ENVIRONMENT FRIENDLY CONTROL OF ARGULOSIS IN BROOD STOCK POND

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ENVIRONMENT FRIENDLY CONTROL OF ARGULOSIS IN BROOD STOCK POND

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Environment Friendly Control of Argulosis in Brood Stock Pond

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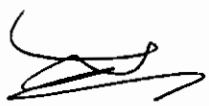
DECLARATION

The results submitted in this project thesis are entirely the candidate's own investigations and no part of the results has been accepted for any degree, nor it is being concurrently submitted for any degree.

Supervisor



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DEDICATED
TO
MY BELOVED PARENTS

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First of all, I wish to express my sincere gratefulness to Almighty Allah, who gave me the ability to complete this modest piece of work.

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CHAPTER-1

INTRODUCTION

INTRODUCTION

To increase fish production, it is necessary to reduce mortality through various contributory factors. Healthy, without disease and better quality brood is the major demand for produce better offspring, especially in large number and healthy seeds. *Argulus* sometimes referred to as "fish lice" is an obligatory ectoparasite infecting many fresh water fish species (Kollatsch, 1959). It represents major threat to health, both as a result of direct tissue damage and secondary infections. While describing the pathogenicity of *Argulus*, it could kill a larval eel with a single sting by injecting cytotoxic toxin into the host (Hoffman and Meyer, 1974) reported that. The parasites and diseases constitute one of the important factors in regulating the populations in natural environments and in fish (Tripathi, 1969). *Argulus* can survives only a few days (less than 48 h) without fish host and even shorter period in juveniles (Kollatsch, 1959). As juveniles hatch from eggs laid on the substrate, they require a fish host at least once during their short life span. This ectoparasite feeds by piercing the skin of its host, injecting a toxin and drawing off blood (Shimura and Inoue, 1984).

The fresh water louse, *Argulus* Müller a branchiuran ectoparasite causing the disease Argulosis has become a major hazard to the fish farmers as it is causing mortality, morbidity and growth loss to the carp ponds in different fish farms and hatcheries in Bangladesh. The mortality of carp due to *Argulus* infection in an experimental pond in Chadpur Fisheries Research Station done by Rahman (1968). Since then there have been many reports of Argulosis from fish ponds in the country. *Argulus* from fresh water wild fish was also reported by Ahmed and Sanaullah (1976). The maximum prevalence and intensity of infestation of *Argulus* sp. in carp pond occurred in the month of July and the female fishes are more susceptible to infestation and highest occurrence on pelvic region have been reported by Ahmed and Chowdhury (2002). The larger proportion of the female *Argulus* on the fish host, whereas free-swimming males are more frequent (Kollatsch, 1959 and Stammer, 1959). When *Argulus foliaceus* and its related species eggs attach to fish can be seen with the naked eye. *Argulus* is transparent and may take on the color of the host. The attached area is often red and irritated- the fish hemorrhagic reaction to the affection. Gravid females detaching from the hosts to lay eggs have to find a suitable substratum in the vicinity. Cyprinids and percids are the main hosts for *Argulus foliaceus* (Kennedy and Stage, 1982). Infected fish swam in an erratic way (Mikheev *et al.* 1998). 80.5 percent

infestation of *Argulus foliaceus* on young coregonid fishes during July to August and correlated to the water temperature (Zhilyukas and Rautskis, 1982).

Argulosis, a major threat to the fish hatchery owners in our country as it is increasing mortality, growth loss and economic loss to the carp farms and hatchery owners. Heavy infestation of *Argulus* sp. also been reported from many fish hatcheries like BCDM (BRAC Center for Development Management) at Gazipur, Pabna, Rajshahi, Dinajpur, Luxmipur (Raipur hatchery) and from few local hatcheries in Jessore like Monirampur Fish Farm, Mamoni hatchery and T.R hatchery at Sunamganj, Sylhet, Mitha and M.R. Hatchery at Manikganj. *Argulus* sp. made injures on the skin, the secondary bacterial or fungal infection, resulting reduces the commercial value of the fish (Kimura, 1960). After treating with some pesticides like Cymbush and Sumithion Argulosis continues. Although, some control has been achieved with chemicals, most commercial chemicals are costly to farmers. Moreover, these chemicals are toxic to aquatic fauna particularly the zooplanktons. Randomly use of pesticides to treat fish diseases like Argulosis over a period, develop to protect pesticides effect and cause even mortalities of the host (Baticados and Paclibare, 1992). Since *Argulus* is now resistant to some of the commonly used chemicals, the uses of more chemicals are unlikely to control this. *Argulus* are also reported to be controlled by dip treatments using potassium permanganate, common salt (Nacl), glacial acetic acid, gammaxene, as well as organophosphate pesticides and quicklime in water (Jafri and Ahmed, 1994; Padmavathi and Prasad, 1998). Since the chemical treatments carry some ecological risks, attempts also have been made by Shimura (1981) and Northcott *et al.* (1997) to control *Argulus* by the manipulation of pond ecology.

Most of the hatchery farmers reported that Argulosis as a major problem in the brood stock ponds of carps. Cymbush, Sumithion, Marshal, Imithion Nagos Resent, V-tex etc. pesticides used to prevent Argulosis. Randomly use of pesticide, the pond environment has been deteriorating as well as also negative on the productivity of pond. The *objectives* of the study were

- to know the condition, occurrence, intensity and impact of Argulosis;
- clinical symptoms of Argulosis;
- to know the biological control by using predatory species and mechanical control by using some substrate for collection and destruction of *Argulus* sp. egg clusters and
- dip treatment as means of control measure.

CHAPTER-2

REVIEW OF LITERATURE

2.1 Review of Literature on Research Work

The review was concerned with the taxonomic identification, occurrence, symptomatology, pathology, intensity and infestation of the *Argulus* sp. in relation to physico-chemical parameters, host specificity, control measures of the parasites and successful administration of pesticides. Emphasis was given to the researches of the Asian region with other important research works of the different parts of the world. This chapter reviewed the available literature.

Ramakrishna (1953) redescribed the *A. siamensis* in India, which was described earlier by South well, and Hora as *A. foliaceus*. He also reported the occurrence of a new sub-species *Argulus siamensis* subsp. *Peninsularis* of the same and two new species *Argulus giganteus* and *Argulus bengalensis*.

Central inland Fisheries Research station. Calcutta-Annual Report for the year 1953 (1955) reported that in a pond near Calcutta large numbers of fishes (Rohu, Catla, Mrigal) were died from the infection with *Argulus siamensis*. The infection was controlled by the application of 2 ppm of Gammexane in methylated spirit.

Gopalkrishan (1964) reported Argulosis in several stocking tanks of West Bengal and found that *Labeo rohita* was more susceptible to Argulosis than *Catla Catla* and *Cirrhina mrigala*. He also treated the argulosis by using W.D.P Gammexane in pond treatment to a strength of 0.2ppm repeating twice or thrice at weekly intervals and found it very effective.

Rahman (1967, 1968) reported that fish lice (*Argulus*) caused a mass mortality of major carps in a small pond at the Chadpur.

Tripathi (1969) reported on the treatment of *Argulus* sp. by repeated application at weekly intervals of W.D.P Gammexane in the water.

Shoskov and Kolarova (1977) studied on the action of neguvon and chlorophos aginst *Argulus foliaceus* in carps.

Thomas and Devaraj (1977) reported on the two new species of *Argulus*,Muller(*Argulus fluvitilis* and *Argulus cauveriensis*) from river Cauvery..

Canic *et al.*, (1977) reported *Argulus foliaceus* L.1758 as the causal agent of deaths of carp offsprings on a fishpond in Yogoslavia.

Inoue *et al.* (1980) studied the pesticidal effects of trichlorfon against *Argulus coregoni* parasitic on yearling Yamame (*Onchorhynchus masou*) and found that trichlorfon caused 100% mortality of *Argulus coregoni* at 20^0c and p^H 7.0 were obtained by the immersion in 6.25ppm for 1 hr, 12.5-50ppm for 30 min, 100ppm for 10 min. He also studied the lethal effects of trichlorfon on four species of juvenile salmonoids such as Yamame, Brook trout (*Savelinus fontanalis*), Rainbow trout (*Salmo gairdneri*) and Brown trout (*Salmo trutta*). The immersion in the solution up to 800 ppm for 1hr at 16-17^0c caused neither death nor abnormal behavior of each test fish. However the higher mortality occurred in the immersion for longer period up to 18 hrs in the solutions ranging 6.25 to 100 ppm at 16-18^0c.

Das *et al.* (1980) carried out experiment in 3 treating Argulosis with Gammexane applied in 3 doses-first and second doses at the rate of 0.2 ppm and a third dose at the rate of 0.5 ppm which completely controlled the lice.

Sharma and Pant (1980) reported on the occurrence of *Argulus foliaceus* in kumaon lakes (mesotrophic) in India.

Silva (1980) reported the occurrence of *Argulus spinulosus* in freshwater fishes from the river Rio Grande do Sul in Brazil.

Shimura and Egusa (1980) reported on some ecological notes on the egg deposition of *Argulus coregoni* Thorell.

Puffer and Beal (1981) worked on the control of Argulosis in killifish (*Fundulus parvipinnis*). They treated the fishes with 0,0, dim ethyl 2,2,2,-trcahloro –1- hydroxy ethyl phosphonate (DTHP) at the rate of 2.50 mg/l for 1 hour once only & found it effective. Toxicity testing indicated that repetitive doses of 4.20 mg/l were toxic and that static exposure of more than 1 hour to doses of 7.50 mg/l was lethal to the fish.

Natarajan (1982) described a new species of *Argulus, Argulus mangalorensis* collected form the estuarine stretch of Netravathy river of Mangalore,India.

Shimura *et al.* (1983) studied the hematological changes caused by *Argulus coregoni*. He found that after 10 days of infection, erythrocyte and leukocyte counts, hemoglobin concentration, haematocrit value plasma total protein, total cholesterol and calcium concentration decreased.

Shimura (1983) analyzed the seasonal occurrence of *Argulus coregoni* on Onchorhynchus masou and *Salmo gairdneri* indicated that the parasites overwintered as eggs and that there were 1 or 2 generations/year. Larvae which hatched from the overwintered eggs were abundant from May through July. Individuals hatched in May and June attained maturity and laid eggs in August. Larvae of the second generation were abundant in September and they laid eggs in October to November which overwintered. Hatched larvae from overwintered eggs might mature in July and deposited overwintering eggs in September.

Shimura and Inoue (1984) studied the toxic effects of extracts from the mouthparts of *Argulus coregoni* Thorell, which were taken from rainbow trout and masu trout.

Das and Srivastava (1985) reported the occurrence of *A. japonicus* on *Puntius* sp. *Tor* sp. *Cyrinus* sp. *Schizothorax* sp in lake Naini Tal.

Ahne (1985) reported that *Argulus foliaceus* acts as mechanical vectors of spring viremia of carp virus (SVCV).

Singhal *et al.* (1986) reported that *Argulus indicus* was found in Catla, Rui, Mrigal, Grasscarp and Silvercarp. They studied the mortality rate and found that mortality rate in untreated *C. catla, L.rohita* and *C.idella* was 30% and in *C.mrigala* and Siver carp was 35-40%. They also applied chemotherapy as follows: 0.2 mg $^{l-1}$ gammexane spread on the pond three times at weekly intervals which resulted in a 97% reduction of *Argulus*. Early stages of infection were killed on fish immersed in 0.01 mg^{l-1} glacial acetic acid for 5 min. For Catla, Silver carp and Rui dipping in 30 mg^{l-1} sodium chloride for 2-5 min was effective while in Grass carp and Mrigal only 1-2 min were necessary. A bath in 0.5 mg $^{l-1}$ potassium permanganate for 5 min reduced *Argulus* by 60%.

Bai Sundara *et al.* (1998) studied the oviposition and sex ratio of *Argulus siamensis* var. *siamensis* and *Argulus siamensis* var. *hessarghattaris* parasitic on Catla, Grass carp and Guppies.

Rao *et al.* (1992) reported on the occurrence of *Argulus* sp. and their control by chemotherapeutic agents. Malathion at 0.15 to 0.25 ppm or Dichlorvos at 0.05-0.1 ppm applied in 2-4 installments at 4-7 day interval.

Baticados and Paclibare (1992) reported *Argulus* infections on Tilapia which were controlled when the fishes were bathed with 5ppm potassium permanganate for 5-10 min. He also reported the use of Neguvon^{R} for the treatment of *Argulus* sp. infections as a 2-3 min dip at 3% concentration for juveniles and adults of different fish species.

Gresty *et al.* (1993) studied the fine structure and function of the cephalic appendages of the *Argulus japonicus* Thiele.

Jafri and Ahmed (1994) made some observations on mortality in major carps; *Labeo rohita, Catla catla* and *Cirrhinus mrigala* infected with *Argulus indicus* and *Argulus japonicus* and eradicate them by using 10kg/acre (2.6 ppm) of powdered quicklime. Three treatments during a period of 10 days were sufficient for the control of fish lice. Similarly potassium permanganate solution applied twice as 5 kg/acre (1.3ppm) after 3 days interval was equally effective for the eradication of lice.

Rushton-Mellor and Boxshall (1994) studied the developmental sequences of *Argulus foliaceus.*

Rahman (1995) studied the complete life cycle of the *Argulus foliaceus* in laboratory and all stages of development including egg, copepoids, juveniles and adult were described.

Rahman (1996) studied the effects of freshwater fish parasite, *A. foliaceus* Linn. Infection on common carp, *Cyprinus carpio.*Linn.

Benz *et al.* (1995) redescribed adult male and female *Argulus melanostictus* (Wilson 1935) based on detailed examinations of a syntype and recently obtained specimens

of both sexes collected from California grunion, *Leuresthes tenuis* captured in near shore Pacific waters at Monterey, California. They also treated Argulosis in a 14-16 hr seawater bath containing .5µl/l trichlorfon administered once weekly for 3 weeks killed *Argulus melanostictus* while not noticeably harming grunion.

Avenant-Oldewge and Oldewage (1995) reported a new species of *Argulus*, *Argulus smalei* from leatherjacket *(Aluterus monoceros)* in Algoa Bay, South Africa.

Northcott *et al.*(1997) made an ecological and management note on the outbreak of freshwater fish lice *Argulus foliaceus* L. seriously affecting a Scottish Stillwater fishery. They reported that *Argulus* heavily infected the sticklebacks, *Gasterosteus aculeatus* L.rainbow trout, *Onchorhynchus mykiss* (Walbaum), brown trout, *Salmo trutta* L. roach, *Rutilus rutilus* (L.), grass carp, *Ctenopharyngodon idella* (Valenciennes), eels, *Anguilla anguilla* (L.). They found that the level of infestation decreased with the fall of temperature and reduction of aquatic weeds.

Mikheev *et al.* (1998) studied the host searching in *Argulus foliaceus*: the role of vision and selectivity. In laboratory experiments, the swimming behavior of the Argulus foliaceus and its infection rates on juvenile perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) were examined. The highest infection rate and a preference for perch juveniles were obtained in darkness, the lowest infection rate and a lack of preference in the light, when aquaria with glass walls (high reflectivity) were used. In the light, when aquaria were lined with black plastic (low reflectivity) an intermediate level of infection for perch and the highest for roach was recorded.

Mikheev *et al.* (2000) studied the light mediated host searching of *Argulus foliaceus*. A change in the illumination produced a clear difference in the searching behavior of adult *Argulus* females. The mean swimming speed and the area explored

were 3-4 times higher in the dark, when the parasite employed a cruising search strategy. This changed to an ambush strategy in the light. The swimming activity if accompanied by changes in metrabolic costs. The most pronounced light induced differences in host searching behavior took place in moderately hungry parasites. Less motivated or exhausted animals did not exhibit any clear differences in the swimming speed. Among the external signs tested, fish smell, from both perch and roach induced an elevated swimming speed of the parasite. Periodic water movements caused similar but weaker effects. The effects of these stimuli were observed under both light and dark conditions.

Pasternak *et al.* (2000) studied the life–history characteristics of *Argulus foliaceus*. Demographic characteristics of *Argulus foliaceus* from a commercial fish farm with a water supply from the same lake system differed significantly from those of the lake population where the hosts are scarce. Extended periods of egg laying and hatching were documented in the lake population which possibly prevented the appearance of summer peaks in juveniles that were observed in the farm. The elevated activity of the males, which was most clearly observed in their high attachment, is suggested to result in lower survival and growth than in females. The flexibility, in both behavior and life cycle strategy, allows *Argulus foliaceus* to utilize a broad spectrum of habitats of differing host abundances.

2.2 Review of Categories and Structure of Pesticides

2.2.1 Classification of Pesticides (Nimmo *et al.* 1981):

According to Von Rumker *et al.* (1975), pesticides are classified in the following way:

1. Insecticides	: Aldrin, Diazinon, Sumithion, Cymbush.
2. Herbicides	: Alachlor, Atrazine, Bromacil
3. Fungicides and preservatives	: Captan, Creosote.
4. Fumigants	: Methylbromide.

Lincer (1975), categorized pesticides on the basis of chemical structure of the pesticides:

2.2.1.1. Insecticides:

a. Chlorinated hydrocarbons (organochlorines)-------	DDT, Aldrin, Dieldrin, Heptachlor, and Toxaphene.
b. Organophosphate----------	Malathion,Sumithion. Cymbush and Diazinon
c. Carbamates----------	Sevin, Zectran and Baygon.
d. Pyrethrins (house and garden sprays)----------	Allethrin and Cyclethrin.

2.2.1.2. Herbicides:

a. Phenoxy acids----------	2,4-D and 2,4,5-T.
b. Aquatic herbicides----------	Endothal and Diquat.

2.2.1.3. Fungicides:

a. Dithiocarbamates---------- Febram, Ziram and Maneb.

b. Nitrogen containing compound---------- Phenylmercuric acetate, Quinones, Heteroctclics etc.

c. Hexachlorobenzene.

2.2.2 Common Form of Pesticides:

The various forms in which pesticides are applied illustrated by the following definition (Farm Chemical Hand Book, 1981).

Emulsifiable Concentrate: They are produced by dissolving the pesticides and an emulsifying agent in an organic solvent. A solvent substantially insolvent in water is usually selected. The strength is usually stated in pounds of pesticides per gallon of concentrate.

Emulsifier: A surface active substance that stabilizes (reduces the tendency to separate) a suspension of droplets of one liquid in another liquid, which otherwise would not mix.

Formulation: The process used by the manufacturer to prepare a pesticide is termed as formulation. The prepared, or formulated, mixture concocted to give proper results.

Surface-active agent: A substance that reduce the interfacial of two boundary layers. Most pesticides adjuvant can be considered surface active agent. They are also known as surfactants.

Synergist: A material that exhibits synergism, the joint action of different agents such that the total effects is greater than the sum of the independent effects. The efficiency of one or more components of a mixture is greatly heightened or potentate by a synergistic components.

Technical Material: The pesticide chemical in pure form (usually 95-100% active ingredient) as manufactured by a chemical company prior to its formulation into wettable powder that are easily added to water make a slurry, dust, emulsifiable.

2.2.3 Organophosphate pesticides:

They are modern broad spectrum poison and have high insecticidal and acaricidal activity.They are broken down in the biological system in a few hours or few days and also rapidly decomposed in the soil and water.

2.3 Properties and use of Sumithion®

Sumithion (fenitrothion) is a contact insecticide and selective acaricide of low ovicidal properties. It is considered a cholinesterase inhibitor. It is non-systemic and non-persistent.

Common name: Fenitrothion.

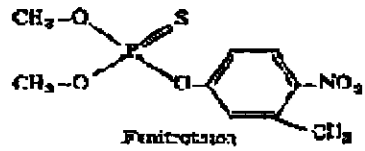
Chemical name: *O,* O-dimethyl-*O* (3-methyl-4-nitrophenyl) phosphorothioate.

Trade name: Sumithion ®, Folithion ®, Novathion ®

CAS Registry No.: 122-14-5.

Empirical formula: $C_9 H_{12} NO_5 PS$

Structural formula:



Molecular weight: 277.2

Physical form: oily liquid

Color: yellow-brown

Melting point: 0^0/C, pure

Boiling point: 140^0-14^0/C

Vapor pressure: 2.14 x 10^{-4} mm Hg at 20^0/C

Octanol/Water partition coefficient (KOW): 3800

Volatility :0. 09 mg/m^3.

Solubility: water: 14 mg/L at 3$\tilde{0}$C

Specific gravity: d 25 = 1.32 - 1.34 25

Density: 1.32 to 1.34 @ 25 deg C/25 deg C

Use: Contact and stomach insecticide; Acaricide for controlling chewing and sucking insects on cereals, cotton, orchard fruits, vegetables and forest. Fly, Mosquito and cockroach residual contact sprays for farms and in public health programs.

Aquatic Fate: Volatilization of fenitrothion from water should be a slow process as indicated by a volatilization half-life of 64 days in still water. The volatilization was further retarded by dissolved fulvic acid in water. However, volatilization from surface slicks following spray application may be very fast as indicated by a half-life of 18 mins at 20 deg C. Based on log Koc value of 2.40-3.19, fenitrothion should

moderately to strongly adsorb to suspended solids and sediments in water (2-3,SRC). This is confirmed by the presence of a large portion of fenitrothion in suspended solids and sediments following application to a stream. Experimentally determined log BCF of 2.35-2.66 in fish muscle tissues (5-7) suggests that fenitrothion should moderately bioconcentrate in aquatic organisms.

Source:(Worthing, 1983 and WHO, 1989)

2.4 Properties and use of Cymbush®

Cypermethrin is a Synthetic Pyrethroid insecticide.

Cypermethrin is a non-systemic insecticide, which has contact and stomach action properties. It has an excellent quality of action towards a variety of crop-damaging insects. It is quite versatile and is used extensively in agricultural crop protection.

Cypermethrin formulations are synthetic pyrethroid insecticides, which have Cypermethrin as the active ingredient. They are mainly used as a residual treatment for the control of flies, mosquitoes and cockroaches and a wide range of insects on crops.

Common Name: Cypermethrin

Chemical name: (IUPAC): (RS9-alpha-cyano-3-phenoxybenzyl (1RS) *cis*-trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane carboxylate

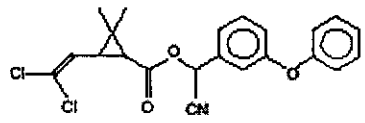
Chemical name: (CAS) (RS)-cyano(3-phenoxyphenyl)methyl (1RS)- *cis-trans*-3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropane carboxylate

CAS registry number: 52315-07-8 (formerly 69865-47-0)

Chemical classification: Pyrethroid

Molecular formula: $C_{22}H_{19}O_3NCl_2$

Molecular structure:



cypermethrin
(+/-) alpha-cyano-(3-phenoxyphenyl) methyl (+)-cis, trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

Physical state: Varies from a viscous yellow liquid to a semi solid crystalline mass at ambient temp

Relative Molecular mass: 416.3

Melting point: 80.5°C

Boiling Point: Decomposes > 220°C

Solubility (25°C): 0.004mg/lit in water

Density: 1.12g/ml (22°C)

Vapour pressure: 1.9 x 10^{-7} Pa

Stability: Cypermethrin is highly stable to light and at temperatures below 220°C. It is resistant to acidic rather than alkaline media with an optimum stability at pH Cypermethrin hydrolyses under alkaline conditions in a similar way to simple aliphatic esters. Dilute aqueous solutions are subject to photolysis, which occurs at a moderate rate.

Mode of Action: Cypermethrin®, like all synthetic pyrethroids, kills insects by disrupting normal functioning of the nervous system. In in-sects, as well as all other animals including humans, nerve impulses travel along nerves when the nerves become momentarily permeable to sodium atoms, allowing sodium to flow into the nerve. Pyrethroids delay the closing of the "gate" that allows the sodium flow. This results in multiple nerve impulses instead of the usual single one. In turn, these impulses cause the nerve to release the neurotransmitter acetylcholine and stimulate

other nerves. Cypermethrin® has other effects on the nervous system. It inhibits the g-aminobutyric acid receptor, causing excitability and convulsions. In addition, it inhibits calcium uptake by nerves 8 and inhibits monoamine oxidase, an enzyme that breaks down neurotransmitters. Cypermethrin® also affects an enzyme not directly involved with the nervous system, adenosine triphosphatase. It is involved in cellular energy production, transport of metal atoms, and muscle contractions.

Uses: Control of soil- dwelling and foliar feeding insects (including wireworms, white grubs, millipedes, symphylids, fruit flies, bean seed flies, root flies, flea beetles, weevils, sciarid flies, aphids, thrips, etc.) and nematodes in vegetables, ornamentals, beet, maize, sorghum, sunflowers, oilseed rape, potatoes, alfalfa, peanuts, soya beans, sugar cane, rice, cotton, coffee, cucurbits, tobacco, lavender, citrus, vines, strawberries, bananas, mushrooms, and other crops.

Source: (Meister *et al.* 1984)

CHAPTER-3

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation was conducted from November 2005 to April 2006 in three distinct sampling areas (Map 1), namely 1. BCDM hatchery (Gazipur; Map 2), 2. Mitha and M.R. Hatchery (Manikganj; Map 3) and 3. Raipur Hatchery (Laximpur; Map 4).

BCDM (BRAC Center for Development Management) at Rajendrapur (Map 2) is about 14 kilometers away from Joydevpur under Gazipur district in Dhaka division. There are 21 earthen ponds and 1 mini lake of which 9 ponds are under fish culture. Semi-intensive culture system, density moderate. Used supplementary feed, drainable pond, water exchange weekly and application of manure and fertilizer is also regular in brood ponds. In this hatchery 3 pond no. 6, 8 and 10 were regularly investigated. Pond no. 9 and the lake were investigated only to check the presence of *Argulus*. Source of water in the hatchery is shallow tube-well. Of the three ponds, the major carps *viz*. Katla (*Catla catla*), Rui (*Labeo rohita*), Mrigal (*Cirrhinus mrigala*), as well as *Puntius goniontus* and Chinese carps -Silver carp (*Hypophthalmicthys molitrix*) and Bighead (*Aristichthys nobilis*) were cultivated together. It has a carp and a prawn hatchery.

Pond no. 6 & 8 was selected as pond no. 1& 2 and a mixed culture food fish pond no. 10 not applying pesticides was selected as pond no. 3 (Table-1) for regular investigation. The pesticides dose used varies from 0.095 ppm to 0.125 ppm depending on the intensity of infection.

Table 1. Brief information on the study ponds and Lake of BCDM.

Pond no.	Area (ha)	Length (m)	Width (m)	Water depth (m)	Stocking density (kg/ ha)
1(6)	0.303	60.00	50.50	1.50	2470
2(8)	0.299	55.00	54.40	1.50	3705
3(10)	0.214	48.00	44.60	1.50	4940
9	0.303	60.00	50.50	1.50	2470
Lake	0.606	150.00	40.40	0.60-1.00	4940

Mitha hatchery is situated at Manikganj District (Map 3) about 1.5 kilometers away from the Manikganj town (in Dhaka division). There are 22 earthen cultural-able ponds and a hatchery. Semi-intensive culture system and maintained moderate stocking density. Water exchange system is not well-develop. Application of manure and chemical fertilizer is somewhat regular. During investigation, four commercial brood stock ponds were investigated (pond no. 1, 2, 3 and 6) (Table 2). Two ponds (1& 2) of them were treated with pesticides like Sumithion, Immithion and Marshal at a dose of 0.085 to 0.125 ppm and the other two ponds no. 3 & 6 were non-treated but limed.

Table 2. Brief information on the study ponds of Mitha Hatchery

Pond no.	Area (ha)	Length (m)	Width (m)	Water depth (m)	Stocking density (kg/ ha)
1	0.204	50.50	40.40	0.75	3211
2	0.486	70.00	69.4	0.80	4693
3	0.546	70.00	78.00	0.80	2964
6	0.405	67.5	60.00	0.70	1729

On the other hand, M.R. hatchery at Rasulpur union in Manikganj District (Map 3), non-treated commercial brood stock pond about 5-6 kilometers away from Manikganj town was also investigated (Table 2). This hatchery used pesticides like Sumithion and mixture of Nogos and Rejent at a dose of around 0.130 ppm. Water source is shallow tube-well.

Table 3. Brief information on the study pond of M.R. Hatchery

Pond no.	Area (ha)	Length (m)	Width (m)	Water depth (m)	Stocking density (kg/ ha)
1	0.202	46.00	44.00	0.75	3458

Raipur hatchery, the biggest hatcheries in this subcontinent. It is situated at 35 kilometers away from Laximipur district (in Chittagong division) town. About 66 ponds and a big hatchery present here. During investigation four commercial culturable brood stock ponds were investigated (pond no. 3, 8, 10 and 30) and one natural culture pond (not using pesticides) of the hatchery was also investigated. Semi-intensive culture system and

moderate stocking density. Water exchange regularly and application of fertilizer and pesticides (Sumithion and V-tex) are applied at 7 days intervals up to 3/4 weeks. Water source shallow tube-well as well as from Jamuna river. The dose Sumithion used varies from 0.120 ppm to 0.125 ppm depending on the intensity of infection.

Table 4. Brief information on the study ponds of Raipur Hatchery

Pond no.	Area (ha)	Length (m)	Width (m)	Water depth (m)	Stocking density (kg / ha)
1(3)	0.243	49.8	48.8	0.60	7407.40
2(8)	0.142	44.00	32.3	0.65	7407.40
3(10)	0.364	62.00	58.7	0.75	7407.40
30	0.324	62.00	52.30	0.65	7407.40

Basic data collection survey on Argulosis, impacts of pesticides, disease occurrence etc. were conducted by using different data collection formats (Annex-1, 2, 3, 4 and 5 A-F).

Fishes were collected from different ponds by seine net for investigation on weekly and monthly basis at a regular interval (Fig. 1). A total of 2300 fishes were investigated (451 fishes were infected) on weekly basis in which 540 were *Catla catla,* 180 *Hypophthalmicthys molitrix,* 180 *Aristichthys nobilis*, 700 *Labeo rohita,* 590 *Cirrhinus mrigala,* and 110 *Cyprinus carpio*. *Argulus* were collected from external body surface of the host fishes by rubbing with sponge and also by thumbs and forceps and were first placed into a jar containing pond water and allowed them to swim around the container for several hours which allowed the fish slime to be washed from the argulid before preservation. After the washing, the live specimens were dropped into a vial containing 70% alcohol. Then the *Argulus* were brought to the laboratory of the Department of Zoology, University of Dhaka, cleared in lactic acid and made temporary mount for taxonomic study. Collected *Argulus* were studied by using a binocular compound microscope and photomicrographs were taken by the Olympus (CH30 RF200) microscope. The *Argulus* was identified following the descriptions and figures of

Tokioka (1939), Wilson (1944), Ramakrishna (1953), Yamaguti (1963) and Thomas and Devaraj (1975).

Gross clinical symptoms of infected fishes were studied in the field just after netting. Prevalence and intensity were determined according to the formulas used by Margolis *et al.* (1982).

$$\text{Prevalence (\%)}: \frac{\text{Infected}}{\text{Examined}} \times 100$$

$$\text{Intensity}: \frac{\text{No. of } \textit{Argulus} \text{ sp.}}{\text{Infected}}$$

The host fishes were divided into three length groups: 30-44.9cm, 45-59.9cm and 60-74.9cm for the purpose of studying the intensity of infestation in different size groups.

To study the abundance of zooplanktons, samples were regularly collected from ponds of the four hatcheries by filtering 50 liters of sub-surface water (10 cm below the surface) collected from each pond by means of a 500 ml container (plastic bucket) and passing through the plankton net made of silk bolting cloth No. 25 (mesh size 60 μm). The filtrate containing the plankton was transferred to a 50 ml plastic bottle and preserved immediately in 5% formalin and the enumeration of the zooplankton was done by using Sedgwick-Rafter Cell placed in a compound microscope. The number of organisms was expressed as individuals/liter calculated by using the formula $N = \frac{A \times C \times X}{L \times Y}$ where, N = number of zooplankton/ml of original water, A = average number of zooplankton observed in 20 sub cells of the S-R cell, C = volume of the concentrated sample in ml, L = volume of the original water expressed in liter, X = Number of total cells in the S-R cell and Y = Number of counted sub cells of the S-R cells. Physical variables like water temperature was determined by a centigrade thermometer, transparency by a secchi disc and chemical variables of were color, temperature, transparency, pH (water), DO, hardness, alkalinity, CO_2, nitrate nitrogen, ammonia nitrate and conductivity were measured by using HACH's universal aquaculture testing kit, Model no. FF-2, USA. Soil pH was measured by a hand soil pH tester (Model DM-13, Takemura electric works, Ltd. Tokyo, Japan)

Lime treatment: The idea of using lime came from the report of Jafri and Ahmed (1992) but could be tried only in a pond of Mitha Hatchery (Manikganj) where it was used at regular interval of once a month mainly to removal turbidity of water. Other hatcheries also used lime but very irregularly, whenever necessary mainly to improve water quality.

Biological control: It was reported from Mitha Hatchery (Manikganj) and Raipur Hatchery (Laximpur) that fresh water scale feeder Chanda fish (*Chanda nama*) feeds on *Argulus* sp. Observation was made on one pond each of these two hatcheries on the control of *Argulus* Chanda fish (Elongate glass-perch let), *Chanda nama* was tried in pond no. 6 of Mitha hatchery at Manikganj and pond no. 2 at Raipur hatchery. Chanda fish collected from near by freshwater bodies were released in the pond and gradually removed by netting during harvesting the brood fish. In BCDM Lake Polyculture of fish with giant fresh water prawn *Macrobracium rosenburgii* was going on. Both fishes and bamboo splits were checked in the lake and *Argulus* on fish and their eggs on bamboo splits were found. Due to scarcity of prawn separate experiments could not be conducted.

Mechanical control: The idea of using Pyrex sheet comes from the Shimura and Egusa (1980) report. But Pyrex sheets available only at Dhaka market was very expensive Tk. 6000/- per sheet (1.5mx1.0mx.0.03mm) and so it was considered not suitable for the rural farmers. Therefore, bamboo splits were considered suitable, as it is easily available and affordable by the farmers. It also serves as the substrate for periphyton feed production and very much environment friendly.

Bamboo splits were made by splitting the bamboo poles into 4 splits dressed by removing branches and sharp edges and was cut into 2-meter long pieces. It was placed in the pond 2 meters away from the pond (Fig. 2) edges at an interval of 1 meter each around all sides of the pond. It was checked, once in a week for four weeks (Fig. 3). The eggs were destroyed by hand and sun drying of the bamboo splits over a day or two. Checking of fish and bamboo splits were always done at the same time (Figs. 1 & 4). Usually the

farmers use pesticides, which are easily available at the time where *Argulus* infection occurs. They use the dose determined on the basis of intensity of infestation. When prevalence and intensity is less they use low dose (0.085 to 0.125 ppm) and when infestation is serious the dose is higher (0.200 ppm). But incase of low dose reoccurrence of *Argulus* is quicker (25 to 30 days) and with repeated use the parasite become resistant to some extent requiring higher dose. Often because of higher dose mortality of fishes takes place. Particularly *Labeo rohita* and *Cirrhinus mrigala* are very much sensitive to pesticides. As the farmers using the pesticides whenever they thought it necessary, experimental strategy had to change to adjust with their plan.

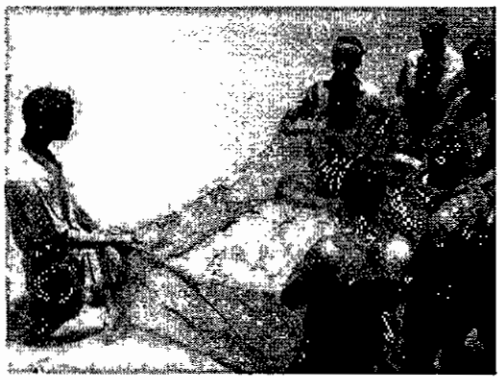


Fig. 1: Checking carps to find *Argulus* infestation.

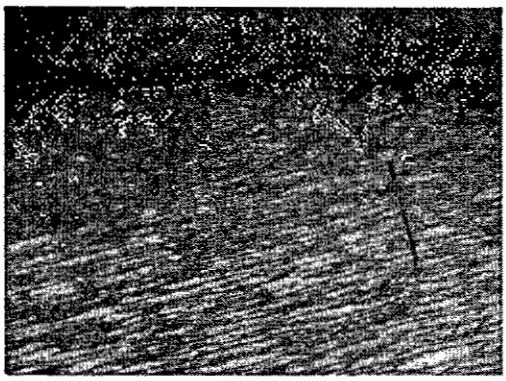


Fig. 2: Bamboo splits placed in BCDM brood carp ponds to collect egg clusters.

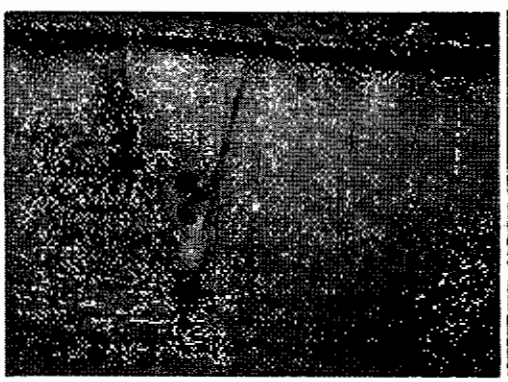


Fig. 3: Taking out bamboo splits from the pond to check Argulus egg cluster.

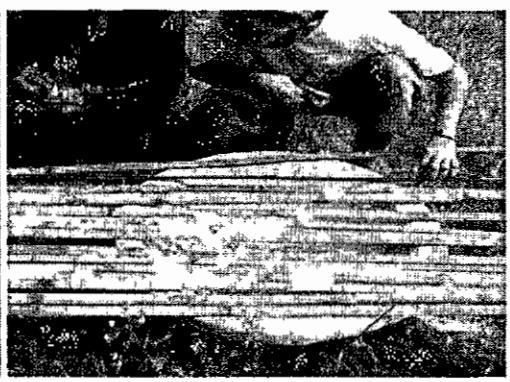
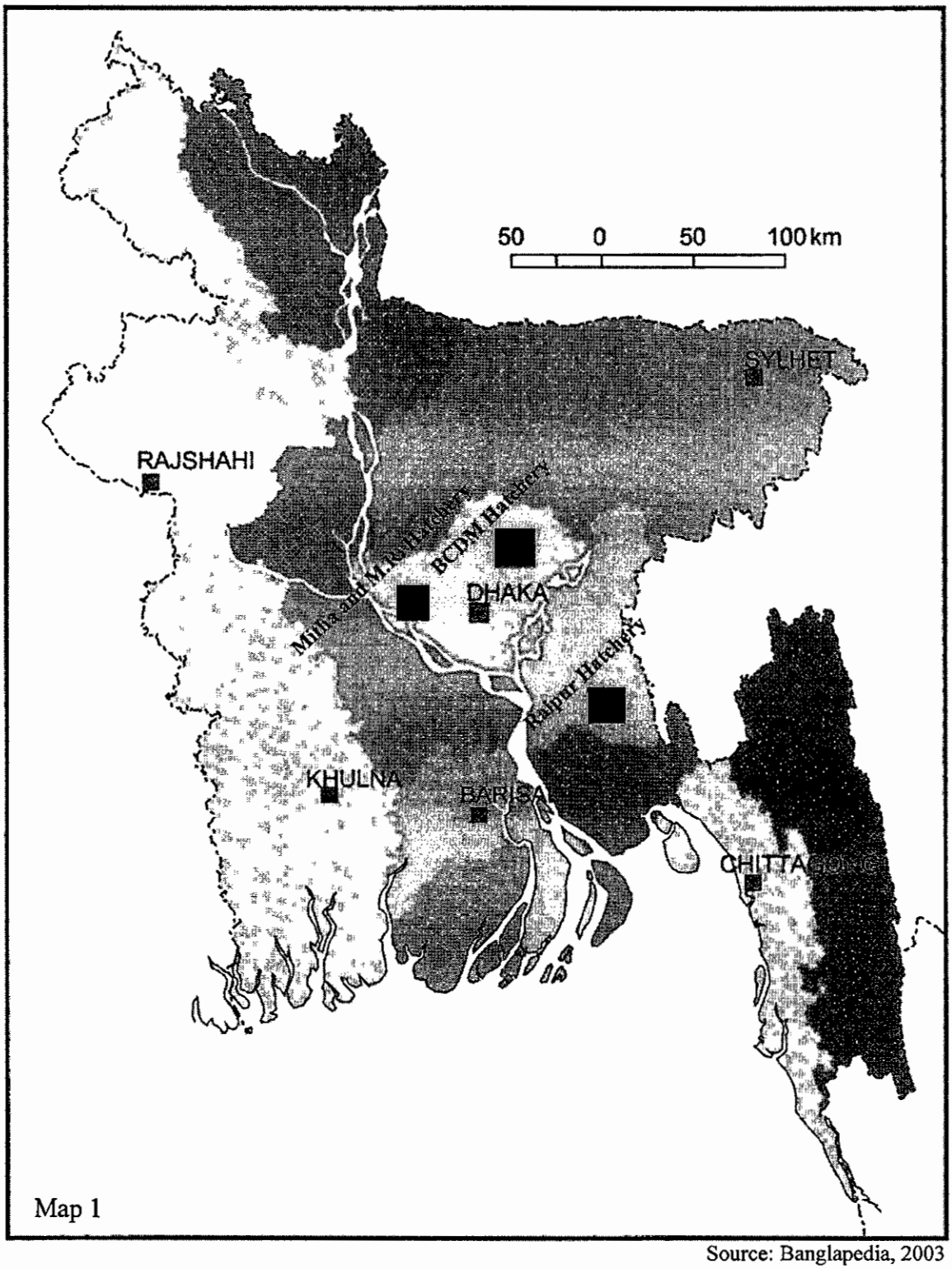
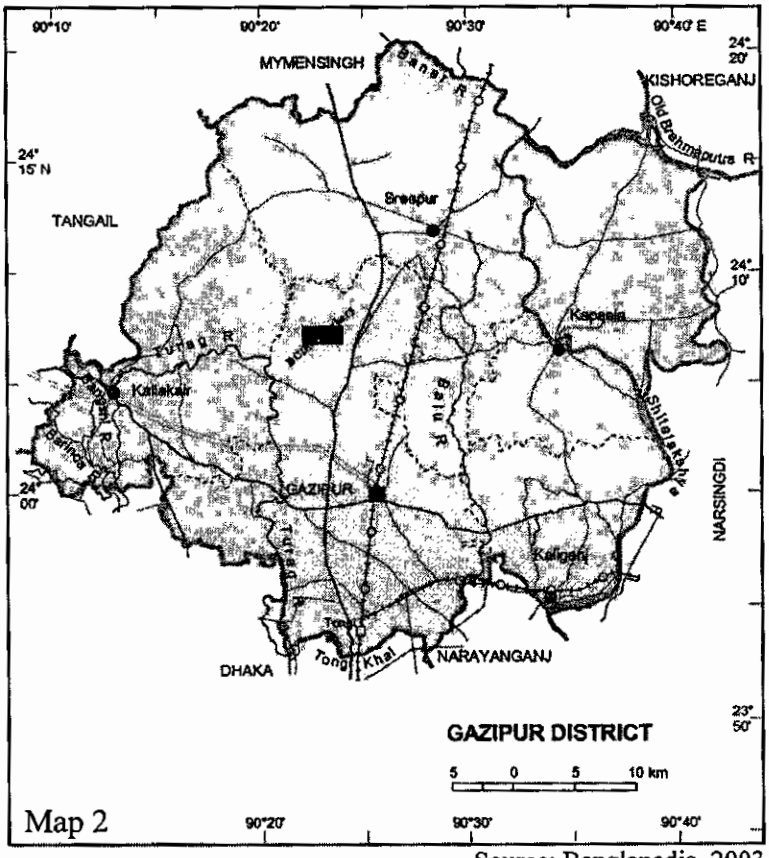


Fig. 4: Checking egg cluster attachment on bamboo splits.



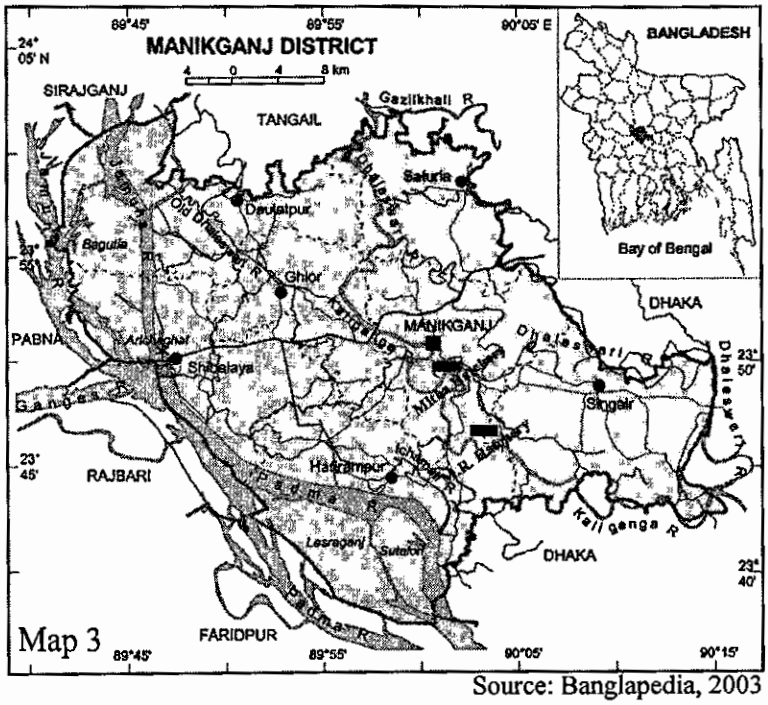
Map 1

Source: Banglapedia, 2003



Map 2

Source: Banglapedia, 2003



Map 3

Source: Banglapedia, 2003

CHAPTER-4

RESULTS AND DISCUSSION

4 RESULTS AND DISCUSSION

4.1 Status of Argulosis

To know the present status of Argulosis in carp culture in Bangladesh investigations were conducted at 3 major carp culture dominated areas- BCDM-BRAC hatchery at Gazipur (Dhaka division), Mitha and M.R. hatcheries at Manikganj (Dhaka division) and Raipur hatchery at Laximpur (Chittagong division) (Map 1) by using different data collection formats (Annex 1-4) and data input and record sheets (Annex 5 a-e). Monthly sampling on water quality variables and fish infection was also conducted and the results have been summarized as follows:

4.1.1 Prevalence and intensity of infestation:

It was found from the studies that of the four hatchery ponds studied about 17.11 to 79.17 % fishes were infested by the *Argulus* sp. with intensity of 10.91 to 41.34 parasites per fish (Table 5). In Raipur hatchery most of the time they treated the pond with higher dose of Sumithion. Before sampling date all the ponds was treated by pesticides (Table 6c). So the result was totally negative (Annex 9). Highest infestation was in M.R. hatchery pond using lower dose of pesticides where as lowest rate in BCDM ponds might be due to regular use of higher dose of Sumithion. Table 6 and Annexes 6-8 show that only *Labeo rohita, Cirrhinus mrigala* and *Cyprinus carpio* were found infested during over six months study but *Catla catla, H. molitrix and A. nobilis* were not affected by argulus species (Table 6 b). Of them overall highest rate of prevalence (65.71 %) and intensity (25.86 per fish) was in *Labeo rohita* followed by *Cyprinus carpio* (60.00 % and 12.00 per fish) and *Cirrhinus mrigala* (38.61 % and 5.02 per fish). However, in earlier study (Ahmed and Chowdhury, 2002) *Catla catla*

also had mild infestation. The farmers reported that in case of heavy infestation in the pond Catla and other fishes like Silvercarp and Bighead are also infected but the rate is very low. Gopalakrishnan (1964) found *Labeo rohita* to be more susceptible to Argulosis than *Catla catla* and *Cirrhinus mrigala*. The rate of infestation was higher in Mitha and M.R. hatchery ponds in Manikganj as they are not well managed and they use lower dose of pesticides. Overall, infestation rate (prevalence and intensity) was depending mainly on the use of pesticides dose, duration and management practices like maintenance of water quality, use of fertilizers and feed, water exchange etc. It was also difficult to assess the loss of fish production due to Argulosis as the farmers always use pesticides before reaching the situation of mortality. However, it came out from the farmers that about 10-20 % loss is caused by the lower production of eggs, growth loss, death caused due to over dose and heavily infected fishes becoming unfit for use.

Table 5: Overall prevalence and intensity of infestation of *Argulus* sp. in the host fishes of four hatcheries (all ponds combined).

Name of hatchery	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
	examined	infected			
BCDM	900	154	1680	17.11	10.91
Mitha	540	202	2660	37.41	13.17
M.R	120	95	3927	79.17	41.34
Raipur	540	00	00	00	00
Total	2100	451	8267		

Table 6a: Overall prevalence and intensity of infestation of *Argulus* sp. in the affected host fishes of three hatcheries (BCDM, Mitha and M.R.).

Name of Fish	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
	examined	infected			
Labeo rohita	420	276	7137	65.71	25.86
Cirrhinus mrigala	360	139	698	38.61	5.02
Cyprinus carpio	60	36	432	60.00	12.00
Total	840	451	8267	53.69	18.33

Table 6b: Overall prevalence and intensity of infestation of *Argulus* sp. in the non affected host fish of the four hatcheries (BCDM, Mitha, M.R.).

Name of Fish	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
	examined	infected			
Catla catla	360	0	0	0	0
Hypopthalmicthys molitrix	180	0	0	0	0
Aristichthys nobilis	180	0	0	0	0
Total	720	0	0	0	0

Table 6c: Overall prevalence and intensity of infestation of *Argulus* sp. in the Raipur hatchery.

Name of Fish	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
	examined	infected			
Labeo rohita	180	0	0	0	0
Catla catla	180	0	0	0	0
Cirrhinus mrigala	180	0	0	0	0
Total	540	0	0	0	0

4.1.2 Distribution of *Argulus* sp. on host body:

Carp host body was divided into seven regions (Head region, scapular region, dorsal region, pectoral region, pelvic region, anal region and caudal region; Annex 5e) and *Argulus* sp. was counted organoleptically. Representative samples of all the three host species were counted and result of 100 *Labeo rohita* from the three infected hatchery has been presented in Fig. 5a which shows that highest infestation (50 %) of *Argulus* sp. was in the pelvic region and lowest infestation on head (0%). The pattern of infestation (Figs. 5 b & c) was more or less same in other two hosts (*Cirrhinus mrigala,* and *Cyprinus carpio*) (Annex 10a-c). Similar pattern of distribution was also observed by Shimura (1981) in case of *Argulus coregoni* on *Masu salmon.*

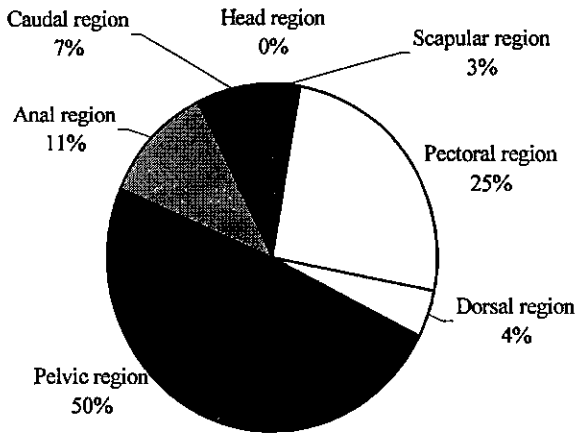


Fig 5a. Percentage distribution of *Argulus* sp. on *Labeo rohita.*

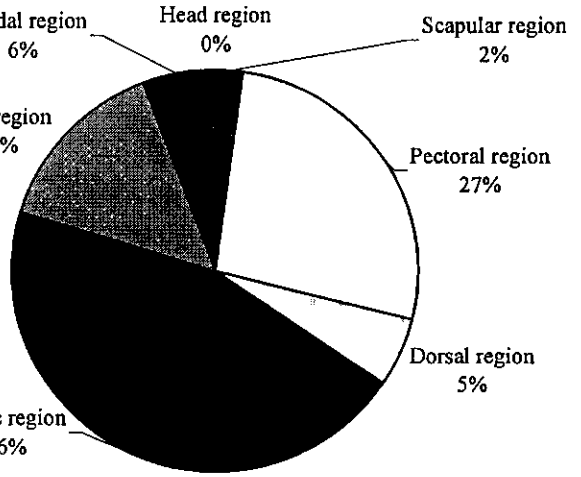


Fig 5b. Percentage distribution of *Argulus* sp. on *Cirrhinus mrigala.*

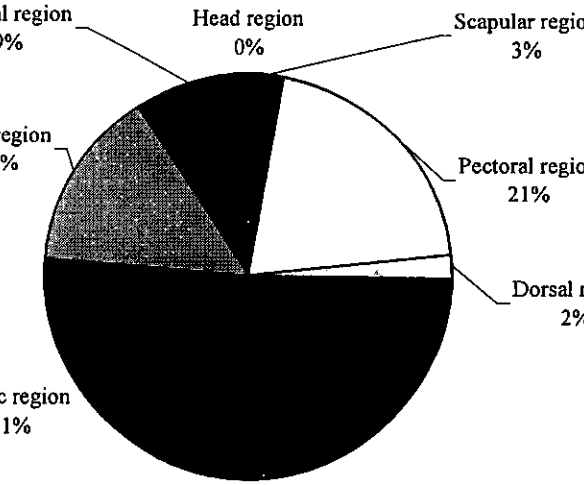


Fig 5c. Percentage distribution of *Argulus* sp. on *Cyprinus carpia.*

4.1.3 Sex wise distribution of *Argulus* sp:

Table 7 shows that prevalence and intensity of *Argulus* sp. was always higher on the female carps than male. Annex 11-13 shows that in all ponds *Labeo rohita* had the highest rate of infestation and females were most infested; same results also followed by *Cyprinus carpio* and *Cirrhinus*. For getting the significant result omit Raipur hatchery.

Table 7: Overall sex wise prevalence and intensity of infestation of *Argulus* sp. in the host fishes of BCDM, Mitha and M.R. hatchery (all ponds combined).

Name of Fish	Sex	No. of host fishes examined	infected	No. of *Argulus* sp	Prevalence (%)	Intensity (Mean)
Labeo rohita	Male	210	127	2882	60.48	22.69
	Female	210	149	4255	70.95	28.56
Cirrhinus mrigala	Male	180	63	238	35.00	3.78
	Female	180	76	460	42.22	6.05
Cyprinus carpio	Male	30	16	186	53.33	11.63
	Female	30	20	246	66.67	12.30

4.1.4 Fish length group wise distribution of *Argulus* sp:

The host fishes were divided into three-length groups (30-44.9 cm, 45-59.9 cm and 60-74.9 cm) to study the prevalence and the intensity of infestation on different size groups. Of the host fishes studied from the 3 hatcheries pond average prevalence and intensity of infestation of *Argulus* sp. varied from 27.50 to 76.43 % and 2.85 to 34.08 per fish being highest in the largest length group (60-74.9 cm) in all the 3 host fishes (Table 8 a-c and Annex 14-16). Highest prevalence and intensity was again in *L. rohita* and in M.R. and Mitha hatcheries of Manikganj (Annex 15-16). Similar observation was also made by Shimura (1983) in fresh water salmonid fish. As to the reason why brood fish in rearing ponds are more susceptible to Argulosis, it is assumed that repeated use of the same fish and hormone treatment for induced breeding as well as degraded environment due to use of pesticides might weaken the fish community. Pesticides also damage and weaken the host fish.

Table 8a: Lengthwise prevalence and intensity of infestation of *Argulus* sp. on *Labeo rohita* of three hatcheries.

Length groups (cm)	No. of host fish		No. of *Argulus*	Prevalence (%)	Intensity (Mean)
	examined	infected			
30-44.9	140	77	1295	55.00	16.81
45-59.9	140	93	2236	66.43	24.04
60-74.9	140	107	3647	76.43	34.08

Table 8b: Lengthwise prevalence and intensity of infestation of *Argulus* sp. on *Cirrhinus mrigala* of three hatcheries.

Length groups (cm)	No. of host fish		No. of *Argulus*	Prevalence (%)	Intensity (Mean)
	examined	infected			
30-44.9	120	33	94	27.50	2.85
45-59.9	120	47	175	39.17	3.72
60-74.9	120	59	380	49.17	6.58

Table 8c: Lengthwise prevalence and intensity of infestation of *Argulus* sp. on *Cyprinus carpio* of M.R. hatchery.

Length groups (cm)	No. of host fish		No. of *Argulus*	Prevalence (%)	Intensity (Mean)
	examined	infected			
30-44.9	20	9	52	45.00	5.78
45-59.9	20	12	135	60.00	11.25
60-74.9	20	15	245	75.00	16.33

4.1.5 Clinical signs of Argulosis:

The infected fish shows both behavioral stress and physical damage. The *Argulus* feed by first inserting a pre-oral string (stylet) which injects toxic digestive enzymes into the body flesh and then suck out liquidized body fluid with their proboscis-like mouth tube. This feeding activity causes intense skin irritations and the fish start flashing and rubbing the body on hard substrates. Localized inflammation is caused by constant piercing of the skin by the stylet (Fig. 6). Various spines, suckers and hooks that *Argulus* use for attachment also cause tissue damage and frayed fins (Fig. 7). Often blood is found oozing out from the minute punctures made by the lice the opportunistic bacteria like *Aeromonas* or *Pseudomonas* sometimes infects secondarily causing red skin lesions (Fig. 8). Secondary parasite and fungal infection may also takes place. The combined attack on stressed and weakened fish results in high

number of fatalities like anemia, thin and darkened soft body (Fig. 9). The fish starves, become slim, weak and sluggish at later stage. In case of heavy infestation skin also becomes opaque having red and black spots all over, the fins frayed and eye sunken. The fish become morbid and unfit for breeding as well as table fish thus causing economic loss, which has been estimated 10-20% of total production.

The *Argulus* infected host carps showed irritation because the parasites always tried to suck blood and body fluid of the host fishes by puncturing the skin with the help of pre-oral sting and suckers. This was due to the fact that they penetrate some toxins through the sting, which develop strong hemorrhagic reaction (Shimura and Inoue, 1984). The host ceases feeding and loses weight. This kind of clinical signs were also observed by Das *et al.* (1980). Opaqueness of the skin and erosion of the fins was also observed by Stammer (1959) who reported that host fish could generally carry many *Argulus foliaceus* with little sign of diseases. Shimura (1983) reported that *Argulus japonicus* infected host showed occasional abnormal behavior of rubbing their bodies against the wall of the pond, which caused damage to the body surface leading to secondary infection. Rahman (1968) reported mortalities of carps due to the heavy infection of *Argulus* sp. but mentioned nothing about treatment. The original source of this *Argulus* considered being the infected brood fishes collected from the river and reared in the pond.

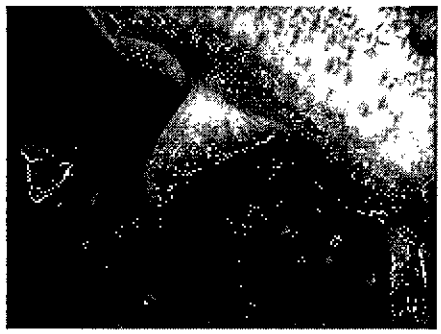


Fig. 6: Inflamed pelvic fin base infested by Argulus and secondary pathogenic infection.

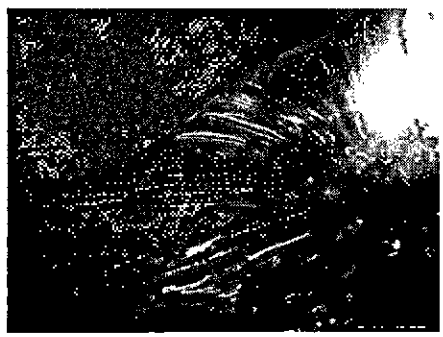


Fig. 7: Heavily infected frayed pelvic fin with numerous Argulus sp.

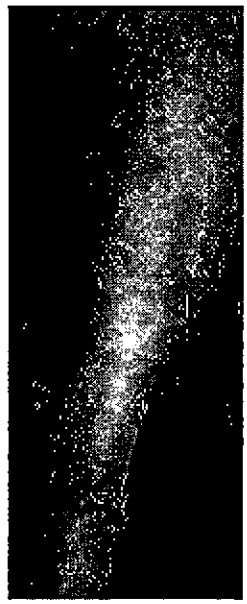


Fig. 8: Argulus sp. induced hemorrhagic lesions and spots on ventral side.

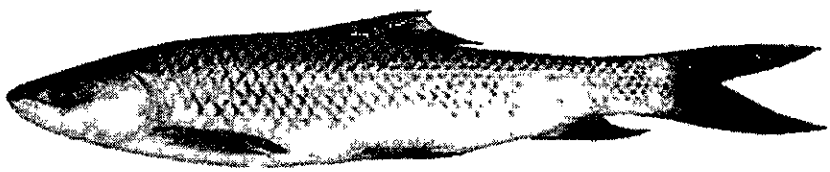


Fig. 9: A typical Argulus sp. infected morbid Labeo rohita.

4.2 Impacts of pesticides:

The farmers use organophosphate pesticides as soon as they find Argulosis. In BCDM and Raipur hatcheries they use mainly Sumithion and some times Cymbush at the rate of about 0.125 ppm. but as the dose fails to stop reinfestation they increases the dose with the increase of infestation rate and occasionally observe abnormal behavior, morbidity and even death of some fishes. In Mitha hatchery they use Marshal and Immithion at the rate of 0.85 ppm and in M.R. hatchery they use mixture of Nogos and Rejent at the rate of 0.103ppm. As these hatcheries use lower dosages the reappearance of *Argulus* is quicker.

Continuous as well as gradually higher dose use of pesticides was assumed to adversely affecting the water quality and abundance of zooplanktons particularly the crustacean populations. To study the impacts of pesticides on pond ecology and zooplanktons and abundance of zooplankton in 2 treated (pesticides used) ponds and 1 non-treated pond in or around the hatcheries were monitored. It should be mentioned here that the non-treated ponds available were mainly the mixed culture food fish ponds and usually non-feed and non-fertilized ponds, where as the treated ponds are manured and fertilized and provided with supplementary feeds to improve the brood quality. It was interesting to note that the *Argulus* sp. infestation was higher in the organic manure loaded ponds with shade and substrate for shelter and egg laying.

4.2.1 Zooplankton abundance:

During the monthly sampling to check *Argulus* infection and water and soil quality parameters, abundance of zooplanktons (individuals/liter) were also checked and the accumulated data are presented in table 9. It shows clear variation in the abundance of zooplanktons in the pesticides treated and non-treated ponds particularly in Mitha and Raipur hatcheries where non-treated ponds having very higher abundance of

zooplanktons. Again the variation is wider in crustacean groups than the rotifers. It should be again mentioned here that huge amount of cow dung mixed with urea and occasional liming are done in pond no. 1 and 2 of BCDM ponds to replenish plankton production. That is why the plankton production and water qualities are rather better in the treated ponds (Fig. 10).

Table 9: Abundance of zooplankton (indiv/l) in chemically treated and non treated brood pond.

Group of Zooplankton	BCDM		Mitha		M.R.	Raipur	
	Treated	Non-treated	Treated	Non-treated	Treated	Treated	Non-treated
Rotifers	72.125	96.75	504.5	440	380.5	36.5	514
Copepods	10.625	16	329.5	1051.5	1025.5	177.5	312
Cladocera	9.875	15	102.75	421.5	65	212.5	410
Ostracoda	0	0	55.5	187	53.5	3.5	7
Total	**92.625**	**127.75**	**992.25**	**2100**	**1524.5**	**430**	**1243**

Overall, higher abundance of zooplankton (Fig. 10 and Annex 17a-d) in Mitha and M.R. hatchery might have some relationship lower dose of pesticides used. Notable differences observed in crustaceans groups might be due to their strong sensitivity with pesticides like the *Argulus*. There was also a trend of overall increase of zooplankton abundance with the increase of time gap from the date of application of pesticides in the ponds (Annex 17a). Maximum numbers of zooplanktons were recorded in the month of April in both treated and non-treated ponds. Similar observation of peak periods of zooplankton abundance was observed by Das and Srivastava (1985) and Begum and Alam (1987).

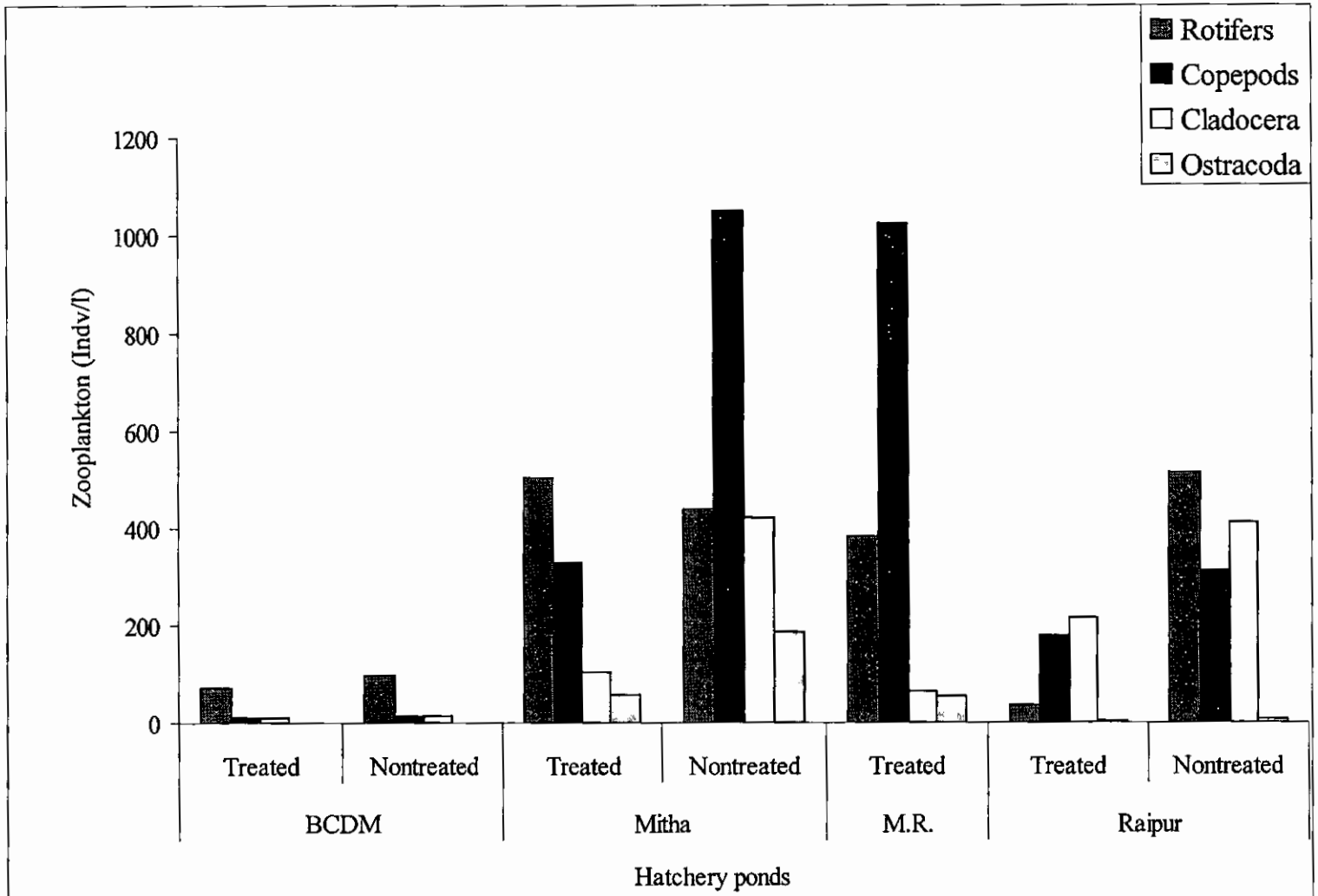


Fig. 10. Comparison of zooplankton abundance in treated and non-treated ponds

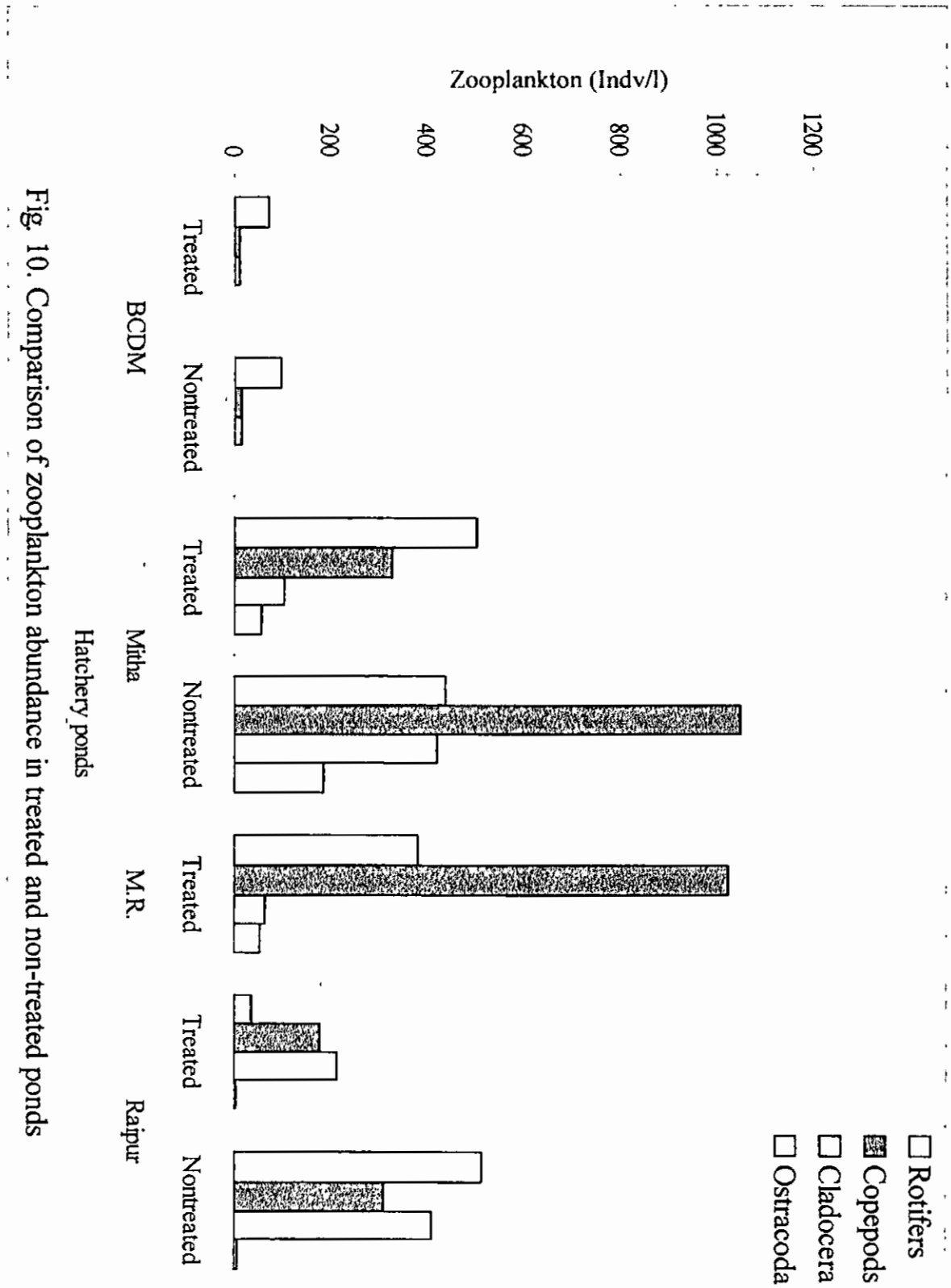


Fig. 10. Comparison of zooplankton abundance in treated and non-treated ponds

4.3 Control measures:

4.3.1 *Bamboo splits introduction*: As stated earlier bamboo splits were placed in all the study ponds at BCDM hatchery and checked after 7/15 day's interval. Simultaneously fishes were also checked by catching through seine net. The result shows (Table-10 & Fig. 16) that the presence of egg cluster on the bamboo splits gradually declined with the collection and destruction of egg cluster. It should be noted that first sampling was taken one week after treatment and sixth just two days after treatment. The deposition of eggs on bamboo splits gradually declined due to collection and destruction of egg clusters. Similar observation was made by Balarin and Hatton (1979).

Table 10: Percentage occurrence of egg clusters on bamboo splits at BCDM hatchery ponds.

Weeks	Pond no.	No. of splits observed	No. of splits attached by *Argulus* sp. eggs	Percentage
First	1	20	15	75
	2	20	11	55
	3	20	17	85
Second	1	20	13	65
	2	20	11	55
	3	20	15	75
Third	1	20	7	35
	2	20	6	30
	3	20	8	40
Fourth	1	20	4	20
	2	20	3	15
	3	20	4	20
Fifth	1	20	2	10
	2	20	2	10
	3	20	1	5
Sixth	1	20	1	5
	2	20	0	0
	3	20	1	5

Fig. 11 and 12 shows that the egg clusters as well as a egg laying *Argulus* sp. on the bamboo splits. It also shows the accumulation of periphyton on bamboo substrates, which is a very good feed source for *Labeo rohita*. Wahab *et al.* (1999) demonstrated that periphyton production on bamboo substrate in fish pond potentially contribute in the production increase of rohu (*Labeo rohita*) fish.

The ribbon like egg clusters are arranged in 2-6 rows of eggs (Fig. 11 a & b) and each row comprising of 60-260 eggs. The eggs as observed on the aquarium glass are whitish and translucent when obviposited but turns yellowish within about 12 hours and later brown till hatching. The eggs are oval in shape and glued by thin gelatinous cementing substance which hardens in water and fasten the eggs to the substratum (Fig. 11). The egg cluster deposition on bamboo splits substrate was found within 10 to 54 cm below from pond water surface. Shimura and Egusa (1980) however, reported more abundance of clusters nearer to the bottom and the *Argulus* preferred rough substrates than transparent glass plates. In the aquarium when stressed or treated with lower dose of pesticides, it was noticed that *Argulus* laid eggs on the glass before dying. These eggs are however resistant to pesticides. So afterwards these eggs hatch out and the juveniles actively attach to a fish host body and continue its development there. Therefore, to destroy eggs there are two options, either by collection and destruction through use of substrates like bamboo splits/poles which are easily available near fish farms and are also comparatively cheaper. The other option might to be to destroy the eggs by using lime, which also improves pond environment.

4.3.2 *Bio-control*:

Crustacean treatment: In the BCDM hatchery pond no. 18 was culture *Macrobrachim rosenbergii* with carp fish and *Argulus* sp. Infection decrease gradually.

Chanda fish treatment: In BCDM pond no. 14 *Argulus* sp. Infection was moderate and after join the Elongate-glass-perchlet (*Chanda nama*) in the pond also get good result (Fig. 13). Same result also found by using Climbing perchlet (*Anabas testudenius*) (Fig. 14).

From the present study it was found that farmers treat Argulosis by using organophosphate pesticides, which is banned in many countries like UK, on environmental and human health grounds, though very effective in immediate killing of *Argulus*. As a result of continued and indiscriminate use of pesticides, adverse impact on pond environment as well as on brood fish where egg producing potentialities of fish gradually decreases and growth impaired were noticed. *Argulus* also develop resistance and re-infestation takes place. In the above circumstances no single biological, chemical or mechanical means can completely remove the lice under sustainable environment friendly condition. Therefore, it is believed that by

using bamboo splits/ poles as substrate for egg collection, not only to monitor the presence or absence of *Argulus* in the pond but also to destroy the eggs by sun drying and hand picking, the outbreak can be prevented. Simultaneously, periodic liming when the infestation is higher might be good strategy to keep *Argulus* out break under control if not totally eradicated. Introduction of Chanda fish in the pond will also strengthen the control measure. Therefore this package of bamboo splits, liming and Chanda fish introduction in the pond can be recommended to control Argulosis.

Dip treatment by bath of infested carp into hatchery tank by pesticides was also considered as a method to treat fish out of the pond and also conducted some treatments but the treated fish was found stressed and complete harvesting of fish from pond was difficult. Moreover free moving *Argulus* and total removal of eggs are also not possible. So, liming is preferred over dip treatment.

4.3.3 *Lime treatment*: In Mitha hatchery (Manikganj) pond no. 3 was regularly limed (Fig. 16) by spraying dissolved lime stone ($CaCO_3$) at the rate of 25 kg/ ha (2.5 ppm) twice a month mainly to remove turbidity of water. It was noticed that Argulosis out break was under control and water quality was within normal range in that pond. It is believed that lime sprayed over pond water surface destroys eggs and juveniles of *Argulus*. Jafri and Ahmed (1994) reported eradication of *Argulus* from major carp ponds in Pakistan by applying quick lime in ponds in three treatments during ten days time. Thus liming can be a good mendicant for environment friendly treatment of Argulosis as well as other disease like EUS. It was reported that in BCDM ponds they also use lime occasionally to improve water quality.

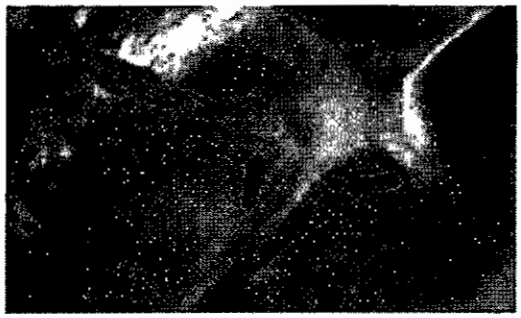


Fig. 11 a: Egg cluster on bamboo split with periphyton on the smooth surface (For appeoved collected in the bottle).

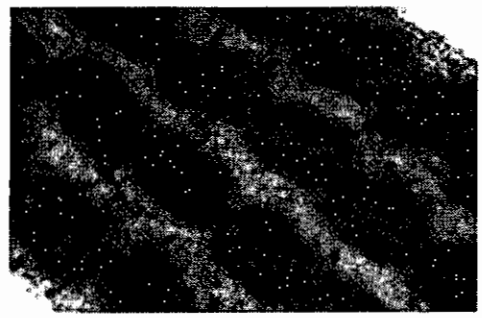


Fig. 11 b: Haematoxylin-eosin stained egg cluster of *Argulus* sp. (20x).

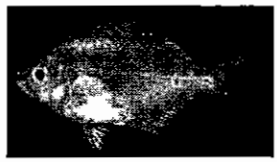


Fig. 13: Elongate glass-perch let (*Chanda nama*) used for controlling *Argulus* sp.

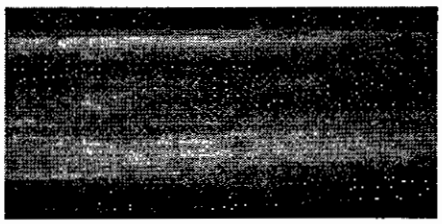


Fig. 12: *Argulus* sp. laying eggs on bamboo substrate.

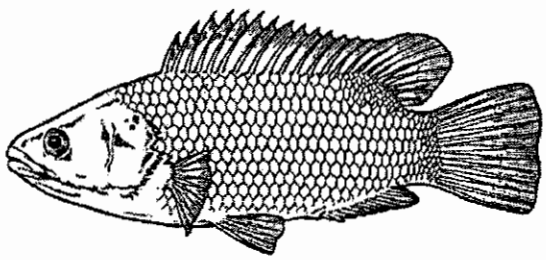


Fig. 14: Climbing-perch (*Anabas testudenius*) used for controlling *Argulus* sp.

Fig. 15: Liming in pond no 3 at Mitha hatchery at Manikganj.

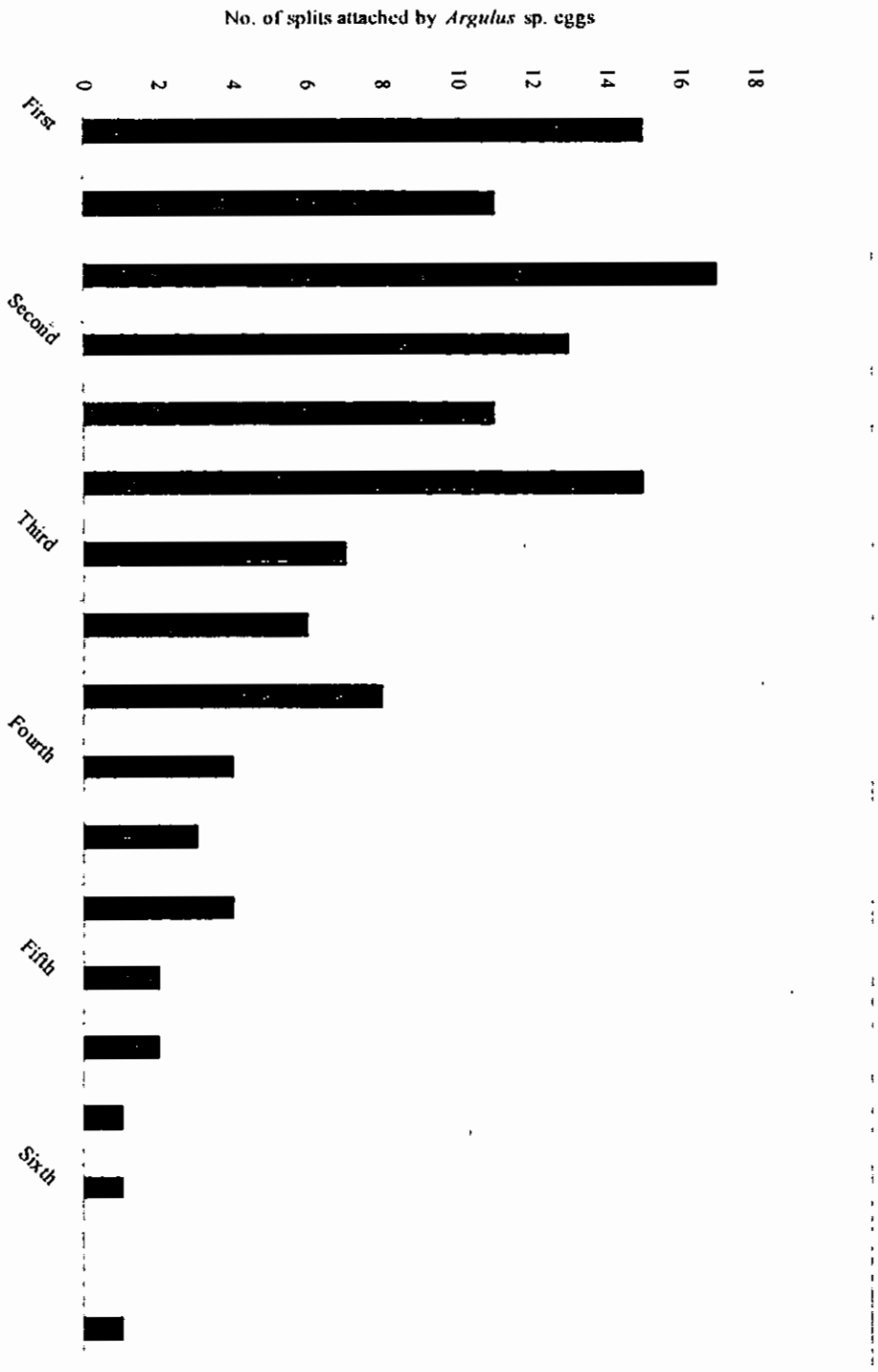


Fig. 16. Percentage occurrence of egg clusters on bamboo splits at BCDM hatchery ponds

CHAPTER-5

CONCLUSION AND RECOMMENDATION

5 CONCLUSION AND RECOMMENDATION

With the gradual intensification of carp culture disease outbreak particularly Argulosis is expected to increase and spread in carp farms of the country. To face these problem pesticides may be a quick selection but not at all a permanent and safe remedy. In this situation present study is an important venture. But this is a very brief and preliminary study which needs detailed long term trail at different problem faced areas in Bangladesh where carp culture is rapidly increasing.

Though encouraging results have been achieved to control the disease by the biological methods as described, it will need further detail study and refinement before application. The farmers can easily adopt the technology as it is simple, cheaper and raw materials are easily available. It serves dual purposes of control of *Argulus* as well as improvement of pond ecology and productivity.

The recommendations are as follows:

1. As the main source of *Argulas* infection in the brood ponds is the introduction of wild and infected broods from different sources, the broods must be given chemical bath treatment before stocking in hatchery ponds.
2. The ponds using water from canals, rivers, beel etc. natural surface water sources must be lime treated.
3. Regular monitoring of *Argulus* infection must be monitored by netting as well as use of bamboo splits/poles.
4. In *Argulus* infected ponds liming should be initiated once a week for three consecutive weeks and Chanda fish should be introduced in the pond as well as Climbing perchlet.
5. Regular collection and destruction of egg clusters to continue to keep the *Argulus* infection under control.

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ANNEXES

Format-1

Annex-1: Basic data collection survey forms on Argulosis:

Date:	Farm/Hatchery name:
Address:	Owner's name: Pond no.:
Pond size:	Type of pond:
Disinfection: Yes / No	Culture species:
Source of fishes:	Stocking density:

:

Epidemiology/ Status of infection:

i) Starting year		ii) Peak season	
iii) Susceptibility of species:	a)	b)	c)
Probable sources of *Argulus*:			
Duration of disease:			
Clinical symptoms: (Behavioral and physical signs)			
Production in non-infected and infected ponds:			
Loss due to Argulosis:			

Format-2

Annex 2: Data collection on pesticide use:

Name of pesticide used: Type of pesticide

Source: Amount/Dose:

Duration or frequency of use: Method of application:

Persistant period:

Negative and positive impacts:

a) Fish behavior:

b) Reoccurrence:

c) Etc.

Format-3

Annex 3. Water quality study format:

a) Color:

b) Temperature:

c) Transparency:

d) pH: i) water:

ii) soil:

e) DO:

f) Hardness:

g) Alkalinity:

h) CO_2:

i) Nitrate N_2:

j) Ammonia nitrate:

k) Conductivity:

Format-4

Annex 4: Basic data collection on disease occurrence:

Name of infected fish (e.g. *Labeo rohita*):

Sex: Male/Female: Length: Weight(Kg):

No. of *Argulus* attached per fish:

Organal distribution:

Other ecto parasites present:

Format-5

Annex 5: Data input and record sheet:

Annex 5a: Monthly data record format of prevalence and intensity of *Argulus* infected and non-infected ponds.

Months	No. of host examined	No. of host infected	No. of host non-infected	No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
January						
February						
March						
April						
May						
June						
July						
August						
September						
October						
November						
December						

Annex-5b: Host specific data record format:

Host fishes	Sex	No. of host examined	No. of host infected	No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)

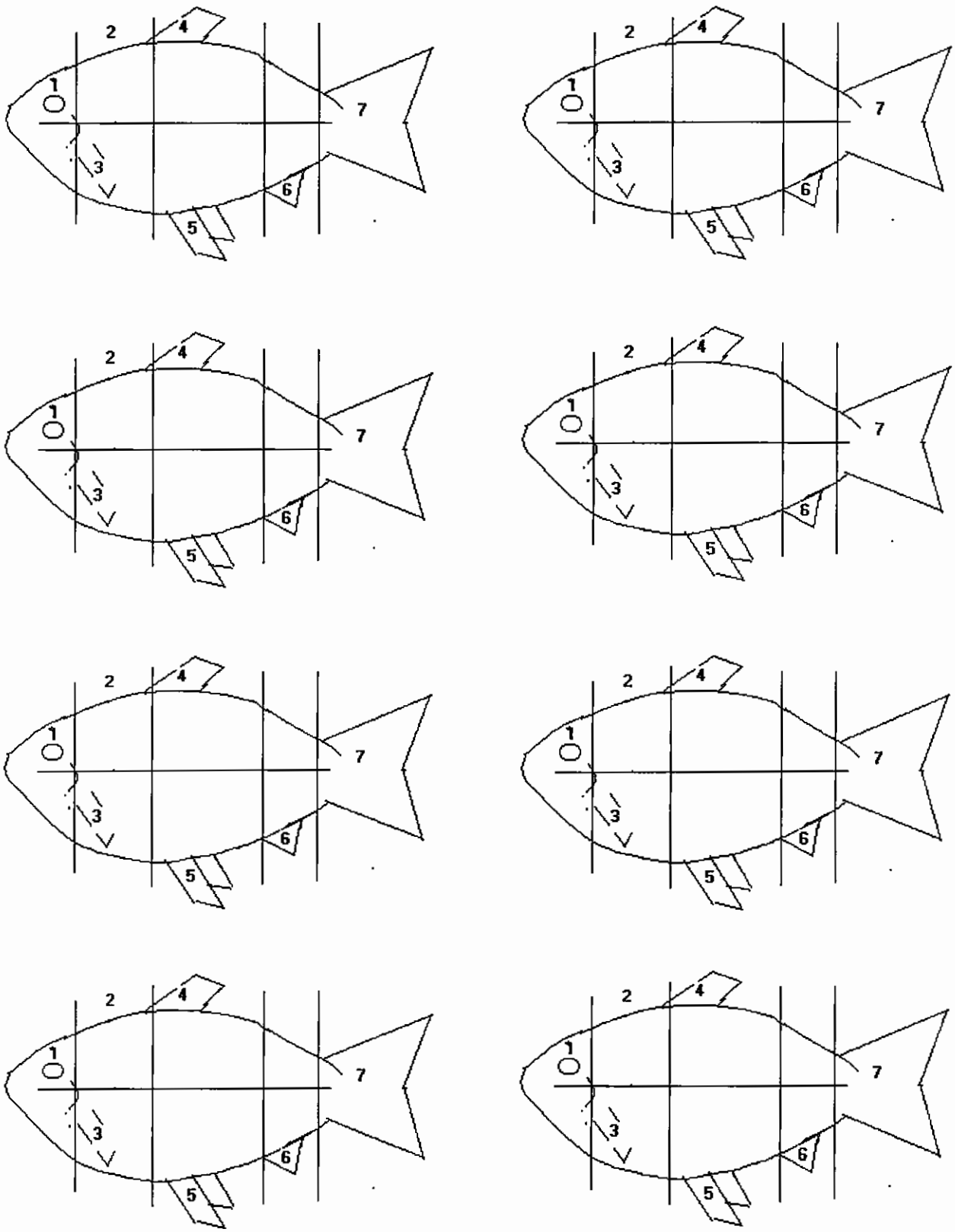
Annex-5c: Length group wise data record format:

Length Groups	No. of host examined	No. of host Infected	No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)

Annex-5d: Diversity and abundance (ind/l) of zooplankton in ponds:

Zooplankton	Number of indiv/l in months											
	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
Rotifers												
Nauplius												
Copepods												
Cladocera												
Ostracoda												

Annex-5e: Distribution of *Argulus* sp. in seven-body regions (Head region, Scapular region, Pectoral region, Dorsal region, Pelvic region, Anal region and Caudal region)



Annex 6a: Prevalence and intensity of infestation of *Argulus* sp. in the host fishes of BCDM hatchery (pond no. 1).

Name of Fish	No. of host fish		No. of *Argulus* sp	Prevalence (%)	Intensity (Mean)
	examined	infected			
Catla catla	60	00	00	00	00
Hypophthalmicthys molitrix	60	00	00	00	00
Aristichthys nobilis	60	00	00	00	00
Labeo rohita	60	44	732	73.33	16.64
Cirrhinus mrigala	60	23	117	38.33	5.09

Annex 6b: Prevalence and intensity of infestation of *Argulus* sp. in the host fishes of BCDM hatchery (pond no. 2).

Name of Fish	No. of host fish		No. of *Argulus* sp	Prevalence (%)	Intensity (Mean)
	examined	infected			
Catla catla	60	00	00	00	00
Hypophthalmicthys molitrix	60	00	00	00	00
Aristichthys nobilis	60	00	00	00	00
Labeo rohita	60	48	651	80	13.56
Cirrhinus mrigala	60	30	146	50	4.67

Annex 6c: Prevalence and intensity of infestation of *Argulus* sp. in the host fishes of BCDM hatchery (pond no. 3).

Name of Fish	No. of host fish		No. of *Argulus* sp	Prevalence (%)	Intensity (Mean)
	examined	infected			
Catla catla	60	00	00	00	00
Hypophthalmicthys molitrix	60	00	00	00	00
Aristichthys nobilis	60	00	00	00	00
Labeo rohita	60	9	34	15	3.78
Cirrhinus mrigala	60	00	00	00	00

Annex 7a: Prevalence and intensity of infestation of *Argulus* sp. in the host fishes of Mitha hatchery (pond no. 1).

Name of Fish	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
	examined	infected			
Catla catla	60	00	00	00	00
Labeo rohita	60	56	1107	93.33	19.77
Cirrhinus mrigala	60	41	182	68.33	4.44

Annex 7b: Prevalence and intensity of infestation of *Argulus* sp. in the host fishes of Mitha hatchery (pond no. 2).

Name of Fish	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
	examined	infected			
Catla catla	60	00	00	00	00
Labeo rohita	60	51	1108	85	21.72
Cirrhinus mrigala	60	44	212	73.33	4.82

Annex 7c: Prevalence and intensity of infestation of *Argulus* sp. in the host fishes of Mitha hatchery (pond no. 3).

Name of Fish	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
	examined	infected			
Catla catla	60	00	00	00	00
Labeo rohita	60	10	51	16.67	5.10
Cirrhinus mrigala	60	00	00	00	00

Annex 8: Prevalence and intensity of infestation of *Argulus* sp. in the host fishes of M.R. hatchery (pond no. 1).

Name of Fish	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
	examined	infected			
Labeo rohita	60	59	3495	98.33	59.24
Cyprinus carpio	60	36	432	60.00	12.00

Annex 9a: Prevalence and intensity of infestation of *Argulus* sp. in the host fishes of Raipur hatchery (pond no. 1).

Name of Fish	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
	examined	infected			
Catla catla	60	00	00	00	00
Labeo rohita	60	00	00	00	00
Cirrhinus mrigala	60	00	00	00	00

Annex 9b: Prevalence and intensity of infestation of *Argulus* sp. in the host fishes of Raipur hatchery (pond no. 2).

Name of Fish	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
	examined	infected			
Catla catla	60	00	00	00	00
Labeo rohita	60	00	00	00	00
Cirrhinus mrigala	60	00	00	00	00

Annex 9c: Prevalence and intensity of infestation of *Argulus* sp. in the host fishes of Raipur hatchery (pond no. 3).

Name of Fish	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
	examined	infected			
Catla catla	60	00	00	00	00
Labeo rohita	60	00	00	00	00
Cirrhinus mrigala	60	00	00	00	00

Annex-10a: Percentage distribution of *Argulus* sp. on different body regions of *Labeo rohita*.

No. of fish studied	Total number of parasite	Investigated area	*Argulus* sp.	
			Number of parasite	%
100	2595	Head region	0	0
		Scapular region	72	2.77
		Pectoral region	654	25.21
		Dorsal region	116	4.47
		Pelvic region	1269	48.90
		Anal region	291	11.21
		Caudal region	193	7.44

Annex-10b: Percentage distribution of *Argulus* sp. on different body regions of *Cirrhinus mrigala*.

No. of fish studied	Total number of parasite	Investigated area	*Argulus* sp.	
			Number of parasite	%
50	238	Head region	0	0
		Scapular region	5	2.10
		Pectoral region	64	26.89
		Dorsal region	13	5.46
		Pelvic region	108	45.38
		Anal region	34	14.29
		Caudal region	14	5.88

Annex-10c: Percentage distribution of *Argulus* sp. on different body regions of *Cyprinus carpio*.

No. of fish studied	Total number of parasite	Investigated area	*Argulus* sp.	
			Number of parasite	%
50	596	Head region	0	0
		Scapular region	16	2.69
		Pectoral region	123	20.64
		Dorsal region	12	2.01
		Pelvic region	305	51.17
		Anal region	86	14.43
		Caudal region	54	9.06

Annex 11a: Sex wise prevalence and intensity of infestation of *Argulus* sp. in the host fishes of BCDM hatchery (pond no. 1).

Name of Fish	Sex	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
		examined	infected			
Catla catla	Male	30	00	00	00	00
	Female	30	00	00	00	00
Hypophthalmicthys molitrix	Male	30	00	00	00	00
	Female	30	00	00	00	00
Aristichthys nobilis	Male	30	00	00	00	00
	Female	30	00	00	00	00
Labeo rohita	Male	30	20	291	66.67	14.55
	Female	30	24	441	80.00	18.38
Cirrhinus mrigala	Male	30	10	46	33.34	4.60
	Female	30	13	71	43.33	5.46

Annex 11b: Sex wise prevalence and intensity of infestation of *Argulus* sp. in the host fishes of BCDM hatchery (pond no. 2).

Name of Fish	Sex	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
		examined	infected			
Catla catla	Male	30	00	00	00	00
	Female	30	00	00	00	00
Hypophthalmicthys molitrix	Male	30	00	00	00	00
	Female	30	00	00	00	00
Aristichthys nobilis	Male	30	00	00	00	00
	Female	30	00	00	00	00
Labeo rohita	Male	30	22	228	73.33	10.36
	Female	30	26	423	86.67	16.27
Cirrhinus mrigala	Male	30	13	53	43.33	4.08
	Female	30	17	93	56.67	5.47

Annex 11c: Sex wise prevalence and intensity of infestation of *Argulus* sp. in the host fishes of BCDM hatchery (pond no. 3).

Name of Fish	Sex	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
		examined	infected			
Catla catla	Male	30	00	00	00	00
	Female	30	00	00	00	00
Hypophthalmicthys molitrix	Male	30	00	00	00	00
	Female	30	00	00	00	00
Aristichthys nobilis	Male	30	00	00	00	00
	Female	30	00	00	00	00
Labeo rohita	Male	30	2	5	6.67	2.50
	Female	30	7	29	23.33	4.14
Cirrhinus mrigala	Male	30	00	00	00	00
	Female	30	00	00	00	00

Annex 12a: Sex wise prevalence and intensity of infestation of *Argulus* sp. in the host fishes of Mitha hatchery (pond no. 1).

Name of Fish	Sex	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
		examined	infected			
Catla catla	Male	30	00	00	00	00
	Female	30	00	00	00	00
Labeo rohita	Male	30	26	446	86.67	17.15
	Female	30	29	667	96.67	23.00
Cirrhinus mrigala	Male	30	19	67	67.00	3.53
	Female	30	23	115	76.67	5.00

Annex 12b: Sex wise prevalence and intensity of infestation of *Argulus* sp. in the host fishes of Mitha hatchery (pond no. 2).

Name of Fish	Sex	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
		examined	infected			
Catla catla	Male	30	00	00	00	00
	Female	30	00	00	00	00
Labeo rohita	Male	30	24	506	80.00	21.08
	Female	30	27	561	90.00	20.78
Cirrhinus mrigala	Male	30	21	72	70.00	3.43
	Female	30	23	181	76.67	7.87

Annex 12c: Sex wise prevalence and intensity of infestation of *Argulus* sp. in the host fishes of Mitha hatchery (pond no. 3).

Name of Fish	Sex	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
		examined	infected			
Catla catla	Male	30	00	00	00	00
	Female	30	00	00	00	00
Labeo rohita	Male	30	4	13	13.33	3.25
	Female	30	6	38	20.00	6.33
Cirrhinus mrigala	Male	30	00	00	00	00
	Female	30	00	00	00	00

Annex 13: Sex wise prevalence and intensity of infestation of *Argulus* sp. in the host fishes of M.R. hatchery (pond no. 1).

Name of Fish	Sex	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity (Mean)
		examined	infected			
Labeo rohita	Male	30	29	1399	96.67	48.24
	Female	30	30	2096	100.00	69.87
Cirrhinus carpio	Male	30	16	186	53.33	11.63
	Female	30	20	246	66.67	12.30

Annex-14a: Lengthwise prevalence and intensity of infestation of *Argulus* sp. in *Labeo rohita* of BCDM hatchery (pond no. 1).

Length groups (cm)	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity
	examined	infected			
30-44.9	20	10	111	50.00	11.10
45-59.9	20	15	230	75.00	15.33
60-74.9	20	19	391	95.00	20.58

Annex-14b: Lengthwise prevalence and intensity of infestation of *Argulus* sp. in *Labeo rohita* of BCDM hatchery (pond no. 2).

Length groups (cm)	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity
	examined	infected			
30-44.9	20	13	115	65.00	8.85
45-59.9	20	16	181	80.00	11.31
60-74.9	20	19	355	95.00	18.68

Annex-14c: Lengthwise prevalence and intensity of infestation of *Argulus* sp. in *Labeo rohita* of BCDM hatchery (pond no. 3).

Length groups (cm)	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity
	examined	infected			
30-44.9	20	2	3	10.00	1.50
45-59.9	20	3	12	15.00	4.00
60-74.9	20	4	19	20.00	4.75

Annex-14d: Lengthwise prevalence and intensity of infestation of *Argulus* sp. in *Cirrhinus mrigala* of BCDM hatchery (pond no. 1).

Length groups (cm)	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity
	examined	infected			
30-44.9	20	5	7	25.00	1.40
45-59.9	20	7	19	35.00	2.71
60-74.9	20	11	91	55.00	8.27

Annex-14e: Lengthwise prevalence and intensity of infestation of *Argulus* sp. in *Cirrhinus mrigala* of BCDM hatchery (pond no. 2).

Length groups (cm)	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity
	examined	infected			
30-44.9	20	6	15	30.00	2.50
45-59.9	20	10	37	50.00	3.70
60-74.9	20	14	94	70.00	6.71

Annex-15a: Lengthwise prevalence and intensity of infestation of *Argulus* sp. in *Labeo rohita* of Mitha hatchery (pond no. 1).

Length groups (cm)	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity
	examined	infected			
30-44.9	20	17	184	85.00	10.82
45-59.9	20	19	309	95.00	16.26
60-74.9	20	20	614	100.00	30.70

Annex-15b: Lengthwise prevalence and intensity of infestation of *Argulus* sp. in *Labeo rohita* of Mitha hatchery (pond no. 2).

Length groups (cm)	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity
	examined	infected			
30-44.9	20	15	130	75.00	8.67
45-59.9	20	17	308	85.00	18.12
60-74.9	20	19	670	95.00	35.26

Annex-15c: Lengthwise prevalence and intensity of infestation of *Argulus* sp. in *Labeo rohita* of Mitha hatchery (pond no. 3).

Length groups (cm)	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity
	examined	infected			
30-44.9	20	1	3	5.00	3.00
45-59.9	20	3	14	15.00	4.67
60-74.9	20	6	34	30.00	5.67

Annex-15d: Lengthwise prevalence and intensity of infestation of *Argulus* sp. in *Cirrhinus mrigala* of Mitha hatchery (pond no.1).

Length groups (cm)	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity
	examined	infected			
30-44.9	20	11	31	55.00	2.82
45-59.9	20	14	49	70.00	3.50
60-74.9	20	16	102	80.00	6.38

Annex-15e: Lengthwise prevalence and intensity of infestation of *Argulus* sp. in *Cirrhinus mrigala* of Mitha hatchery (pond no. 2).

Length groups (cm)	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity
	examined	infected			
30-44.9	20	11	41	55.00	3.78
45-59.9	20	15	70	75.00	4.67
60-74.9	20	18	101	90.00	5.61

Annex-16a: Lengthwise prevalence and intensity of infestation of *Argulus* sp. in *Labeo rohita* of M.R. hatchery (pond no. 1).

Length groups (cm)	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity
	examined	infected			
30-44.9	20	19	749	95.00	39.42
45-59.9	20	20	1182	100.00	59.10
60-74.9	20	20	1564	100.00	78.20

Annex-16b: Lengthwise prevalence and intensity of infestation of *Argulus* sp. in the *Cyprinus carpio* of M.R. hatchery (pond no. 1).

Length groups (cm)	No. of host fish		No. of *Argulus* sp.	Prevalence (%)	Intensity
	examined	infected			
30-44.9	20	9	52	45.00	5.78
45-59.9	20	12	135	60.00	11.25
60-74.9	20	15	245	75.00	16.33

Annex 17a: Abundance of zooplankton (ind/l) in BCDM ponds in different sampling dates.

Group of Zooplankton	First sampling (January, 06)			Second sampling (February, 06)			Third sampling (March, 06)			Fourth sampling (April, 2006)		
	Pond no. 1	Pond no. 2	Pond no. 3	Pond no. 1	Pond no. 2	Pond no. 3	Pond no. 1	Pond no. 2	Pond no. 3	Pond no. 1	Pond no. 2	Pond no. 3
Rotifers	45	51	89	73	24	93	68	91	95	132	93	110
Copepods	6	7	14	10	6	9	9	15	20	17	15	21
Cladocera	12	9	15	7	8	11	9	9	15	13	12	19
Ostracoda	0	0	0	0	0	0	0	0	0	0	0	0
Total	**63**	**67**	**118**	**90**	**38**	**113**	**86**	**115**	**130**	**162**	**120**	**150**

Annex 18b: Abundance of zooplankton (ind/l) in Mitha hatchery ponds in different sampling date.

Group of Zooplankton	First sampling (January, 2006)			Second sampling (February, 2006)		
	Pond no. 1	Pond no. 2	Pond no. 3	Pond no. 1	Pond no. 2	Pond no. 3
Rotifers	510	545	445	465	498	435
Copepods	355	317	1085	301	345	1018
Cladocera	91	125	412	74	121	431
Ostracoda	50	68	160	28	76	214
Total	**1006**	**1055**	**2102**	**868**	**1040**	**2098**

Annex 17c: Abundance of zooplankton (ind/l) in M.R. hatchery ponds in different sampling date.

Group of Zooplankton	First sampling (January, 2006)	Second sampling (February, 2006)
	Pond no. 1	Pond no. 1
Rotifers	316	445
Copepods	1009	1042
Clodocera	55	75
Ostracoda	38	69
Total	**1418**	**1631**

Annex 17d: Observation of Zooplankton (ind/l) at Raipur hatchery ponds in Laxmipur.

Group of Zooplankton	First sampling March, 2006		
	Pond no.1	Pond no.2	Pond no.3
Rotifers	25	48	514
Copepods	308	47	312
Clodocera	397	28	410
Ostracoda	5	2	7
Total	**735**	**125**	**1243**