Cell* vol.88, pp.323-331

Li, M., McGrail, M., Serr, M., Hays, T. S. (1994). *Drosophila* cytoplasmic dynein, a microtubule motor that is asymmetrically localized in the oocyte. *J Cell Biol* vol.126, pp.1475–1494

Lok, E., Scott, F. W., Mongeau, R., Nera, E. A., Malcolm, S., Clayson, D. B. (1990). Calorie restriction and cellular proliferation in various tissues of the female Swiss Webster mouse. *Cancer Lett* vol.51, pp.67–73

Lu, H., Guo, X., Meng, X., Liu, J., Allen, C., Wray, J., Nickoloff, J. A., Shen, Z. (2005). The BRCA2-interacting protein BCCIP functions in RAD51 and BRCA2 focus formation and homologous recombinational repair. *Mol Cell Biol* vol.25, pp.1949–1957

Lu, M. H., Hinson, W. G., Turturro, A., Sheldon, W. G., Hart, R. W. (1993). Cell proliferation by cell cycle analysis in young and old dietary restricted mice. *Mech Ageing Dev* vol.68, pp.151–162

Maier, A. B., Westendorp, R. G., VAN Heemst, D. (2007). Beta-galactosidase activity as a biomarker of replicative senescence during the course of human fibroblast cultures. *Ann N Y Acad Sci* vol.1100, pp.323–332

Masoro, E. J., McCarter, R. J., Katz, M. S., McMahan, C. A. (1992). Dietary restriction alters characteristics of glucose fuel use. *J Gerontol* vol.47, pp.B202–B208

McGrail, M. and Hays, T. S. (1997). The microtubule motor cytoplasmic dynein is required for spindle orientation during germline cell divisions and oocyte differentiation in *Drosophila*. *Development* vol.124, pp.2409–2419

Meng, X., Liu, J., Shen, Z. (2004). Inhibition of G1 to S cell cycle progression by BCCIP beta. *Cell Cycle* vol.3, pp.343–348

Modrich, P. (1997). Strand-specific mismatch repair in mammalian cells. *J Biol Chem* vol.272, pp.24727–24730

Modrich, P. and Lahue, R. (1996). Mismatch repair in replication fidelity, genetic recombination, and cancer biology. *Annu Rev Biochem* vol.65, pp.101–133

Mori, S. (1998). *Doubutsu no Seitai (Animal Ecology),* 2nd edn. Kyoto University Press, Kyoto in Japanese

Reynders, H., van der Ven, K., Moens, L. N., van Remortel, P., De Coen, W. M., Blust, R. (2006). Patterns of gene expression in carp liver after exposure to a mixture of waterborne and dietary cadmium using a custom-made microarray. *Aquat Toxicol* vol.80, pp.180–193

Ryan, E. D., Tracy, J. W., Kohlhaw, G. B. (1973). Subcellular localization of the leucine biosynthetic enzymes in yeast. *J Bacteriol* vol.116, pp.222–225

Shikata, K., Sasa-Masuda, T., Okuno, Y., Waga, S., Sugino, A. (2006). The DNA polymerase activity of Pol epsilon holoenzyme is required for rapid and efficient chromosomal DNA replication in *Xenopus* egg extracts. *BMC Biochem* vol.7, pp.21

Shirotani-Ikejima, H., Kokame, K., Hamuro, T., Bu, G., Kato, H., Miyata, T. (2002). Tissue factor pathway inhibitor induces expression of JUNB and GADD45B mRNAs. *Biochem Biophys Res Commun* vol.299, pp.847–852

Soetaert, A., Moens, L. N., Van der Ven, K., Van Leemput, K., Naudts, B., Blust, R., De Coen, W. M. (2006). Molecular impact of propiconazole on *Daphnia magna* using a reproduction-related cDNA array. *Comp Biochem Physiol Part C* vol.142, pp.66–76

Stoica, C., Carmichael, J. B., Parker, H., Pare, J., Hobman, T. C. (2006). Interactions between the RNA interference effector protein Ago1 and 14-3-3 proteins: consequences for cell cycle progression. *J Biol Chem* vol.281, pp.37646–37651

Sugino, A. (1995). Yeast DNA polymerases and their role at the replication fork. *Trends Biochem Sci* vol.20, pp.319–323

Swan, A., Nguyen, T., Suter, B. (1999). *Drosophila* Lissencephaly-1 functions with Bic-D and dynein in oocyte determination and nuclear positioning. *Nat Cell Biol* vol.1, pp.444–449

Verdone-Smith, C., Enesco, H. E. (1982). The effect of dietary restriction on cell division potential, DNA content and enzyme levels in the rotifer *Asplanchna brightwelli*. *Exp Gerontol* vol.17, pp.463–471

Wang, D. S., Shaw, R., Hattori, M., Arai, H., Inoue, K., Shaw, G. (1995). Binding of pleckstrin homology domains to WD40/beta-transducin repeat containing segments of the protein product of the Lis-1 gene. *Biochem Biophys Res Commun* vol.209, pp.622–629

Wang, L. and Wu, X. (2007). Identification of differentially expressed genes in lipopolysaccharide- stimulated yellow grouper *Epinephelus awoara* spleen. *Fish Shellfish Immunol* vol.23, pp.354–363

Yoshinaga, T., Hagiwara, A., Tsukamoto, K. (2000). Effect of periodical starvation on the life history of *Brachionus plicatilis* O.F. Müller (Rotifera): a possible strategy for population stability. *J Exp Mar Bio Ecol* vol.253, pp.253–260

Yoshinaga, T., Hagiwara, A., Tsukamoto, K. (2001a). Why do rotifer populations present a typical sigmoid growth curve? *Hydrobiologia* vol.446, pp.99–105

Yoshinaga, T., Hagiwara, A., Tsukamoto, K. (2001b). Effect of periodical starvation on the survival of offspring in the rotifer *Brachionus plicatilis*. *Fish Sci* vol.67, pp.373–374

Yoshinaga, T., Kaneko, G., Kinoshita, S., Tsukamoto, K., Watabe, S. (2003). The molecular mechanisms of life history alterations in a rotifer: a novel approach in population dynamics. *Comp Biochem Physiol Part B* vol.136, pp.715–722

Effects of Nitrogen Fixing Bacteria, *Azotobacter* spp. and *Azospirillum* spp. on the Growth of Rice *Oryza sativa* L.

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Abstract

In order to evaluate the effect of *Azotobacter* and *Azospirillum* inoculants on the growth of rice, pot experiment with four treatments (*Azotobacter* sugarcane 1-T_1, *Azotobacter* maize 2- T_2, *Azospirillum* sugarcane 3-T_3 and *Azospirillum* maize 2 -T_4) and one control (without bacteria) each with six replicates was carried out in Zoology Department, University of Mandalay during March to November 2011. The results indicated that the shoot length of treatment increased over control with a range of 8.04 to 33.14% and the root length of treatments increased over control with a range of 2.13 to 132.89%. The fresh shoot weight and fresh root weight of treatments increased over control with a range of 0 to 1160% and 0 to 462.92% respectively. The dry shoot and root weights of treatments at maturity stage increased significantly ($p<0.01$) over control with a range of 53.31 to 109.28% and 163.89 to 280.56% respectively. In this study, panicle length and total seed numbers per panicle of treatments increased significantly ($p<0.01$) over control with a range of 18.75 to 35.02% and 96.55 to 114.83% respectively. *Azotobacter* sugarcane 1 is found to be the most effective species for the growth of rice and all tested bacteria could be useful as biofertilizers to increase the productivity of crops.

Key words: *Azotobacte, Azospirillum,* nitrogen fixing bacteria, rice

Introduction

Rice (*Oryza sativa* L.) is the staple food for half of the world's population especially in oriental countries (Kannan and Ponmurugan, 2010). In the next three decades, the world will need to feed the extra billion people. Nitrogen is the major nutrient limiting rice production. Rice requires 1 kg of nitrogen to produce 15-20 kg of grain. Increased future demand for rice will entail increased application of fertilizer N (Ladha and Reddy, 2003). Finding an alternative for such a nutrient has become important. Soil microorganisms like *Azotobacter* and *Azospirillum* are free living N_2 fixing bacteria which can successfully grow in the rhizosphere zone of crops and fix 10-20 kg N ha^{-1} cropping season (Yasari *et al.*, 2008).

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Various kinds of cereals were tested by using a member of nitrogen fixing bacteria viz., *Azotobacter*, *Nitrosomonas* and *Azospirillum* to increase yield under controlled conditions (Kannan and Ponmurugan, 2010).

Several authors have shown the beneficial effects of *Azotobacter chroococcum* on vegetative growth and yield of maize (Mishra *et al*., 1995; Pandey *et al*., 1998; Radwan, 1998), as well as the positive effect of inoculation with this bacterium on wheat (Elshanshoury, 1995; Pati *et al*., 1995; Fares, 1997) (cited in Aquilanti *et al.*, 2004).

In numerous studies, *Azospirillum* inoculations have been reported to reduce the use of chemical fertilizers in particular nitrogen by 20% to 50% (Attitalla *et al*., 2010). Inoculation of plants with *Azospirillum* strains alters root morphology, increases numerous plant shoot growth parameters, and eventually increases the yield of many cereal crops (Patriquin *et al*., 1983), vegetables, and other agricultural plants (Sala *et al*., 1985; Crossman and Hill, 1987; Bashan *et al*., 1989) (cited in Bashan *et al*., 1990).

Inoculation of rice with *Azospirillum* strains (10^7 CFU ml^{-1}) can be increased in plant growth and grain yield of rice (Zaw Lwin Oo, 2010).

Based on the above information, it was realized that the isolated bacteria should be investigated to determine their effects on the growth of plants. This paper aimed to determine the effects of bacteria on the growth of rice.

Materials and Methods

Experimental Site and Study Period

Pot experiment was conducted in the net house of Zoology Department, University of Mandalay during March to November 2011 (Plate 1.A).

Source of Bacteria

Bacteria were isolated from the root of sugarcane and maize. Among them, the best growth species; *Azotobacter* sugarcane 1 (T_1), *Azotobacter* maize 2 (T_2), *Azospirillum* sugarcane 3 (T_3) and *Azospirillum* maize 2 (T_4) were selected and used to inoculate the paddy seed.

Preparation of Soil for Pot Experiment

The soil for pot experiment was collected from the field site near Tada-U Town, Mandalay Region. For soil analysis, soil was collected at a depth of 15 cm with V-shaped method and Zig-zag pattern. These were dried at room

temperature and transported to the Laboratory of Soil Science Section, Department of Agricultural Research, Yezin. Sterilization of soils for pot experiment were made by autoclaving (121°C, 1.05 $kgcm^{-2}$, 30 minutes) and then placed into oven for one night and again sterilized by 180°C for one hour. After sterilization, 5 kg of soil was placed into each of sterilized earthen pot and sealed with plastic bag before sowing (Plate 1.A).

Source of Paddy Seeds and Sterilization of Seeds

Paddy seeds (*Oryza sativa* L.) were obtained from Seed Division, Department of Agriculture Service, Mandalay Region. The seeds were surface sterilized by treatment with 0.1% $HgCl_2$ for 2 min which were then washed 5 times with sterile distilled water.

Pot Experiment

Before sowing, pots with 5 kg of sterile soil were watered and stirred to mix ingredients of soil thoroughly. The following four inocula (bacteria) and one control with six replicates were imposed: *Azotobacter* sugarcane 1 (T_1), *Azotobacter* maize 2 (T_2), *Azospirillum* sugarcane 3 (T_3) and *Azospirillum* maize 2 (T_4) and control (C) without bacteria. Both inoculated and un-inoculated (control) seeds were then sown in the pots (30 seeds per pot) of net house under natural condition. Pots were arranged in complete randomized design and placed 23 cm apart from each other (Plate 1.B). Thinning was done after 7 days of sowing and 15 seedlings were kept. Inoculations of each diluted bacterial suspension (10^6 CFU ml^{-1}) and sterile diluted nutrient broth (one ml per seedlings) to pots were made from 10^{th} day onwards at 10 day intervals until harvesting period. One liter of filtered tap water was used to water each pot two times a day. No chemical fertilizer was applied during the experiment.

Parameters of Growth Employed

A single seedling was selected at random from each pot on the 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th}, 60^{th}, 70^{th}, and 80^{th} days of sowing and necessary measurements were taken. At maturity, the crops were harvested and fresh and dry weights of shoot and root were recorded. The dry weights were determined after placing the plants into the oven at 80ºC for 48 h. The length of panicles, the numbers of seed per panicle and 1000 grains weight were also recorded.

Statistical Analysis

The measured data were subjected to analysis of variance and means of sample were compared by least significance difference (LSD) using SPSS (Statistical Package for Social Science) software version 17.0.

Results

The experimental soil was texturally made of silty clay and measured 8.86 of pH, 61 ppm of available N, 9 ppm of available P, 147 ppm of available K, 165% organic matter and 0.81% moisture.

Inoculation with *Azotobacter* and *Azospirillum* species showed noticeable effects on the growth of paddy plants. Mean values of shoot length, root length, shoot weight, root weight, panicle length, seed numbers per panicle and 1000 grains weight are presented in Tables 1, 2,3,4,5 and 6 respectively.

The percentage of treated plant height increase over control ranged from minimum 8.04% at 10 DAS (days after sowing) to maximum 33.14% at 30 DAS. Significant effects of inoculation on plant height were observed at 30 DAS to 80 DAS ($p<0.05$) (Table 1). The percent increase of root length of treated plants over control ranged from minimum 2.13% at 20 DAS to maximum 132.89% at 80 DAS. Significant effects were observed at 40 DAS, 50 DAS and 80 DAS ($p<0.05$) (Table 2). The percent increase of fresh shoot weight of inoculated plants over control ranged from 0% at 10 DAS to 1160% at 50 DAS. Shoot weights were significantly different ($p<0.01$) between control and inoculated plants at 20 DAS to 80 DAS, but not significant at 10 DAS (Table 3). The percent of fresh root weight increase over control ranged from 0% to 266.67%. Root weights were not significantly different between control and inoculated plants at 10 DAS, 30 DAS and 40 DAS. But significant effects were observed at 20 DAS, 50 DAS, 60 DAS and 80 DAS ($p<0.05$) (Table 4).

At maturity stage, the effects of inoculation were significant ($p<0.01$) over control in all plant growth and yield parameters (Plate 1.C) (Table 5). The percent of panicle length increase over control ranged from 18.75% to 35.02%. Significant ($p<0.01$) effect of *Azotobacter* and *Azospirillum* inoculation on panicle length were observed in this work. The longest panicle length was observed in *Azotobacter* maize 2 (T_2) inoculated plant (22.17 cm) (Plate 1.D). The percent of total and fertile seed numbers per panicle increase significantly ($p<0.01$) over control ranged from 96.55% to 144.83% and 142.86% to 209.52% respectively. The weights of 1000 grains were not significantly different between control and

treatments. But the 1000 grain weights of inoculated plants (a range of 22.802 g to 23.05g) were higher than the control (21.83g) (Table 6).

Table 1. Mean shoot length of paddy plant at 10th, 20th, 30th, 40th, 50th, 60th, 70th and 80th days after sowing in control and treatment (n = 6)

Treatment	Shoot length (Mean ± SD (cm))							
	10 DAS	20 DAS	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	80 DAS
Control	13.69^{a} ±4.86	29.52^{a} ±5.86	39.53^{a} ±3.71	42.80^{a} ±1.33	61.73^{a} ±8.56	65.68^{a} ±2.17	67.32^{a} ±8.72	68.42^{a} ±13.75
T_1	16.73^{a} ±3.97	34.62^{a} ±5.44	52.63^{c} ±5.28	55.47^{b} ±5.66	77.83^{c} ±5.10	78.50^{b} ±7.53	80.08^{b} ±6.09	83.28^{b} ±5.38
T_2	15.95^{a} ±4.66	34.2^{a} ±3.10	45.13^{a} ±4.03	51.70^{b} ±5.33	69.67^{b} ±11.27	75.68^{b} ±5.77	78.88^{b} ±4.97	87.17^{b} ±10.75
T_3	15.00^{a} ±4.09	34.72^{a} ±3.18	43.78^{a} ±6.19	51.60^{b} ±7.09	70.08^{b} ±4.85	78.17^{b} ±7.22	79.40^{b} ±8.96	81.97^{b} ±4.36
T_4	16.57^{a} ±4.14	36.62^{a} ±2.27	47.12^{b} ±4.62	52.53^{b} ±6.49	74.70^{b} ±2.94	77.58^{b} ±5.18	77.60^{b} ±7.55	82.48^{b} ±6.88

Table 2. Mean root length of paddy plant at 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th}, 60^{th}, 70^{th} and 80^{th} days after sowing in control and treatment (n = 6)

Treatment	Root length (Mean ± SD (cm))							
	10 DAS	20 DAS	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	80 DAS
Control	2.5 [a] ±0.63	3.7 [a] ±1.29	4.83[a] ±1.08	5.05[a] ±0.54	7.67[a] ±1.70	7.73[a] ±1.79	7.90[a] ±0.39	8.33[a] ±5.42
T_1	3.17 [a] ±0.82	3.98 [a] ±0.95	6.05[a] ±0.93	8.37[b] ±4.37	12.18[b] ±7.61	12.88[b] ±4.46	14.62[b] ±3.66	15.58[b] ±8.84
T_2	3.73 [b] ±1.32	4.58 [a] ±2.56	5.08[a] ±1.07	7.22[b] ±1.18	8.88 [b] ±2.59	12.57[b] ±3.89	14.58[b] ±8.79	16.12[b] ±4.29
T_3	3.25 [a] ±0.52	3.83 [a] ±1.20	5.23[a] ±3.50	7.08[b] ±1.30	11.22[b] ±4.87	13.62[b] ±3.98	14.12[b] ±3.08	19.40[b] ±6.77
T_4	3.33 [a] ±0.88	3.92 [a] ±1.77	6.43[a] ±2.56	9.80[b] ±1.94	10.33[b] ±2.52	12.60[b] ±3.72	14.20[b] ±2.88	15.28[b] ±2.61

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

DAS = Days after sowing

Control = Dilute nutrient broth without inoculum

T_1 = *Azotobacter* sugarcane 1

T_2 = *Azotobacter* maize 2

T_3 = *Azospirillum* sugarcane 3

T_4 = *Azospirillum* maize 2

Table 3. Mean shoot weight of paddy plant at 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th}, 60^{th}, 70^{th} and 80^{th} days after sowing in control and treatment (n = 6)

Treatment	Shoot weight (Mean ± SD (g))							
	10 DAS	20 DAS	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	80 DAS
Control	0.02^{a} ±0.01	0.04^{a} ±0.01	0.08^{a} ±0.030	0.14^{a} ±0.06	0.20^{a} ±0.05	1.45^{a} ±0.26	1.47^{a} ±0.15	2.09^{a} ±1.21
T_1	0.02^{a} ±0.01	0.09^{c} ±0.04	0.40^{c} ±0.13	0.42^{b} ±0.21	2.52^{b} ±0.67	3.10^{b} ±1.13	3.70^{b} ±0.85	4.13^{b} ±0.95
T_2	0.02^{a} ±0.01	0.07^{b} ±0.01	0.23^{b} ±0.07	0.29^{a} ±0.08	1.87^{b} ±0.48	2.43^{b} ±0.39	3.40^{b} ±1.29	4.97^{b} ±1.35
T_3	0.02^{a} ±0.004	0.07^{a} ±0.014	0.22^{b} ±0.08	0.31^{b} ±0.11	1.90^{b} ±0.61	3.06^{b} ±0.75	3.46^{b} ±1.00	3.60^{a} ±1.56
T_4	0.02^{a} ±0.007	0.08^{b} ±0.03	0.29^{b} ±0.03	0.45^{b} ±0.08	1.93^{b} ±0.37	2.69^{b} ±0.43	3.54^{b} ±1.12	3.79^{b} ±1.57

Table 4. Mean root weight of paddy plant at 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th}, 60^{th}, 70^{th}, 80^{th} days after sowing in control and treatment (n = 6)

Treatment	Root weight (Mean ± SD (g))							
	10 DAS	20 DAS	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS	80 DAS
Control	0.02^{a} ±0.002	0.02^{a} ±0.01	0.02^{a} ±0.015	0.03^{a} ±0.01	0.04^{a} ±0.01	0.05^{a} ±0.02	0.06^{a} ±0.01	0.10^{a} ±0.06
T_1	0.02^{a} ±0.01	0.03^{a} ±0.01	0.04^{a} ±0.02	0.06^{b} ±0.04	0.10^{b} ±0.05	0.10^{b} ±0.03	0.22^{b} ±0.02	0.28^{b} ±0.09
T_2	0.03^{a} ±0.002	0.03^{a} ±0.01	0.03^{a} ±0.01	0.07^{b} ±0.05	0.08^{b} ±0.02	0.10^{b} ±0.05	0.12^{a} ±0.04	0.30^{b} ±0.02
T_3	0.02^{a} ±0.01	0.03^{a} ±0.002	0.03^{a} ±0.01	0.04^{a} ±0.02	0.07^{a} ±0.03	0.09^{b} ±0.03	0.13^{a} ±0.03	0.26^{b} ±0.03
T_4	0.02^{a} ±0.01	0.03^{a} ±0.005	0.04^{a} ±0.02	0.05^{a} ±0.03	0.07^{a} ±0.01	0.10^{b} ±0.03	0.12^{a} ±0.05	0.25^{b} ±0.05

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

DAS = Days after sowing T_1 = *Azotobacter* sugarcane 1 T_3 =*Azospirillum* sugarcane 3

Control = Dilute nutrient broth without inoculum T_2 = *Azotobacter* maize 2 T_4 =*Azospirillum* maize 2

Table 5. Mean shoot and root lengths, fresh and dry weights of shoot and root of paddy plant at maturity stage (n=6)

Treatments	Mean length ± SD (cm)		Mean weight ± SD (g)			
	Shoot	Root	Fresh shoot	Fresh root	Dry shoot	Dry root
Control	70.67^{a} ±13.06	8.42^{a} ±1.02	2.38^{a} ±1.00	0.089^{a} ±0.031	0.711^{a} ±0.14	0.036^{a} ±0.004
T_1	104.75^{b} ±14.19	16.97^{b} ±6.82	6.92^{b} ±1.26	0.437^{b} ±0.077	1.488^{c} ±0.33	0.137^{b} ±0.060
T_2	95.33^{b} ±12.57	16.83^{b} ±3.67	6.34^{b} ±1.31	0.394^{b} ±0.108	1.201^{b} ±0.09	0.099^{b} ±0.033
T_3	89.13^{b} ±4.88	19.63^{b} ±0.95	4.95^{b} ±1.83	0.501^{b} ±0.073	1.090^{b} ±0.07	0.095^{b} ±0.034
T_4	91.70^{b} ±14.54	16.17^{b} ±2.58	5.47^{b} ±1.47	0.410^{b} ±0.082	1.212^{b} ±0.35	0.102^{b} ±0.041

Table 6. Mean panicle length, seed numbers per panicle and 1000 grains weight

Treatments	Panicle length mean±SD(cm)	Total seed numbers/panicle mean±SD	Fertile seed numbers/panicle mean±SD	Sterile seed numbers/panicle mean±SD	1000 grains weight (g)
Control	16.42^{a} ±1.28	29^{a} ±10	21^{a} ±8	8^{a} ±3	21.828^{a}
T_1	21.17^{b} ±1.94	71^{b} ±17	65^{b} ±17	7^{a} ±4	23.048^{a}
T_2	22.17^{b} ±2.40	60^{b} ±14	53^{b} ±15	7^{a} ±3	22.960^{a}
T_3	19.50^{b} ±1.98	57^{b} ±17	51^{b} ±14	6^{a} ±4	23.004^{a}
T_4	21.67^{b} ±2.73	63^{b} ±12	55^{b} ±10	8^{a} ±2	22.804^{a}

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

Control = Dilute nutrient broth without inoculum T_2 = *Azotobacter* maize 2 T_4 =*Azospirillum* maize 2

T_1 = *Azotobacter* sugarcane 1 T_3 = *Azospirillum* sugarcane 3

A. Pots in net house before sowing

B. Pots with paddy seedlings

C. Paddy plant at maturity stage

D. Panicle at maturity stage

Plate 1. Pot experiment in net house and effect of bacteria on the paddy plant at maturity stage

Discussion

The best grown bacteria were selected as inoculums for pot experiment of paddy plant. The bacterial concentration for the inoculums used in this work is 10^6 CFU/ml. This is the optimal concentration for many plant species (Bashan, 1986 and Bashan *et al.,* 1989) (cited in Puente and Bashan, 1993).

The results indicated that the growth of *Azotobacter* spp. and *Azospirillum* spp. treated seedlings excelled over the untreated ones. Effects of bacterial inoculation on plant height at different stages of growth were observed in this study with an increase over control ranged from minimum 8.04% at 10 DAS to maximum 33.14% at 30 DAS. At 20 DAS, *Azospirillum* maize 2 was found to be effective in increasing plant height and caused 24.05% increase over control. At 30 DAS, 40 DAS, 50 DAS, 60 DAS and 70 DAS, *Azotobacter* sugarcane 1 was found to be highly effective in increasing plant height. At 80 DAS, *Azotobacter* maize 2 inoculated plants showed the maximum height (87.17 cm) with 27.40% increase over control. At maturity stage, *Azotobacter* sugarcane 1 inoculated plants showed the maximum height (104.75cm) with 48.22% increase over control. At 20 DAS, the significant effects of treatment on the plant height were observed in *Azospirillum* sugarcane 3 ($p<0.05$) and *Azospirillum* maize 2 ($p<0.01$) inoculated plants. Significant differences of plant height were observed between the treatments and control at 30 DAS to 80 DAS and at maturity stage ($p<0.05$). However, the difference in plant height among four treatments was not significant ($p>0.05$). The same condition was also reported by Gunarto *et al.* (1999) in that the inoculation of indigenous strains of *Azospirillum* to rice led to increase plant height at some growth stages.

In this study, root length of treated increased over control with the range of 2.13% to 132.89%. At 80 DAS and maturity stage, *Azospirillum* sugarcane 3 inoculated plants showed the maximum length (19.40 cm and 19.63 cm) with 132.89% and 133.14% respectively increase over control. Significant effects were observed at 40 DAS to 80 DAS.

Kannan and Ponmurugan (2010) reported that 10^{th}, 20^{th} and 30^{th} days of sowing revealed that *Azospirillum* treated seeds of rice had higher productivity than control. The seedlings from this particular biofertilizer treated seeds had longer shoot and root lengths than the untreated ones. So, the results in this work are in agreement with the previous work.

The percent increase of fresh shoot weight of treated plants over control ranged from 0 to 1160% at 10 DAS and 50 DAS respectively. Significant effects were observed at 20 DAS to 80 DAS and at maturity stage ($p<0.01$). The percent increase over control were very height at 50 DAS in all treated plants and highly significant ($p<0.01$) over the control. At maturity stage, the highest fresh and dry shoot weights were observed in *Azotobacter* sugarcane 1 inoculated plant.

The percent increase of fresh root weight of treated plants over control ranged from 0% to 266.67%. Significant ($p<0.05$) effects were observed at 20 DAS, 50 DAS, 60 DAS, 80 DAS and at maturity stage. At maturity stage, *Azospirillum* sugarcane 3 inoculated plants possessed maximum fresh and dry root weights.

Kannan and Ponmurugan (2010) stated that the fresh weights of root and shoot system of paddy varieties were also found to be increased to a considerable extent in *Azospirillum* treated seedlings.

The percent increase of fresh weight of treated wheat plants over control ranged from 24.71 to 106.22 and 43.89 to 57.23 at pre-flowering stage (30-35 days after sowing) and post-flowering stage (45-50 days after sowing) respectively (Mubassara *et al.*, 2008).

In this work, the percent of fresh weight of treated plants over control ranged from 175% to 400% and 835% to 1160% at 30 DAS and 50 DAS respectively. So, the fresh weights of treated plants were more enhanced than the control and are in agreement with the previous works.

In this study, panicle lengths were significantly ($p<0.01$) increased over control with the percentage of 18.75% to 35.02%. Total and fertile seed numbers per panicle were also significantly ($p<0.01$) increased over control of 96.55% to 144.83% and 142.86% to 209.52% respectively. The highest total and fertile seed numbers per panicle were observed in *Azotobacter* sugarcane 1 inoculated plant and the lowest in uninoculated (control) plant.

So, *Azotobacter* sugarcane 1 is the most effective in all plant growth and yield parameters followed by *Azotobacter* maize 2, *Azospirillum* maize 2 and *Azospirillum* sugarcane 3 respectively.

Zaw Lwin Oo (2010) also stated that the inoculation with selected strains of *Azospirillum* sp. causes significant increase in length of panicle of rice with ranges of 3.95% to 7.01%. Total seeds per panicle were significantly different from control.

A significant positive response with single inoculation of *Azotobacter chroococcum* in all growth parameters of both vegetative and reproductive stages of rice plants can be attributed to the ability to fix atmospheric nitrogen (Prajapati *et al.*, 2008).

The rice plant inoculated with *Azotobacter* spp. and *Azospirillum* spp. showed significant beneficial effect on all the growth parameters and the findings are in agreement with the results of above works.

In this work, 1000 grains weights of inoculated plants were not significantly different from the control. This result is same with the report of Zaw Lwin Oo (2010).

Conclusion

In conclusion, the inoculation of isolated bacteria from rhizosphere of sugarcane and maize, into paddy plants showed the enhancement of the plant growth parameters. So, the isolated bacteria of this work were not plant specific bacteria and can be used as biofertilizers for the growth and yield parameters of commercially important cash crops and other plants. But field experiment will be needed to confirm the effect of these isolated bacteria. Therefore, the increase use of the various biological processes in soil will decisively contribute to make agriculture more productive with less harm to the environment. This fact may be of importance for developing countries where the use of fertilizers is costly. It is hoped for substantial increase in food production in order to eliminate undernourishment and poverty, which is the main goal to be achieved by using biofertilizers.

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References

Aquilanti, L., F. Favilli, and F. Clementi, (2004). Comparison of Different Strategies for Isolation and Preliminary Identification of *Azotobacter* from Soil Samples. *Soil Biology and Biochemistry*, 36: 1475-1483.

Attitalla, I.H., A.M. Alhasin, M.A. Nasib, A.H. Ghazali, L. Zakaria, H.M. Jais, I.A.A. Balal, and B. Salleh, (2010). Occurrence and Microbiological Characteristics of *Azospirillum* Strains Associated with Leguminous and Non-leguminous Plants in Al Jabal Al Akhdar Eco-Region, Libya. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 8(6): 617-625.

Bashan, Y., S.K. Harrison, and R.E. Whitmoyer, (1990). Enhanced Growth of Wheat and Soybean Plants Inoculated with *Azospirillum brasilense* is not Necessarily Due to General Enhancement of Mineral Uptake. *Applied and Environmental Microbiology*, 56(3): 769-775.

Gunarto, I., K. Adachi, and T. Senboku, (1999). Isolation and Selection of Indigenous *Azospirillum* spp. from a Subtropical Island and Effect of Inoculation on Growth of Lowland Rice Under Several Levels of N Application. *Biol. Ferti. Soil*, 28: 129-135.

Kannan, T. and P. Ponmurugan, (2010). Response of Paddy (*Oryza sativa* L.) Varieties to *Azospirillum brasilense* Inoculation. *Journal of Phytology*, 2(6): 8-13.

Ladha, J.K. and P.M. Reddy, (2003). Nitrogen Fixation in Rice Systems: State of Knowledge and Future Prospects. *Plant and Soil*., 252: 151-167.

Mubassara, S., Z.U.M. Khan, M.M. Rahman, F.K. Patwary, and M.A. Akond, (2008). Seeds Inoculation Effect of *Azospirilum* spp. on Growth, Biomass and Yield Parameters of Wheat. *Academic Journal of Plant Sciences*, 1(4): 56-61.

Prajapati, K., K.D. Yami, and A. Singh, (2008). Plant Growth Promotional Effect of *Azotobacter chroococcum*, *Piriformospora indica* and Vermicompost on Rice Plant. *Nepal Journal of Science and Technology*, 9: 85-90.

Puente, M.E and Y. Bashan, (1993). Effect of Inoculation with *Azospirillum brasilense* Strains on the Germination and Seedlings Growth of the Giant Columnar Cardon Cactus (*Pachycereus pringlei*). *Symbiosis*, 15: 49-60.

Yasari, E., A.M.E. Azadgolch, H. Pirdcushti, and S.Mozafari, (2008). *Azotobacter* and *Azospirillum* Inoculants as Biofertilizers in Canola (*Brassica napsu* L.) Cultivation. *Asian Journal of Plant Sciences*, 7(5): 490-494.

Zaw Lwin Oo, (2010). Isolation of *Associative Azospirillum* Strains from *Saccharum spontaneum* L., *Saccharum officinarum* L. *and Sorghum bicolor (L.)* Moench and Their Effects on Growth and Development of Ma-Naw-Thu-Kha Rice. PhD Dissertation, Department of Botany, University of Mandalay (Unpublished).74 pp.