2020

Environmental Impacts of Entrepreneurial Fisheries in Barind Tract

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ENVIRONMENTAL IMPACTS OF ENTREPRENEURIAL FISHERIES IN BARIND TRACT

A Dissertation
submitted to the Institute of Environmental Science, University of Rajshahi in partial fulfillment of the Requirements for the degree of Doctor of Philosophy

Submitted by

Md. Mokhlesur Rahman

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DECLARATION

*This is to declare that the whole work of my PhD dissertation entitled '**Environmental Impacts of Entrepreneurial Fisheries in Barind Tract'** to the Institute of Environmental Science (IES), University of Rajshahi, Bangladesh for the **Doctor of Philosophy** is the result of my own investigation and findings. I conducted the research work under the supervision of Professor Dr. M. Nazrul Islam, Department of Zoology, University of Rajshahi, Bangladesh. None of this research work has been submitted to any other institute or university for any other degree. Relevant findings of other researchers have been duly acknowledged whenever cited in the dissertation.*

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CERTIFICATE

*This is to certify that the dissertation entitled '**Environmental Impacts of Entrepreneurial Fisheries in Barind Tract**' submitted for the degree of **Doctor of Philosophy** to the Institute of Environmental Science (IES), University of Rajshahi, Bangladesh, is based on independent, original research work done by Md. Mokhlesur Rahman, under my guidance and supervision. Neither the whole thesis nor any part thereof has been presented for any other diploma or degree to any other institute or university.*

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ACKNOWLEDGEMENT

First of all my sincere thanks to my family whose cooperation has enabled me to spend time for the study over last three and half years; who have made sacrifices and gladly accepted my shortcomings that had arisen due to me not being able to provide enough care and time to the family.

My sincere gratitude to my supervisor, Professor Dr. M. Nazrul Islam, Department of Zoology, University of Rajshahi for his encouragement, proper guidance and motivation on day to day basis for last four years that has enabled me to go through the course of the study.

I would also like to acknowledge the valuable contribution of my teachers in the Institute of Environmental Science, especially Dr. Golam Mostafa and Dr. Md. Redwanur Rahman for their cooperation, affection and encouragement that they have offered me even during the weekends and off days.

I'm very grateful to my employer, Country Representative of MCC Bangladesh, Mr. David Hall (former Country Representative) and Mr. George Epp, for their kind permission and cooperation that has allowed me to spend enough time for the purpose of study, without which I couldn't even get involved in this.

I'm very thankful to my colleagues for their cooperation and assistance, my Lab. mates in the water quality management Lab. of Institute of Environmental Science, University of Rajshahi; Md. Mahbubur Rahman, Assistant Professor, Biochemistry Department of Rajshahi Medical College, Rajshahi and Md. Alauddin, Lab. assistant in water quality Lab. of Faculty of Fisheries of Bangladesh Agricultural University in Mymensingh.

I would also like to thank all the fisheries entrepreneurs who shared their information and allowed me to conduct several experiments and sampling during the course of my study; Soil Resource Development Institute (SRDI) Lab., Rajshahi and Central Laboratory of Rajshahi University for their cooperation and services.

I'm also grateful to the Ministry of Science and Technology, for its financial support through National Science and Technology Scholarship for year 2017-18, which was very helpful for bearing partial expenses of this PhD research work.

The Author

ABSTRACT

The gradual increase in fish production of Bangladesh is primarily attributed to the increased inland aquaculture especially from the entrepreneurial fisheries. Entrepreneurial fisheries in Barind Tract has also gained traction in the last several years like the few other top fish producing districts of Bangladesh. In four Barind districts (Bogura, Joypurhat, Naogoan, and Rajshahi), pond area and fish production have increased by 41.39% and 134.25% respectively in 2017-18 than that of 2007-08. In this process, thousands of hectares of the seasonal floodplain, paddy fields, and perennial wetlands have been purposely converted into large fishponds for entrepreneurial fisheries in Barind Tract. Since entrepreneurial fisheries are commercial ventures, these are also capital and input-intensive. Not only the intensity (quantity/unit area) of inputs but also the sheer volume (spread) of entrepreneurial fisheries has made it a new but significant farming system in Barind Tract hence deserves a closer look in terms of environmental impacts.

Since a large number of paddy fields have been converted into entrepreneurial fishponds, input use was compared between entrepreneurial fisheries and paddy production in this study through a questionnaire-based survey. Additionally, the faunal diversity, especially aquatic invertebrates (aquatic insects, zooplankton and benthos) and water quality parameters, macro, and micro soil nutrients and heavy metal contents of the bottom sediments of entrepreneurial fishponds were compared with those of natural wetlands. Several experiments were conducted to know the impacts of various pesticides, hormones, and fish toxicants used in entrepreneurial fisheries on aquatic invertebrates and human health. To know the social impact of entrepreneurial fisheries, focus group the discussion was conducted.

The current study revealed that the entrepreneurial fishponds were purposely built hence large (average size of more than an acre water area) in size with 5 to 10 feet water depth depending on the season and rearing of large-sized Indian major carps and Chinese carps. Of all the entrepreneurial fishponds, 61.5% of the ponds were located in areas that used to be seasonal floodplain, whereas 23.1% of the ponds were constructed by transforming paddy fields and 15.4% ponds were constructed through the transformation of perennial wetland.

Being an intensive venture, more inputs were required for entrepreneurial fisheries (7158.4 kg/year/acre water area) compared to the paddy fields (4611 kg/acre). Of all the inputs used in entrepreneurial fisheries, 69.34% was feed followed by fertilizers (26.79%), different disinfectant chemicals (3.45%) and others. For paddy fields, almost all (99.98%) the inputs

were fertilizers (mostly organic manure) and very negligible percentage (0.02%) of pesticide, fungicides and herbicides.

Of the surveyed fish feed, 50% of the feed contained lower protein than the reference value mentioned in the respective feed bags. 57.14% of the feed had crude protein levels between 24 and 27% which is desirable for the good growth of Indian major carps. All the fish feeds (14 different types) were tested for heavy metals (chromium, cadmium, and lead) and only 7.1% of the feed had more cadmium concentration than the maximum permissible limit. Five different brands of TSP fertilizer samples and three MOP fertilizer samples contained chromium, cadmium, and lead within the permissible limit as per Bangladesh standard.

The higher concentrations of organic carbon, nitrogen, potassium, phosphorus, sulfur, zinc, and copper were found in entrepreneurial fishpond sediments than natural wetlands and pond dikes. Pond dikes without agriculture had the highest concentration of calcium and magnesium. On the other hand, the highest quantity of boron, iron, and manganese was found in natural wetland (perineal static water) sediments. Of the heavy metals, entrepreneurial fishpond sediments contained the highest concentration of lead. Sediments of natural wetland had a higher concentration of chromium and cadmium than the pond bottom sediments. Regarding water quality parameters, water in natural wetlands was more transparent (in terms of Secchi disk reading) than the water of entrepreneurial fishponds. On the other hand, water in entrepreneurial fishponds had higher conductivity, pH, TDS, and turbidity (ntu).

Entrepreneurial fishponds were found to be having a higher density of zooplankton than the natural wetlands. Though the diversity of zooplankton was the same, the community structure of the zooplankton in the natural wetland was more balanced indicating a homogeneous distribution of zooplankton than entrepreneurial fishponds where the community structure of zooplankton was dominated by few zooplankton types like *Keratella*, *Brachionus*, nauplius etc.

Higher numbers of aquatic insects (per unit water volume) were present in entrepreneurial fishponds than the natural wetlands. The diversity of insects was the same in both the fishponds and the natural wetlands. However, the community structure of the aquatic insects in the natural wetland was more balanced which means that various types of aquatic insects were homogeneously distributed. On the other hand, the community structure of aquatic insects in entrepreneurial fishponds was dominated by few insect types (water boatman, backswimmer, damselfly larvae, etc.). Entrepreneurial fishponds also supported a very high density of benthos (*Chironomid* larvae and *Tubifex*) than natural wetlands.

Farmers in entrepreneurial fisheries were found to be using emamectin benzoate for the control of fish parasites, especially for treating external parasites like fish lice and anchor worms. But unlike the salmon industry where emamectin benzoate is permitted for feed treatment; farmers use emamectin benzoate exclusively in entrepreneurial fisheries as a water treatment agent. Use of emamectin benzoate as a water treating agent at the rate of 2µg/L pond water had negatively affected zooplankton (declined by 29.11% after 48 hours) and benthos population (58.57% decline after 24 hours), compared to in-feed use of emamectin benzoate at the rate of 50 µg/kg feed which had no impact on the zooplankton. Moreover, the choice of application method led to a great difference (water treatment requires many times more) in the amount of emamectin benzoate required for the treatment.

In the process of being very efficient, entrepreneurial fish farmers have used selective poisoning for getting rid of the undesired species through the use of quinalphos pesticides at 0.02mg/liter water, while keeping their desired species (Indian and Chinese major carps) unaffected in the culture ponds. Quinalphos sustained and bioaccumulated in fish up to10 days after the use. Zooplankton count declined by 31.41% on day two after the use of quinalphos. Zooplanktons were impacted for a short duration and were able to regenerate within 5 days of the use of quinalphos. Aquatic insects count had declined slowly and loss of insect diversity was observed till the 28 days after the use of quinalphos. On the other hand, benthos count was declined by 49.65% one day after use of quinalphos then stabilized after a slight increase.

Agricultural insecticides especially cypermethrin, deltamethrin, abamectin, and lambda-cyhalothrin have been used regularly for the control of aquatic insects in entrepreneurial fishponds. Aquatic insect number count declined by 89.87% and by 86.24% for the treatment of cypermethrin and deltamethrin respectively within one day after treatment at the rate of 5µg (active ingredient)/liter pond water in presence of fish. The use of lambda-cyhalothrin at 0.5 µg/liter pond water had caused 93%, 60%, and 79% decline in water insect, zooplankton, and benthos respectively. Along with the decline in number, diversity loss for aquatic insects was also observed in cypermethrin, deltamethrin, abamectin, and lambda-cyhalothrin treated waters, while diversity loss of zooplankton was observed when abamectin and lambda cyhalothrin were used.

In entrepreneurial fisheries in the northwest Bangladesh where pond ownership (using rights due to lease) changes frequently (every few years), use of fish toxicants is common and crucial. Along with some traditional fish toxicants (rotenone and aluminium phosphide), unconventional toxicants and insecticides like fenpropathrin (not approved for aquaculture use) were also used by fish farm owners. Zooplankton and aquatic insect population were least impacted by fenpropathrin than other fish toxicants, while the benthos population was least impacted by rotenone in terms of mortality and quick recovery time for the population, due to the high turbidity (suspended soil particle) of the pond water.The total insect count in the fenpropathrin and aluminium phosphide treatments declined by 86% and 82% respectively compared to their pretreatment level.

Fish killed by farmers using fish toxicants in commercial fisheries in northwest Bangladesh were being sold in the fish markets for human consumption. Therefore, there is always a concern about the food safety of the fish killed using a fish toxicant. Using convenience, quick killing, cheaper price, short duration of toxicity, and no potential long-term damage of the water body contributes positively to fenpropathrin as fish toxicant except for the severe potential public health concern from eating fish killed by fenpropathrin due to very high bioconcentration factor of fenpropathrin; hence, demands regulation of fenpropathrin's use as fish toxicants for food fish.

Entrepreneurial fisheries have a long supply chain where many stakeholders are involved, and it has a great impact on the livelihoods of the people of nearby villages. The type of work that many of the actors are involved in the entrepreneurial fisheries value chain are often intense, usually for a short duration and at odd times (especially for actors involved forward linkage, including fish harvester group), mainly at early hours from 3.00 am to 9.00 am for selling their commodity to the consumers that result in a high payment (up to 400% depending on the season) for the workers than conventional agriculture work.

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CHAPTER ONE

GENERAL INTRODUCTION AND REVIEW OF LITERATURE

According to FAO (2018) statistics, fin fish production from inland aquaculture in Bangladesh started increasing sharply since 2010 and stood ranked fifth only after China, India, Indonesia and Vietnam in 2016 when Bangladesh produced 1.9446 million tonnes of finfish from inland aquaculture sources. In 1995 Bangladesh produced 317 thousand tonnes of aquaculture food fish, 1.3% of the total aquaculture food fish produced in the world in 1995, whereas in 2016 Bangladesh contributed 2.8% (2.204 million tonnes) of the total world aquaculture food fish production (FAO, 2018).

Bangladesh has extensive and diversified fisheries resources. Department of Fisheries (DoF) (2018) statistics shows that the total fish production in Bangladesh in 2017-18 was about 4.277 million metric tonnes, of which 2.4 million metric tonnes (56.24% of the total fish production in Bangladesh) came from inland closed water fisheries, and 1.21 million metric tonnes (28.45% of the total fish production in Bangladesh) came from inland open water fisheries. Therefore, a total of 84.69% of the total fish production in Bangladesh came from inland fisheries in 2017-18 as opposed to the 80.59% in 2007-08, where inland closed water fisheries contributed 39.23% of the total production.

As food, fish are rich in nutrients including good quality protein with all the essential amino acids, long chain poly-unsaturated fatty acids and micronutrients (vitamins and minerals) (FAO, 2019; Mohanty, 2011; Tilami and Samples, 2017). Hence, regular consumption of fish is instrumental in preventing nutritional deficiency diseases and high cholesterol, high blood pressure, Alzeimer’s disease and cardiovascular diseases (Barberger-Gateau *et al.*, 2002; Verbeke & Vackier, 2005; McNaughton *et al.*, 2008).

Fish have remained the dominant protein source among all protein sources of animal origin in Bangladesh. In synchrony with the increase in fish production, per capita per day fish consumption in 2016 reached 62.58g, an increase of 51.07% of the per capita consumption in 2005 (BBS, 2019). However, a gap between rural and urban per capita per day fish consumption does exist, where per day per capita fish consumption in rural areas is 60.59g, a total of 7.32g less than the urban consumption of 67.91g per capita per day (HIES-2016, 2017). In a similar trend, fish consumption from capture fishery between 1991 and 2010 declined by 33% (Bogard, 2017). On the other hand, the increased consumption from culture fishery compensated the reduction of consumption from capture fishery and per day per capita fish consumption has increased by 51.07% over the period of last 10 years (BBS, 2019).

Entrepreneurial fisheries

Belton *et al.* (2011) in their review of aquaculture and fish consumption of Bangladesh, differentiated between three main forms of pond aquaculture in Bangladesh; 'homestead pond culture', 'commercial semi-intensive carp culture' and 'entrepreneurial pond culture', estimated that each type accounts for approximately 30% of total aquaculture production in 2011; where entrepreneurial fisheries was defined as purposely initiated enterprise of fish cultivation that involves generally intensive management, utilization of pelleted feed, employment of hired labor, high operational costs and high yield. According to Belton *et al.* (2011) entrepreneurial fisheries is relatively a recent development in Bangladesh, starting from the late 1990s, and primarily dominated by *pangasius* and Nile tilapia, as well as climbing perch, stinging catfish and walking catfish.

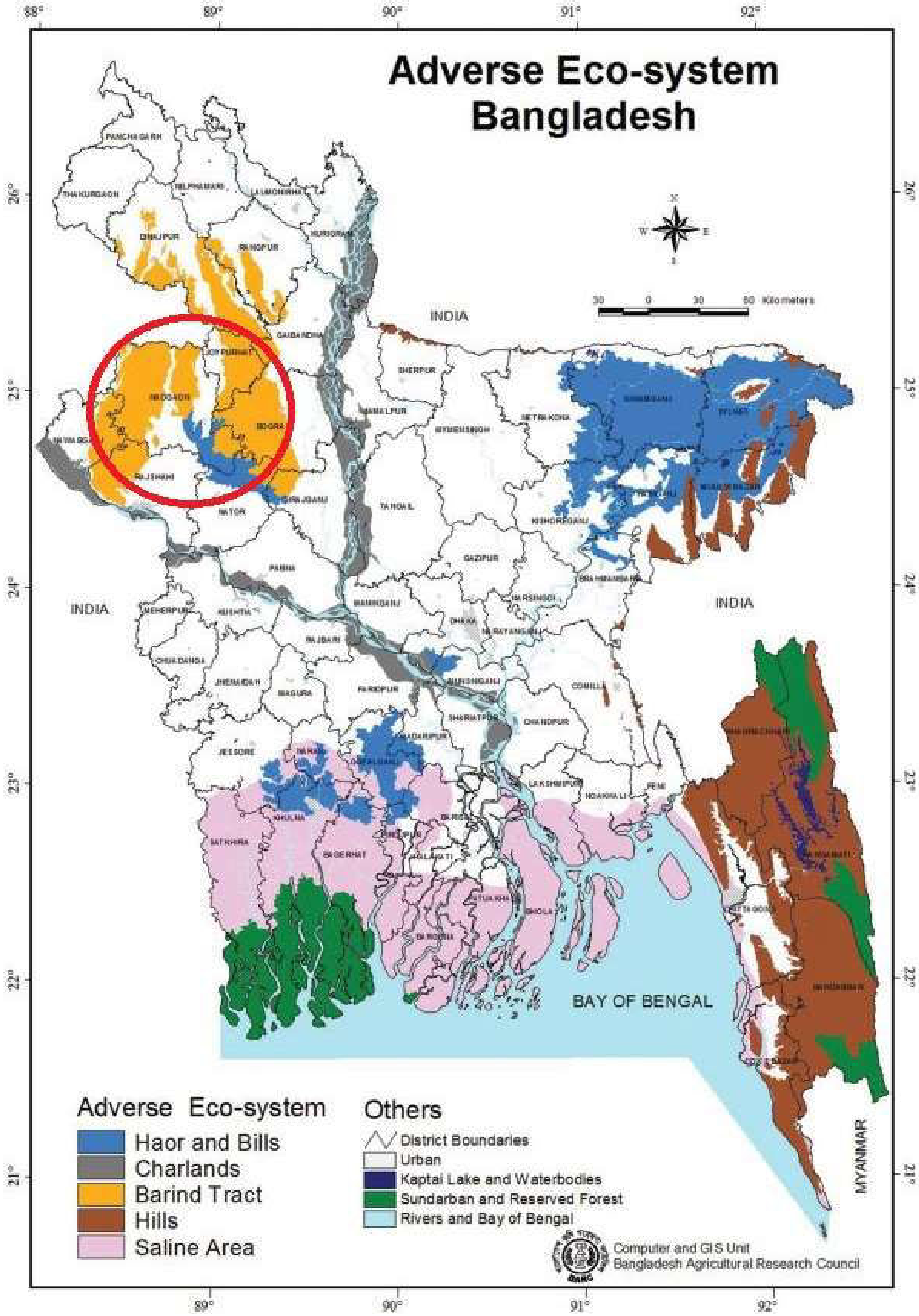
Therefore, entrepreneurial fisheries are literally enterprises/businesses that deal with the commercial production and selling of fish. To do it profitably, all aspects of a business are taken into account, including a purposefully built ponds of the right size and depth to enhance a better growth of fish; reliability of water supply; connectivity to roads for input and fish transport; utilization of various kinds of inputs to maintain and improve water quality, enhance fish growth, and reduce risk of disease/pest infestation; maximization of space use, hired skilled labor, and the provision of physical protection and safety of the culture ponds. Hence, entrepreneurial aquaculture has a very high investment compared to the subsistence level of fish farming. It is primarily a business rather than the intensive farming of fish. Due to its high quantity and various types of input supplies, as well as large volume of fish harvesting and marketing, there are various stakeholders involved in both input supply and fish marketing, forming a complex and long value chain.

Since entrepreneurial fisheries is a business that requires high initial investments, running capital and good managerial and technical skills, it is predominantly wealthier and educated people who are involved in this kind of enterprise. Various studies on entrepreneurial fisheries in Mymensingh region, the pioneering district of entrepreneurial fisheries in Bangladesh (Belton *et al.,* 2011) validates the clam made above. Ali (2009), in a study of two villages of Mymensingh, found that 80 percent of the households involved in *P. hypophthalmus* culture were in the richest category, but none of the poorest 116 fish farming households specifically raised *P. hypophthalmus*. Haque (2009) found that most of the *P. hypophthalmus* farmers had another primary or secondary occupation other than farming in Mymensingh. This means that *P. hypophthalmus* farmers operate a business or are formally employed in another sector and were not involved in fish farming as a primary and exclusive income source, rather they entered in farming as an entrepreneurial venture. Munir (2009) found a very strong positive correlation between educational qualification of the farm owners and farm size of *P. hypophthalmus* in Trishal Upazila of Mymensingh, where farmers who own bigger *P. hypophthalmus* farms were significantly more educated than those who had smaller farms of same type.

The growth of intensive aquaculture over last 10 years from 2007-08 in terms of hectares of ponds and fish production in the Barind Tract is indicative of the fact that in the past ten years new ponds have been built for aquaculture and primarily as entrepreneurial fisheries. Unlike the entrepreneurial fisheries of top three pond fish producing districts (Mymensingh, Jessore and Comilla) in Bangladesh, which are dominated by monoculture of *P. hypophthalmus*, *O. niloticus*, *Anabus testudineus* and other cat fishes, the entrepreneurial fisheries of the Barind Tract is primarily dominated by Indian and Chinese Carps (DoF, 2018). There is only a small presence of *P. hypophthalmus* and *O. niloticus* monoculture in the Barind Tract, particularly in the areas of Joypurhat, Bogura and Naogoan districts. Fisheries in Mymensingh, Jessore and Comilla are characterized by a monoculture condition with a density as high as the species types allow. This is one of the reasons (apart from greater pond hectarage) that has put those districts at the top of the list of the highest fish producing districts of Bangladesh (DoF, 2018).

The Barind Tract

The Barind Tract is a Pleistocene terrace which spreads over the area of 9,324 sq km in the greater Rajshahi, Dinajpur, Rangpur and Bogura districts in Bangladesh and about 2650 sq km of the Maldah district of West Bengal (Rashid *et al*., 2014). It is made up of a few fragmented and separate sections in the northwestern part of Bangladesh (Banglapedia, 2019). The Barind Tract lies northwest of the confluence of the upper Padma and the Jamuna rivers, bordered by flood plains of Mahananda river in the west and Karotoa river in the east (Encyclopaedia Britannica, 2019). It is also crisscrossed by a few other rivers mostly flowing roughly N-S, NE-SW and SE directions (Rashid *et al*., 2014). The Barind Tract is a relatively high, undulating region with reddish and yellowish clay soil (Encyclopaedia Britannica, 2019) and is tilted upwards in the West and slopes down towards the South and Southeast (Rashid *et al*., 2014). However, in this writing, the Barind Tract refers primarily to the geographical region consisting of district of Rajshahi, Naogoan, Joypurhat and Bogura.



Source: BARC, 2019

Map 1.1 Different eco-systems of Bangladesh with the Barind Tract within the red circle.

The Barind Tract is generally hot and humid, since the Tropic of Cancer is in the south side of the region. Four different weather conditions, namely pre monsoon, monsoon, post monsoon and winter, are observed in this region based on the humidity, rainfall, temperature and wind pressure. The average temperature during hot months ranges from 25^0C to 35^0C and 9^0C to 15^0C during cold months, but temperatures can climb as high as 45^0C or drop as low as 5^0C on the extremes. Rainfall in this region varies from year to year and is generally low compared to other parts of Bangladesh. The rainfall average is 1971mm/year, most of which occurs during the monsoon (Encyclopaedia Britannica, 2019).

Considering the inherent sedimentological characteristics of depositional environment and after a detailed study of the soil, the Barind Tract soil is identified as “Barind Clay” (Rashid *et al*., 2014). Recent study shows that 85% of the area has low groundwater recharge potentiality and the rest has moderate. Only 8.6% of the total average annual precipitated water (1685mm) percolates into subsurface and ultimately contributes to recharge the groundwater (Adham *et al*., 2010).

Wetland in Barind Tract

Wetlands are not only among the most productive but are also one of the most threatened ecosystems of the world (Ducks Unlimited Canada, 2008). Throughout Bangladesh 4.16 million hectares of floodplain was reduced due to the flood protection structures built in the 1980s (ADB, 2004). The drastic reduction of flood plain wetland throughout the country was the direct result of Flood Control, Drainage and Irrigation (FCDI) projects, reflecting the tradeoff between increased crop production and reduced flood plain including the ecological and fisheries services that these ecosystems provide (ADB, 2004).

Shopan *et al*. (2013) after analyzing Landsat satellite images, found a reduction of 25.25% in wetland area in northwest Bangladesh during the dry season between years 1989 and 2000. Dry season wetland was reduced by 305.18 square kilometer from 1208.72 square kilometer to 903.54 square kilometer in northwest Bangladesh in the decade of 1990’s. On the other hand, the dry season reduction of wetland in northwest Bangladesh was slower in the next decade, reducing to 867.18 square kilometer in 2010 from 903.54 square kilometer in 2000 with a decline of 4.02% in 10 years. In Bogura district, dry season wetland reduction was 16.23% between 1989 and 2000 but 9.78% between 2000 and 2010. On the other hand, in Rajshahi district the dry season wetland reduction was higher (22.18%) between 2000 and 2010 compared to 15.41% between 1989 and 2000 (Shopan *et al*., 2013).

Pond aquaculture with special reference to the Barind Tract in Bangladesh

Pond aquaculture in the form of entrepreneurial fisheries in Bangladesh and particularly in the Barind Tract increased during the last decade, in contrast to the trend of natural wetland reduction during the same period. In the Bogura district of Bangladesh in 2017-18, a total of 14188 hectares of ponds were under aquaculture of which 528 hectares (3.72%) were under highly intensive aquaculture, 3687 hectares (25.99%) under intensive aquaculture, 9553 hectares (67.33%) under semi intensive aquaculture and 420 hectares (2.96%) under extensive aquaculture (DoF, 2018). These ponds produced 67093 metric tonnes fish, as opposed to a total of 9477 hectares of ponds under aquaculture in 2007-2008 that produced 25808 metric tonnes fish (DoF, 2009). In last 10 years in Bogura district alone, pond area has increased by 4711 hectares, an increase of 49.71% of the 2007-2008 pond area. The production has increased by 41284 metric tonnes, an increase of 159.97% of the 2007-2008 production. In Bogura, the total inland (culture plus capture) fish production was 33878 metric tonnes of which 76.18% came from pond aquaculture in 2007-2008. However, in 2017-2018, the total inland culture and capture fish production was 75827 metric tonnes of which contribution from pond aquaculture was 88.48%. Therefore, the increase in contribution from pond aquaculture to the total inland fish production in Bogura district was 12.30% in between 2007-08 and 2017-2018.

In Joypurhat District of Bangladesh, a total of 4635 hectares of ponds were under aquaculture of which 2499 hectares (53.92%) were under intensive aquaculture, 2105 hectares (45.41%) under semi intensive aquaculture and 31 hectares (0.67%) under extensive aquaculture in 2017-2018 (DoF, 2018). The ponds produced 21679 metric tonnes fish, as opposed to a total of 3062 hectares of ponds under aquaculture in 2007-2008 that produced 9117 metric tonnes fish (DoF, 2009). In last 10 years in Joypurhat district alone, pond area has increased by 1573 hectares, an increase of 51.37% of the 2007-2008 pond area, and the production has increased by 12561 metric tonnes, an increase of 137.77 % of the 2007-2008 production. In Joypurhat the total inland culture plus capture fish production in 2007-2008 was 16178 metric tonnes of which 56.35% came from pond aquaculture. In 2017-2018, the total inland culture and capture fish production was 22231 metric tonnes of which the contribution from pond aquaculture was 97.52%. Therefore, the increase in contribution from pond aquaculture to the total inland fish production in Joypurhat district was 41.17% in between 2007-2008 and 2017-2018.

In Naogoan District of Bangladesh in 2017-2018, a total of 12750 hectares of ponds were under aquaculture of which 117 hectares (0.92%) were under highly intensive aquaculture, 2799 hectares (21.95%) under intensive aquaculture, 9714 hectares (76.19%) under semi intensive aquaculture and 120 hectares (0.94%) under extensive aquaculture (DoF, 2018). The ponds produced 54470 metric tonnes fish, as opposed to a total of 10768 hectares of ponds under aquaculture in 2007-08 that produced 24069 metric tonnes fish (DoF, 2009). In last 10 years in Naogoan district alone, pond area has increased by 1982 hectares, an increase of 18.41% of the 2007-2008 pond area, and the production has increased by 30401 metric tonnes, an increase of 126.31% of the 2007-2008 production. In Naogoan, the total inland culture plus capture fish production was 40366 metric tonnes of which 59.63% came from pond aquaculture in 2007-2008. In 2017-2018, the total inland culture and capture fish production was 77942 metric tonnes of which contribution from pond aquaculture was 69.89%. Therefore, the increase in contribution from pond aquaculture to the total inland fish production in Bogura district was 10.26% in between 2007-2008 and 2017-2018.

In Rajshahi District of Bangladesh in 2017-2018, a total of 12310 hectares of ponds were under aquaculture of which 321 hectares (2.61%) were under highly intensive aquaculture, 5561 hectares (45.17%) under intensive aquaculture, 6428 hectares (52.22%) under semi intensive aquaculture and no ponds under extensive aquaculture (DoF, 2018). These ponds produced 54859 metric tonnes fish, as opposed to a total of 7729 hectares of ponds under aquaculture in 2007-2008 that produced 25576 metric tonnes fish (DoF, 2009). In last 10 years in Rajshahi district alone, pond area has increased by 4581 hectares, an increase of 59.27% of the 2007-2008 pond area, and the production has increased by 29283 metric tonnes, an increase of 114.49% of the 2007-2008 production. The total inland culture plus capture fish production in 2007-2008 was 41496 metric tonnes of which 61.63% came from pond aquaculture. In 2017-2018, the total inland culture and capture fish production was 75266 metric tonnes of which contribution from pond aquaculture was 72.93%.

In the four Barind districts (Bogura, Joypurhat, Naogoan and Rajshahi) in 2017-2018, a total of 43883 hectares of ponds were under aquaculture of which 966 hectares (2.2%) were under highly intensive aquaculture, 14546 hectares (33.15%) under intensive aquaculture, 27800 hectares (63.35%) under semi intensive aquaculture and 571 hectares (1.3%) under extensive aquaculture (DoF, 2018). The total ponds produced 198099 metric tonnes of fish, as opposed to a total of 31036 hectares of ponds under aquaculture in 2007-2008 that produced 84570 metric tonnes of fish (DoF, 2009). In the last ten years in the four Barind districts (Bogura, Joypurhat, Naogoan and Rajshahi), pond area has increased by 12847 hectares, an increase of 41.39% from the 2007-2008 pond area. The production has increased by 113531 metric tonnes, an increase of 134.25% from the 2007-2008 production. In the four Barind districts (Bogura, Joypurhat, Naogoan and Rajshahi), the total inland culture and capture fish production in 2007-2008 was 131918 metric tonnes of which 64.11% came from pond aquaculture. In 2017-2018, the total inland culture and capture fish production was 251226 metric tonnes of which the contribution from pond aquaculture was 78.85%.

The national total inland culture and capture fish production in 2007-2008 was 2065723 metric tonnes of which 6.39% came from the four Barind districts (Bogura, Joypurhat, Naogoan and Rajshahi) but in 2017-18 the national total inland culture and capture fish production was 3621954 metric tonnes of which the contribution from the four Barind districts was 6.94%. The total national inland fish production has increased 75.34% over the last ten years or 7.5% per year, but the growth of inland fish production in the four Barind districts increased 90.44% over past 10 years or 9.04% per year. The total national production from pond aquaculture was 866049 metric tonnes in 2007-2008 of which 9.77% came from four Barind districts. In 2017-2018 the contribution of the four Barind districts was 10.43% of the total national production from pond aquaculture of 1900298 metric tonnes. A total of 305025 hectares of ponds were available nationally for aquaculture in 2007-2008, of which 10.18% were in four Barind districts. However, in 2017-2018 nationally ponds covered 391753 hectares of which 11.20% were in the four Barind districts (DoF, 2009 and DoF, 2018). Therefore, the share of pond hectarage, production from pond aquaculture and contribution to total inland fish production has increased in the Barind Tract at a greater rate than the national average and most other parts of the county, except the top three fish producing districts (Mymensingh, Jessore, and Comilla) in last ten years.

In 2007-2008, Bogura, Joypurhat, Naogoan, and Rajshahi ranked 6th, 37th, 4th and 13th respectively among all districts of Bangladesh in terms of aquaculture pond hectarage. In 2017-2018, the four Barind districts ranked 5th, 31st, 8th and 9th respectively among all districts of Bangladesh in terms of aquaculture pond hectarage. Of the four Barind districts only Naogoan has slipped in ranking related to pond hectarage, while Bogura, Joypurhat and Rajshahi have made progress in ranking (Appendix Table 1.2).

In terms of fish production from pond aquaculture, Bogura, Joypurhat, Naogoan, and Rajshahi ranked 7th, 34th, 10th and 9th respectively in 2007-2008 among all districts of Bangladesh. While in 2017-2018, the ranking of all the four Barind districts progressed and stood at 4th, 25th, 7th and 6th respectively among all districts of Bangladesh. However, compared to the top three aquaculture fish producing districts (Mymensingh, Jessore and Comilla), the progress in fish production from aquaculture sources and expansion of pond hectarage in the four Barind districts over last 10 years has been relatively slow. This is due to the top three districts having the natural and geographical advantage of low-lying land topography, loamy soil, and higher rainfall as compared to the Barind Tract districts. Despite the geographical and natural disadvantages, the growth in production and hectarage of pond aquaculture over the last 10 years in these four Barind districts is second highest in the nation, only after the group of top three aquaculture fish producing districts.

Of the four Barind districts the highest expansion of aquaculture ponds occurred in Rajshahi district, showing an expansion of 59.25% over the past 10 years. There, 45.17% of the total ponds were under intensive aquaculture in 2017-2018, second only to Joypurhat where 53.92% of the ponds were under intensive aquaculture. However, the hectarage of aquaculture ponds in Joypurhat is only 37.65% of Rajshahi ponds (DoF, 2018).

As an enterprise, entrepreneurial fisheries are dynamic in terms of the share of fish species that are cultivated in Barind Tract as well as in other top three fish producing districts (Mymensingh, Jessore and Comilla). Off the many fish species, share of *L. rohita* and *C. mrigala* has increased in terms of pond production (yields) in Joypurhat, Naogoan and Rajshahi in 2017-2018 compared to 2007-2008 (App. Table 1.3). Share of *C. catla* and *H. molitrix* in terms of production diminished in all four Barind districts of Bogura, Joypurhat, Naogoan and Rajshahi from 2007-2008 to 2017-2018. Contribution of *C. carpio* and other exotic carps also increased in all four Barind districts over the past 10 years. Contribution of *O. niloticus/ mossambicus* and *P. hypophthalmus* increased drastically in Bogura, Joypurhat and Naogoan in the last ten years since 2007-2008. In Rajshahi, contribution of both *O. niloticus/mossambicus* diminished drastically over the last 10 years, but the contribution of *p. hypophthalmus* did not change much. On the other hand, contribution of *O. niloticus* and *P. hypophthalmus* has increased drastically in Bogura, Joypurhat and Naogoan districts over the period of last ten years. Indian, Chinese, and common carps contributed 92.57% of the total pond aquaculture production of Rajshahi district in the year 2017-2018 (App. Table 1.3).

Share of *L. rohita*, *C. catla*, *C. mrigala*, *H. molitrix* reduced drastically in Mymensingh in 2017-2018 compared to their production percentage in 2007-2008 (App. Table 1.4). On the other hand, production of *P. hypophthalmus* has increased to reach a staggering 52.93% in 2017-2018 compared to just 4% in 2007-2008. The share of *O. niloticus*, *C. batrachus*/ *H. fossilis* and *A. testudineus* production has also increased significantly in 2017-2018 compared to its share in 2007-2008 in Mymensingh. A similar trend has also been observed in both Jessore and Comilla, where the share of Indian and Chinese carp's production declined drastically, and production share of *O. niloticus*, *P. hypophthalmus*, *C. batrachus*, *H. fossilis* and *A. testudineus* increased in 2017-18 compared to that of 2007-2008. The combined share of four types of fish (*O. niloticus*, *P. hypophthalmus*, *C. batrachus*, *H. fossilis* and *A. testudineus*) production contributed a staggering 72.49%, 36.14% and 77.27% of total pond fish production respectively in Mymensingh, Jessore and Comilla in 2017-18, compared to 12.74%, 11.58% and 29.8% respectively in 2007-2008 (App. Table 1.4).

Fish species composition in entrepreneurial fisheries in the Barind Tract as well as in other parts of Bangladesh is still evolving and is influenced by the geographical, physical advantages and business opportunities as perceived and grabbed by the fisheries entrepreneurs. But currently (2017-2018) entrepreneurial fisheries in the Barind Tract is dominated by Indian and Chinese carps, while entrepreneurial fisheries in top three fish producing districts (Mymensingh, Jessore, Comilla) in Bangladesh is dominated by catfish and tilapia.

Though the Barind Tract is a special agro-climatic zone with generally low rainfall unlike the rest of the country but the natural wetlands in this region is generally declining like the other parts of the country. On the other hand, entrepreneurial fisheries are flourishing in this region in higher rate than most part of Bangladesh that has made this region an aquaculture hub. In this context, it is only justified that the environmental impact assessment of entrepreneurial fisheries in the Barind Tract is done.

The formal definition of environmental impact assessment usually deals with the assessment of environmental impacts before the implementation of the project. But in this case the environmental impacts of entrepreneurial fisheries that are already in place and going on in the Barind districts have been studied.

Specific objectives of the study

The study was conducted to fulfill the following objectives:

1. To know the production system and input supplies of entrepreneurial fisheries.
2. To identify and know the environmentally sensitive practices of entrepreneurial fisheries.
3. To assess the impact of entrepreneurial fisheries on soil and water resources.
4. To know the impact of entrepreneurial fisheries on aquatic faunal diversity.
5. To assess the immediate impact of harmful chemicals used in entrepreneurial fisheries on aquatic invertebrates and social implications of entrepreneurial fisheries on the surrounding community.

The first four objectives are dealt in the chapter two ‘Characterization of entrepreneurial fisheries in the Barind Tract’ and the fifth objective is explored in chapter three ‘Impacts of entrepreneurial fisheries’.

CHAPTER TWO

CHARACTERIZATION OF ENTREPRENEURIAL FISHERIES IN THE BARIND TRACT

Introduction

The Barind Tract is at odds with the rest of the deltaic flood plain of Bangladesh due to its geological characters defined as Pleistocene terrace with higher elevation and reddish or yellowish clay soil; and environmental characters of relatively higher temperature combined with lower rainfall compared to the rest of the country (Rashid *et al*., 2014; Encyclopaedia Britannica, 2019). Despite of these challenging geo-environmental conditions, Barind Tract has become the nest of entrepreneurial fisheries over the last decade, where primarily carp fattening is practiced in the form of commercial aquaculture; larger size, overwintered fish is stocked at lower density to overcome some of the challenges resulting from the geo-environmental conditions (Hossain *et al.,* 2020) of this region and obtain faster growth (Alam *et al.*, 2002; Jobling, 2010); that leads into attaining maximum fish biomass within shorter period of time (Grover *et al.*, 2000).

This chapter presents a vivid written description of the distinctive characteristics like, water quality, culture mechanism, soil quality and heavy metal concentration, fish diversity, feed, feed additives, water use, fertilizers, water disinfectants, fish toxicants, pesticides, herbicides, antibiotics, therapeutants, aquatic insects, zooplankton and benthos population etc. of entrepreneurial fisheries in the Barind Tract. This depiction will include not only the core features of entrepreneurial fisheries in the Barind Tract but will also compare it with relevant proxy production systems and/or ecosystems to make greater sense of the facts, information and figures in the relative context. Because entrepreneurial fisheries take place in a large physical space, it has replaced the original geographical features of the physical space that existed before it was converted into an aquaculture pond for entrepreneurial fisheries. Therefore, to make a comparison regarding various aspects of entrepreneurial fisheries with the previous system which has been replaced by entrepreneurial fisheries, the prior proxy production system or ecosystem is used for comparison. To be more specific, when it comes to understanding the use and impact of agrochemicals in entrepreneurial fisheries, a comparison is made with rice cultivation. On the other hand, when it comes to understanding faunal diversity, primarily the diversity of aquatic insects, zooplankton and benthos, fish species and other vertebrates and water quality parameters in entrepreneurial fisheries, these are compared with the diversity and water quality of natural *beel*s (lakes) and canals of the same region.

Materials and Methods

Site selection for the study

To locate the study site based on the selection criteria of entrepreneurial fishpond clusters in the Barind tract, searching was done, on the basis of information received from literature review and interviews of secondary sources related to the fisheries sector. As mentioned in chapter one, a functional definition of the Barind Tract for this study is defined as the geographical region consisting of the administrative districts of Bogura, Joypurhat, Naogoan and Rajshahi. Also, the general discussion about entrepreneurial fisheries in Chapter one, reveals that there are more similarities among these four administrative Barind districts than dissimilarities in terms of entrepreneurial fisheries. On the other hand, the entrepreneurial fisheries of the Barind Tract, is more homogenous in terms of the composition of cultured species and pond structures than that of entrepreneurial fisheries of Mymensingh, Jessore and Comilla. Throughout the Barind Tract primarily one system that is 'intensive culture of Indian and Chinese carps' is followed. Based on the information, any site would be suitable for study if it falls under the coverage of the Barind Tract. To narrow it down to several potential locations, help from google earth map was taken to locate potential sites where a good number of purposely built aquaculture ponds were in proximity with each other. Each of the locations identified from google map were personally visited. During the visit, local people, who have knowledge of entrepreneurial fisheries and natural wetlands were consulted about various matters regarding entrepreneurial fisheries and natural wetlands of that area.

Accordingly, 3 sites were selected as follows:

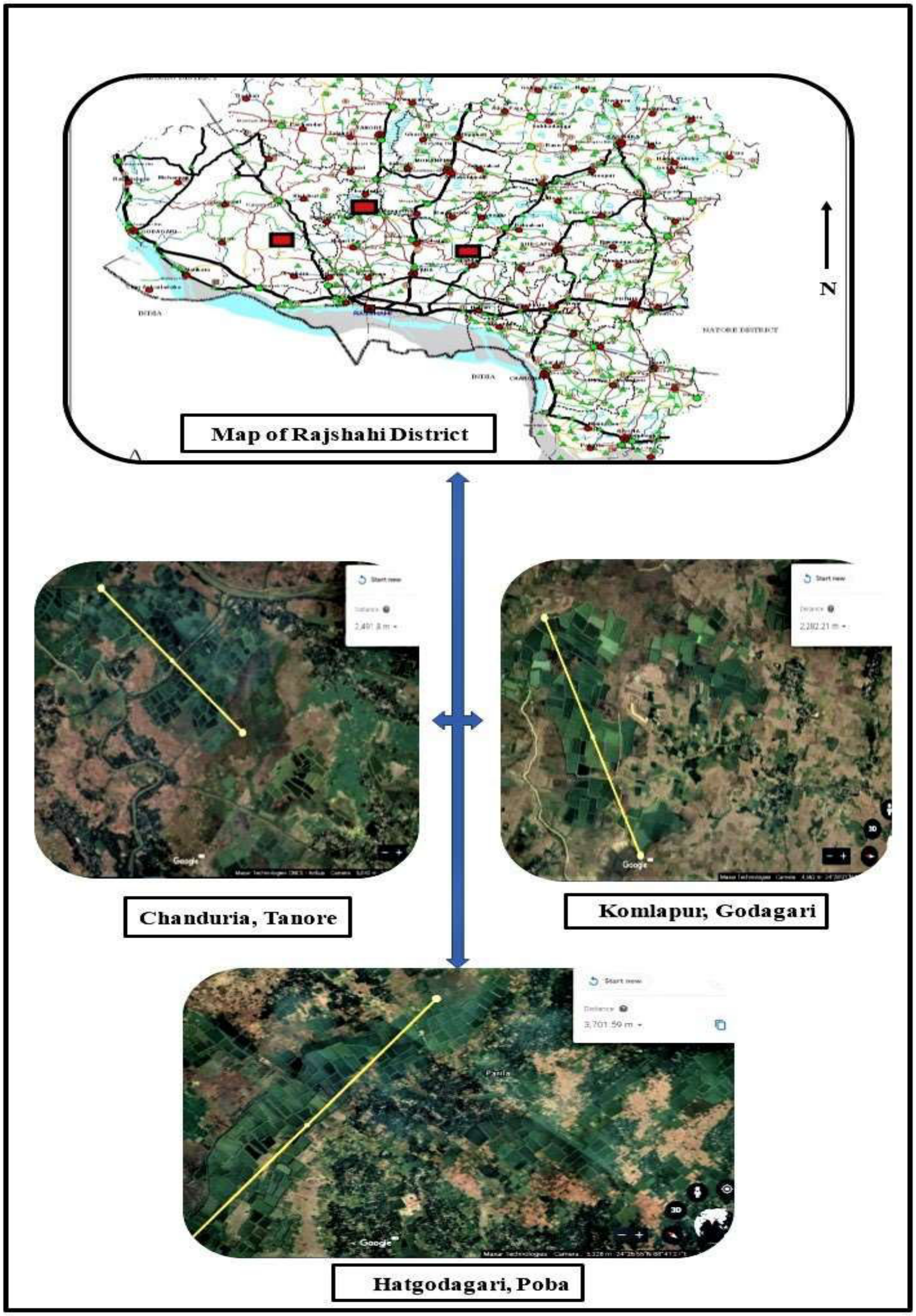
- Komlapur, near Kakon hat of Godagari Upazila of Rajshahi District
- Near Chanduria of Tanore Upazila of Rajshahi District
- Near Hatgodagari area of Poba Upazila of Rajshahi District

For collecting the data to use in comparison with the entrepreneurial fisheries, the following locations were selected as follows:

For rice cultivation data- Komlapur near Kakon hat of Godagari Upazila of Rajshahi District

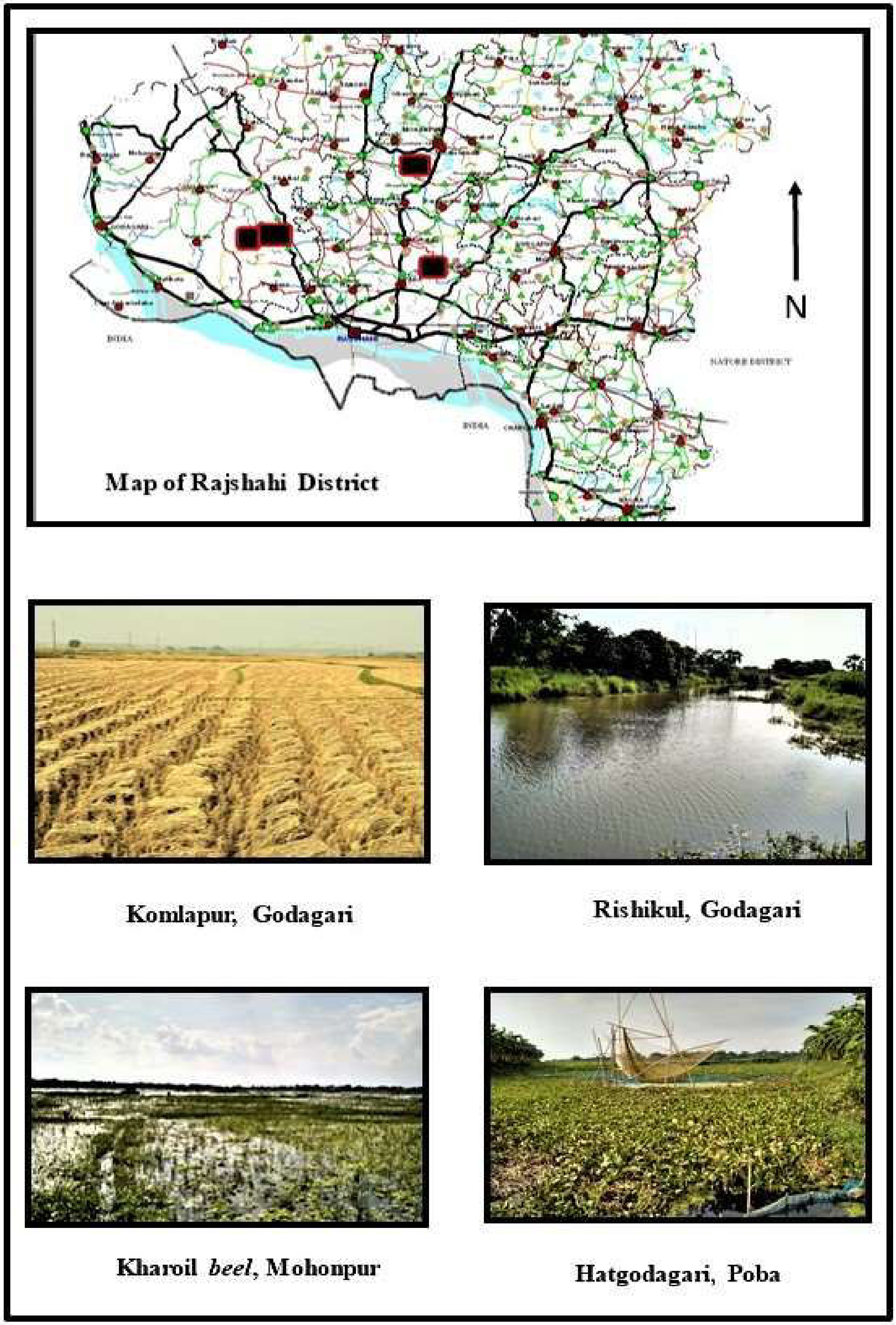
For water quality and faunal diversity

- Natural canal in Rishikul Union of Godagari Upazila of Rajshahi District.
- Kharoil *beel* of Mohonpur Upazila of Rajshahi District
- Natural canal near Hatgodagari area of Poba Upazila of Rajshahi District.



Source: LGED, Bangladesh and Google Earth, accessed on 5 September 2019.

Figure 2.1. The location of the entrepreneurial fishponds in blue color along the yellow line marks that came under survey for this study.



Source: LGED, Bangladesh, accessed on 5 September 2019.

Figure 2.2. Location of the paddy fields and natural water bodies that were surveyed in this study for data to compare with entrepreneurial fisheries.

Description of the study sites

Komlapur, Godagari site is located at $24^{0}27'27''$ N and $88^{0}28'34''$E, Chanduria of Tanore Upazila site is located at $24^{0}30'12''$ N and $88^{0}34'00''$ E and Hatgodagari, Poba site is located at $24^{0}26'55''$ N and $88^{0}41'06''$ E. The natural canal in Rishikul Union of Godagari Upazila is located at $24^{0}25'59''$ N, $88^{0}30'25''$E, Kharoil *beel* of Mohonpur Upazila is located at $24^{0}31'04''$ N and $88^{0}38'10''$E and the natural canal near Hatgodagari area of Poba Upazila is located at $24^{0}27'00''$ N and $88^{0}41'05''$E.

Determination of sample size

For surveying the ponds being used for entrepreneurial fisheries, the total number of ponds in the selected clusters was counted from the google map image. Based on the counting, the Komlapur cluster in Godagari Upazila was found to consist of approximately 90 ponds, Chanduria cluster of Tanore Upazila was found to consist of nearly 200 ponds, and Hatgodagri cluster of Poba Upazila was found to consist of nearly 165 ponds. To determine the sample size for each cluster, the online statistical sample size calculator 'Raosoft®' was used. For calculating the sample size, ''Raosoft®" was set with a 5% margin of error and 90% level of confidence. Based on the total number of ponds in each cluster, 'Raosoft®" calculated the sample size as 68, 116 and 103 respectively for the Komlapur, Chanduria, and Hatgodagari clusters. An equal number of ponds under entrepreneurial fish farming were surveyed. During the survey it was found that often the same farmer owned many ponds in the cluster or sometimes even in more than one cluster. Ponds under the same ownership had the same management. If one farmer owned 10 ponds, all the ponds received the same treatment from that farmer. Therefore, surveying one pond meant surveying all the ponds under the same management. Thus, the total number of ponds, determined statistically as sample size, were surveyed from all three clusters. In order to gather information about the production system of the original use for the land or water body and to use that data for comparison with entrepreneurial fisheries, fifty paddy farmers were surveyed from Komlapur of Godagari Upazila.

Development of questionnaire for aquaculture entrepreneur

In the process of site selection, the author spoke with many people from the selected study site. However, that interaction was not deep enough to know the information that was required for the study. Therefore, to gather detailed information from the owners of the entrepreneurial fisheries ponds, a questionnaire survey form was designed. It was tested first interviews with two fisheries entrepreneurs. Based on that experience, the questionnaire was modified new relevant questions were added, and the questionnaire was finalized.

Development of questionnaire for paddy farmers

In consultation with agriculturalists and the Upazila Agriculture Officer of Godagari Upazila, a questionnaire survey form was developed for surveying the paddy farmers. After the development of the questionnaire, it was tested among a few farmers. Based on the test, questionnaire was updated and finalized for use in surveying paddy farmers.

Survey and handling of data

Between November 2017 and February 2018, the pond owners were surveyed from each of the clusters using the questionnaire. In most cases one pond owner owns many of the ponds within the same cluster, therefore, 11 pond owners were required to be surveyed to cover the 68 ponds in the Komlapur cluster. Similarly, in the Chanduria and Hatgodgri clusters, 17 and 14 farmers were surveyed respectively to cover the statistically determined sample size of 116 and 103 ponds respectively. Similarly, 50 paddy farmers were surveyed from the Komlpur area of Godagri Upazila. After the survey, data from the survey forms were entered in an Excel spreadsheet, organized and analyzed as required.

Soil sample

To know whether entrepreneurial fisheries have any effect on the soil, a total of 19 soil samples were collected. Soil samples were collected from pond bottoms under commercial fish culture, pond dikes under intensive agriculture, pond dikes not under agriculture (or newly built), natural wetlands that have static water, and natural canals with flowing water. All the samples were processed and prepared for analysis as described below.

A total of six samples were collected from the bottom of ponds under commercial aquaculture at three culture sites per cluster in Komlapur of Godagari Upazila, Chanduria of Tanore Upazila, and Hatgodagari of Poba Upazila. Due to the intensive fish culture, the ponds have received inputs over the period of last two to five years since they were built.

A total of three samples were collected from bond dikes that were either newly built or not under agriculture, which means they have received few if any agricultural inputs.

A total of five samples were collected from pond dikes that were under intensive agriculture and have received agricultural inputs over last two to five years since the ponds were built.

A total of three samples were collected from the bottom of three different natural water bodies with static water *(beels):* a seasonal water body from the Komlapur area of Godagari, the perennial Kharoil *beel* of Mohonpur Upazila, and the perennial *beel* from the Hatgodagari area of Poba Upazila that receives surface runoff from a nearby catchment area.

A total of two samples were collected from the bottom of two different natural canals of flowing water from the Rishikul area of Godagari Upazila, and near the Kharoil *beel* of Mohonpur Upazila.

Soil sample collection

All soil samples were collected within five days of each other in the middle of August 2017. According to the soil sample collection guidelines of the Soil Resource Development Institute (SRDI) of Bangladesh, a final soil sample must be comprised of soil from nine different places within the specific sampling site. This is to ensure the true representation of the soil of the pond bottom, or other sites by minimizing the internal variations and collection error. Therefore, soils from nine different places of the particular pond or wetland bottom were collected, mixed and taken in a Ziploc bag clearly marked with pond identification.

Soil sample preparation for nutrient analysis

All the collected samples were put in separate ceramic bowls, placed on a tray, and dried separately in an incubator for 48 hours at a constant temperature of 70^0C. Then the samples were ground separately using a hard-wooden grinder. In this way, a homogenous mixture of powdered soil for each sample was prepared.

Each of the 19 samples was coded to hide their identity at the analyzer Lab., in order to prevent any unwanted human biases or assumptions regarding a certain soil sample. Two hundred and fifty grams (250g) of each sample were bagged in clear plastic (poly propylene) bags clearly marked with the code name. Then the soil samples were submitted to the regional Lab. of the Soil Resource Development Institute (SRDI) in Rajshahi for the analysis of soil pH, organic Carbon, P, S, K, Ca, Mg, Cu, Fe, Mn, Zn, B. After two months the results were collected from the Lab.

Feed sample collection

From the questionnaire survey and informal discussions with the commercial fish farmer in the study areas, it was discovered that 13 different brands of pelleted fish feed were being used by the farmers. Approximately one kg of feed was collected for each of the fish feed type from the fish farmers. During the collection it was made sure that none of the feed were produced more than 15 days prior to the collection date. In addition to this, the feed samples were collected from the intact feed bag to ensure the freshness of the feed in the sample. The feed samples were collected in between October and November 2017 in plastic Ziploc bags. Additional air in the bags was pushed out by squeezing the bags and each bag was ziplocked to make them airtight. The sample bags were labeled with their respective feed brand for proper identification immediately after the collection of each of the samples. The feed samples were preserved in the refrigerator until it used in various tests.

Proximate composition analysis of feed sample

After bringing the feed samples into the Lab., from each type of feed sample, 250g were taken and repacked in a plastic bag in an airtight condition. Prior to sending the sample to the Lab. for analysis of proximate composition, each of the 250g bags was labeled with numbers instead of the feed brand name.to ensure the avoidance of any potential bias by the person analyzing the proximate composition. This number then becomes the identity for each type of the feed. A separate note was maintained by the researcher for each feed brand and their corresponding number, so that once the results of proximate composition are received, they can be set against the correct brand name based on the sample number. The samples were sent to the Nutrition Lab. in the Faculty of Fisheries in Bangladesh Agricultural University for the analysis of the proximate composition on November 8, 2017.

In the Lab., moisture content of the feed sample was determined by measuring the weight difference of feed before and after drying in the thermostat incubator at 105^0C over 24 hours. Protein content was determined based on the Nitrogen content determined by following the Kjeldahl method of Nitrogen analysis. Lipid content was determined by solvent extraction of lipids and measurements, where acetone was used as solvent. Ash content was determined by burning the feed samples in a muffle furnace for 6 hours at 550^0C. Then the percentages of each of the elements (Moisture, Protein, Lipid and Ash) were calculated for all the feed samples.

Physical properties of the feed

To describe the physical properties of the feed 25 pellets from each feed sample were measured for their length and diameter using a slide caliper.

Pellet stability of feed sample

To know the stability of the feed pellets in pond water when used for feeding, a test was conducted in the Lab. Simulating the situation of the pond where many fish coming to eat the feed makes shaking and turbulence in the water, a shaker was used to see the stability of the pellets in water. Twenty-five grams (25g) of each feed sample were placed in a conical flask of 500ml size. One hundred fifty ml tap water at 30^0C temperature was added to the flask and the flask was then put in the shaker at 75rpm and again at 150rpm. The time was counted until the pellet was mostly disintegrated or dissolved. The longer time required to disintegrate or dissolve the pellet, the more stable the pellet is in water.

Chemical fertilizer sample collection

From the questionnaire survey it was revealed that both entrepreneurial fisheries and rice cultivation use a large quantity of chemical fertilizer. From the market of different clusters, a total of five different Triple Super Phosphate (TSP) samples and three Muriate of Potash (MOP) samples were collected. The samples were collected from fertilizer dealers in plastic bags. The sample bags were clearly labeled with the brand names as follows: TSP-Bulgaria, TSP-Lebanon, TSP-Bangladesh (Potenga), TSP-Tunisia, TSP-Morocco, MOP-Belarus, MOP-BADC (Bangladesh) and MOP-Canada. All the samples were kept in an airtight condition until used for analysis.

Soil sample preparation for heavy metal analysis

Because the soil samples had no standard moisture content, all soil samples were made moisture free. To make the soil sample moisture free, all grounded soil samples were taken in ceramic bowl and kept in incubator for 48 hours at 105^0C for the second time. After that the soil samples were allowed to cool down within the incubator for a few hours and put into plastic bags in an airtight condition until prepared for heavy metal analysis. Fish feed samples and chemical fertilizer samples were used as it is (moist basis) for heavy metal analysis.

Digestion of samples for heavy metal analysis

Half gram (0.5g) of each of the soil, ground fish feed, and ground fertilizer samples were taken into graduated tubes separately. Then a three ml mixture of pure HCL and HNO_3 (1:3) was added (USEPA 3051A) to each tube and heated on a hot plate at 95^0C for 1 hour. Then it was cooled to room temperature. The mixture was transferred into a beaker and 20ml of distilled water was added and the mixture was allowed to settle overnight. The following morning each of the mixtures was filtered using filter paper. Distilled water was added to bring the amount to 30ml. The filtered samples were given to the central science Lab. of Rajshahi University where each of the samples was analyzed for Lead (Pb), Cadmium (Cd) and Chromium (Cr) using the flame method with the Atomic Absorption Spectrophotometer (AAS).

Sampling for water quality parameters

Common water quality parameters like water pH, dissolved oxygen, electrical conductivity, total dissolved solids, turbidity, and Secchi disk reading were taken once in a month from the entrepreneurial fisheries ponds and natural water bodies. Since the dissolved oxygen measure varies with many factors, such as temperature, light, and agitation, dissolved oxygen from the waterbodies was measured before sunrise directly with the dissolved oxygen meter. Because of the physical distance of the sampling sites and the sensitive timing of the collection, the sampling was done on three consecutive days in each month at different locations. In the same way, the Secchi disk reading was taken directly from the water bodies by using a Secchi disk. Water samples from all the water bodies were taken in half liter plastic bottles and carried to the water quality Lab. of Institute of Environmental Science in Rajshahi University, where the water samples were tested for pH using a pH meter, electrical conductivity and total dissolved solids using an EC meter, and turbidity using a turbidity meter. The data was duly recorded.

Sampling of zooplankton

Plankton net made of silk bolting cloth with the specifications of 200 US with 75µm to 85µm mesh size were used for the sampling of zooplankton. To minimize variations, sampling was done at a certain place of the water body between 10.30am and 11.30am. A specific length of the water body was marked for traversing with the plankton net. A collecting bottle with a valve was attached to the tapering end of the plankton net. The plankton net was traversed along the designated length one foot below the surface of the water during each sampling. After traversing the net, plankton were taken into a plastic bottle by opening the valve of the collecting bottle of the net. This volume of plankton sample in the bottle represented the total water volume sieved through the plankton net. Immediately after collection, formalin was added at 5ml for each 100ml sample in order to fix the zooplankton. The zooplankton were identified and counted using the Sedgewick-Rafter cell counter (Welch 1948) and observing under the microscope. Water volume that traversed through the rounded net was calculated by using following formula:

Volume of water (L)= л (3.14) X square of radius (square meter) of the rounded net X length of water column traversed by the net (meter) X 1000

Sampling of aquatic insects

A net one square meter in size made up of Silk bottling cloth with the specification of- 35 US with 500µm mesh size was fitted into a bamboo frame. This was used for insect sampling. The net was sieved through a three-meter horizontal distance of water towards the edge of the pond to catch the aquatic insects. After harvesting, the insects were transferred into a plastic bottle with some water. Formalin was added in the bottle for the fixation of the insects. Later, the insects were identified and counted in the Lab.

Sampling of benthos

A metal scoop (2.75-inch diameter and 1.5-inch maximum depth) was used to collect the soil samples containing benthos populations from bottom of the pond. The mud was then transferred into plastic bag. It was taken to the Lab. and sieved under running water using a fine mesh sieve, where all the dirt was washed, and all the benthos insects remained in the sieve. The insects were then transferred into a large Petri dish with some water. Then each type of the benthos insect was separated and counted under bright light.

Results and Discussion

Production system and input supplies of entrepreneurial fisheries

The entrepreneur's engagement in entrepreneurial fish farming in the Barind Tract ranged from 2 to 22 years, with the average experience being 9.7 years of engagement. The average pond size (with dike) was found 4.5 acres where average water area was 3.4 acres per pond under entrepreneurial fisheries.

Ponds under entrepreneurial fisheries

The survey result showed that 61.5% of the ponds under entrepreneurial fisheries are located on lands that used to be the seasonal flood plain before the ponds were constructed, meaning it used to be submerged under water during the monsoon season and under winter rice cultivation when water receded in the month of January. 23.1% of the ponds under entrepreneurial fisheries are located on lands that used to be regular paddy fields before the ponds were constructed, meaning that two paddy crops (monsoon rice and winter rice) were grown on those lands. Only 15.4% of the ponds under entrepreneurial fisheries are located on lands that used to be a perennial water body-a natural depression in the floodplain that never dried up regularly for paddy cultivation. Rai *et al.* (2017) and Hasan *et al.* (2013) reported that over the period of last 40 years, low-lying agricultural land in Bangladesh converted into aquaculture ponds has resulted in increased aquaculture area, contrary to the significant reduction of total agricultural land and natural wetland.

Equipment used in construction of ponds

Almost all ponds were constructed using heavy construction equipment like excavators, Bulldozers, and dump trucks. For the construction of a pond (one acre of water area) equipment was required for 269.4 hours, of which the excavator was used for 131.5 hours (48.8% of the total duration), the bulldozer was used for 27.9 hours (10.4% of the total duration) and the dump truck was used for 109.9 hours (40.8% of the total duration required) (App. Table 2.1). Entrepreneurial fish ponds are large and done as part of the commercial venture therefore civil engineering construction equipment like excavators, bulldozers and dump trucks are needed (Freshwater Habitats Trust, 2020).

Use of water

Ponds under entrepreneurial fisheries are only as good as the amount of water it can contain. The survey found that only 23.1% ponds accessed surface water from the canal that was directly fed in into the ponds. Sixty-nine point two percent (69.2%) of the ponds used water from deep tube wells to keep enough water for fish culture. A total of 9.8% of ponds used both the surface water and deep tube well water during 2018. Only 17.5% pond did not receive or require any water from outside sources other than the rainfall. In addition to the rain fall, on an average one acre of pond water area used all of the water over a one-year period that was lifted by a deep tube well in operation for a total of 31.2 hours (App. Table 2.2). Jahan *et al.* (2015) found close to half of the carp aquaculture ponds throughout Bangladesh used ground water and little over half of the carp aquaculture ponds were dependent on rain water. Given the lower level of annual precipitation (Jahan *et al.*, 2010) in the Barind Tract it is justifiable that most of the entrepreneurial carp aquaculture fish ponds under this study used ground water as primary source of water.

On the other hand, winter rice is completely dependent on irrigated water, almost all of which comes from underground sources in the Barind Tract. In addition to the winter rice, due to irregular rainfall, *Aman* rice is also often required to be irrigated with underground water. Altogether for winter rice and *aman* rice, on average an acre of paddy fields used water lifted by a deep tube well operated for 74 hours (App. Table 2.3). Deep tube wells in Barind Tract are generally lifting less than half (0.75 to 1.00 cusec/hour as opposed to 2 cusec/hour) of the optimum capacity of the pump due to the lowering of the water table and the blockage of the filters (personal communication with BMDA). Most of the tube wells are 20 to 30 years old and are performing poorly in terms of water lifting. Based on the average water lifting performance (0.75 cusec/hour) and using hours of deep tube wells in the Barind Tract it was found that entrepreneurial fisheries used 3182 cubic meters of water/acre/ year compared to 7548 cubic meters of water/acre/year used in paddy cultivation.

Paddy plant is very water hungry and categorized as semi aquatic that grow best when at least 75% of usable soil moisture is available (IRRI, 2020) and it takes up to 5000 liter of water for producing one kg of rice (Bauman, 2009), which is equivalent to 8000 cubic meter of water/acre/cropping season depending on the yield of rice. Around 90% of the total precipitation in the Barind Tract occurs in between June and August (Bulbul and Rahman, 2014) leaving the winter rice production solely dependent on irrigated water. Ground water irrigation covers around 80% of all agricultural land in Bangladesh (Rahman and Mahbub, 2012) and in Barind Tract no winter paddy cultivation is possible without irrigation water, making it consuming almost double the water than used in entrepreneurial fisheries.

Fish species composition

Of all the surveyed ponds revealed that only 4.3% of ponds were used for monoculture of tilapia. The rest of the ponds were used for poly culture of carp fish. Of the ponds under the polyculture system, 100% of the ponds used Indian major carps (Rui (*Labeo rohita*), Catla (*Catla catla*), and Mrigel (*Cirrhinus mrigala*), and Chinese carps (Silvercarp (*Hypophthalmichthys molitrix*), Grass carp (*Ctenopharyngodon Idella) and Black* carp (*Mylopharyngodon piceus*). Eighty-four point six percent (84.6%) of ponds used for Common carp (*Cyprinus carpio*), 15.4% ponds for big head carp *((Hypophthalmichthys nobilis),* 61.5% ponds for Chithol (*Notopterus chitala*), 46.2% ponds for Pholi (*Notopterus notopterus*), 23.1% pond for Air (*Sperata aor*) and 6.3% ponds for Magur (*Clarias batrachus)*. In the polyculture system, Indian major carps are the primary species with which Chinese carps used for ecological balance and better utilization of the production system. Predatory species are the added to control the small indigenous fish species. The average fish density was 938fish/acre in the polyculture system under entrepreneurial fish production in the Barind Tract. Of all the species *L. rohita* had the highest density (533/acre) followed by *C. mrigala* (187/acre), *C. catla* (100/acre), *C. carpio* (30/acre), *H. molitrix* (29/acre), *C. batrachus* (26/acre) and *C. Idella* (13/acre). Other species were used in numbers ranged between 1 to 8/acre (App. Table 2.4).

Similar to the findings of this study Jahan *et al*. (2015) also found that 100% carp farmers all over the country stocked Indian major carps and exotic carps (Chinese and European carp), 50% household stocked Indian minor carps, 24% stocked tilapia, 14% stocked Shing (*H. fossilis*) and 5% stocked other species in carp aquaculture ponds. Species composition of pond fish production (by weight) in Rajshahi District shows that *L. rohita* contributed 30.54%, *C. Catla* contributed 17.33%, *C. mrigala* contributed 13.38%, *L. calbasu* contributed 3.73%, *H. molitrix* contributed 17.20%, *C. Idella* contributed 1.38%, *C. carpio* contributed 7.86%, *O. niloticus* contributed 2.05% (DoF, 2018) which is analogous to the species composition of fish stock of entrepreneurial fisheries found in this study in Barind Tract.

Use of inputs in entrepreneurial fisheries

Being a commercial venture, many inputs were used in entrepreneurial fisheries. On an average a total of 7158.4kg inputs are used per acre water body/year excluding fish seeds. Of the inputs 69.34% is feed, 0.19% feed additives, 26.79% fertilizers, 3.45% different disinfecting chemicals, 0.04% pesticides, 0.01% antibiotics, 0.1% different therapeutants, 0.05% fish toxicants and 0.02% herbicides for pond dikes (Figure 2.3). Mohsin *et al.* (2012)

found that lime, fertilizers, medicines, antibiotics, fish toxicants were used as inputs in their study of carp aquaculture in Rajshahi District and that increased yield is positively correlated with increased use of inputs. Hossain *et al.* (2017) also reported similar use of inputs in traditionally managed carp aquaculture ponds in Rajshahi. Similarly, Jahan *et al.* (2015) reported that 40% carp farmers throughout Bangladesh controlled predatory species, 86% used pre-stocking liming, 73% used organic fertilizer, 95% used inorganic fertilizers, 95% used raw feed, 40% used pelleted feed and 89% took preventive measures against diseases. Given the entrepreneurial nature of the carp aquaculture in Barind Tract it is justifiable that it is input intensive.

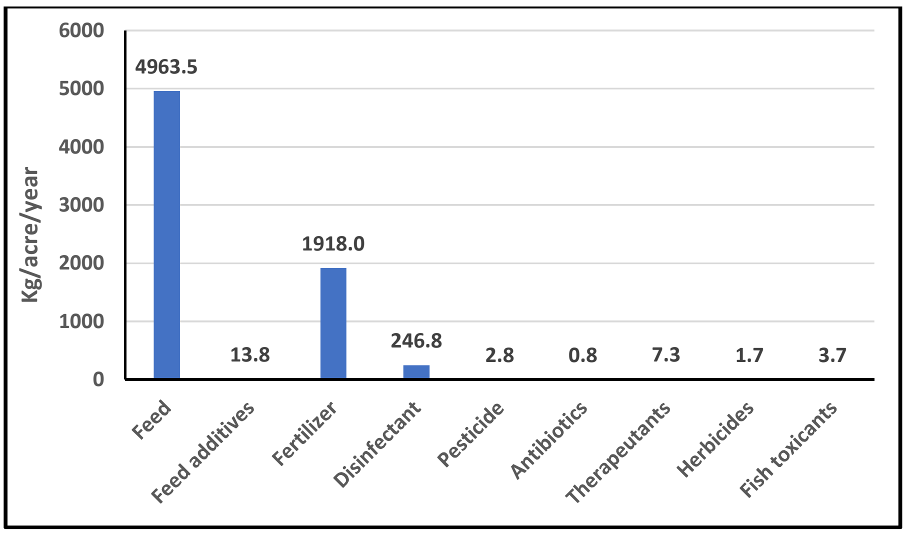


Figure 2.3. Average quantity (kg/acre/year) of all inputs used in entrepreneurial fish production in the Barind Tract.

Comparisons of inputs used in entrepreneurial fisheries and paddy production

Some of the inputs used in entrepreneurial fisheries and paddy production are the same, but there are many differences since entrepreneurial fisheries and paddy production are completely different production systems (Figure 2.4). Some of the inputs are similar in both production systems but often used in different quantities. Since entrepreneurial fish production is a commercial and intensive production venture, overall higher amount of inputs except water and organic fertilizer are used/unit area compared to the paddy production.

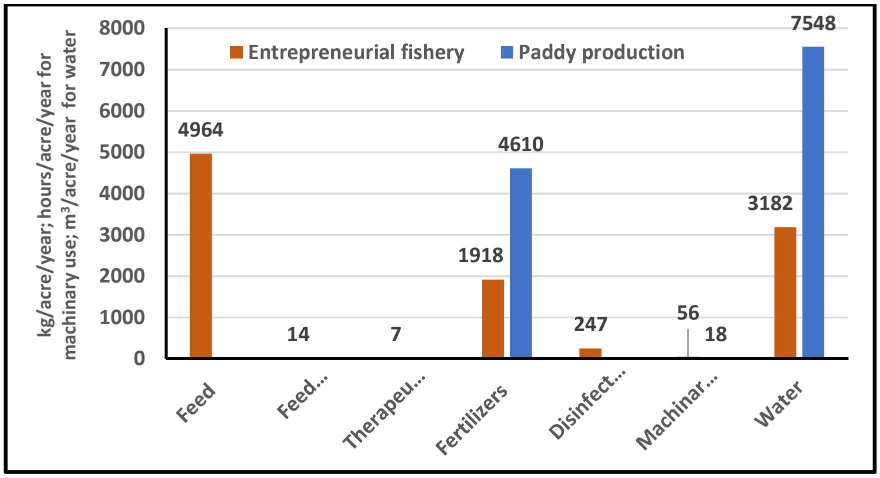


Figure 2.4. Comparative use of major inputs in entrepreneurial fish production and paddy production.

Fish feed comprises the largest input in entrepreneurial fish production, outperforming other inputs in both production systems except the amount of irrigation water used in paddy production. Feed, feed additives, pond disinfectants including anti-fungal agents, and therapeutants are four inputs that are only used in entrepreneurial fisheries. Fertilizers are used both in entrepreneurial fisheries and paddy production. The total quantity of fertilizers used in paddy production is much higher than used in entrepreneurial fisheries, primarily due to the higher quantity of cow dung-compost used in the paddy production system. More chemical fertilizers are used in entrepreneurial fish production compared to the paddy production (App. Table 2.7 and 2.8). Use of machinery is also more than three times higher in entrepreneurial fish production than paddy production, because the entrepreneurial fisheries relies on regular transportation, compared to the paddy production system (App. Table 2.17 and 2.18).

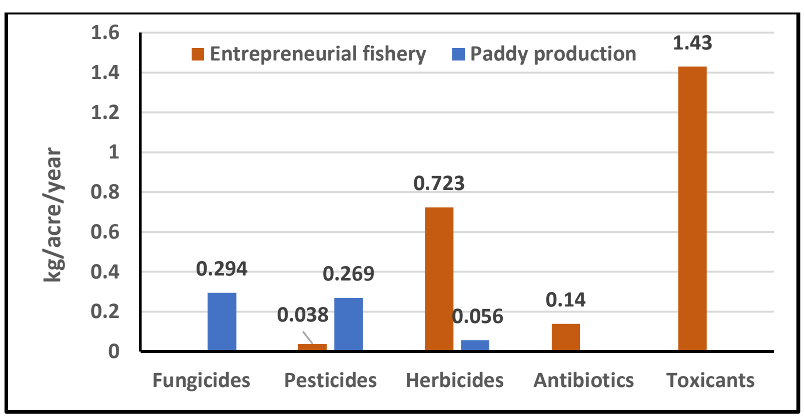


Figure 2.5. Comparative use of other inputs in entrepreneurial fisheries and paddy production.

Antibiotics and fish toxicants are also used only in the entrepreneurial fisheries. On the other hand, dedicated fungicides are used only in paddy production (Figure 2.5). Use of pesticides and herbicides are common in both production systems. Pesticide use (active ingredient/acre) was higher in paddy production compared to the entrepreneurial fisheries, but primarily due to the higher use of carbofuran pesticide in paddy production which is not used in entrepreneurial fisheries (App. Table 2.11 and App. Table 2.12). On the other hand, total herbicide used (active ingredient/acre) was several times higher on pond dikes than the paddy field, primarily due to the different nature of the two fields, and relatively short duration of paddy production.

Feed and feeding of fish

All the ponds under the survey used pellet feed for fish. In addition to the pellet feed, mustard oilcake was fed in 46.2% ponds, wheat bran in 38.2% ponds, rice bran in 23.1%, corn in 14.4%, and biscuits were fed in 30.8% ponds. The biscuits that were fed to fish were date expired biscuits originally intended for human consumption. Having become expired, they were sold for animal consumption. On an average a total of 4963.5kg feed was fed to the fish per acre of water area per year of which 75.6% was pellet feed, 13.1% was mustard oilcake, 5.8% was wheat bran, 3.3% was rice bran, 2.2% was corn and 0.6% was biscuit (App. Table 2.5). On an average 2.5 different types of feed or feed ingredients were fed to the fish in each pond. Fish were only fed with pellet feed in 46.1% of the ponds. In 15.4% of the ponds fish received in total two kinds of feed or feed ingredients including pellet feed. In 38.5% of the ponds fish received a total of 3 or more kinds of feed or feed ingredients including pellet feed.

Several determinants were identified that influenced farmers choice of fish feed brand. Quality of the feed was the most important determinants that eighty-four point six percent (84.6%) farmers referred to. Feed price was an important factor for 7.7% farm owners. Availability of feed in credit was a priority for 46.2% farmers, similarly flexible payment option for feed was important for 23.1% farm owners. Feed being the single most used input, can cost up to 79% of total operational expense of intensive *P. hypophthalmus* farming (Ahmed, 2007). Belton *et al.* (2011) mentioned selling of fish feed in credit in Mymensingh is most common among entrepreneurial fish farmers, which is also very similar to the findings of this current study.

Use of feed additives

Additional to the feed, feed additives were used in 76.9% ponds in entrepreneurial fisheries in Barind Tract. A total of 13.77 kg/acre/year feed additives were fed to the fish, of which 64.63% was multivitamin premix, 23.97% DCP, 7.99% domperidone, 2.18% vitamin C, 0.43% probiotics and 0.8% non-antibiotic growth promoters (App. Table 2.6). Of the feed additives, fish were fed vitamin C in 17.4% cases, premix multivitamin in 38.5%, domperidone in 23.1%, dicalcium phosphate (DCP) in 24.7%, probiotics in 30.8% and non-antibiotic growth promoters in 15.3% ponds. Fish in 30.8% ponds were fed at least one kind of feed additive, fish in 23.1% ponds were fed two types of feed additives, and fish in 23.1% of the ponds fed more than two types of feed additives. Ali *et al*. (2016) found the use of feed additives like vitamin premix, vitamin-C and herbal growth promoters in aquaculture in Bangladesh. Mohsin *et al.* (2012) also reported about use of vitamin premixes in *P. hypophthalamus* farming in Rajshahi. The entrepreneurial fish farmers in Barind Tract were found using more feed additives than reported previously.

Use of fertilizers in entrepreneurial fisheries

The survey revealed that 100% of the ponds were fertilized with triple super phosphate (TSP), followed by 76.9% of the ponds by urea, 53.8% of the ponds with muriate of potash (MOP), 38.5% of the ponds with poultry litter and 30.8% of the ponds with cow dung. On an average a total of 1918kg of fertilizer was used per acre water area of ponds per year, of which poultry litter constitutes the highest percentage at 55.6%, followed by TSP at 24.5%, cow dung at 14.6%, urea at 4.6% and MOP at 0.6% (App. Table 2.7). On an average each pond under entrepreneurial fish production received three kinds of fertilizers. Only 15.4% of the ponds received only one type of fertilizer, which was TSP. Another 15.4% of ponds received two types of fertilizers. A staggering 69.2% of the ponds received three or more types of fertilizers. The current findings differ from those of Mohsin *et al.* (2012) where it was found that 98% of farmers used Urea, 80% of farmers used TSP and 28% of farmers used MOP in Rajshahi District. Ali *et al.* (2016) reported around 99% carp farmers used fertilizers and around 79% carp farmers used TSP fertilizers in Bangladesh which is more similar to the findings of this study.

Use of fertilizers in rice production

The survey revealed that 100% of rice farmers used TSP, urea, MOP and compost/cow dung. In addition to these, 93.75% of farmers used gypsum, 56.25% used zinc, 50% used boron and 18.75% used poultry litter. On an average a total of 4609.9kg of fertilizer was used per acre of paddy field per year, of which any form of compost and cow dung constituted that highest at 79.7%, followed by 11.9% poultry litter, 3.3% urea, 2.1% TSP, 1.7% MOP, 1.1% gypsum, 0.1% boron and 0.1% zinc (App. Table 2.8). Of the total fertilizers used, 91.6% came from organic sources, and 8.4% was chemical fertilizers. Rasha *et al.* (2018) found lower (2700.8 kg/acre) use of fertilizer in paddy cultivation in Mymensingh where around 92.2% of the fertilizer came from organic sources (manure) and rest 7.8% was chemical fertilizers consisting of 3.7% urea, 2.1% TSP, 1% MOP, 0.8% gypsum and 0.2% zinc which are slightly different than the findings of current study due to the difference in study area.

A comparison between the use of fertilizer in entrepreneurial fish production and paddy production showed that the total amount of fertilizer used in paddy field was double than that of fishponds. This difference is primarily due to the uses of higher amount of compost/ cow dung in paddy field. On the other hand, poultry litter use in fishponds was almost double than that of paddy fields. TSP uses in ponds under entrepreneurial fish production were found four times higher than that in paddy fields (Figure 2.6), whereas gypsum, boron and zinc fertilizers were only used in paddy production but not in entrepreneurial fisheries.

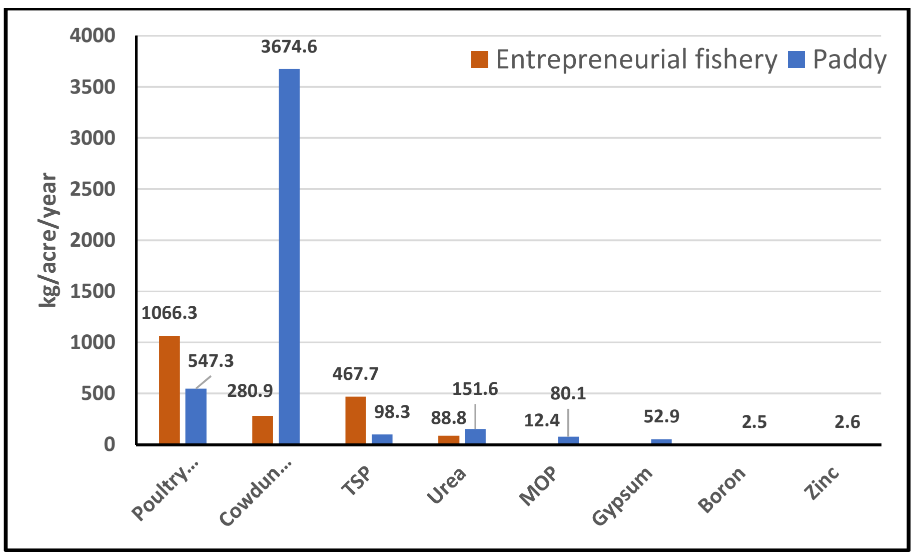


Figure 2.6. Average use of fertilizer (kg/acre/year) in fishponds under entrepreneurial fisheries and paddy cultivation in the Barind Tract.

Use of disinfectants in entrepreneurial fisheries

The survey revealed that 100% of ponds received Lime treatments each year. In addition, zeolite was used in 76.9% of ponds, table salt was used in 61.5% of ponds, benzalkonium chloride and brominated salt containing products were used in 30.8% of ponds, N-alkyl dimethyl benzyl ammonium chloride containing products were used in 46.2% of ponds, potassium permanganate was used in 53.8% of ponds, malachite green was used in 7.7% of ponds and ammonia remover products were used in 30.8% of ponds in a year. On an average a total of 246.82kg of disinfectants were used per acre of water area per year, of which Lime constituted the highest quantity at166.3kg (Figure 2.7) or 67.37%, zeolite at 11.47%, table salt at 20.67%, benzalkonium chloride and brominated salt containing products at 0.12%, n-alkyl dimethyl benzyl ammonium chloride containing products at 0.15%, potassium permanganate at 0.16%, malachite green at0.02% and ammonia removing products at 0.04% (App. Table 2.9). On an average each pond received more than four different types of disinfectant during the last one year. Only 15.4% of ponds received three different kinds of disinfectant treatments. Around 30.8% of ponds received four different kinds of disinfectant treatments and another 30.8% ponds received five or more types of disinfectants.

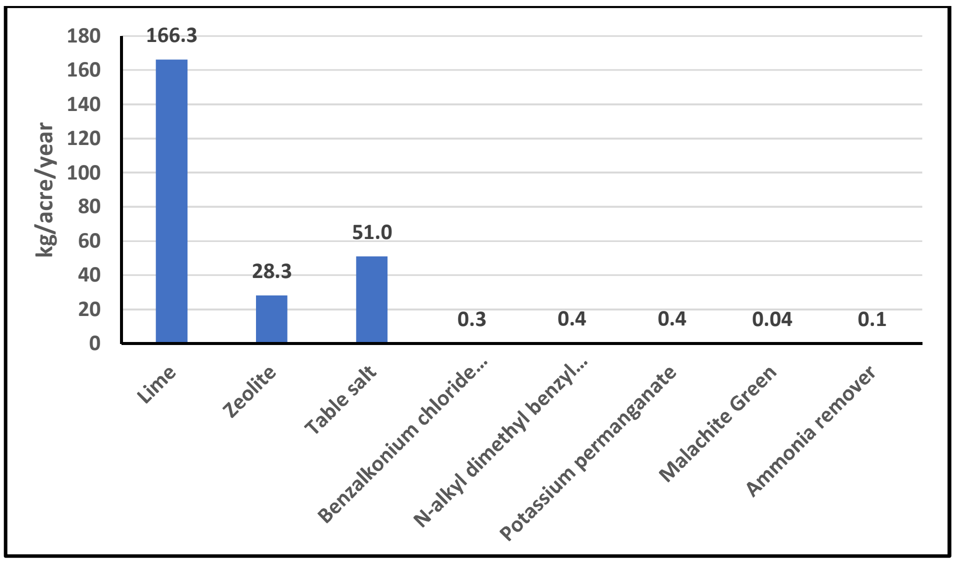


Figure 2.7. Average quantity (kg/acre/year) of disinfectants used in entrepreneurial fish production in the Barind Tract.

Use of lime, zeolite, salts, benzalkonium chloride and brominated salt containing products, N-alkyl dimethyl benzyl ammonium chloride containing products, potassium permanganate, malachite green, and ammonia removing products were also mentioned by other authors (Shamsuzzaman and Biswas, 2012; Rahman *et al*., 2015; Rasul *et al*., 2017; Hossain *et al*., 2018; Mohsin *et al*., 2012; Chowdhury *et al*., 2015, Alam and Rashid, 2014 and Sarker *et al*., 2014) in various parts of Bangladesh ranging from south to the north, and west to the east.

The present study also revealed that most of the chemical inputs and disinfectants are primarily in two seasons, namely prewinter and late winter or pre monsoon. When asked about the reasons for this seasonal use of the chemicals, farmers responded that fish disease generally breaks out in winter. To prevent any fish mortality, they use the disinfectants and chemicals for maintaining the water quality. Parveen *et al*. (2005) observed an outbreak of Epizootic Ulcerative Syndrome (EUS) in between October and January in oxbow lakes of Bangladesh. Similarly, with the incoming monsoon, water volume increases significantly with water depth almost doubling the lowest depth from the months of March and April. Therefore, to maintain water quality optimum for fish growth, chemical inputs and disinfectants are also used. Hasan *et al*. (2013) found 73.3% of farmers responded that fish disease occurred during winter and 18.89% farmers responded diseased occurred during late winter in rural Bangladesh. Similarly, Aftabuddin *et al.* (2016) also noted that 20.85% and 23.96% of farmers that disease outbreak occurs ‘during rainy season’ and ‘just after fish stocking’ respectively.

Use of fungicide in paddy production

Though many disinfectants used in entrepreneurial fisheries have some antifungal properties, there are not as many fungicides used in fishponds as compared to the paddy fields. Paddy farmers use a good number and amount of fungicides in raising paddies. The survey of the paddy farmers revealed that 81.25% of farmers used propiconazole, 68.75% used a mixture of azoxystrobin and difenoconazole, 31.25% used difenoconazole, and 6.25% used carbendazim. On an average a total of 294.2g of the active ingredient of different fungicides were used per acre of paddy field per year, of which 129.4g (44%) came from mixture of azoxystrobin and difenoconazole, 56g difenoconazole, 96.4g propiconazole and 12.4g carbendazim (App. Table-2.10 and Figure 2.8). Parveen and Nakagoshi (2001) in their study in Kustia, Manikgonj and Comilla found that edifenfos, carbendazim and propiconazole were respectively used by 7%, 44% and 9% paddy farmers. But with change in time and availability of combined fungicide (azoxystobin and difenoconazole) more and more paddy farmers (44%) became accustomed to using combined fungicides found in this study.

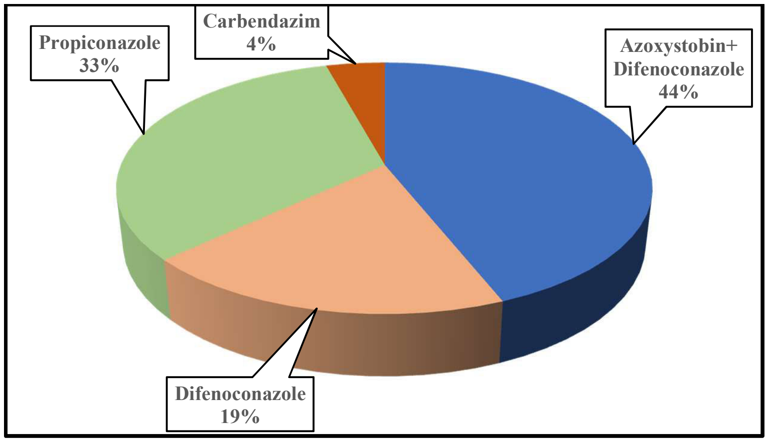


Figure 2.8. Percentage of fungicides used in paddy fields in the Barind Tract.

Use of pesticides in entrepreneurial fisheries

The survey revealed that a total of seven types of pesticides are used in ponds under entrepreneurial fish production for killing aquatic insects and undesired fish species. Ninety-two point three percent (92.3%) of ponds received cypermethrin treatment. On the other hand, Quinalphos was used in 69.2% of ponds, deltamethrin was used at 38.5% of ponds, abamectin was used in 30.8% of ponds, lambda-cyhalothrin was used in 23.1% of ponds, emamectin benzoate was used in 15.4% of ponds and Fenitrothion used in 7.6% of ponds. On an average a total of 37.83g active ingredient of different pesticides was used per acre of water area per year, of which 15.1g (39.9%) was cypermethrin, 0.72g (1.9%) deltamethrin, 0.46g (1.2%) abamectin, 0.64g (1.69%) lambda-cyhalothrin, 6.99g (25.5%) quinalphos, 8.99g (23.76%) fenitrothion and 2.25g (5.94%) emamectin benzoate (App. Table 2.11 and Figure 2.9). All the ponds under entrepreneurial fisheries were found treated with pesticides on an average of nine times per year. On an average 2.8 different types of pesticides were used in each pond. A total of 46.2% of ponds received treatment with two types of pesticides and 53.8% of ponds were treated with three or more types of pesticides.

Shamsuzzaman and Biswas (2012) and Hossain *et al*. (2008) reported use of fenitrothion and active malathion in shrimp farms of southwest Bangladesh. Rahman *et al*. (2015) mentioned use of Trichlorfon, cypermethrin and fenitrothion in fish farms of northeast Bangladesh. Rasul *et al*. (2017) and Chowdhury *et al*. (2012) mentioned the use of trichlorfon, fenitrothion and malathion in fishponds of Sylhet and Noakhali respectively. Hossain *et al*. (2018) and Chowdhury *et al*. (2015) found use of deltamethrin in fishponds of southwest Bangladesh, and in Sylhet respectively. Comparing the pesticide types mentioned by various researchers, use of pesticides in entrepreneurial fish production in the Barind Tract is more intense and diverse. Use of abamectin, lambda cyhalothrin, emamectin benzoate and quinalphos are exclusive in entrepreneurial fish production in the Barind Tract. This has not been reported before by other researchers.

Farmers described the purpose of the pesticide use as follows: control of aquatic insects, control of undesired *Chanda* sp. and *Glossogobius* sp., and control of ectoparasites of fish (argulids and anchor worm). Since the commencement of these generally occur in summer and during the monsoon therefore the pesticides are used between the months of March and November. Farmers also understand that pesticides are toxic, capable of killing aquatic insects, undesired fish species, and fish parasites, and are used in the presence of live fish in the ponds; therefore, despite using safe doses of pesticides, it is stressful to the fish. In winter season fish are generally stressed due to the weather conditions and reduced water volume in the pond; therefore, pesticides are not applied during the winters seasons so as not to cause more stress.

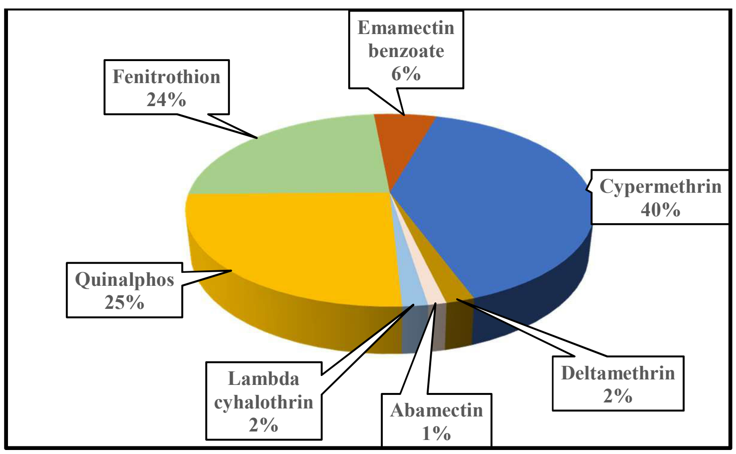


Figure 2.9 Percentage of different pesticides used in entrepreneurial fisheries in the Barind Tract.

Use of pesticides in paddy production

Like the entrepreneurial fishponds, 100% of the paddy plots received pesticide treatments. Eighty-one point two five percent (81.25%) of the paddy plots received more than one type of pesticide treatment, while 12.5% paddy plots received three or more types of pesticide treatment. Specifically, 81.25% plots were treated with carbofuran, 62.5% plots were treated with mixture of thiamethoxam and chlorantraniliprole, 6.25% received dimethoate, 31.25% received lambda-cyhalothrin, and 18.75% received chlorpyriphos. On an average a total of 269.6g of active ingredient of different pesticides were used per acre paddy plots per year, of which 211.4g (78.4%) was carbofuran, 53.7g (19.9%) was a mixture of Thiamethoxam and chlorantraniliprole, 3.0g (1.1%) was chlorpyriphos, 1.0g (0.4%) was dimethoate and 0.4g (0.2%) was lambda-cyhalothrin (App. Table 2.12 and Figure 2.10). Islam *et al.* (2016) found paddy farmers using carbofuran and cypermethrin in Patuakhali District, and a mixture of thiamethoxam plus chlorantraniliprole and lambda cyhalothrin in Camilla District. They also noted that the use of thiamethoxam plus chlorantraniliprole was missing in 2011 but found in 2016. On the other hand, the application rate of carbofuran and lambda cyhalothrin tripled in 2016 from the rate of 2011. Similarly, the most used pesticide in Bangladesh was carbofuran (7000 tons in 2017), which is sold under 255 brand names and mostly used in rice, potato, eggplant and sugarcane cultivation (Hossain, 2018).

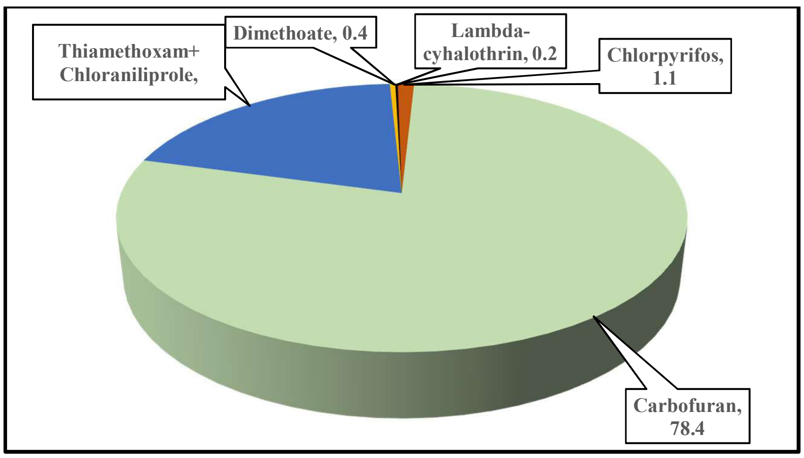


Figure 2.10. Percentage of pesticides used in paddy fields in the Barind Tract

In comparison to the entrepreneurial fishpond, pesticide use in paddy fields per acre area was much higher. The difference is primarily due to the use of carbofuran pesticide. In ponds, pesticides are used to kill aquatic insects therefore, the pesticides are chosen based on their toxicity to the aquatic organisms. However, since there are fish present in the pond while the pond is being treated with pesticides, the doses must be optimized in such a way that the pesticide does not cause any damage to the fish. For paddy fields no such consideration is required, since the pesticide has no negative impact on the paddy itself.

In contrast, the frequency of pesticide use was much higher in ponds than in paddy fields. Ponds are under cultivation year-round, whereas in paddy fields, one to two crops are grown per year and the rest of the time the land remains fallow. Therefore, the duration of the paddy crop may be up to 7 months altogether. Due to this difference in duration of the land/water use, fishponds are found to receive a higher number of pesticide treatments.

Regardless of the impact of use of pesticides in aquaculture ponds on aquatic fauna (described in next chapter), the impact is limited within that pond due to the confinement of the pond water. On the other hand, pesticides used in paddy field often gets distributed by precipitation and surface runoff and usually finds its way into the natural waterbody. Therefore, natural wetlands, where pesticides may not be used, ultimately end up getting pesticides and become polluted via surface runoff that comes from the paddy fields.

Use of herbicides on the pond dikes

All the ponds under survey received herbicide treatments on their dikes. The pond's dike is used for various fruits production, such as banana, papaya, mango and oranges. However, the dikes are also covered with grass and bushy plants, to control those grass and bushy plants, herbicides are used on the pond's dike. Of all the ponds, 61.5% of the pond's dikes were treated with glyphosate, 23.1% of the pond's dikes were treated with 2, 4-dichlorophenoxy acetic acid and 15.4% of the pond's dike received paraquat treatment. Glyphosate are nonselective systemic herbicides, making it effective against most plants regardless of narrow leaves or broadleaves (NPIC, 2019). Paraquat is also a post-emergent, nonselective herbicide that acts quickly upon contact against grass and broad-leafed weeds (Wikipedia, 2019a). 2, 4-dichlorophenoxyacetic acid is a selective, synthetic herbicide that primarily kills broad leafed plants, but not the grasses (Wikipedia, 2019b). On an average a total of 723.38g of active ingredient of different herbicides were used per acre of pond dike per year, of which 376.38g (52.0%) was glyphosate, 304.8g (42.2%) was 2, 4-dichlorophenoxy acetic acid and 42.2g (5.8%) was paraquat (App. Table 2.13 and Figure 2.11). Around 46% of the ponds' dikes received two or more types of herbicides, while the rest received one type of herbicide.

Of all the herbicides used on the pond dike, paraquat is the most concerning in terms of environmental risk. Paraquat is a restricted use pesticide in the USA, which means it needs certified personnel for application and has wide range of ecological risks for humans and other mammals, birds, terrestrial insects, and algae (USEPA, 2019). It is only partially inactivated in contact with the soil (Wikipedia, 2019a) and has the potential to get washed into the aquaculture ponds when applied on pond dikes. It has been banned in the European Union since 2007 (The Court of First Instance, 2007).

Glyphosate is one of the most used herbicides in the world and was considered a benign herbicide compared to paraquat and others (EHN, 2019). However, the International Agency for Research on Cancer (IARC, 2015) has declared glyphosate as possibly carcinogenic to humans. Additional ecological risks for non-targeted small insects and microorganisms in the soil and on other thigs are also listed by Friends of the Earth Europe (2013).

The half-life of 2, 4-dichlorophenoxyacetic acid in soil ranges from 1 to14 days and is not very stable, but may pollute shallow ground water and streams (Jervias *et al*, 2008). Like glyphosate, IARC has also classified 2, 4-dichlorophenoxyacetic acid as a possible carcinogen (IARC, 2015). It is considered a highly volatile ester and is currently banned in Europe and Australia (Wikipedia, 2019b).

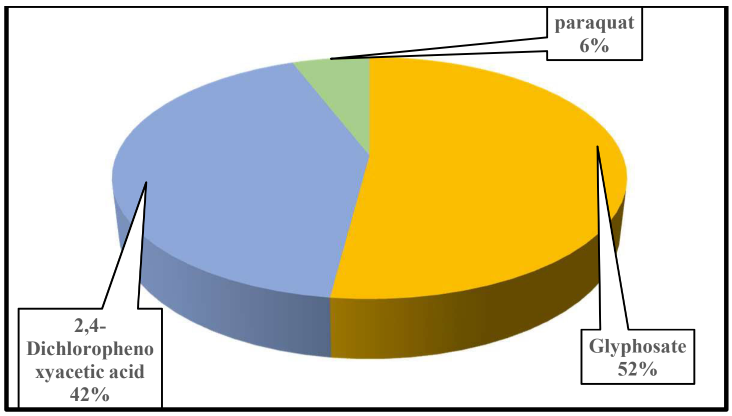


Figure 2.11. Percentage of herbicides used on the dikes of ponds under entrepreneurial fish production in the Barind Tract.

Use of herbicides in paddy production

A total of 43.75% paddy farmers were found using herbicides in paddy fields. Forty-three point four seven percent (43.47%) of plots received treatments with the mixture of bensulfuran methyl and quinclorac (pre-emergent), 12.5% paddy plots received treatments with pretilachlor (pre-emergent), and 6.25% plots received pyrazosulfuran ethyl (post emergent). On an average a total of 56.2g of active ingredient of different herbicides were used per acre of paddy field per year of which 49.4g (87.8%) was a mixture of bensulfuran methyl and quinclorac, 6.3g (11.1%) was pretilachlor and 0.6g (1.1%) was pyrazosulfural ethyl (App. Table 2.14 and Figure 2.12). Only 18.75% farmers used 2 or more types of herbicides. Islam *et al.* (2018) reported the most effective weed management practice was to controlling weeds at the early stage of crop growth. The study found a 76.5% reduction in weed density and 92.0% reduction in weed biomass at 45 days after transplantation of rice when pre and post emergence herbicides were used. Ahmed *et al.* (2014) found pretilachlor 56.56% effective in weed control in rice at 60 days after transplantation into the field.

Compared to the paddy field, herbicide use in pond dikes is higher per acre per year. This is primarily due to four reasons: economic status of paddy farmers, seasonal nature of the paddy field, limited vulnerability of paddy to weeds, and the prevention of grazing on pond dikes. Most of the paddy farmers are in a lower economic status than the fish farming entrepreneurs and are directly working in the paddy fields, often using their own physical labor for weeding rather than using herbicides. Only 43.75% paddy farmers were found using herbicides over the past year as opposed to 100% pond dikes received herbicide treatment. This factor contributed to the use of relatively lower quantity of herbicides per acre land area in paddy fields compared to the pond dikes.

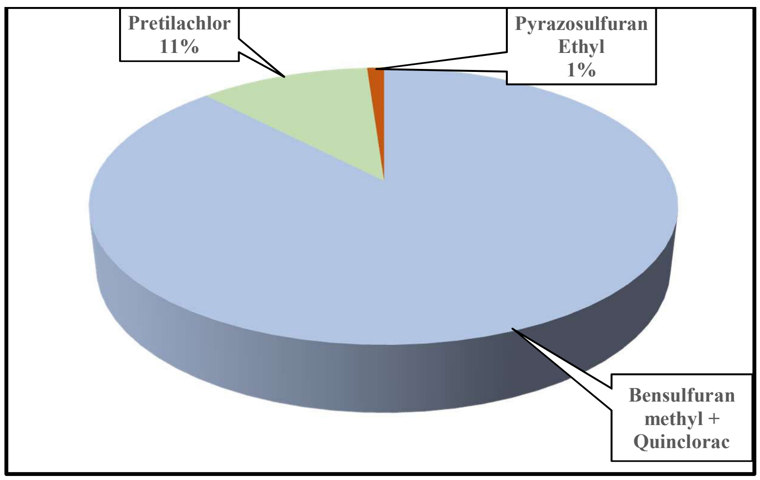


Figure 2.12. Percentage of herbicides used in paddy fields in the Barind Tract.

Most paddy fields have a maximum of two growing seasons which cover up to seven months in total. During these seven months, the paddy is only vulnerable to weeds when the rice plants are small. Once the plants have grown tall enough and are in the flowering stage, the weeds can no longer compete and there is no need for herbicide.

Interestingly, prevention of grazing is an important reason for the use of herbicides by fisheries entrepreneurs. Weeds do not compete with crops on the pond dike like banana and papaya but do end up being attractive to goats. Goats could damage the crops and their herders could graze upon the fruit trees.

The herbicides used in paddy and entrepreneurial fishpond systems are different. The paddy crop itself and weeds that grow in the paddy field are aquatic weeds or weeds that can tolerate standing water. These are different from the terrestrial plants that grow on pond dikes. Therefore, the nature and types of herbicides used in both systems are completely different.

Hossain (2015) noted that in comparison to mechanical weeding, which is used when there is a lack of manual labor, the use of herbicides has several positive attributes (in addition to its negative ones), such as reduced soil erosion, better nutrient conservation and less nutrient drainage, reduced fuel use and greenhouse gas emissions. On the other hand, it is also more efficient weed control mechanism over manual systems, which is usually never done adequately and timely.

Use of antibiotics in entrepreneurial fisheries

A total of 84.6% of the surveyed ponds were found using antibiotics over the past year. Among those ponds, 61.5% were treated with ciprofloxacin, 53.8% were treated with oxytetracycline, 15.4% were treated with doxycycline or doxycycline and colistin, and 7.7% were treated with sulfur drug, mixture of erythromycin thiocyanate, sulfadiazine and trimethoprim. On an average a total of 140.2g of the active ingredient of different antibiotics were used for treatment or prevention of fish disease per acre of water area per year, of which 86.29g (61.54%) was oxytetracycline, 28.07g (20.02%) was ciprofloxacin, 20.22g (14.43%) was sulfur drug, a mixture of erythromycin thiocyanate, sulfadiazine and trimethoprim, and 5.61g (4.01%) was doxycycline or doxycycline plus colistin, (App. Table 2.15 and Figure 2.13). Around 46.2% of ponds received treatment with one type of antibiotic, 30.8% of ponds received treatment with two types of antibiotics, and 7.7% of ponds received treatment with more than two types of antibiotics. The antibiotics are generally used with fish feed during the winter months (December to February) when the temperature is low, water volume in pond has decreased due to lack of rainfall, fish are naturally stressed, and disease outbreak is more common than the summer months. Sometimes, the antibiotics are used in early summer, when disease breaks out in late winter.

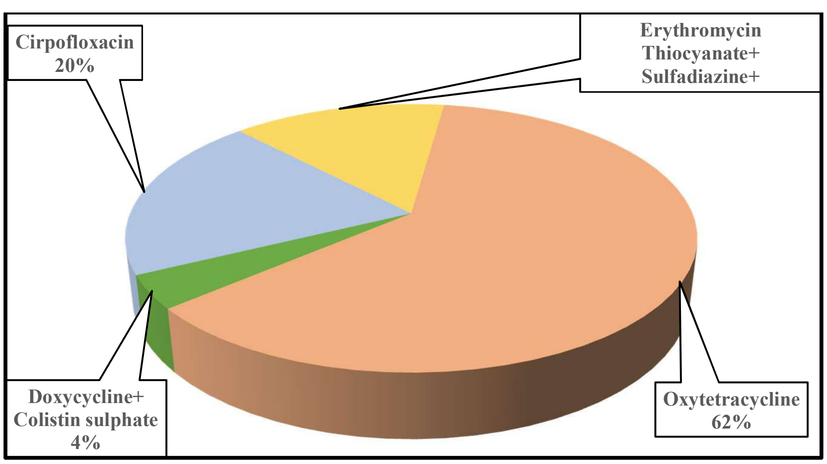


Figure 2.13. Percentage of different antibiotics used in entrepreneurial fisheries in the Barind Tract.

Sarker *et al.* (2014), Shamsuzzaman and Biswas (2012) and Mohsin *et al.* (2012) reported use of oxytetracycline, chlortetracycline, sulphadiazine and amoxicillin in aquaculture ponds and shrimp farms in southern Bangladesh. In addition to those, use of erythromycin was reported by Rasul *et al.* (2017) in Sylhet. Use of oxytetracycline and doxycycline in aquaculture ponds in north east Bangladesh was found by Rahman *et al*. (2015) and Hossain *et al*. (2018). In addition to the mentioned antibiotics, Ciprofloxacin is being used in entrepreneurial fisheries in the Barind Tract as a next generation antibiotic; gradually replacing the old once like doxycycline and chlortetracycline.

Use of therapeutants in entrepreneurial fisheries

A total of three other types of therapeutants (App. Table 2.16) were found being used in entrepreneurial fisheries in the Barind Tract. A total of 68.4% of ponds were using Sodium carbonate peroxyhydrate or Sodium percarbonate as a therapeutant in aquaculture ponds to combat short-term oxygen deficiency in pond water. Three point seven percent (3.7%) of ponds used Copper sulfate for controlling algal bloom and aquatic plants. Four point nine percent (4.9%) of ponds used tea seed cake for getting rid of snails. Since tea seeds are very toxic to fish as well, before use, fish were harvested either to sell or to transfer to another pond. The use of Sodium percarbonate is also common among fish farmers throughout the country (Shamsuzzaman and Biswas, 2012; Rahman *et al*., 2015; Rasul *et al*., 2017; Hossain *et al*., 2018; Mohsin *et al.*, 2012; Chowdhury *et al*., 2015 and Alam and Rashid, 2014). ASEAN National Coordinating Agency of the Philippines (1978) mentions the use of tea seed cake as selective poison for fin fish in shrimp farms in the presence of shrimp, however in the Barind Tract to control the spread of snails, tea seed cake is used in absence of fish. Hossain *et al*. (2008) found tea seed cakes use in Khulna and Mymensingh region for control of weed fish and snail. Chowdhury *et al*. (2012) also mentioned the use of copper sulphate in aquaculture ponds in Noakhali, Bangladesh.

Use of fish toxicants

A total of three different types of fish toxicants were found being used in entrepreneurial fisheries in the Barind Tract. Among the surveyed ponds, 69.2% were treated with phostoxin tablets (Aluminium phosphide), 38.5% were treated with rotenone powder and 15.4% were treated with fenpropathrin. The average frequency of use of fish toxicants was found 0.92 times/year/pond. On an average, a total of 1.43kg of fish toxicants (active ingredient) were used per acre water area per year of which 1.06kg (73.79%) was aluminium phosphide, 0.17kg (11.99%) was rotenone and 0.2kg (14.22%) was fenpropathrin (App. Table 2.17).

Approximately 23% of ponds received a combined treatment with two types of fish toxicants (aluminium phosphide and rotenone). Rasul *et al*. (2017) and Chowdhury *et al*. (2012) reported use of endrin as a fish toxicant in Sylhet and Noakhali region respectively, but only in the context of pond preparation to get rid of undesired fish before stocking of desired fish fingerling; not for harvesting of food fish to sell in the market. Use of rotenone and aluminium phosphide has been mentioned in numerous other studies (Shamsuzzaman and Biswas, 2012; Rahman *et al*., 2015; Rasul *et al*., 2017; Hossain *et al*., 2018; Mohsin *et al*., 2012; Chowdhury *et al*., 2015 and Alam and Rashid, 2014, Chowdhury *et al*., 2012, Hossain *et al*., 2008).

Vehicle/machinery use in entrepreneurial fisheries

All of the entrepreneurial fishponds (100%) used vehicles or machineries that mostly run on fossil fuel. For all ponds (100%), vehicle or machineries were used for fish seedling transportation, fish transport to market, feed transportation, chemical fertilizer transportation, and transportation of other agriculture produce grown on the pond dike. For 53.9% of ponds, vehicles were used for manure (poultry litter and/or cow dung) and for 69% of ponds vehicles used for medicine/disinfectant or plant seedling transportation. On an average, vehicles/ machineries were operated for a total of 55.5 hours per acre of water area per year, of which 47.8 hours (86.13%) for fish transportation to the market, 4.8 hours (8.65%) for feed transportation, 1.3 hours (2.34%) was for fish seedling transportation, 1.1 hours (1.98%) for transportation of agricultural produce grown on pond dike, 0.7 hours (1.26%) for manure transportation, 0.2 hours (0.36%) for chemical fertilizer transportation and 0.03 hours (0.05%) for medicine, disinfectant and plant seedling transportation (App. Table 2.18 and Figure 2.14).

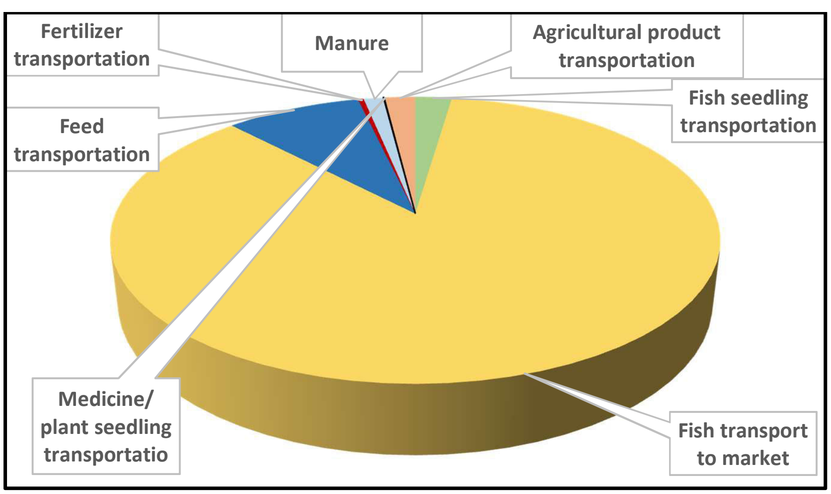


Figure 2.14. Percentage of vehicle use for different activities in entrepreneurial fisheries in the Barind Tract.

Vehicle/machinery use in paddy production

All the farmers used power tillers for plowing their fields in the study areas. Fifty-six point two five percent (56.25%) of farmers used a mechanized thrasher for thrashing the paddy grains, and 51% of the farmers used a mechanized vehicle for transporting their paddy from the field. On an average vehicles/machines were used for a total of 18.28 hours per acre paddy field per year, of which 4.47 hours (24.44%) was for land cultivation, 6.59 hours (36.10%) for thrashing of paddies, and 7.21 hours (39.36%) for transportation of inputs and paddy grains (App. Table 2.19 and Figure 2.15).

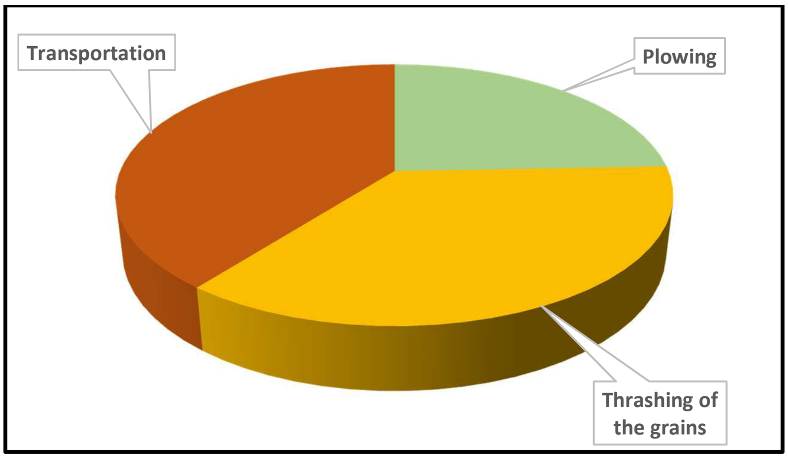


Figure 2.15. Percentage of types of machinery uses in paddy production in the Barind Tract.

In comparison to paddy production, vehicle and machineries use in entrepreneurial fisheries is almost 3 times more per acre per year. This is primarily due to the differences in the quantity of inputs and the output (produce) of the systems that are dealt with, in the two different systems. In addition to the difference in quantity of input and output, the methods of fish transportation: live fish are transported by trucks where truck beds are converted into an artificial tank containing 15 tonnes water and 0.8 tonnes of live fish. Also, many of the fisheries entrepreneurs transport their fish to long distances (e.g. to Dhaka, Chittagong, or Sylhet from the Barind Tract districts), resulting in higher use of vehicles. On the other hand, most of the paddy farmers are subsistence farmers or tenants of the paddy field. Therefore, the paddy farmers do not transport paddy to long distances by themselves (compared to the entrepreneurial fish farmers), usually they bring inputs, bring paddy plants for thrashing and often the actual thrashing is done with their own manual labor. Commercial fish farming is a year-round business venture that requires transportation of inputs and outputs around the year, as opposed to the seasonal rice production system that requires transportation only for a few months. For these reasons, paddy production has resulted in a lower use of the transportation system as compared to entrepreneurial fish production.

Wild fish/weed fish

All the ponds that came under the survey were reported being infested with wild fish or undesired fish species termed weed fish by the entrepreneurs. In entrepreneurial fisheries in the Barind Tract, when the fish seedlings are released into the culture ponds the smaller indigenous fish species (*Chanda* sps., *Puntius* sps.) find their way into the culture ponds along with them. Also, the other hardy species like *Oreochromis niloticus*, *Channa* sp., fingerlings find their way into the ponds. Sometimes, unwanted fish find their way during heavy rain through surface runoff. Fishermen fishing/catching fish with nets in ponds that are side by side often work as an instrument of transfer. Aquatic birds will also transfer the fish from one pond to another. On an average each pond under survey was infested with 3.7 types of undesired fish species. A total of 30.8% ponds had two types of undesired fish, 7.7% ponds had 3 different types of undesired fish, 23.1% ponds had four different types of undesired fish, and 38.5% ponds had five different types of undesired fish. Oftentimes, farmers refer to one type of fish to describe several species within the same genus. Fish types, such as *Chanda* sp., *Channa* sp., and *Puntius* sp. each have 4 to 6 different species found in Bangladesh under the same genus. A total of 86.3% were infested with *Puntius* sp., 53.8% of ponds were infested with *Chanda* sp., 47.6% were infested with *Channa* sp., 36.5% were infested with *Rasbora* sp., 28.3% were infested with *Glossogobius* sp., 16.3% were infested with *Aplocheilus panchax* and 8% ponds were infested with *Oreochromis niloticus*. Due to the available feed supply in the ponds these small fish species thrive, and is the main reason why entrepreneurs release Chithol (*Notopterus chitala*), Pholi (*Notopterus notopterus*) and Air (*Sperata aor*) along with Indian and Chinese carps in entrepreneurial fish production to control the wild fish population.

Plant diversity

Pond dikes account for approximately 24% of the total pond area, half of which consists of the sloped sides and the other half of the top dike. The remaining pond area is water. Since the entrepreneurs pay high rent for those lands, entrepreneurs use the pond dikes for cultivation of high value crops, mainly fruits and some vegetables. On an average, the dikes of each pond have 1.8 types of crops. The use of pond dikes for banana plantation is most popular with 84.6% of pond dikes having banana plantation. Papaya was the second most popular fruit grown, found on 46.2% of pond dikes. Fifteen point four percent (15.4%) of ponds had guava on their dikes, 13.8% ponds had oranges and mangoes on their dikes, and 15% ponds had mixture of vegetables, such as chili, eggplant, and gourd, etc., as well as turmeric. Compared to the paddy fields that are used for the growing of paddy only, pond dikes house diversified types of crops and fruits.

Aquatic birds

The entrepreneurial fisheries sites under the study are unique ecosystems, where stretches of ponds are located over a several square kilometer areas and consist of a perennial water body. Dikes house diverse kinds of crop production along with aquatic vegetation on the shallow part of the water body and terrestrial vegetation on the dikes. Different kinds of terrestrial birds and aquatic birds are part of this ecosystem. The presence of aquatic birds, such as herons, king fishers and black cormorants, are common in the paddy fields. However, due to the diversity in crops and natural plans around the entrepreneurial fishponds, the diversity of terrestrial birds is greater than that found in the paddy fields.

Other animals

The presence and storage of fish feed and feed ingredients near the pond sites combined with the presence of large numbers of smaller indigenous fish species attracts other animals to these sites. Because farmers rear the relatively larger size fish in entrepreneurial fisheries of the Barind Tract, their nets have a bigger mesh size, allowing the undesired small size fish to pass through the net without being caught. The survey revealed that 100% of the ponds have populations of water snakes (*Fowlea piscator)*, Indian water monitor and frogs/tadpoles. A total of 25.4% ponds were reported to have the presence of rats, 38.5% ponds were reported with the presence of Indian rat snakes (*Ptyas mucosa*) and 7.7% ponds were reported with the presence of cobra (*Naja kaouthia*).

Peculiarity

Only 23.1% of the fisheries entrepreneurs followed scientifically proven systems of water quality management, feeding pellet feed to the fish once or twice daily. Forty-six point two percent (46.2%) of the ponds were found to have peculiarity in terms of feeding practices, where entrepreneurs use 'mixtures' as feed. A 'mixture' is defined as 50% pellet fish feed, 25% mustard oilcake, 15% wheat bran or corn, 5% Urea fertilizer, and 5% TSP fertilizer, by weight. The mixture is prepared in such a quantity that would be equal to the feed quantity of a pond for 15 days. Then the entire mixture quantity is put in plastic bags with small holes in the bags that feed can come out through those holes and the bags are hung in the water. No other feed or inputs are provided for 10 to 15 days in the pond. Thirty point eight percent (30.8%) of ponds were found to have peculiarity in terms of using poultry litter on a regular basis along with pelleted feed. Steroid hormones and digestive stimulants were also found incorporated with the feed mixed with poultry litter in many cases. Though not tested through research, farmers described steroid hormones have the side effect of producing fluid accumulation in the body of the fish, hence making the fish bulky in weight for selling.

Risk of feeding of poultry litter

Chemicals and antibiotics are used intensively in poultry sector in Bangladesh. Khan *et al.* (2018) found that all sampled broiler chicken in Mymensingh town had antibiotic residue (amoxicillin, enrofloxacin and ciprofloxacin) in the liver, while the contamination rate was 20% in case of breast meat of broiler chicken. A joint study of Bangladesh Agricultural Research Council (BARC) and Patuakhali Science and Technology University (PSTU) found nearly 50% of 14 poultry feed brands sampled from Dhaka, Barisal, Manikganj and Dinajpur had the presence of broad-spectrum antibiotics including Chlortetracycline, Lincomycin, Epioxytetracycline, Oxytetracycline and Epichlortetracycline (Molla, 2019). A good portion of all the antibiotics fed to poultry pass through the gut and are eliminated with the feces. Since the fish are fed with fresh poultry litters that are not decomposed for pond fertilization, therefore there is a great risk that the residual antibiotic present in poultry litter finds its way into the fish. Poultry litter are generally given to fish during summer and monsoon months. It is not given in winter months when the temperature is low, water volume in ponds has decreased, feed consumption by fish is low and fish are generally more susceptible to disease outbreaks. Fish are fed with antibiotics indirectly during summer and monsoon months by providing fresh poultry litter as feed to them, and directly fed antibiotics during the winter months, therefore, directly or indirectly, fish are fed antibiotics round the year.

Plastic lining of the pond bottom

Plastic lining of the pond bottom is one of the many environmental concerns associated with commercial fish culture as it prevents water seepage into the aquifers. All the ponds surveyed were in naturally low-lying lands of flood plains or former paddy fields in Barind tract. These areas generally retain water for longer periods of time due to their lower elevation. Also, due to their lower elevation, over the many years they have received deposition of silts and clay, creating a fine particle soil that helps with better water retention capacity. Since entrepreneurial fisheries is a commercial venture and water is the sole medium for fish cultivation, entrepreneurs always try to retain as much water as they can in their culture ponds. Of all the surveyed ponds, only 3.8% ponds were found being lined with plastic sheets. Overall, it is not a common practice, which is better for environment.

Greenhouse gasses (GHG) in aquaculture ponds and paddy production

In Bangladesh, while both paddy cultivation and aquaculture ponds are similar in that the land is covered in water, there are key distinctions that impact on the amount of greenhouse gases (GHG) produced in each system. Due to the shallowness and transparency of water in paddy fields, these environments are usually anaerobic. Contrary to that, the earthen aquaculture pond water, which is rich in phytoplankton, maintains good aerobic conditions to support animal productivity in the ponds. This primary different of aerobic and anaerobic water in aquaculture ponds and paddy fields respectively determines the GHG production in both systems.

Given that the fish culturing practice remains the same, where there is regular supply of inputs, consistent fish species diversity, feeding etc., the organic matter content in the pond sediments usually reach to the highest level generally after four to five production cycles and an equilibrium condition occurs. The addition of organic matter and the decomposition of organic matter in aquaculture pond sediments roughly becomes the same (Avnimelech *et al.*, 1984 and Boyd, 1995), hence organic matter content in pond sediments does not increase indefinitely and the equilibrium will remain the same year after year (Boyd *et al.*, 2002). Oxygen in the pond water cannot enter easily into the soil at the bottom of the pond because it has to diffuse through the water filled tiny pores of the soil particle in the bottom sediments, at depths of few millimeter from the sediment surface the demand for oxygen by the microbial community becomes higher than the availability. Hence a thin layer of anaerobic sediments develops in aquaculture ponds (below the top aerobic layer of sediments) especially when large quantity of organic matter is added (Blackburn, 1987). Some microorganisms in the anaerobic sediments at the pond bottom produce alcohols, ketones, aldehydes and other organic metabolites. Another group of microorganisms in the anaerobic sediments below the aerobic sediments layer produces nitrogen gas, ammonia, hydrogen sulfide and methane as metabolites (Blackburn, 1987). The aerobic sediment layer on top prevents the toxic metabolites from being released by oxidizing them to nontoxic forms (nitrite to nitrate and hydrogen sulfide to sulfate). Although some methane and nitrogen gases do pass through the pond water and are diffused into the atmosphere, it is a small amount (Boyd *et al.*, 2002).

An estimated amount of 36 million metric tons of methane is produced from global paddy cultivation and is responsible for approximately 2.5% of all global atmospheric warming associated with methane production (Kritee *et al*., 2018). Flooding of the paddy fields creates an anaerobic condition, leading to an anaerobic decomposition of organic matters in the paddy fields. This, in turn, produces methane (Carlson *et al*., 2017). However, paddy production not only produces methane, but also nitrous oxide which traps more heat over time due to the longer lifecycle of nitrous oxide (121 years) in the atmosphere than methane (12 years) (Kritee *et al*., 2018). Given the methane production in completely flooded paddy fields, intermittent flooding of paddy fields has been considered as a remedy, however, Kritee *et al*. (2018) found in their experiments that intense intermittent flooding of paddy fields leads into staggering 30 to 45 times more nitrous oxide production. They have suggested that it is possible to reduce the GHG emissions from paddy fields by the ecomanagement of water, nitrogen, and carbon.

Given that 84.6% of the entrepreneurial fishponds under this study were constructed on paddy fields (single crop and two crops), entrepreneurial fisheries as a whole have reduced GHG emissions per unit area, by shifting the land use to aquaculture ponds from paddy fields.

Proximate analysis of the fish feed

A total of 14 different pellet feeds were found being used in the ponds under this study. All the feeds were carp grower feed optimized for fish in their growing stages for Indian major carps. The feed samples were found to contain a higher moisture level than the reference value mentioned on the feed bags. Moisture content ranged from 11.12% to 14.39% in fish feed whereas the reference value ranged from 10% to 12%. The relative higher moisture content than the reference value may be attributed to the time difference between when the feed was prepared and packaged and when the sampling was done, between when the sampling was done and when the analysis was done, and the exposure of the feed to the open air during sampling and during handling at the Lab. Fourteen point two eight percent (14.28%) of feed types had moisture levels just below 12 percent, another 14.28% of feed types had moisture levels between 12 and 13 percent, 50% of feed types had moisture levels between 13 and 14 percent, and 21.44% of feed types had moisture levels between 14 and 15 percent (App. Table 2.20).

Actual crude protein percentage of the feed samples ranged from 14.4% to 27.88% against the reference value range of a minimum of 22% to 26% as per the labeling on the feed bag. A total of 50% of feed types had lower protein content than that was mentioned as the reference value on their respective feed bags. Belton *et al.* (2011) also reported that crude protein content in fish feed is generally lower than the level mentioned by the feed company. Seven point one four percent (7.14%) of feed types had crude protein levels just below 15 percent, 28.57% of feed types had crude protein levels between 16 and 17 percent, 7.14% of feed types had crude protein levels between 21 and 22%, and 57.14% of feed types had crude protein levels between 24 and 27% (Figure 2.16 and App. Table 2.20). Kader *et al.* (2005) found protein level in five different carp (grower) feed brands ranged from 20.63% to 29.79%.

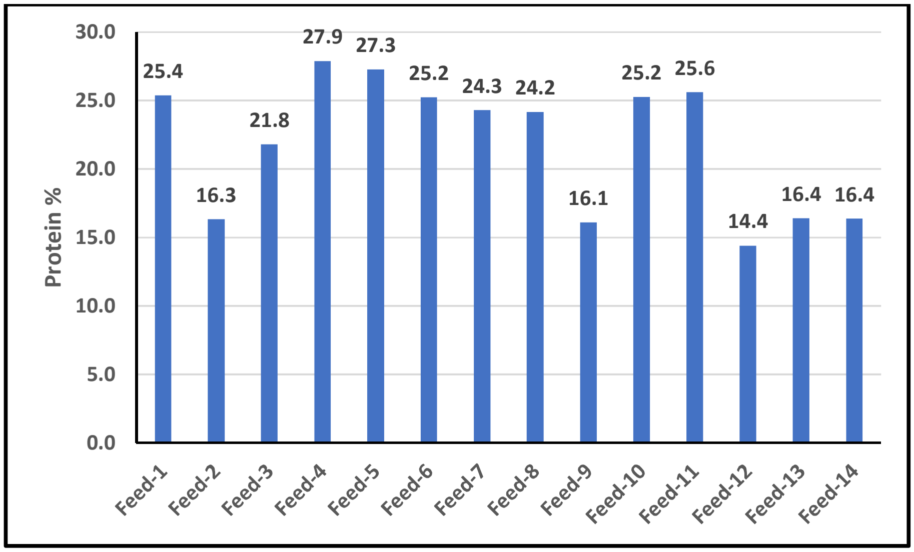


Figure 2.16. Protein percentage in different pellet feed analyzed for proximate composition.

Mohanty (2006) reported that with the increase in size, the protein requirement for *Labeo rohita* decreases and generally requires 25 to 30% protein in their diet for pond cultivation. Given the protein requirement, only 43.83% of the feed found in this research had the optimized level of protein to satisfy the nutritional requirements of *Labeo rohita*. The contribution of natural food is important in meeting the dietary protein requirement. Nandeeshsa *et al.* (1994) found that with the use of 10 tonnes of poultry manure/hectare/year the growth rate of *L. rohita* increased to the level of growth attained with 25% dietary protein, but with use of 20 tonnes of poultry manure/hectare/year the growth rate for *L. rohita* was decreased to a level achieved with 15% dietary protein. In 30.8% of ponds under this study, farmers were compensating for the lower amounts of dietary protein in the pellet feed by adding poultry litter.

The actual Lipid percentage of the feed samples ranged from 5.2% to 7.3% against the reference value range listed on the feed bag of a minimum of 3% to 6% percent. A total of 21.43% of feed types were found to have lower lipid content than the reference value on the feed bag, and 78.7% of the feed types were found to have the lipid content as per their reference value. A total of 28.6% of feed types had a lipid content just below 6 percent and the rest (71.4%) had lipid content above 6 percent (Figure 2.17 and App. Table 2.20). Kader *et al.* (2005) found dietary lipid ranged 6.24 to 9.98% in five reputed carp (grower) feed brands. For optimum growth of *L. rohita* a dietary requirement of 6 to 9% lipid in feed has been reported by Mohanty, 2006; Gangadhar *et al.,* 1997; and Mishra and Samantaray, 2004. Although, 28.6% of feed types in this study were found containing a lower dietary lipid level than recommended, 46.2% of the ponds under this study received mustard oilcakes along with the pellet feed which fills up the gap in dietary lipid content, since mustard oilcake is rich in lipids.

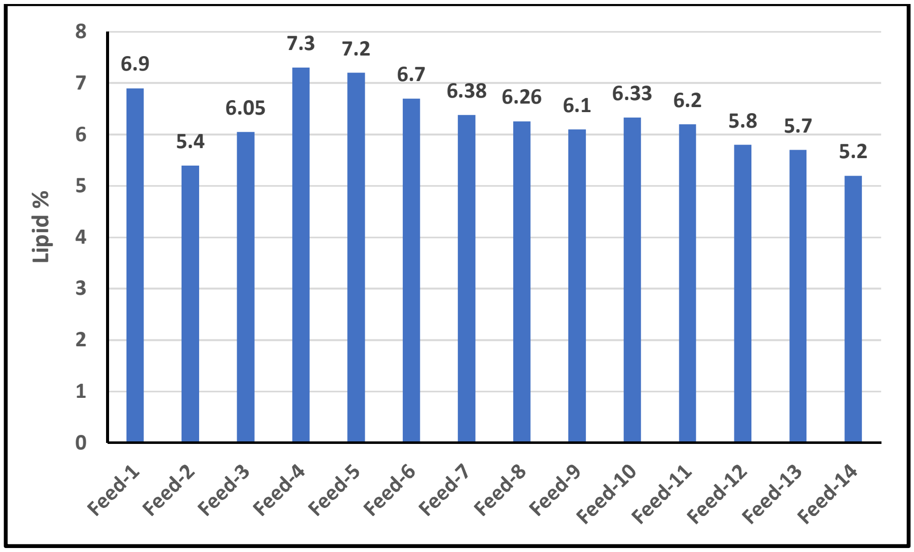


Figure 2.17. Lipid percentage in different pellet feed analyzed for proximate composition

The actual ash percentage in feed samples ranged from 9.72% to 29.14% against the reference value range of a maximum of 11% to 21%. However, for many samples, there were no reference value mentioned on the bag. A total of 30% of feed types that had reference value mentioned on the packet, exceeded the reference value. A total of 35% of feed types had no mention of ash reference value in their bags (App. Table 2.20). Kader *et al.* (2005) found the ash percentage of five different reputed carp feed (grower) brand ranged between 11.99% and 26.32%.

Actual crude fiber in feed samples ranged from 5.88% to 8.52%, against the reference value range maximum of 4.5 to 11%, while 14.28% feed did not mention the reference value. In 21.3% of feeds, the actual crude fiber value exceeded the reference value mentioned on the label, while the others were within the range listed on the label. The Bureau of Indian Statistics (BIS, 2013) noted that the dietary crude fiber requirements for carps in growing stages is 8%. Only 14.28% feed had crude fiber below the level of 6%, while for the rest, the crude fiber was in between the range of 6 and 9%. In a similar study, Kader *et al.* (2005) found crude fiber ranged from 8.54% to 11.36% for five different reputed brands of carp grower feeds.

The Nitrogen free extract (NFE) content of the feeds ranged from 30.46% to 39.57%. A total of 64.28% of the feeds had an NFE content of less than 35%. Herbivorous fish like *L. rohita* can tolerate a higher level of carbohydrate in diet, therefore adding carbohydrate as a cheap source of energy makes the feed economically viable. The dietary carbohydrate requirement for *L. rohita* for various age groups ranged from as low as 26% to 40% (Sen *et al.*, 1978; Satpathy and Ray, 1998; Saha and Ray, 2001).

Physical appearance of the feed pellet

The size and shape of the pellets of all feeds were different from each other. Length of pellets varied within and among different feed brands, whereas the maximum pellet length of all the feed samples ranged from between 12mm to 53mm in length. The average diameter of the different feed pellets ranged from 2.7mm to 4.5mm (App. Table 2.21). Pellet size in entrepreneurial fisheries in the Barind Tract does not matter much because larger fish that are cultivated in entrepreneurial fisheries can eat larger size pellets, since farmers mostly raise fish larger than 0.75kg in the culture ponds.

Raw materials used in feed preparation

Of the 14 feed types, only 42.85% mentioned on the feed bag that raw ingredients were used in the feed preparation. The remaining percentage did not mention the addition of raw materials on the labels of the feed package. Among the feed types that did mention raw materials, 100% used rice polish, soymeal, mustard oilcake and vitamin and mineral premix. Eighty three point thee three percent (83.33%) used corn and wheat flour, 66.66% added fish meal, meat and bone meal, and salts, 50% used vegetable oil, 33.33% used pellet binders, limestone or dicalcium phosphate, full fat soybean and amino acids, 16.66% used shrimp meal, de-oil rice bran, enzymes, preservatives, or wheat brans as raw material (App. Table 2.22). The use of meat and bone meal in the fish feed is a concern, since it is likely that contain various biological and chemical contaminants.

Stability of the feed pellets

Higher pellet stability means greater consumption of the feed by fish reduced feed loss, because fish will have more time to consume it before the feed pellet is melted. Lower pellet stability increases feed loss since it disintegrates very quickly and is therefore wasted. In the Lab. test, the stability of feed pellets was found to vary. Stability was measured as the time it took for a pellet to disintegrate completely in water. When 75 rpm shaking was used in a shaker, the stability of the feed pellet was as low as 10 minutes to as high as 180 minutes at

30^{0}C water temperature. When shaking increased, the stability reduced. When 150 rpm shaking was used in a shaker the stability of the feed pellet reduced to as low as 5 minutes and as high as 128 minutes (Figure 2.18 and App. Table 2.23). Twenty-one point three percent (21.3%) of feed (Feed-2, Feed-3 and Feed-5) required more than 170 minutes to disintegrate at 75rmp of shaking. As the threshold disintegrating time to qualify as stable was predetermined as 40 minutes to disintegration, then 49.87% of the feed types (Feed-2, Feed-3, Feed-5, Feed-7, Feed-10, Feed-12 and Feed-13) qualify as having stable pellets, requiring a minimum of 40 minutes of shaking until disintegration occurs.

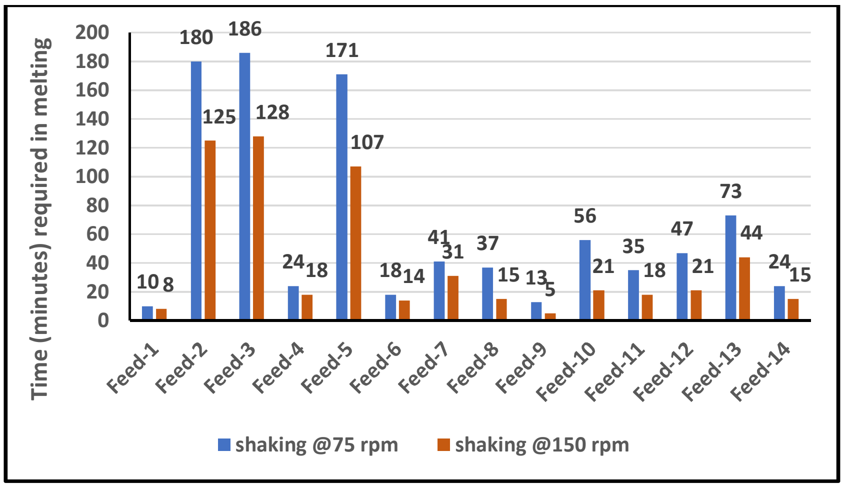


Figure 2.18 Pellet stability in terms of time (in minutes) required for disintegration in a shaker at different shaking rate.

Heavy metals in fish feed

Lead compounds are hazardous and neurotoxic to both fish and human beings (Bondy, 1988). Of all the feed tested for heavy metal content, none found exceeding the maximum permissible limit of Lead content (5mg per kg feed with 12% moisture content) in complete fish feed set by the European Commission (EC, 2015). The highest amount of lead content found in the feed samples was 1.66ppm which is well below the permissible limit for complete fish feed (App. Table 2.24). The results in this study is also found a lower than the average lead content (8.49ppm) of the forty fish feed brands tested from the southern districts of Bangladesh by Sabbir *et al.* (2018), and the lead content (3.58ppm) in shrimp feed described by Shamshad *et al.* (2009).

Cadmium is a non-essential heavy metal that usually accumulates in the vital organs of the body like the liver and kidney (Li *et al*., 2005). The lowest concentration of cadmium found in the sample fish feed was 0.025ppm feed and the highest was 2.84ppm (App. Table 2.24). The

European Commission (2015) approved a limit of Cadmium content in fish feed at 1.0ppm. Of all the fish feed tested, only one type of feed (7.11% of all feeds) exceeded the maximum permissible limit of cadmium concentration.

Unlike cadmium and lead, chromium is an essential heavy metal that plays very important role in the metabolism of carbohydrate fat and protein in the body (Anderson, 1997). However, excessive exposure to chromium can damage the vital organs of the kidney and liver (Dayan and Paine, 2001). Of all the feed sample tested, the lowest chromium concentration was 0.54ppm, and the highest value was 13.98ppm. Given the maximum permissible value of chromium in fish feed is 5ppm, a total of 3 of the feeds or 21.33% exceeded this (App. Table 2.24). This rate of excessive chromium concentration is lower than the findings of Sabbir *et al.* (2018) where 77% of 40 feed samples had chromium content beyond the limit.

Heavy metal contamination in TSP fertilizers

Triple super phosphate (TSP) fertilizers analyzed for heavy metals were found to contain lead levels near each other, ranging up to a maximum of 0.396ppm fertilizer (App. Table 2.25 and Figure 2.19). As per the European Union (2019) fertilizer regulations, the maximum permissible limits of heavy metals in inorganic macronutrient fertilizers are as follows: lead 120ppm (dry matter) fertilizer, hexavalent chromium 2ppm (dry matter) fertilizer and for cadmium 60ppm P_2O_5 which is equivalent to 27ppm TSP fertilizer considering a 45% P_2O_5 content in TSP fertilizer. Therefore, the lead content in all the fertilizers were well below the EU permissible limit. Mohiuddin *et al.* (2017) analyzed 9 TSP samples from different parts of Bangladesh, found lead contamination ranged from 71.4ppm to 168.5ppm, and 4 of those 9 samples exceeded the limit set by EU.

The lowest value for cadmium in TSP fertilizers was 1.5ppm while the highest value was 5ppm. All the studied TSP fertilizer had cadmium contamination well below the EU limit for cadmium contamination (27ppm TSP). The level of chromium contamination in all the TSP fertilizer was the highest of all three heavy metals. The lowest chromium contamination rate was 11.6ppm while the highest contamination level was 18.2ppm (App. Table 2.25 and Figure 2.19). Mohiuddin *et al.* (2017) found chromium in 9 TSP samples with levels ranging from 260 to 302ppm, approximately 20 times higher than the findings in the current study. Sarker *et al.* (2017), in analysis of TSP fertilizer from the Mymensingh region, found chromium contamination ranged from 168.4 to 206.6ppm, and cadmium contamination ranged from 14.7 to 15.6ppm. Chromium in aqueous conditions are found in two forms, namely trivalent chromium and hexavalent chromium. Trivalent chromium is essential for metabolic processes in human body, but hexavalent chromium is toxic and carcinogenic to humans, and is primarily used in plastic, leather, dye, paint, wood, and steel industries (Thermo Fisher Scientific, 2019). The maximum allowable limit (MAL) for heavy metals set by the Ministry of Agriculture (MoA, 2007) in Bangladesh is as follows: lead 100ppm, chromium 500ppm and cadmium 10ppm. Since in this study the total chromium amount was measured, it was not possible to compare with EU standards, which has set limits for hexavalent chromium. Based on the maximum allowable limit for chromium contamination according to Bangladesh standards, all findings were well within the maximum allowable limit.

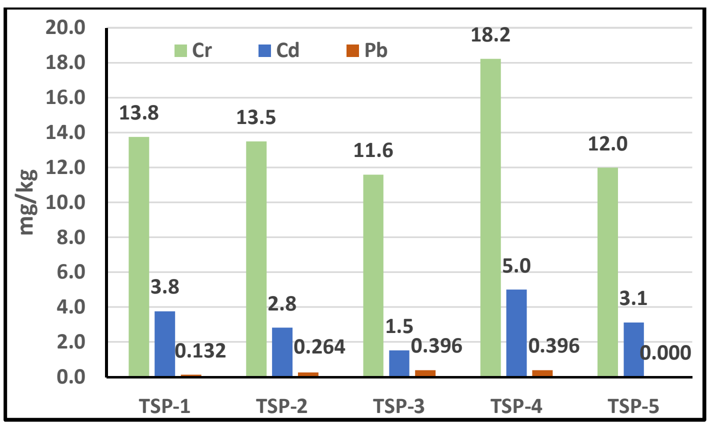


Figure 2.19. Heavy metal content (mg/kg) in TSP fertilizers available in the market in the Barind Tract.

Heavy metal contamination in MOP fertilizer

Lead contamination in MOP fertilizer samples ranged from below the detection level to a maximum level of 0.264ppm. Cadmium contamination in MOP fertilizer samples ranged from 0.204ppm to a maximum of 0.474ppm (App. Table 2.25 and Figure 2.20). Lead and cadmium contamination levels in all three tested MOP fertilizer is well below the EU limit as well as the Bangladesh limit for lead and cadmium contamination of inorganic macronutrient fertilizers.

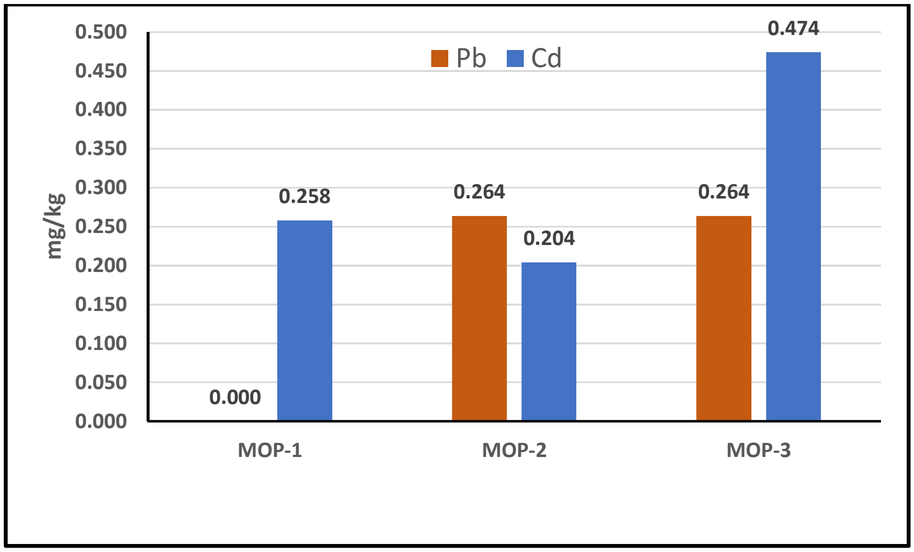


Figure 2.20. Lead and cadmium contamination (mg/kg) in MOP fertilizers available in the market in the Barind Tract.

Compared to lead and cadmium, the chromium contamination level in MOP fertilizers was high. The chromium contamination level ranged from 5.4ppm to a staggering 249ppm (Figure 2.21 and App. Table 2.25). Mohiuddin *et al.* (2017) studied five MOP fertilizers, found lead contamination ranged from 148.6ppm and 188.6ppm, chromium contamination ranged from 296 to 310ppm and cadmium contamination below detection range. Sarker *et al.* (2017) found in their study of MOP fertilizers from the Mymensingh region that lead contamination levels were between 33.5 to 101.2ppm, chromium contamination levels between 15.8 to 16.1ppm and cadmium contamination levels between 0.13ppm to 3.9ppm. Chromium contamination levels in all MOP fertilizers examined in this study were well within the limit as per the Bangladesh standard.

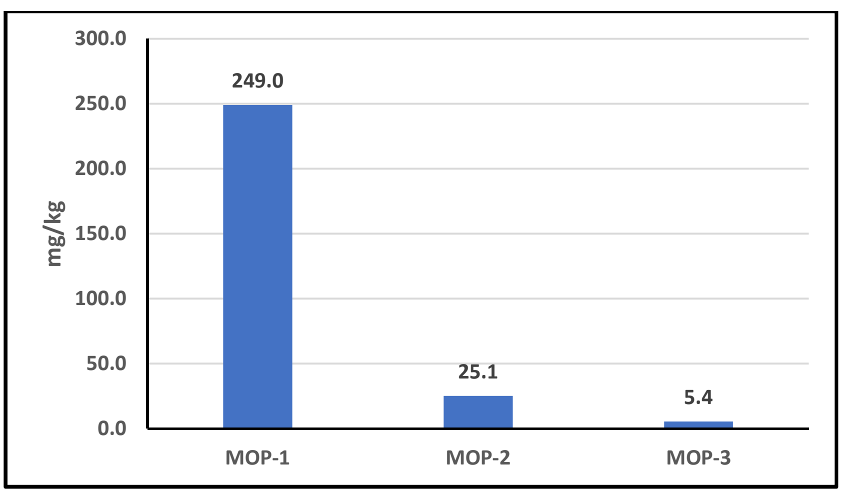


Figure 2.21. Chromium contamination (mg/kg) in MOP fertilizers available in the market in the Barind Tract.

Environment sensitive practices of entrepreneurial fisheries

Environment positive practices

- Culture of multiple fish species, leads into better ecological balance and better use of habitat
- Transportation of live fish (large fish seedlings) with water leads into unknowingly giving opportunities to other small indigenous species being transported to the new ponds with large fish seedlings, ultimately leads into greater fish diversity in culture ponds.
- The desire of fish farmers to capture as much water as possible in their pond leads into use of surface water and almost 100 percent of the precipitation, and relatively small portion (compared to the precipitation) of water from underground source.
- Large ponds with high dikes, creates large littoral zone for each of the ponds creating suitable zones where various types aquatic plants grow (not much by volume).
- Pond dikes are used for various fruit production, hence covered by vegetation that reduces soil erosion and supports terrestrial fauna near water.
- Large earthen (only 3.8% ponds with plastic lining in the bottom for prevention of water seepage) and perennial ponds allow water seepage and very helpful for recharging the ground water table especially in the context of dwindling natural wetland of the country.
- Regular and enough use of lime and salts in fishponds helps maintaining the water quality that supports faunal diversity and higher quantity.

Environment negative practices

- Regular use of pesticides for controlling of aquatic insects based on the assumption that the aquatic insects do harm/compete for space and food with large fish (usually large fish seedling, >0.75kg in size are released and reared in pond) deserves justification, how much damage do these aquatic insects do to large fish.
- No withdrawal period is maintained for antibiotics used in commercial aquaculture ponds. Almost half of the cases antibiotics are used as preventive option, which is wrong way of using antibiotics.
- Regular wipe out of all fish from the pond using fish toxicants primarily to eliminate the undesired small indigenous fish species that is present in the pond along with some leftover food fish that weren’t caught by netting.

- A considerable percentage of fish farmers regularly use fresh poultry litter in the ponds that may very well contain antibiotic residue of antibiotics fed to the chickens.
- Use of herbicides on pond dikes and dikes for controlling of undesired vegetation of the pond dikes may often finds its way into the pond water, especially during monsoon when it rains frequently, and vegetation growth is higher.
- Occasional use of steroids and digestive stimulants were also reported primarily due to the ill motivation of the farmers.

Impacts of entrepreneurial fisheries on soil and water resources

A comparative study of soil nutrients

Nutrient analysis of pond sediments in commercial fishponds, pond dikes with no crops of newly built ponds, pond dikes used for agriculture, sediments from perennial and static water *beels* (natural wetlands) and sediments from canals in *beels* with flowing water showed that pond sediments had the highest value of four macronutrients: nitrogen, potassium, phosphorus and sulfur. Pond dikes without agriculture had the highest value for the macronutrients, calcium and magnesium, closely followed by pond sediments (App. Table 2.26).

On the other hand, the levels of micronutrients boron, iron and manganese were highest in *beel* (perennial static water) sediments. Pond sediments had the highest concentrations of zinc and copper, closely followed by *beel* (perennial, static water) sediments.

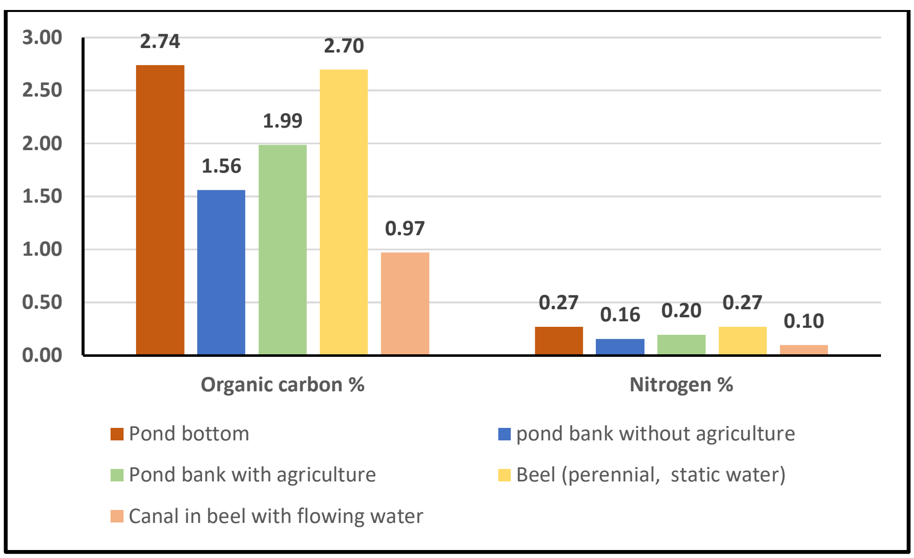


Figure 2.22. Organic carbon and nitrogen content of different soil samples in Barind Tract.

Bangladesh soils are generally low in organic matter. Being a tropical country, the conservation of soil organic matter is a soil management challenge (Bhuiya, 1987). On the contrary, organic carbon content was high in all the soil samples under this study and was highest in entrepreneurial fishpond sediments. Given the fish feed (discussed earlier in this chapter) and organic manure used in entrepreneurial fisheries, it is not surprising that pond bottom sediments were rich in organic carbon. Muendo *et al.* (2014) found 1.5 to 5 tonnes of organic matter was accumulated per hectare area in semi intensive tilapia farming in earthen ponds over a period of four and half months, and the accumulation was not affected by input type or stocking density of fish. While Avnimelech *et al.* (1999) reported organic matter concentration in fishpond sediments at 4.98 to 6.2%, Rahman *et al.* (2002) reported 6.47% organic matter in pond sludge. Using 1.9 as the conversion factor for converting organic carbon into organic matter (Pribyl, 2010), organic matter in entrepreneurial fishponds stands as 5.21%. Boyd *et al.* (2002) found that organic matter in pond sediments does not increase indefinitely once it has reached to a certain maximum level. After the initial few years organic matter concentration reaches an equilibrium where the deposition and decomposition rates becomes almost equal. Soil that contains 1 to 3 percent organic carbon is categorized as 'mineral soil with moderate organic matter content' and is the best range for aquaculture (Boyd *et al.*, 2002). The ponds under entrepreneurial fish production of this study perfectly fit in that category.

On the other hand, perennial *beel* that naturally have been receiving surface runoff and retaining those sediments for centuries have accumulated organic matter with the second highest concentration (2.70%) of organic carbon. Sayeed *et al.* (2015) reported sediments of Chalan *beel* contained 2.59±0.52% organic matter. On the other hand generally, all of the fishponds under entrepreneurial fish production are in low lying areas and previously were either perennial *beel*s or seasonal floodplains, hence the pond dikes of both types (newly built and under intensive agriculture) were also rich in organic carbon content. Canals in the *beel*s that usually carry surface runoff to the *beel* and often get connected to the major river system during the monsoon and seasonal flooding, had the lowest organic carbon concentration (0.97%) in their sediments (Figure 2.22). Alam *et al.* (2014) found 0.83±0.372% and 0.78±0.292% organic carbon in Hilna *beel* and Kumari *beel* respectively in Rajshahi District. Like organic carbon, the nitrogen content in different soil sediments followed a similar pattern of concentration (Figure 2.22) where both the pond sediments and *beel* sediments contained the same levels of nitrogen at 0.27%. Rahman *et al.* (2002) reported 0.28% total nitrogen content in pond sludge, but Sayeed *et al.* (2015) recorded 0.09±0.04% total nitrogen content in the Chalan *beel* sediments, which is much lower than the findings of this study.

This is primarily due to the perennial nature of the *beels* where sediments were used in this study. Alam *et al.* (2014) reported 0.082±0.027% and 0.068±0.02% nitrogen in Hilna *beel* and Kumari *beel* respectively in Rajshahi, which is more like the nitrogen content (0.1%) of the canals in the *beel* with flowing water in this study. Regular urea fertilization of the aquaculture ponds under entrepreneurial fish production and surface runoff are the major sources of nitrogen in ponds and *beel*s respectively. Acosta-Nassar *et al.* (1994) reported that around 65% of applied nitrogen as fertilizer in ponds gets accumulated in the sediments. The regular use of fertilizers for crop production on pond dikes added to the organic carbon as well as nitrogen content of the pond dike soil (Figure 2.22) under cultivation.

The potassium content compared to calcium and magnesium was quite low in all soil samples. The highest potassium content (0.90 mM/100g soil) was in pond sludge while the lowest was in the sludge from canals in the *beel* with flowing water (0.37 mM/100g soil) (Figure 2.23). Rahman *et al.* (2002) reported the potassium content in perennial pond sediments as 51 mg/100g soil. Alam *et al.* (2014) found 615±213 mM/100g soil and 594±184 mM/100g soil in Hilna *beel* and Kumari *beel* respectively in Rajshahi, the levels in both places being high compared to the findings of the current study. The second highest potassium content (0.86 mM/100g soil) was found in the pond dike soil under agriculture, probably due to the potassium fertilization applied for production of banana and papaya on pond dikes. The pond dike potassium has possibly contributed the high pond sediment potassium by being washed away into the pond through surface runoff.

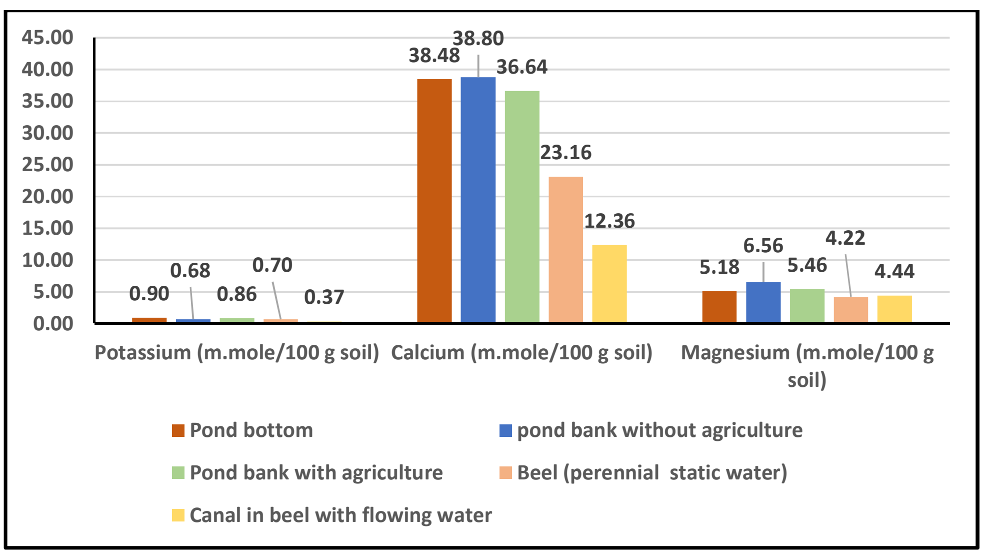


Figure 2.23. Potassium, calcium and magnesium content of different soil samples in Barind Tract.

Calcium content was generally high in all the soil samples under this study, indicative of generally high calcium content in the soil of that region. The highest calcium concentration (38.8 mM/100g soil) was found in the pond dikes without agriculture (Figure 2.23). The second highest calcium content (38.48 mM/100g soil) was found in pond sediments under entrepreneurial fish production. Despite the use of calcium in biomass production, the regular application of lime in ponds is also responsible for the higher calcium content in pond sediments. Wahab *et al.* (1994) found sediments from perennial ponds had calcium concentrations of 24.2 to 47.8ppm which is equivalent to 96.8 mM/100g soil to 191.2 mM/100g soil much higher than the present study. Pond dikes with agriculture had the third highest calcium content (36.64 mM/100g soil), lower than that of pond dikes without agriculture, possibly due to the uptake by plants grown on the pond dikes. Compared to the pond and dike sediments, perennial *beel* and canals in the *beel* with flowing water had relatively lower calcium content. Other than the surface runoff, there is no additional calcium being added into the *beel* systems. On the other hand, calcium outflow is continuing in terms of the production of biota.

Like the calcium content, the highest magnesium content (6.56 mM/100g soil) was found in the soil from the pond dikes without agriculture, followed by pond dikes with agriculture (5.46 mM/100g soil) and pond sediments (5.16 mM/100g soil) under entrepreneurial fish production (Figure 2.23). Perennial *beel* sediments had 4.22mM magnesium/100g soil and sediments in the *beel* canals had 4.44 mM magnesium /100g soil. Therefore, there was not much difference in the magnesium content of soils from different system/sources.

The highest phosphorus content (81.12 µg/g soil) was found in pond sediments and the lowest (6.70 µg/g soil) was found in soils from pond dikes without agriculture (Figure 2.24). Because of the heavy use of phosphorus fertilizer used in biomass production in pond systems, pond sediments had the highest phosphorus content. This result of phosphorus content in pond sediment matches with the findings from Wahab *et al.* (1984) of 70 to 110 µg/g soil phosphorus content in perennial pond sediments. Perennial *beel* sediments had the second highest phosphorus content (19.40 µg/g soil) followed by the phosphorus content (18.04 µg/g soil) in the sediments from canals in the *beel*. Phosphorus content of 11.92±4.014 and 16.02±3.548 µg/g soil respectively in sediments of Hilna *beel* and Kumari *beel* respectively in Rajshahi have been reported by Alam *et al.* (2014), similar to the findings of this study. Phosphorus content in the soil from pond dikes under agriculture was higher (15.87 µg/g soil) than that of pond dikes without agriculture, because phosphorus fertilizers were added for crop production on pond dikes with agriculture.

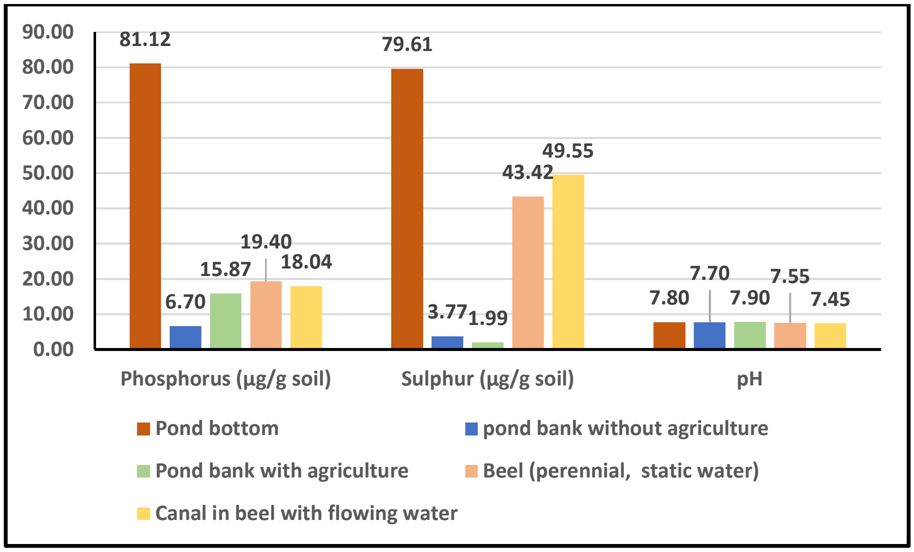


Figure 2.24. Phosphorus, sulfur and pH of different soil samples in Barind Tract.

The highest sulfur content (79.61 µg/g soil) was found in pond sediments, the second highest sulfur content (49.55 µg/g soil) was found in the sediments from *beel* canals followed by the sediments of perennial *beel* (43.42 µg/g soil) (Figure 2.24). Alam *et al.* (2014) found lower sulfur content (24.05±7.85 and 22.17±7.24 µg/g soil in Hilna *beel* and Kumari *beel* respectively) than found in this study. However, the sulfur content in bottom sediments of Posna *beel* in Kalihati, Tangail was found similar (51.20 µg/g soil) to this study by Alam *et al.* (2007). The lowest sulfur content (1.99 µg/g soil) was found in the soil of pond dikes under agriculture. It was lower than that of pond dikes without agriculture (3.77 µg/g soil), possibly due to the uptake of sulfur by plants grown on the pond dike under agriculture that has not been replenished with fertilizer.

Soils from the pond dikes under agriculture had the highest pH (7.90), followed by pond sediments (7.80) and pond dikes without agriculture (7.70) (Figure 2.24). Perennial *beel* sediments had a pH of 7.55 and sediments from canals in the *beel* had the lowest pH (7.45). Despite having the highest organic carbon content, pond sediment pH was higher, primarily due to the liming that is done in the ponds under entrepreneurial fish production. A high pH of the soil of pond dikes without agriculture shows that there is generally a high pH in that area, but the relatively lower pH in *beel* sediments is primarily due to the higher organic carbon content.

The perennial *beel* sediments had the highest concentrations of boron, iron and manganese. On the other hand, the highest concentration of copper and zinc was found in pond sediments under entrepreneurial fisheries, followed by *beel* sediments (App. Table 2.27).

Boron concentration was highest (0.50 µg/g soil) in the perennial *beel* sediments, while the sediments from canals in the *beel* had a concentration of less than half of that (0.23 µg/g soil) (Figure 2.25). Alam *et al.* (2007) found similar a boron concentration (0.57 µg/g soil) in the pond bottom sediments of Posna *beel* in Kalihati, Tangail. In the pond systems, the pond dikes with agriculture had higher (0.45 µg/g soil) concentration of boron than pond sediments (0.41 µg/g soil) and pond dikes without agriculture, possibly due to the boron fertilization in banana and papaya plantations on the pond dike under agriculture.

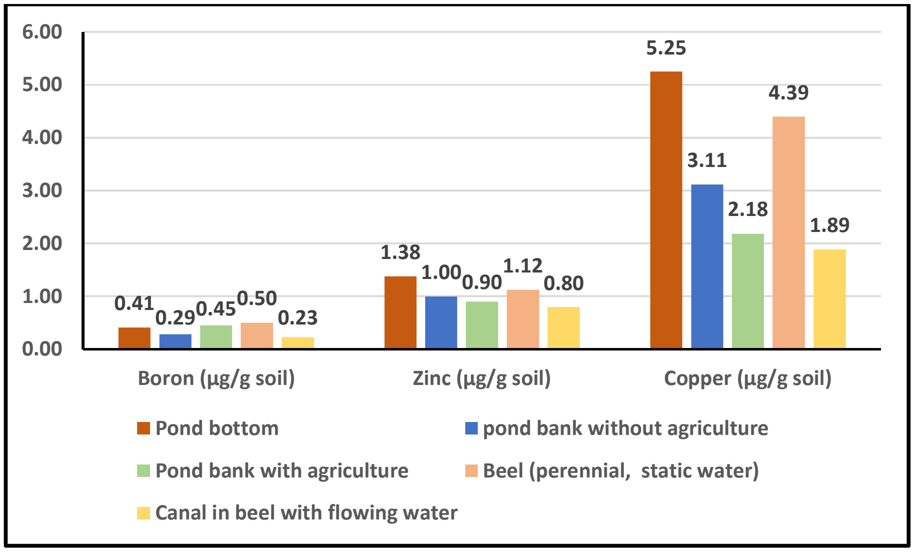


Figure 2.25. Boron, zinc and copper content in different soil samples in Barind Tract.

Zinc concentration was the highest (1.38 µg/g soil) in pond sediments followed by perennial *beel* sediments (1.12 µg/g soil). Soil from the pond dikes without agriculture had higher zinc (1.00 µg/g soil) than the soil from pond dikes under agriculture (0.90 µg/g soil), followed by sediments from canals in the *beel* (0.80 µg/g soil) (Figure 2.25). Alam *et al.* (2007) found 1.36 µg/g soil zinc in sediments of Posna *beel* in Kalihati, Tangail, which is slightly higher than the zinc concentration in *beel* sediments of this current study.

Copper concentration followed the similar pattern as zinc, the highest concentration (5.25 µg/g soil) was found in pond sediments under entrepreneurial fish production followed by the concentration in perennial *beel* sediments (4.39 µg/g soil) (Figure 2.25). Alam *et al.* (2007) found higher copper concentration (9.90 µg/g soil) in Posna *beel* in Kalihati, Tangail. Soil from pond dike without agriculture had higher copper concentration (3.11 µg/g soil) than that of soil from pond dikes under agriculture (2.18 µg/g soil) followed by sediments of canals in the *beel* (1.89 µg/g soil).

Iron concentration was highest (213.5 µg/g soil) in perennial *beel* sediments followed by sediments from pond (147.3 µg/g soil) under entrepreneurial fish production (Figure 2.26). Alam *et al.* (2007) reported Posna *beel* sediments in Kalihati, Tangail had iron concentration of 422.00 µg/g soil, a higher concentration than found in this current study. Sediments from canals in the *beel* had higher iron concentration (95.6 µg/g soil) than that of pond dikes without agriculture (35.9 µg/g soil). The lowest iron concentration (27.4 µg/g soil) was found in pond dikes under agriculture, possibly due to the uptake of iron by plants grown in pond dikes under agriculture.

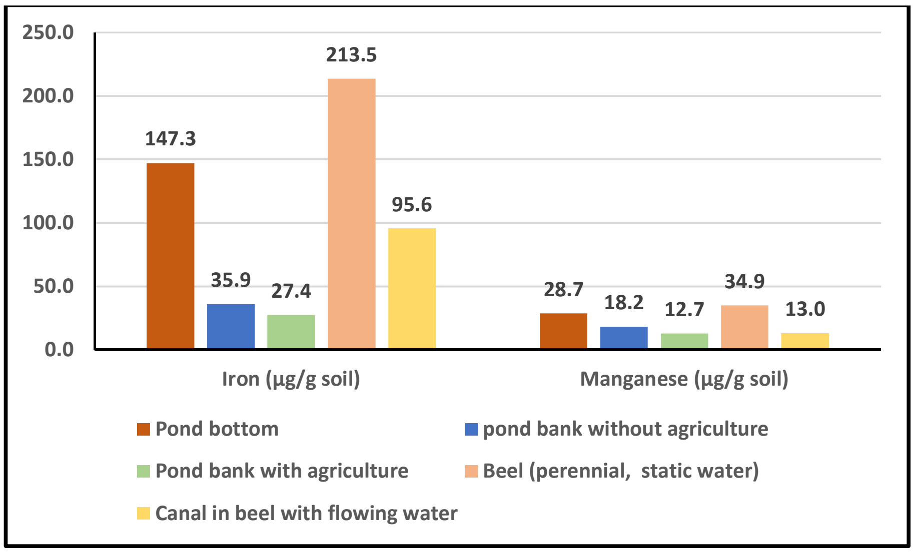


Figure 2.26. Iron and manganese concentration in soils from different sources in Barind Tract.

Like the iron concentration, the highest manganese concentration (34.9 µg/g soil) was also found in perennial *beel* sediments, followed by pond sediments under entrepreneurial fisheries (28.7 µg/g soil). Alam *et al*. (2007) reported similar manganese concentration (46.10 µg/g soil) in the sediments of Posna *beel* in Kalihati, Tangail. Manganese concentration was higher (18.2 µg/g soil) in pond dikes without agriculture than that (12.7 µg/g soil) of pond dikes under agriculture, possibly due to the uptake of manganese by plants grown in pond dikes under agriculture. Even the sediments from canals in the *beel* retained higher concentration (13.0 µg/g soil) of manganese than pond dikes under agriculture.

Heavy metal concentration in soils from different sources

Of all the heavy metals (lead, chromium and cadmium) tested, lead was more abundant in all soils systems, followed by chromium and then cadmium (App. Table 2.28). The highest concentration of lead was found in sediments from ponds under entrepreneurial fish production (8.54ppm) (Figure 2.27). Priyamvada *et al.* (2013) found that fish culture in experimental ponds led to increased lead and cadmium concentrations. Flowra *et al.* (2012) found the highest concentration of lead (7.64ppm) in pond water contaminated with industrial waste in Rajshahi city. The second highest concentration (5.22ppm) of lead was found in sediments of the perennial *beel*, closely followed by pond dikes under agriculture (5.16ppm). Lead concentrations was relatively low (1.83ppm) in pond dike without agriculture, while the concentration was below the detection level in sediments from canals in *beel* (Figure 2.27).

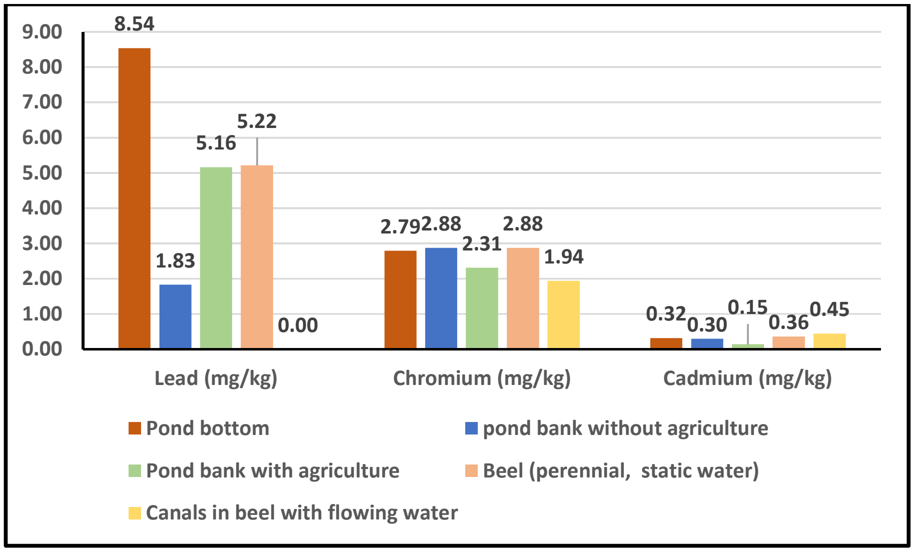


Figure 2.27. Heavy metal concentration in soils from different sources in Barind Tract.

Chromium concentrations in all different soil samples were similar (Figure 2.27). The highest concentration of Chromium was found in sediments of the perennial *beel* (2.88ppm) and pond dikes without agriculture (2.88ppm). Sediments from ponds under entrepreneurial fish production had chromium concentrations of 2.79ppm, followed by pond dikes with agriculture (2.31ppm). The lowest concentration of chromium (1.94ppm) was found in sediments from canals in the *beel*. Chromium concentrations in all samples were well below the average chromium concentration of 2753.2 ± 4598.86ppm in surface agricultural soil and 1039.2 ± 1763.69ppm in sub surface agricultural soil from areas near the Dhaka Export Processing Zone (DEPZ) (Hasnine *et al.,* 2017). Uddin *et al.* (2019) reported lead and cadmium concentration of 11.53 and 0.68ppm in vegetable production plots, both values being well above the concentrations of lead and cadmium found in this study.

Of all the heavy metals, cadmium concentration was the lowest in all soil samples. The highest cadmium concentration (0.45ppm) was found in sediments from canals in the *beel*, followed by perennial *beel* sediments (0.36ppm), pond sediments (0.32ppm), pond dikes without agriculture (0.30ppm) and pond dikes under agriculture (0.16ppm). Despite receiving regular fertilization for crop production, pond dikes under crop production had the lowest chromium and cadmium concentration. It is likely that the plant uptake may have caused a reduced chromium and cadmium concentration in pond dikes under crop production.

Harikumar *et al.* (2009) found heavy metals in core sediments in the Vembanad wetland system in India ranged from 15.65 to 54.42ppm for lead, 1.79 to 2.36ppm for chromium and 0.26 to 0.73ppm for cadmium. Chromium and cadmium concentration in *Beel* sediments in this current study was similar to the concentration in Vembanad wetland system but the lead concentration was considerably lower.

Despite the high level of input used in the form of feed, feed ingredients, poultry litter and fertilizers in fishponds under entrepreneurial fish production, the heavy metal content in the pond sediments was not exceptional. Das *et al.* (2017) found lead, chromium and cadmium concentration in pond sediments of commercial tilapia farms in Noakhali ranged from 6.34 to 7.92ppm, 6.60 to 9.16ppm and 0.2 to 0.36ppm respectively, which is very similar to the findings of this study. Karak and Bhattacharyya (2010) found total lead, chromium and cadmium concentration in roadside pond sediments that received road runoffs along with fish feed supplements as 121, 13 and 15ppm respectively of which plant bioavailable concentration was 58, 1.3 and 2.3ppm respectively in West Bengal, India. Islam *et al.* (2018) confirmed the similar incidence of higher concentration of heavy metals and metalloids in soils near high traffic and industrial areas in Bangladesh. Overall, heavy metal concentration in sediments from the different sources in this study was very much within the limits and guidelines (App. Table 2.29) set by National Oceanic and Atmospheric Administration (NOAA) of US department of Commerce (2008).

While Karak and Bhattacharyya (2010), after using the pond sediments in agricultural soil amendment for wheat production found agricultural soils having heavy metals within the common range of lead 2 to 200ppm, chromium 1 to 1000ppm, cadmium 0.01 to 0.7ppm in soil as guideline suggested by Lindsay (1979). Therefore, pond sediments from entrepreneurial fisheries and *beel*s that have lower heavy metal concentrations than the concentration of pond sediments used by Karak and Bhattacharyya (2010) for wheat production would be safe for using in agricultural soil amendment as a nutrient source.

Comparative study of physicochemical properties of water

Water pH

The water pH level in aquaculture ponds and natural wetlands generally followed similar trends throughout the year (Figure 2.28). Water pH in aquaculture ponds remained around 7.5 and above, while for natural wetlands the pH value remained around 7. The lowest average pH value for aquaculture ponds was 7.31 in the month of July. On the other hand, the lowest average pH value for natural wetlands was 6.65 in the month of October. The highest average pH value for natural wetlands was recorded as 7.59 in the month of December, while the highest average pH value for commercial aquaculture ponds was recorded as 7.96 in the month of January.

Mannan *et al*. (2012) reported water pH in semi intensive aquaculture ponds in Tangail, Bangladesh ranged from 6.5 to 9.2. Hossain *et al.* (2007) found water pH in 12 different *beel*s in Natore, Bangladesh ranged from 6.8 to 8.6 in between the months of September and December in 2006. Islam and Chowdhury (2013) recorded water pH ranged from 7.2 to 7.5 in between the months of January and December in Trimohini *beel*, Rajshahi, Bangladesh. Sayeed *et al.* (2015) recorded a pH of 9.7±0.47 in the Chalan *beel* in between July 2007 and June 2008. Natural phenomena like rainfall, surface runoff, reduction of water level in dry months, fluctuations of temperature and human activity played a role in dictating the water pH level fluctuations throughout the year, while for commercial fishponds the use of chemicals and other inputs are the primary factors for having a more steady water pH, since fish entrepreneurs try to maintain the pond water pH above 7.

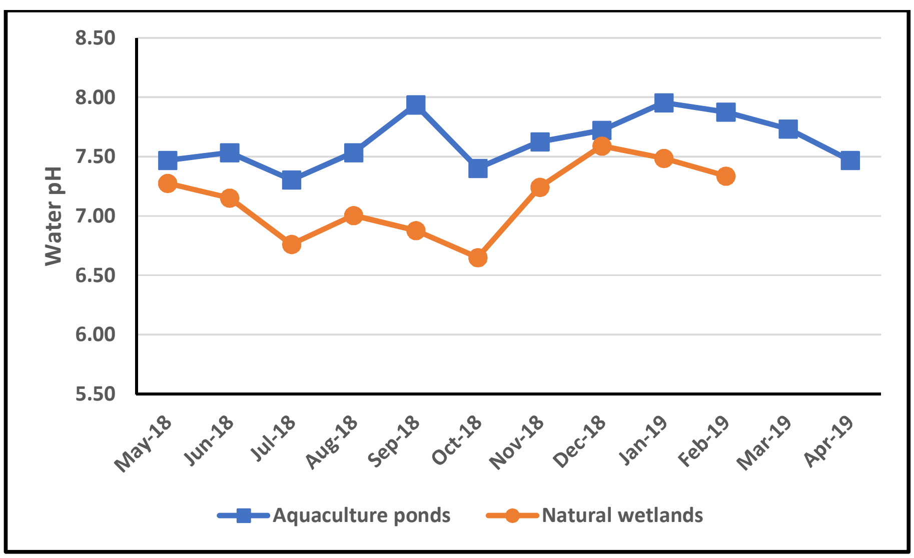


Figure 2.28. Year-round comparison of water pH in aquaculture ponds under entrepreneurial fish productions and in natural wetlands in the Barind Tract.

Water pH is an interdependent factor and closely linked with CO_2, water alkalinity and hardness (Philminaq, 2019). Boyd and Tucker (1998) found that a water pH range from 6.5 to 9 is optimum for the health and growth of aquatic animals. The pH levels in aquaculture ponds and natural wetlands in the Barind Tract were within the range suitable for aquatic animals.

Water transparency

Water transparency (measured with Secchi disk reading in inches) was higher in natural wetlands than in commercial fishponds throughout the observation period of one year. Water transparency two to three times higher in natural wetlands indicates lower suspended particles of both an abiotic (like soil particle, season, angle of light) and biotic (plankton density) nature (Reid and Wood, 1979). The transparency measured from the Secchi disk reading ranged from 9.3 to 15.5 inches for aquaculture ponds, and 21.3 to 39 inches in the natural wetlands in the Barind Tract (App. Table 2.31). Mannan *et al.* (2012) found water transparency between 4 to 14 inches in terms of the Secchi disk reading in semi intensive aquaculture ponds. Secchi disk depths ranging from 9 to 12 inches were also recorded in semi intensive earthen fish culture ponds in Rajshahi by Hossain *et al.* (2007). The lowest transparency in commercial aquaculture ponds was found in the month of July, possibly due to the monsoon rainfall and surface runoff (Figure 2.29). On the other hand, the lowest transparency was recorded in the month of May 2018 in natural wetlands due to early monsoon rain and surface runoff. The highest transparency was recorded in the months of October and November after the monsoon for both natural wetlands and aquaculture ponds, primarily due to the increased water depth. Sayeed *et al.* (2015) also reported higher transparency during the months of August to November in the Chalan *beel*.

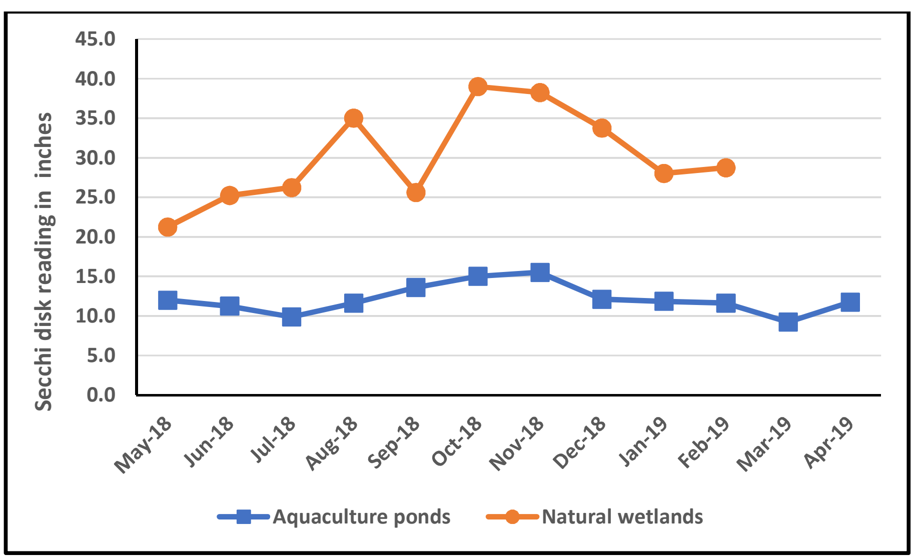


Figure 2.29. Year-round comparison of Secchi disk reading in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Biological activity of aquatic animals is reduced in the winter due to the fall in temperature. This creates less turbulence in the water and allows the suspended abiotic particles to settle on the bottom, making transparency higher during the winter. For natural wetlands, the transparency in winter months would have been higher, but the Secchi disk hit the bottom due to lower water depth. The presence of fish and the turbulence created by them causes higher suspended solid particles in earthen aquaculture ponds especially in summer months when water depth is relatively less, but biological activity is very high. On the other hand, the commercial pond system is very productive biologically and the high plankton population in combination with the suspended solids created lower transparency. A Secchi disk reading of 8 to 16 inches has been suggested as optimum for food fishponds by Boyd and Tucker (1998) because at that level of Secchi disk reading, phytoplankton density is also optimum, maintains a balanced primary productivity but not causing oxygen depletion. Fisheries entrepreneurs in the Barind Tract regularly fertilize their ponds to maintain a maximum level of natural productivity and hence the Secchi disk reading was within the optimum range suggested by Boyd and Tucker (1998).

Electrical conductivity

The electrical conductivity (EC) for aquaculture ponds ranged from 313.3 to 510.3 µS/cm and for natural wetlands was between 184.5 and 308.0 µS/cm (App. Table 2.32). The difference in EC between aquaculture ponds and natural wetlands was consistent year-round except between October and February, when EC in aquaculture ponds declined, and EC in natural wetlands increased (Figure 2.30). Sayeed *et al.* (2015) found an EC of 307±147 µS/cm in the Chalan *beel*, higher than the findings of this study, but reported lower EC in the Chalan *beel* during the months of June to September, which is very similar to the trend found in this study. Islam and Chowdhury (2013) reported the EC in Trimohini *beel* as 84.6-110.5 µS/cm and was the lowest in between April to October, similar to the trend of the current study.

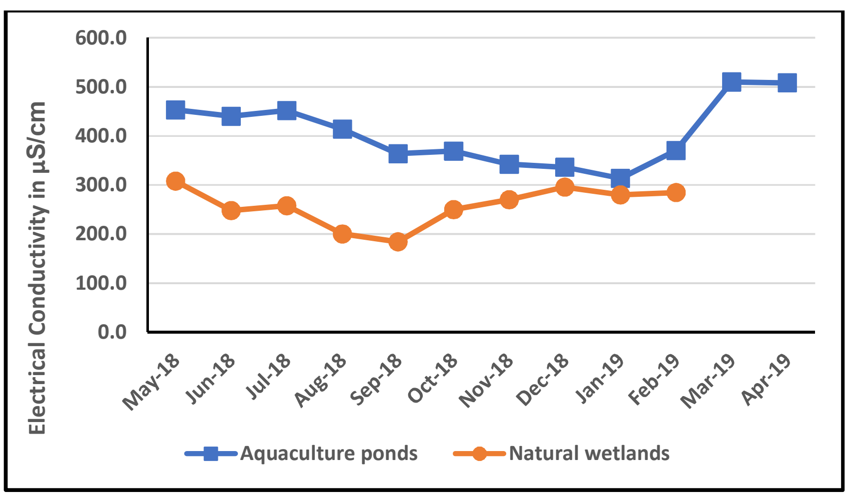


Figure 2.30. Year-round comparison of electrical conductivity of water in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Lucy *et al.* (2016) reported that a reconstructed fishpond at Chittagong University had an EC of 215.38±21.27μS/cm and 128.58±1.10μS/cm. Freshwater fish require some amount of salt in the water for their osmotic balance within the desirable range of 200 to 2000 μS/cm and survive under a wide range (30 to 5000 μS/cm) of EC (Stone and Thomforde, 2019). Since EC is correlated with salt content, EC value in the current study was found within the suitable range for fish culture, primarily due to the use of table salts (discussed earlier in this chapter) in commercial fishponds.

Total dissolved solids

Total dissolved solid (TDS) is the measure of materials dissolved in water, including bicarbonate, sulphate, nitrate, phosphate, magnesium, and sodium (Philminaq, 2019). Year-round TDS content in both aquaculture ponds and natural wetlands followed the similar pattern as the electrical conductivity. Waters of aquaculture ponds had a higher TDS throughout the year than that of natural wetlands, primarily due to the regular use of fertilizers, salts and various inputs in aquaculture ponds. On the other hand, surface run off, soil erosion, fertilizer run off, wastewater and decaying organic matters along with the geology of the area are the primary source of TDS in natural wetlands (Philminaq, 2019). The TDS content of both aquaculture ponds and natural wetlands were within the recommended TDS limits of 1000ppm for freshwater aquaculture (Alam *et al.*, 2015 and Philminaq, 2019). The TDS value and the timing of low and high TDS (Figure 2.31and App. Table 2.33) in natural wetlands in this study were very similar to the findings reported by Alam *et al*. (2015) for the Hilna *beel* (lowest 53.3ppm in September and highest 187.4ppm in May) and the Kumari *beel* (the lowest 55ppm in September and the highest 239ppm in May) in Rajshahi. Sayeed *et al*. (2015) reported the mean TDS values from different sampling sites in the Chalan *beel* taken at different times of the year ranged from 158.64ppm to 217.56ppm, while the highest TDS value was recorded in the month of May. The highest TDS value in aquaculture ponds was found in the month of March and April. The seasonal variation of TDS values roughly coincides with reduced water depth/volume resulting from evaporation where water volume is reduced but the dissolved solids remain. This leads to higher concentrations in the pond system where there is low runoff, but high input of lime and fertilizer that increases the TDS.

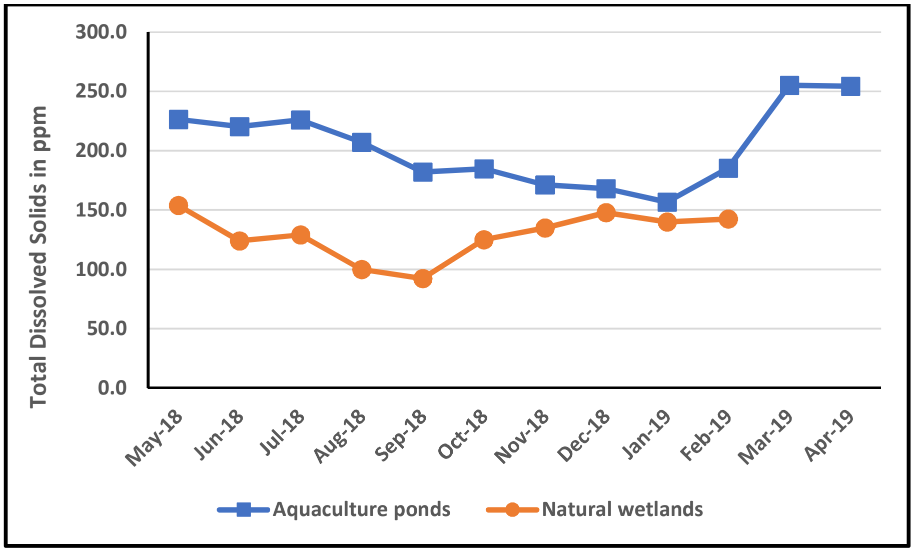


Figure 2.31. Year-round comparison of total dissolved solids in water in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Water turbidity

The visual property of water that absorbs or redirects light from becoming transmitted through the water in a straight line is referred as turbidity. This is primarily caused by suspended soil particles, plankton, organic debris and dissolved colored compounds (Boyd and Tucker, 1998). The highest turbidity in commercial aquaculture ponds was in the month of December; on the other hand highest turbidity in natural wetlands was recorded in September. Turbidity in aquaculture ponds in this study was many times higher than in the natural wetlands throughout the year. Since aquaculture ponds have a higher density of fish, their movement causes water turbulence, leading to a higher amount of suspended soil particles in the pond water. Aquaculture ponds also receive large amounts of fertilizers and other inputs contributing to a higher amount of plankton production, which increases turbidity. This, in combination with the reduced temperature in December, led to reduced feed (including plankton) consumption by fish. However, with a low water depth, the fish movement caused a higher amount of suspended soil particles in the water, possibly resulting in a higher Turbidity for commercial aquaculture ponds. On the other hand, surface runoff from the monsoon rain and seasonal flooding in the month of September resulted in the higher turbidity in the natural wetlands during September.

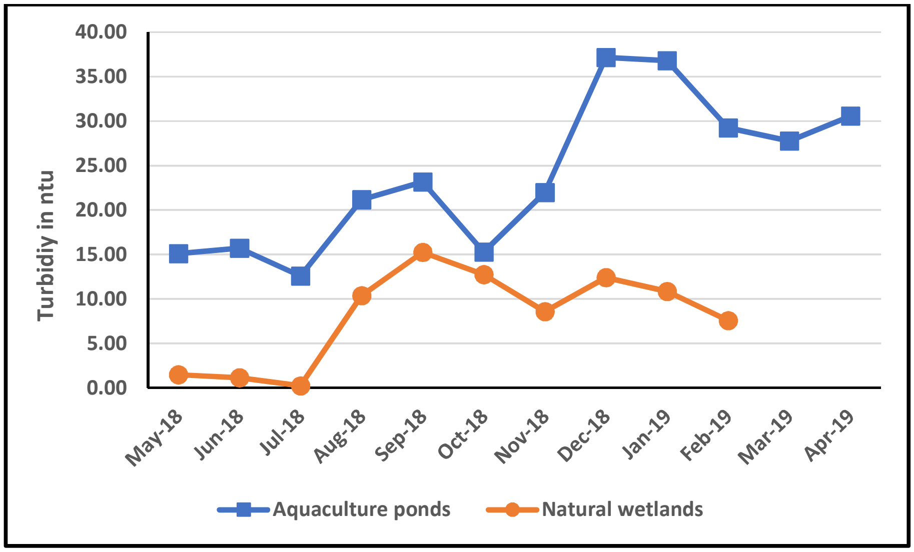


Figure 2.32. Year-round comparison of water turbidity (ntu) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Factors such as surface runoff and water turbulence that impact the amount of suspended solid particles, and factors such as temperature, fertilizer use, light penetration, water depth, and plankton consumption by fish that influence the amount of plankton in the ponds, are primarily responsible for the seasonal fluctuations of turbidity in both commercial aquaculture ponds and natural wetlands. Very low turbidity in natural wetlands was found in this study. The natural wetlands allow the light to penetrate deep into the water and supports macrophyte growth at the bottom of three natural wetlands under this study. Boyd and Tucker (1998) described moderate phytoplankton bloom as the most desirable form of turbidity since it increases productivity, produces oxygen, removes ammonia from the ponds, and prevents the growth of underwater macrophytes by controlling the light penetration. Alam *et al.* (2015) recorded the highest turbidity in the month of April and the lowest turbidity in the month of December in Hilna *beel* and Kurmari *beel* in Rajshahi. On the other hand, Hossain *et al.* (1997) found the highest turbidity in the month of May and lowest in the month of November in Basukhali-Salimpur-Kola-Barnal *beel*. Since the many influencing factors of turbidity are different at different times and in different natural wetlands, therefore, the results may not be directly comparable with each other.

Impacts of entrepreneurial fisheries on faunal diversity

Zooplankton in commercial aquaculture ponds and natural wetlands

Zooplankton count was higher in commercial aquaculture ponds than in natural wetlands throughout the year (Figure 2.33). In commercial aquaculture ponds, zooplankton density was highest (5786±1482/liter) in the month of January and lowest (1261±513/liter) in the month of November. On the other hand, the highest count of zooplankton (2721±405/liter) was found in August in natural wetlands, while the lowest count (536±132/liter) was recorded in October (App. Tables 2.35 and 2.36). On an average aquaculture ponds were found having 119% more zooplankton than that of natural wetlands.

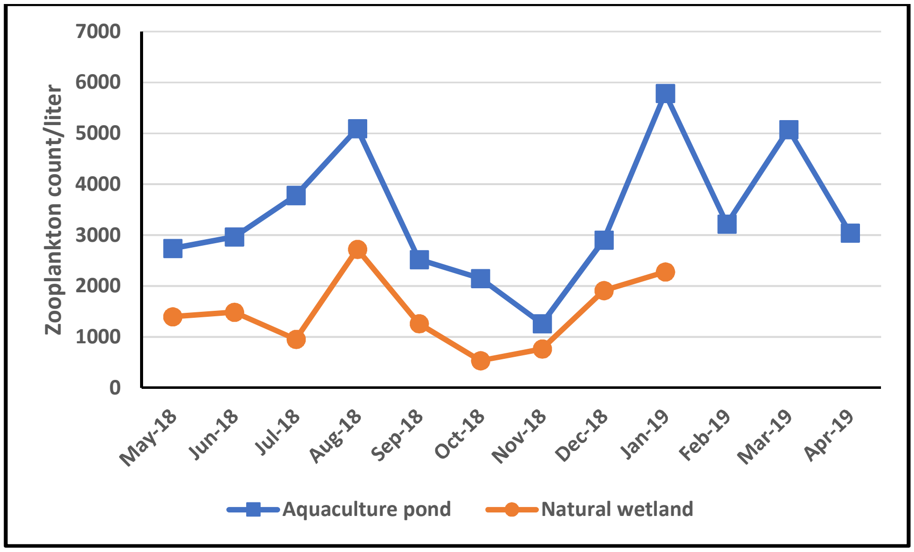


Figure 2.33. Year-round comparison of total zooplankton count (numbers/liter) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

There were total of 11 genera from 3 different groups (rotifera, cladocera and copepods) of zooplankton along with crustacean larvae-nauplius identified in both natural wetlands and aquaculture ponds (Figure 2.34). Zooplankton under rotifera group was comprised of the genera *Brachionus*, *Keratella*, *Asplanchna*, *Filinia*, *Polyarthra* and *Trichocerca*. Zooplankton under the cladocera group was comprised of genera *Daphnia*, *Diphanosoma* and *Moina*. On the other hand, copepods consisted of genera *Cyclops* and *Diaptomus*. Based on the zooplankton count, the dominance of the three groups was as follows: rotifera>copepods>cladocera in both natural wetlands and commercial aquaculture ponds (App. Table 2.37). Ali and Islam (1981) recorded that zooplankton contributes to 47% of the food of *Catla catla* and 6.37% of the food of *Labeo rohita*. Despite having a heavy presence of fish such as *Catla catla, Labeo rohita, Ctenopheringodon Idella* that consume zooplankton, and the regular use of pesticides in fishponds under entrepreneurial fish production, zooplankton density was higher in entrepreneurial fish ponds than in natural wetland. This is primarily due to the regular supply of quality supplementary feed in the ponds. Though there were differences in internal dominance of different zooplankton types, zooplankton diversity in the form of zooplankton genus count was similar in both commercial aquaculture ponds and natural wetlands in the Barind Tract. Margalef (1964) reported that zooplankton diversity is affected by nutrient content of the water along with the other factors. The high abundance of rotifers was observed in eutrophic water by Islam *et al.* (2001) and Islam *et al.* (2005).

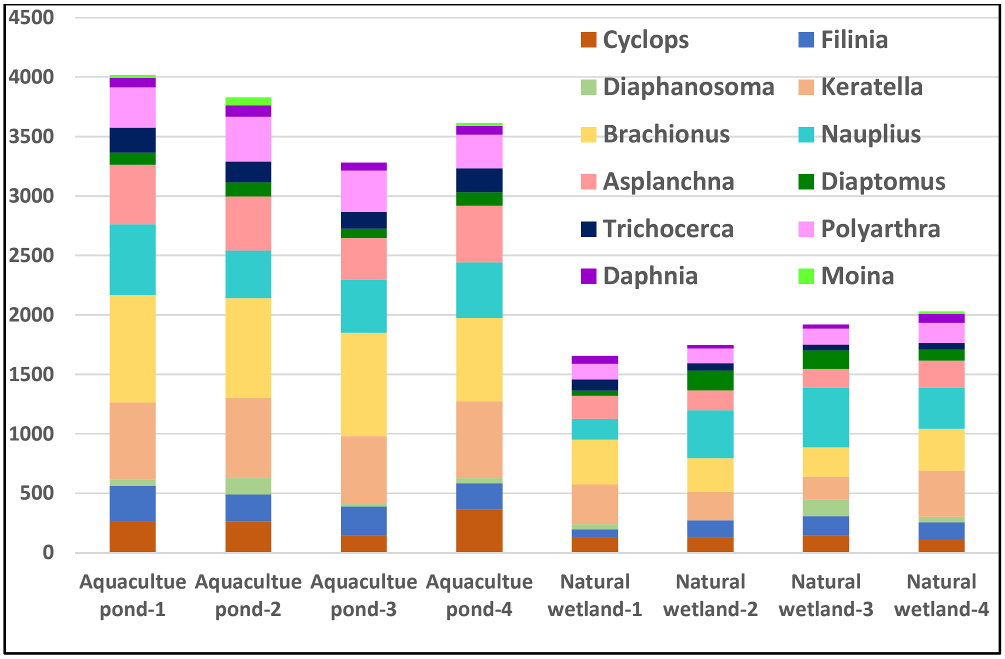


Figure 2.34. Stacked column diagram of recorded zooplanktons (average individual/liter of the whole year) from different aquatic systems.

Roy *et al.* (2010) found the highest six groups of zooplankton in the month of December of which the number of copepoda was dominant followed by rotifera, cladocera, ostracoda crustacean larvae and brysozoa in brood fishponds in Khulna. Like the findings of this study, Hossain *et al.* (2015) identified four different groups (orders) of zooplankton consisting of 14 different genera at an average density of 5550/liter of water in aquaculture ponds in Noakhali, whereas the zooplankton count in unused ponds was 1670 individuals/liter. Hossain *et al.* (2006) found 13 genera of zooplankton in earthen aquaculture ponds in Mymensingh region where the rotifera group was dominant. Average zooplankton density of 3790 individuals/liter water comprising of 21 genera in Ramsagar dighi in Dinajpur was reported by Akther *et al.* (2015). Shil *et al*. (2013) recorded 11 genera of zooplankton of 5 different groups of which copepoda was the dominant group in a semi intensive prawn farm in Bagherhat. The zooplankton abundance there ranged from 1102 to 1570/liter. Rahman and Hussain (2008) found 9 genera of zooplankton in different water bodies on the Rajshahi University campus of which *Cyclops* was most dominant.

Singh *et al.* (2017) recorded a zooplankton density ranging from 589 to 954/liter comprising of 12 genera from the following groups: protozoa, copepoda, rotifera cladocera and a mixed group where protozoans were most dominant in number, in wetland (*beel*) in 24- South Parganas District, West Bengal. Islam and Chowdhury (2013) reported 38 genera of zooplankton belonging to copepoda, cladocera and rotifera orders while the total count varied between 15015/liter in October to 26000/liter in June in Trimohini *beel* of Rajshahi, Bangladesh. Dhanasekaran *et al.* (2017) found 29 species of zooplankton belonging to rotifera, cladocera, copepoda and ostracoda order in the Dharmapuri perennial lake in Tamil Nadu, India, while the lowest total count was 13092/liter during monsoon and the highest total count was 25080/liter in summer. In this current study, three out of four wetland sampling sites dried up completely during the months of February to April 2019. One wetland site (Kharoil *beel*) that did have water had so many macrophytes that traversing the plankton net was very difficult. Therefore, the zooplankton sampling was not done during the months of February to April 2019 for the wetlands.

On an average the *Cyclops* count in commercial aquaculture ponds was 204% higher than that of the count in natural wetlands in the Barind Tract. *Cyclops* count was 7.61% (ranked 6th) and 8.66% (ranked 7th) of mean zooplankton count in commercial fishponds and natural wetland respectively (App. Table 2.37). The highest count of *Cyclops* was found in March and the lowest in April in commercial aquaculture ponds while the highest count in natural wetlands occurred in August and the lowest in July (Figure 2.35).

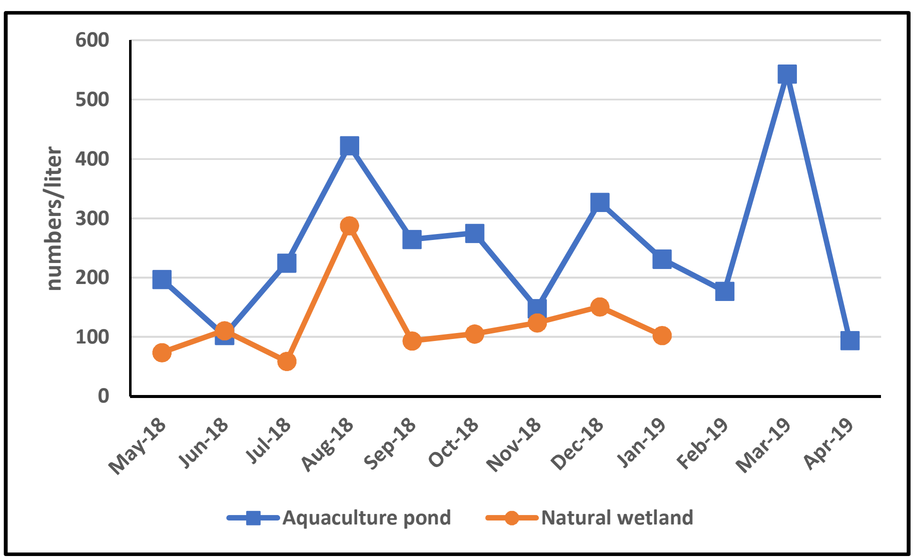


Figure 2.35. Year-round comparison of total *Cyclops* sp. Count (numbers/liter) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

On an average the *Filinia* count in commercial fishponds was 201% higher than that of the count in the natural wetlands. *Filinia* was 7.4% (ranked 7th) and 8.85% (ranked 6th) of total zooplankton count in commercial aquaculture ponds and natural wetland respectively (App. Table 2.37). The highest count of *Filinia* was recorded in January and the lowest in October in commercial fishponds, whereas the highest count was recorded in January for natural wetlands. No *Filinia* was found during sampling in the months of May and October in the natural wetlands (Figure 2.36).

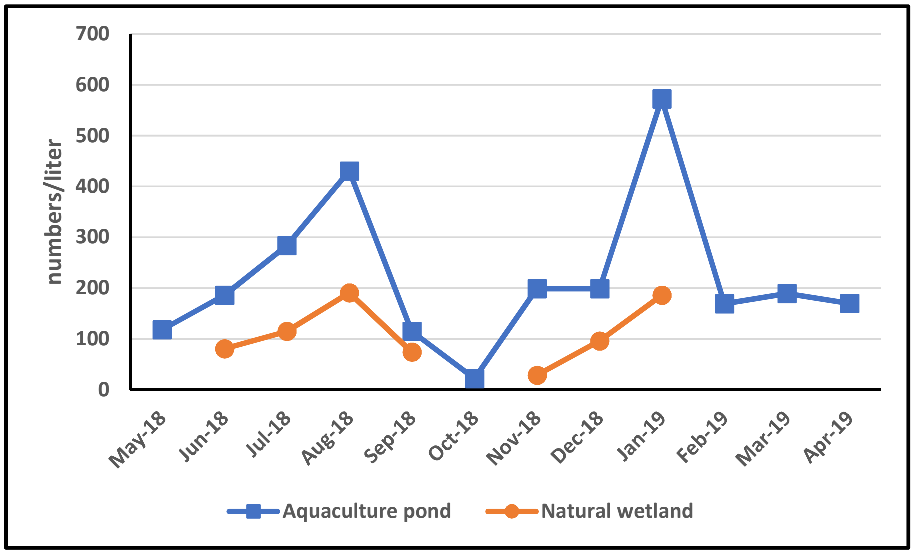


Figure 2.36. Year-round comparison of total *Filinia* sp. count (numbers/liter) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Diaphanosoma sp. was found primarily during the months of May to October (pre monsoon and monsoon) in commercial aquaculture ponds. After that it was not found except for in the month of March. On the other hand, in natural wetlands *Diaphanosoma* was found only in a few months (May, August and November), however at those times they were found in relatively high numbers therefore the average count of *Diaphanosoma* in natural wetlands was 174% over that of commercial aquaculture ponds. The percentage of *Diaphanosoma* was 1.98% (ranked 11th) and 5.21% (ranked 9th) of the average total count of zooplankton in commercial aquaculture ponds and natural wetlands respectively (App. Table 2.37).

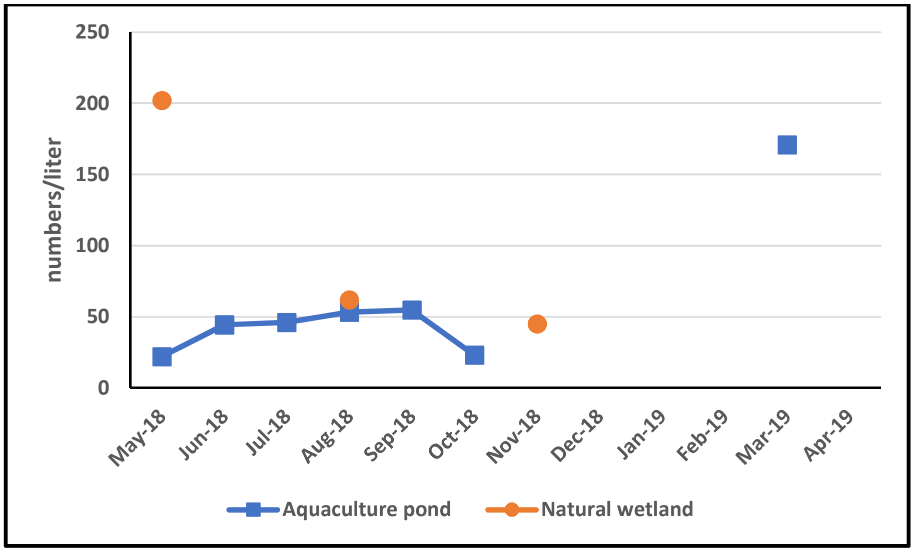


Figure 2.37. Year-round comparison of *Diaphanosoma* sp. count (numbers/liter) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Throughout the year during each sampling, *Keratella* count was higher in aquaculture ponds than in natural wetlands except for in the month of December. On an average the *Keratella* count was 236% higher in aquaculture ponds than in natural wetlands. The percentage of *Keratella* was 18.71% (ranked 2nd) and 19.41% (ranked 3rd) of total zooplankton count in commercial aquaculture ponds and natural wetlands respectively (App. Table 2.37). The highest count of *Keratella* in aquaculture ponds was found in January and the lowest in May. On the other hand, in natural wetlands the lowest count was recorded in October and highest in December (Figure 2.38).

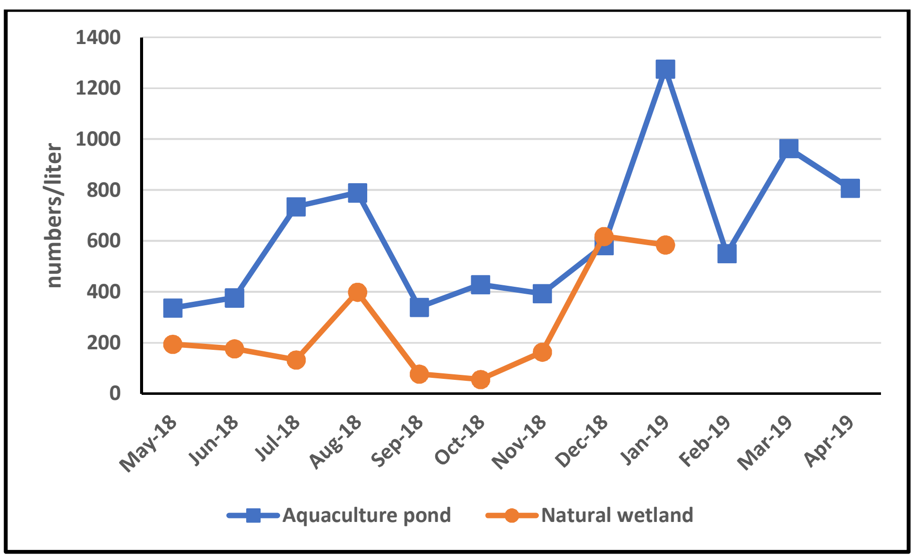


Figure 2.38. Year-round comparison of *Keratella* sp. count (numbers/liter) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Throughout the year the *Brachionus* density pattern was similar in natural wetlands and aquaculture ponds. On an average Brachionus count in aquaculture ponds was 275% higher than that of natural wetlands. The percentage of *Brachionus* was 24.45% (ranked 1st) and 21.29% (ranked 2nd) of the total zooplankton count in aquaculture ponds and natural wetland respectively (App. Table 2.37). The highest count of *Brachionus* in aquaculture ponds was recorded in January and the lowest in November. On the other hand, the highest count of *Brachionus* was recorded in August and the lowest in October in natural wetlands (Figure 2.39).

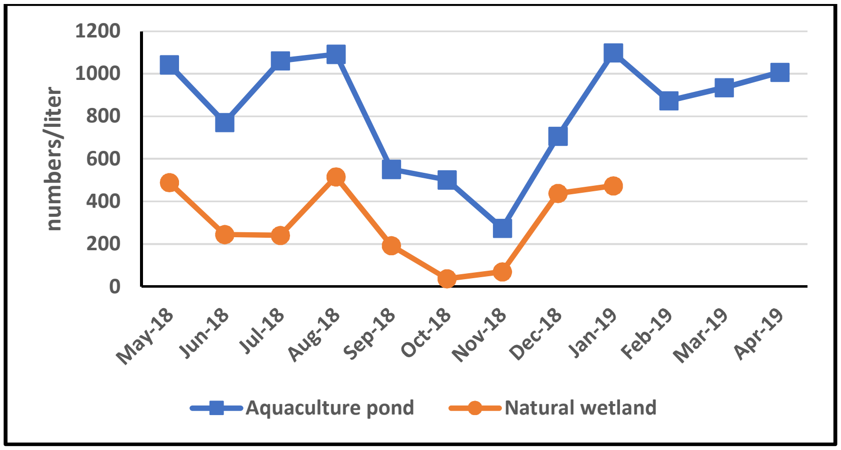


Figure 2.39. Year-round comparison of *Brachionus* sp. count (numbers/liter) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

The year-round concentration of nauplius varied in natural wetlands and aquaculture ponds. On an average the nauplius count in aquaculture ponds was 134% higher than that of natural wetlands. The percentage of nauplius was 14.15% (ranked 3rd) and 24.14% (ranked 1st) of total average zooplankton count in aquaculture ponds and natural wetlands respectively (App. Table 2.37). The highest count of nauplius in aquaculture ponds was found in March and the lowest in November. On the other hand, in natural wetlands the highest count was recorded in June and the lowest in October (Figure 2.40).

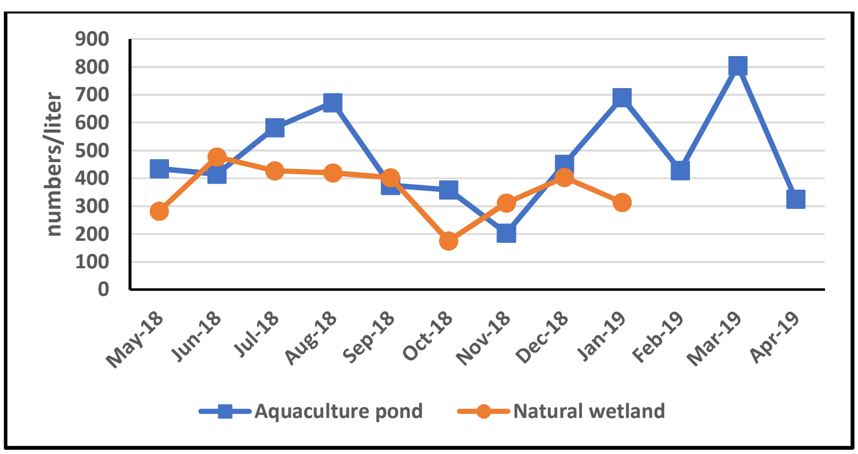


Figure 2.40. Year-round comparison of nauplius count (numbers/liter) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

On an average *Asplanchna* count in aquaculture ponds was 262% that of natural wetlands. The percentage of *Asplanchna* was 13.17% (ranked 4th) and 12.57% (ranked 4th) of the total average density in aquaculture ponds and natural wetlands respectively (App. Table 2.37). The highest count of *Asplanchna* in aquaculture ponds was recorded in January and lowest in November. On the other hand, the highest count of *Asplanchna* was found in August and lowest in July in natural wetlands (Figure 2.41).

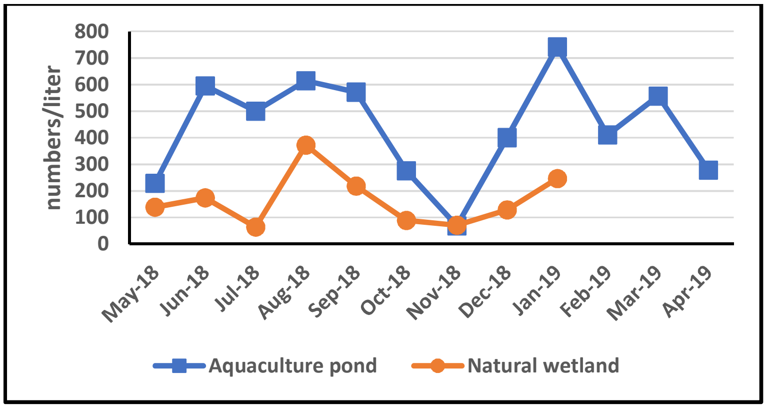


Figure 2.41. Year-round comparison of *Asplanchna* sp. count (numbers/liter) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

During most of the sampling the *Diaptomus* count was higher in natural wetlands than in aquaculture ponds. On an average *Diaptomus* density in natural wetlands was 132% more than that of aquaculture ponds. The percentage of *Diaptomus* was 3.05% (ranked 9th) and 7.78% (ranked 8th) of the total average zooplankton density in commercial aquaculture ponds and natural wetlands respectively (App. Table 2.37). The highest concentration of *Diaptomus* in aquaculture ponds was found in March and the lowest in September. On the other hand, the highest count of *Diaptomus* was recorded in September and the lowest in July in natural wetlands (Figure 2.42).

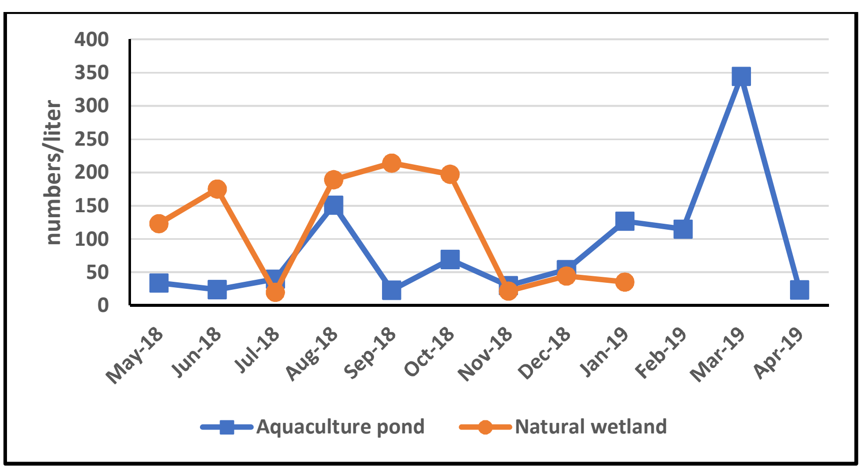


Figure 2.42. Year-round comparison of *Diaptomus* sp. count (numbers/liter) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Throughout the year, during many samplings, *Trichocerca* was missing. However, on an average the *Trichocerca* density in aquaculture ponds was 262% higher than that in natural wetlands. The percentage of *Trichocerca* count was 5.42% (ranked 8th) and 4.39% (ranked 10th) of the total average zooplankton count in commercial aquaculture ponds and natural wetlands respectively (App. Table 2.37). The highest count of *Trichocerca* in aquaculture ponds was recorded in January, while in the natural wetlands the highest counts of *Trichocerca* was recorded both in January and June (Figure 2.43).

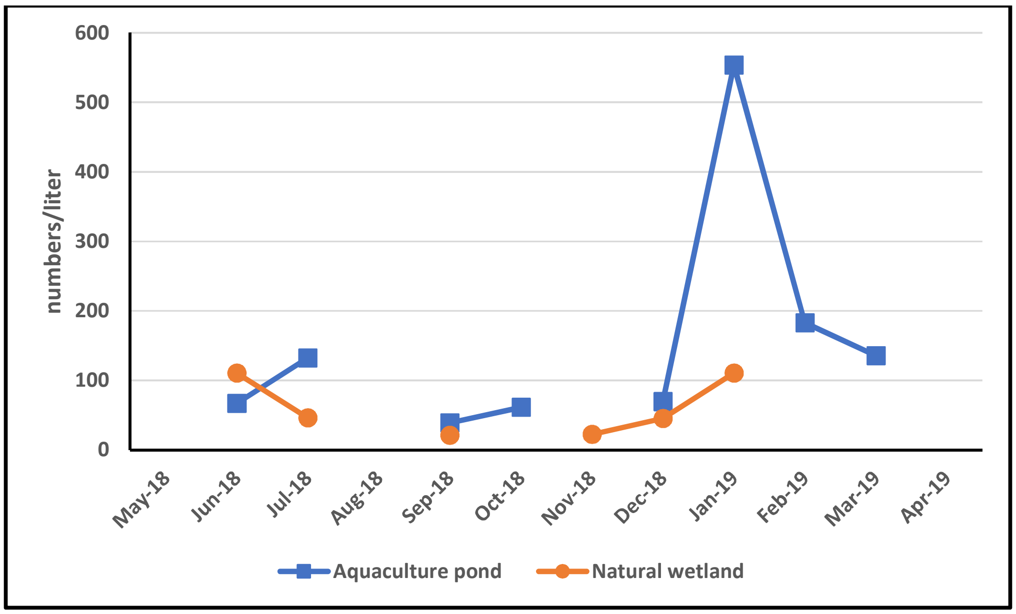


Figure 2.43. Year-round comparison of *Trichocerca* sp. count (numbers/liter) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Throughout the year the *Polyarthra* count followed a similar pattern in aquaculture ponds and natural wetlands. On an average the *Polyarthra* density in aquaculture ponds was 268% higher than that of the natural wetlands. The percentage of *Polyarthra* was 9.98% (ranked 5th) and 9.4% (ranked 5th) of the total average zooplankton count in aquaculture ponds and natural wetlands respectively (App. Table 2.37). The highest count of *Polyarthra* in both aquaculture ponds and natural wetlands was recorded in August and lowest in November (Figure 2.44).

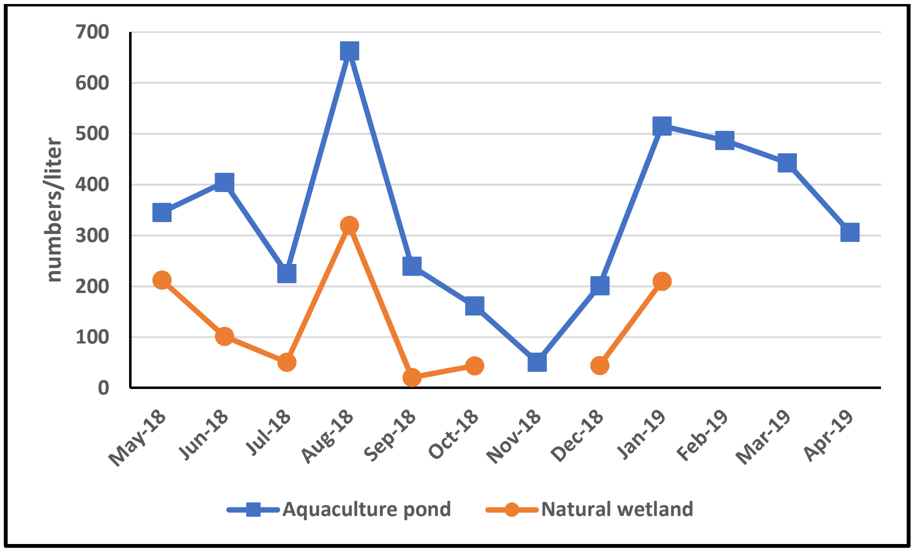


Figure 2.44. Year-round comparison of *Polyarthra* sp. count (numbers/liter) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

On an average *Daphnia* count in aquaculture ponds was 146% higher than that of natural wetlands. The percentage of *Daphnia* was 2.34% (ranked 10th) and 3.58% (ranked 11th) of the total average zooplankton count in aquaculture ponds and natural wetlands respectively (App. Table 2.37). The highest count of *Daphnia* was recorded in August and January for aquaculture ponds and natural wetlands respectively. On the other hand, there were few months when *Daphnia* was not found during zooplankton sampling in both aquaculture ponds and natural wetlands (Figure 2.45).

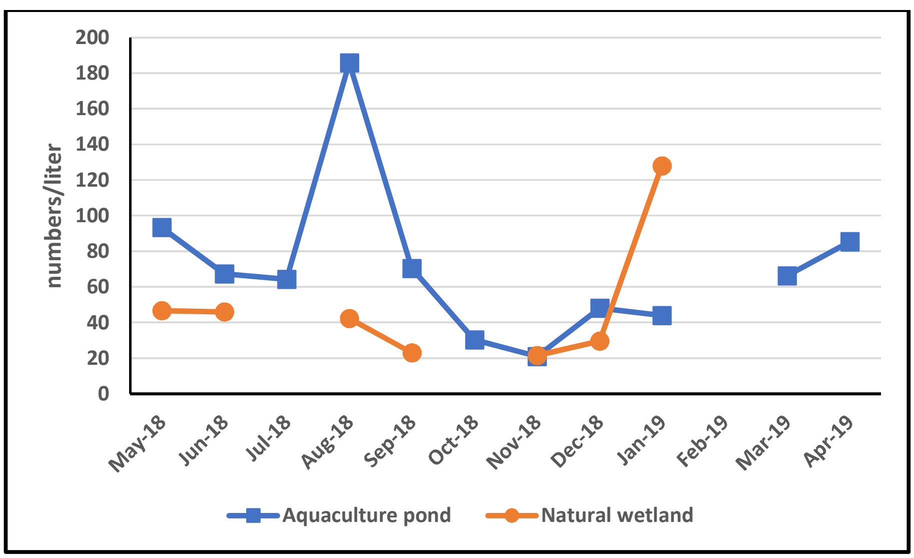


Figure 2.45. Year-round comparison of *Daphnia* sp. count (numbers/liter) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Throughout the year there were only few months when *Moina* was found during zooplankton sampling. On an average the *Moina* count in aquaculture ponds was 179% higher than that in the natural wetlands. The percentage of *Moina* was 1.13% (ranked 12^{th}) and 1.55% (ranked 12^{th}) of the total average zooplankton count in aquaculture ponds and natural wetlands respectively (App. Table 2.37). The highest count of *Moina* was found in August and June respectively in aquaculture ponds and natural wetlands (Figure 2.46).

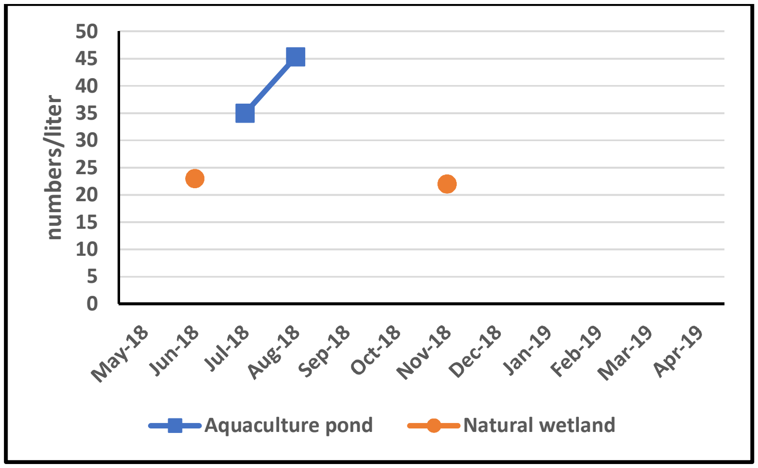


Figure 2.46. Year-round comparison of *Moina* sp. count (numbers/liter) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Aquatic insects in fishponds under entrepreneurial fisheries and natural wetlands

The aquatic insect count was higher in commercial aquaculture ponds than in natural wetlands in the Barind Tract (Figure 2.47). The highest count of aquatic insects (135.5±17.9/3 square meter water area) in commercial aquaculture ponds was recorded in November and the lowest (76.8±16.1/3 square meter water area) in April (App. Table 2.38). On the other hand, the highest count (38.0±11.2/3 square meter water area) of aquatic insects in natural wetlands was recorded in October and lowest (18.6±6.2/3 square meter water area) in May (App. Table 2.39). On an average over the year the total count of aquatic insects in the commercial aquaculture ponds was recorded as 93.2±17.2/3 square meter water area compared to 26.7±6.01/3 square meter water area in the natural wetlands. Nasiruddin *et al.* (2014) reported a higher insect abundance in a pond system rather than in a lake, with the highest number of insects recorded in August and least in July. A higher abundance of aquatic insects during the rainy season in northern Bangladesh was also reported by Ahad *et al.* (2012). On the other hand, Hossain *et al.* (2004) found a higher abundance of aquatic insects during post monsoon months of November & December in the swamps at Rajshahi University.

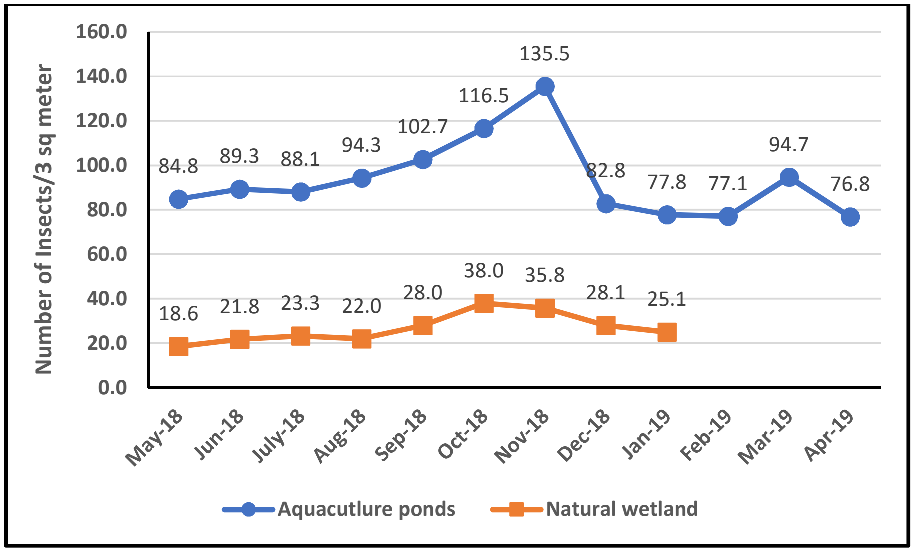


Figure 2.47. Year-round comparison of the aquatic insect count (number/3 square meter water area) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract

There were 11 different kinds of aquatic insects identified in both aquaculture ponds and natural wetlands throughout the year (Figure 2.48). Of all the aquatic insects in the commercial aquaculture pond, the water boatman was the most dominant, with 41.0% of the total count, followed by backswimmer (35.4%), and damselfly nymph (13.6%). These three insects (boatman, backswimmer, and damselfly) were overwhelmingly dominant in commercial aquaculture ponds and collectively contributed to 90.0% of the total count. In the natural wetlands, backswimmer was most dominant (23.4%) followed by water boatman (21.6%), and water strider (9.3%) (App. Table 2.40). The top three aquatic insects collectively contributed 54.2% of the total insect count. Sano *et al.* (2011) found eight different groups of aquatic insects (all of which match with the findings of this study) in aquaculture ponds, where damselfly (49.4%), diving beetle (20.1%) and backswimmer & boatman (19%) collectively contributed 88.5% of the total insect count during August and September in Lao PDR. The three insects with the lowest count in aquaculture ponds (creeping water bug, *Nepa* sp. and *Ranatra* sp.) collectively contributed only 1.42% to the total count. The three least dominant water insects in natural wetlands (*Nepa* sp., water scorpion and creeping water bug) contributed 13.1% to the total insect count. In that regards, the relative share of aquatic insects was more homogeneous means greater evenness in the natural wetlands than in entrepreneurial fishponds. Nasiruddin *et al.* (2014) also found species diversity, species richness and species evenness of aquatic insects was lower in the pond system than the lakes. Choudhury and Gupta (2015) reported an aquatic insect community of 31 species belonging to 18 families and 5 orders in the Deepor *beel* in Assam, India.

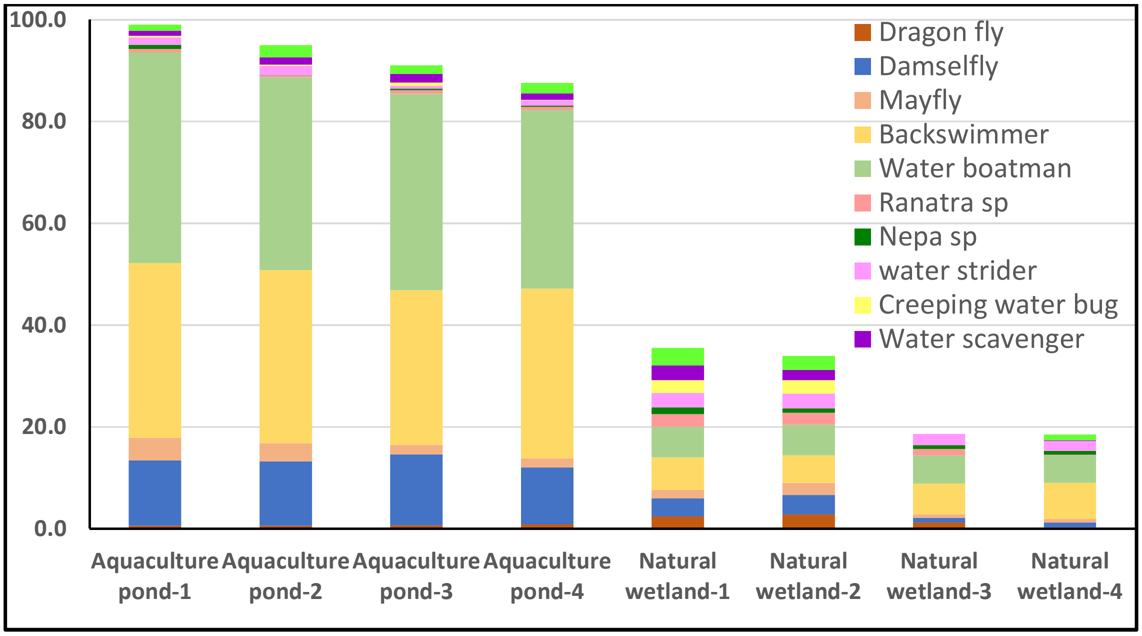


Figure 2.48. Stacked column diagram of recorded mean aquatic insect count (individual/3 square meter water area) throughout the year from different aquatic systems.

Commercial aquaculture ponds were found to contain a moderate amount of aquatic vegetation, primarily emergent, along the water's edge during the monsoon when the water level was higher. The vegetation gradually decreased in the winter and reduced to almost zero in the summer when the water level of the ponds reached the lowest level. In the natural wetlands, the perennial *beel* and water canals that did not dry retained aquatic vegetation almost throughout the year with a variation in types of vegetation throughout the year. Water hyacinth and other floating aquatic plants were more dominant in the monsoon, while rooted macrophytes, including emergent and submerged types, were more dominant in the winter season. The variation of presence of macrophytes in aquaculture ponds and natural wetlands played a role in the distribution of the aquatic insect community that it hosts. Marco *et al.* (1999) reported that ponds with extreme vegetation or without any vegetation had lower insect species richness than the ponds with moderate vegetation. Aquatic insects in aquaculture ponds, when organized in chronology from most dominant to least dominant appeared as follows: water boatman> backswimmer> damselfly> mayfly> diving beetle> water scavenger> water strider> dragonfly, *Ranatra* sp.> *Nepa* sp.> Creeping water bug. The chronology in descending order of aquatic insects in natural wetlands stands as: backswimmer> boatman> water strider> damselfly> dragon fly> diving beetle> *Ranatra* sp.> mayfly> creeping water bug> water scavenger> *Nepa* sp. Ahad *et al.* (2012) confirmed the availability of the above insects in northern Bangladesh. Apart from the aquatic insects mentioned above, the whirligig beetle was also observed in both commercial aquaculture ponds and natural wetlands, but was never caught during sampling, due to their fast-moving capability.

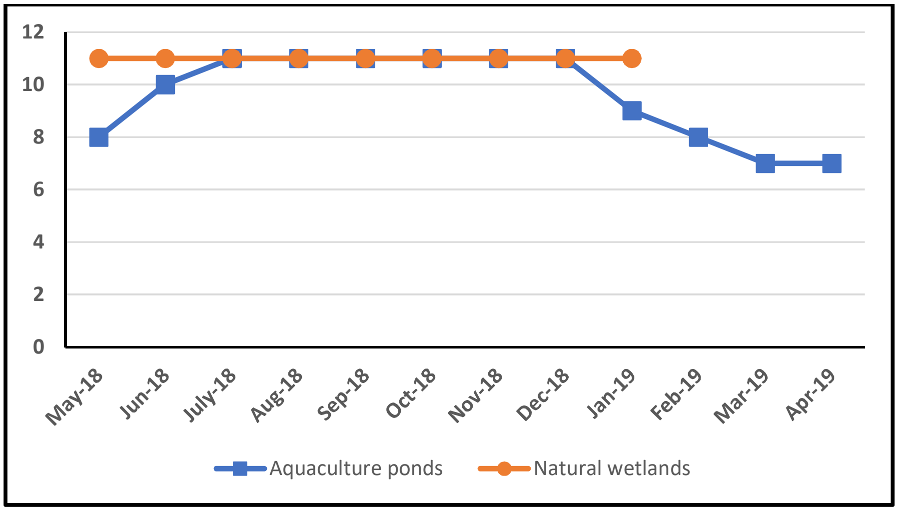


Figure 2.49. Year-round diversity count of aquatic insects in commercial aquaculture ponds and natural wetlands in the Barind Tract.

Although there was some variation in the availability of aquatic insects within the different replications of natural wetlands and aquaculture ponds groups, as a whole, the aquatic insect diversity in natural wetlands remained the same at the highest level throughout the year, while the diversity in the commercial aquaculture ponds varied. Ahad *et al.* (2012) found a greater diversity of aquatic insects in water reservoirs that are less disturbed by human activity and are not under commercial aquaculture in northern Bangladesh. In this study, the highest diversity count in commercial aquaculture ponds was found mostly during the monsoons and until mid-winter, then gradually reduced to its lowest level in summer. The variations in insect diversity also coincided with the variation of aquatic macrophyte density in natural wetlands and commercial aquaculture ponds.

On an average the dragon fly count in natural wetlands was 225% higher than that in aquaculture ponds. The highest count of dragon fly in natural wetlands was found in October, while it was found in September and October for aquaculture ponds. No dragon fly was found in April and May in aquaculture ponds (Figure 2.50). The share of dragon fly of the total insect count was 0.7% (ranked 8^{th}) and 6.4% (ranked 6^{th}) in aquaculture ponds and natural wetland respectively (App. Table 2.40).

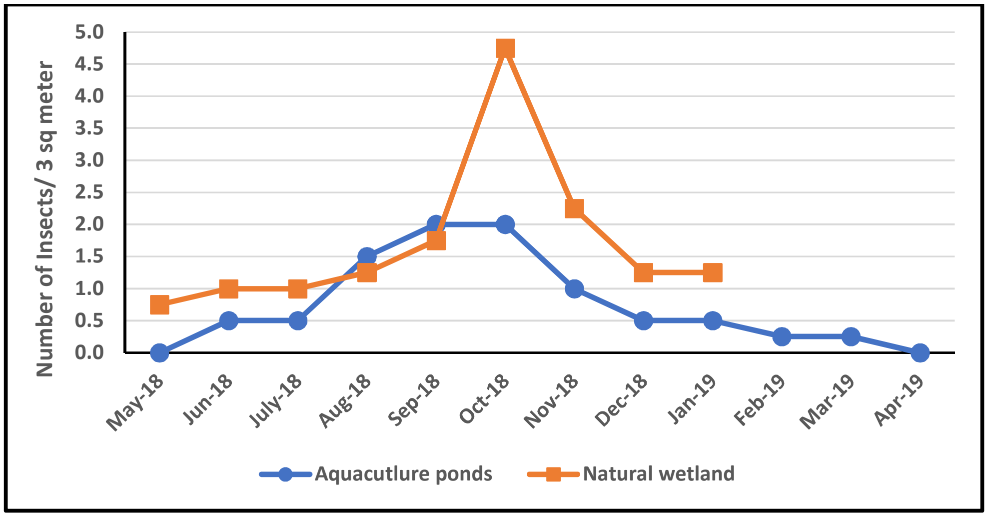


Figure 2.50. Year-round comparison of total dragon fly count (numbers/3 square meter of water area) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

On an average the damselfly count in aquaculture ponds was 541% higher than that in natural wetlands in the Barind Tract. The highest count of damselfly was found in November in both natural wetlands and aquaculture ponds. On the other hand, no damselfly was recorded in March and April in aquaculture ponds (Figure 2.51). The share of damselfly of the total aquatic insect count was 13.6% (ranked 3rd) and 8.7% (ranked 4th) in commercial aquaculture ponds and natural wetlands respectively (App. Table 2.40).

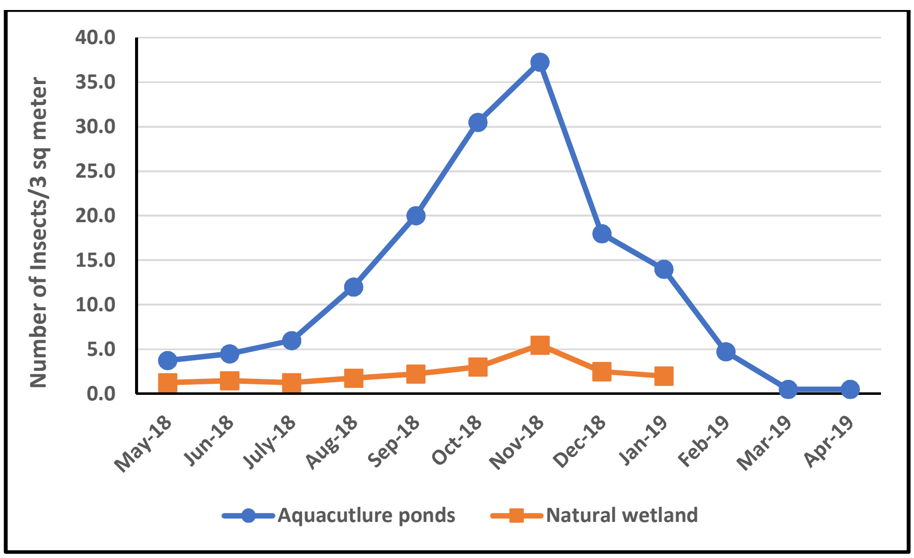


Figure 2.51. Year-round comparison of total damselfly count (numbers/3 square meter of water area) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

On an average the count of mayfly in aquaculture ponds was 218% higher than that in natural wetlands. The mayfly count was higher in October in aquaculture ponds while there was no catch during the months of March and April. On the other hand, in natural wetlands mayfly population maintained a consistent presence throughout the year, while the count was highest in November (Figure 2.52). The share of mayfly of the total insect count was 3.1% (ranked 4th) and 5.0% (ranked 8th) respectively in commercial aquaculture ponds and in natural wetlands (App. Table 2.40).

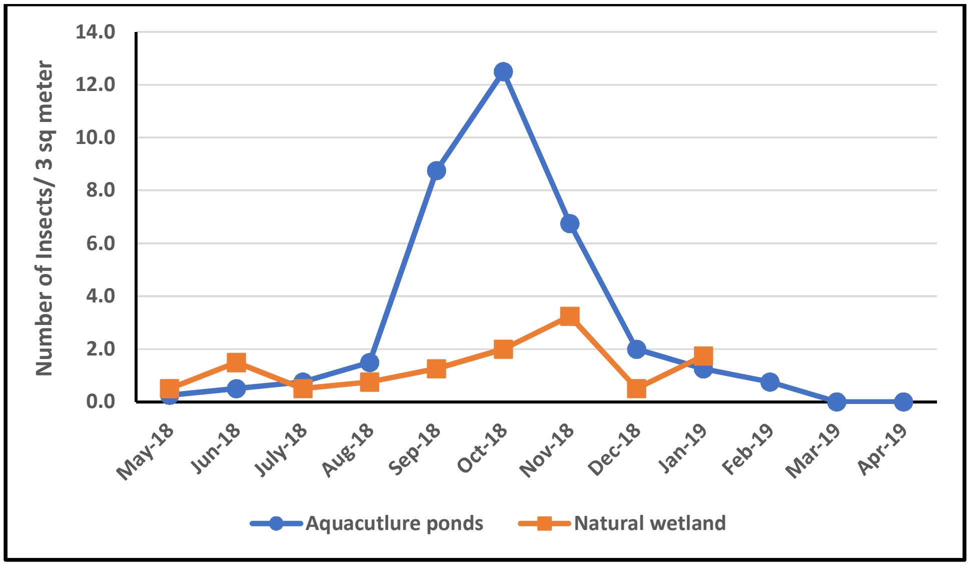


Figure 2.52 Year-round comparison of total mayfly count (numbers/3 square meter of area) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

On an average the count of backswimmer in aquaculture ponds was 529% higher than that in natural wetlands. Though the count of backswimmer was high in aquaculture ponds, it varied throughout the year, where the highest count was recorded in November and the lowest in April. In natural wetlands, the highest count of backswimmer was recorded in July and October and the lowest in May and January (Figure 2.53). The share of backswimmer of the total insect count was 35.4% (ranked 2nd) and 23.4% (ranked 1st) respectively in commercial aquaculture ponds and natural wetlands (App. Table 2.40).

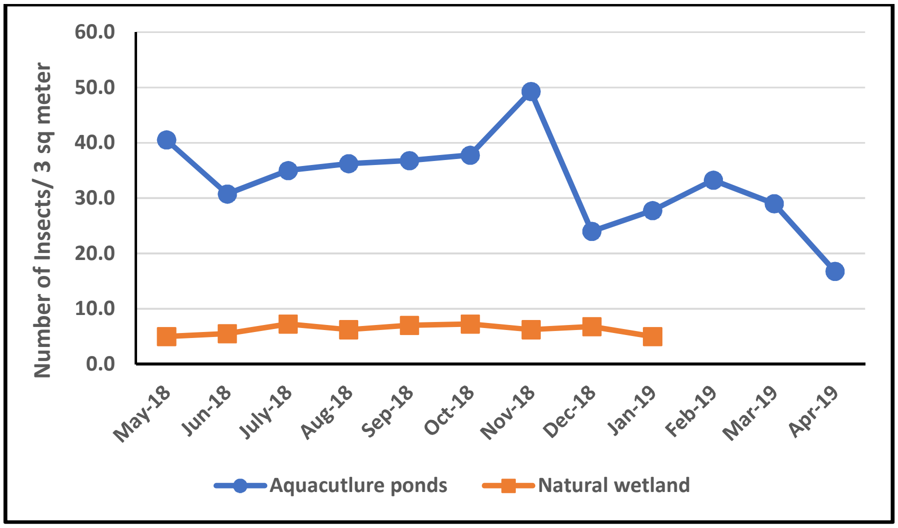


Figure 2.53. Year-round comparison of total backswimmer count (numbers/3 square meter of water area) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

On an average the boatman count in aquaculture ponds was 664% higher than that in natural wetlands. Despite having higher counts, the boatman count in aquaculture ponds varied throughout the year where the highest count was recorded in March and the lowest in October. On the other hand, the highest count of boatman in natural wetlands was recorded in October and lowest in August (Figure 2.54). The share of boatman of the total insect count was 41.0% (ranked 1st) and 21.6% (ranked 2nd) respectively in commercial aquacultue ponds and natural wetlands (App. Table 2.40).

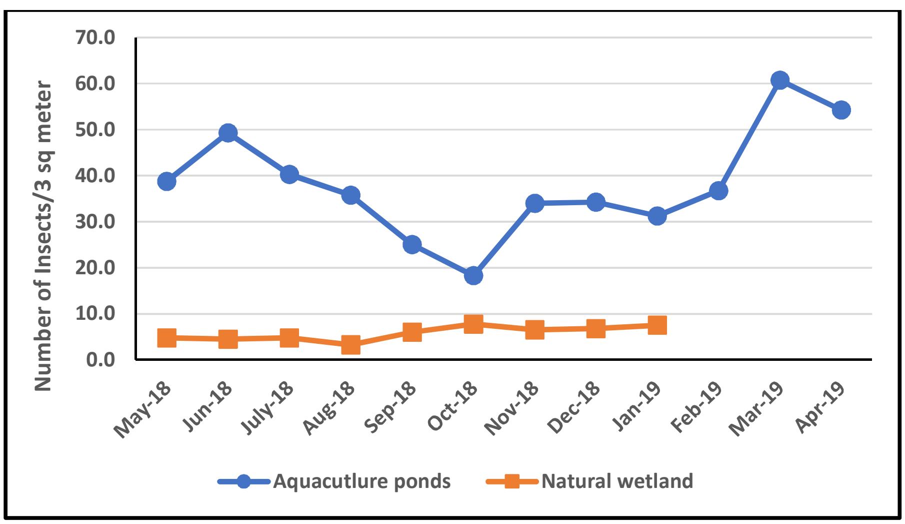


Figure 2.54. Year-round comparison of total boatman count (numbers/3 square meter of water area) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

On an average *Ranatra* sp. count was 265% higher in natural wetlands than in commercial aquaculture ponds. The highest count of *Ranatra* sp. was found in December and lowest in May in natural wetlands. On the other hand, highest count of *Ranatra* sp. in aquaculture ponds was recorded in November, while there was no catch during the months of February to April (Figure 2.55). The shareof *Ranatra* sp. of the total insect count was 0.7% (ranked 8th) and 5.7% (ranked 7th) respectively in aquaculture ponds and natural wetlands (App. Table 2.40).

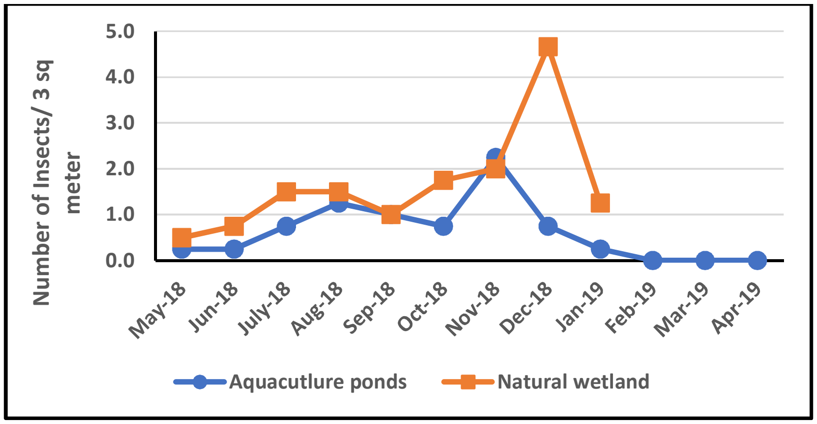


Figure 2.55. Year-round comparison of total water scorpion (*Ranatra* sp.) count (numbers/3 square meter of water area) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

On an average the *Nepa* sp. count in natural wetlands was 261% higher than that in commercial aquaculture ponds. The highest count of *Nepa* sp. in aquaculture ponds was recorded in October, while there was no catch in the months of January, February, March and May. On the other hand, the highest count of *Nepa* in natural wetlands was recorded during the moths of September to November, and the lowest in December, January and May (Figure 2.56). The share of *Nepa* sp. of the total insect count was 0.4% (ranked 9th) and 3.5% (ranked 11th) respectively in aquaculture ponds and natural wetlands (App. Table 2.40).

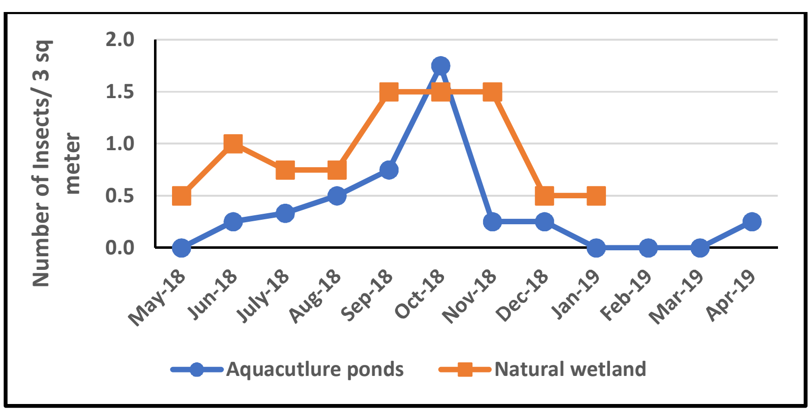


Figure 2.56. Year-round comparison of total water scorpion (*Nepa* sp.) count (numbers/3 square meter of water area) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

On an average *Gerris* sp. count in natural wetlands was 208% higher than that in aquaculture ponds. The highest count of *Gerris* sp. in aquaculture ponds was recorded in October, and the lowest in May and June. On the other hand, the highest count of *Gerris* sp. in natural wetlands was recorded in January and May, while the lowest was in August (Figure 2.57). The share of *Gerris* sp. of the total insect count was 1.3% (ranked 7th) and 9.5% (ranked 3rd) respectively in aquaculture ponds and natural wetlands respectively (App. Table 2.40).

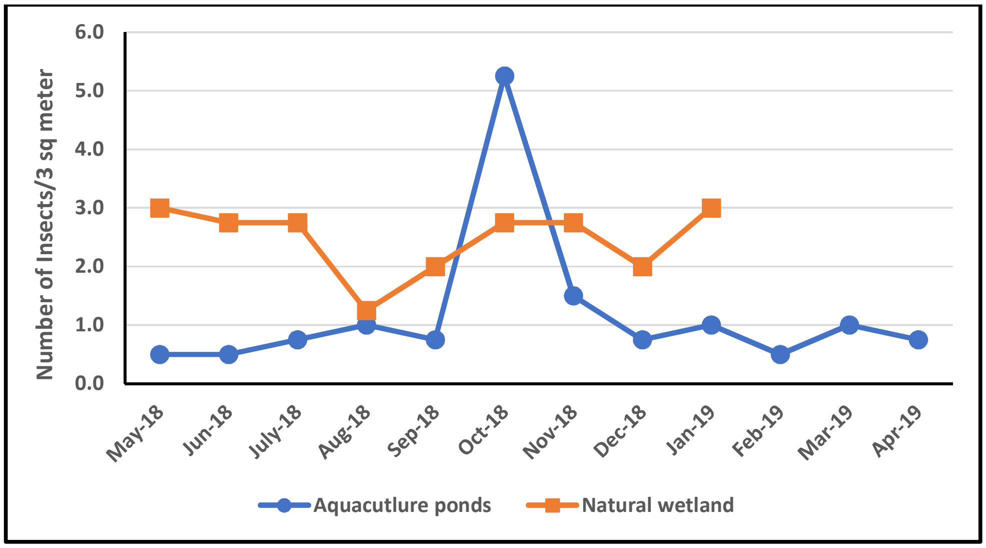


Figure 2.57. Year-round comparison of total water strider (*Gerris* sp.) count (numbers/3 square meter of water area) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

On an average the creeping water bug count in natural wetlands was 417% higher than that of aquaculture ponds. The highest count of the creeping water bug in aquaculture ponds was recorded in September, while there was no catch during the months from January to June. On the other hand, the highest count of creeping water bug in natural wetlands was in October and the lowest in May-June period (Figure 2.58). The share of creeping water bug of the total insect count was 0.3% (ranked 10th) and 4.9% (ranked 9th) respectively in aquaculture ponds and natural wetlands (App. Table 2.40).

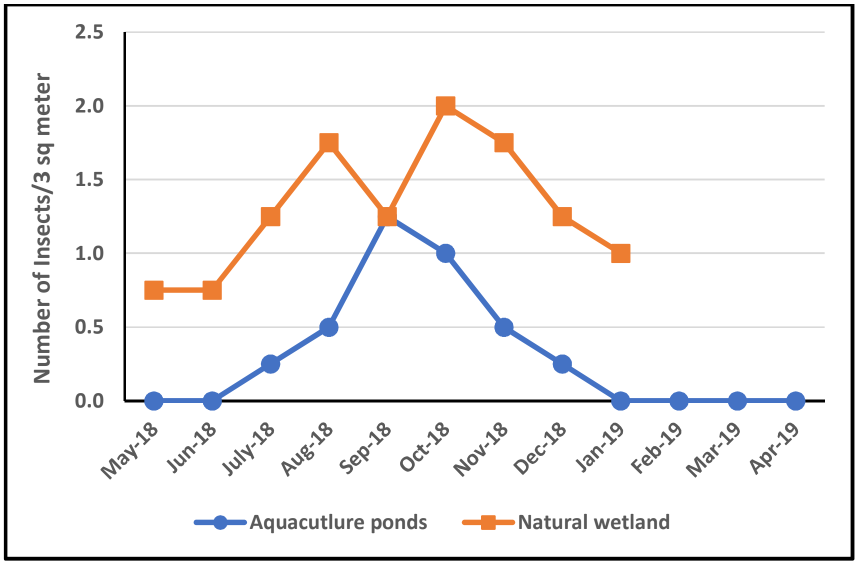


Figure 2.58. Year-round comparison of total creeping water bug count (numbers/3 square meter of water area) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

On an average the water scavenger count in aquaculture ponds was 108% higher than that in natural wetlands. The highest count of water scavengers was recorded in October for both aquaculture ponds and natural wetlands. The lowest count of water scavenger for natural wetlands was recorded in January, and for aquaculture ponds in June and January (Figure 2.59). The share of water scavenger of the total insect count was 1.5% (ranked 6th) and 4.7% (ranked 10th) respectively for aquaculture ponds and natural wetlands (App. Table 2.40).

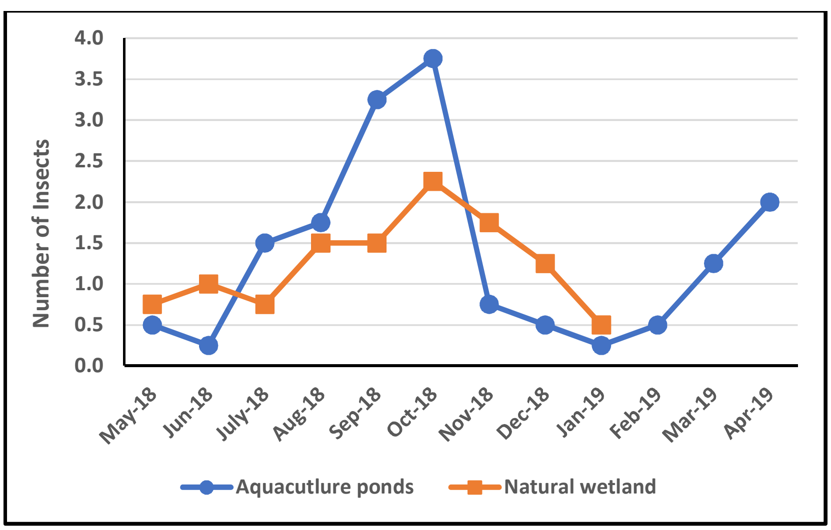


Figure 2.59. Year-round comparison of total water scavenger count (numbers/3 square meter of water area) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

On an average the diving beetle count in aquaculture ponds was 105% higher than that of natural wetlands, though the monthly variation was higher in aquaculture ponds. The highest count of diving beetle in aquaculture ponds was recorded in September and the lowest in February. On the other hand, the highest count of diving beetle in natural wetlands was recorded in October and the lowest in May (Figure 2.60). The share of diving beetle of the total insect count was 2.0% (ranked 5th) and 6.8% (ranked 5th) respectively for commercial aquaculture ponds and natural wetlands (App. Table 2.40).

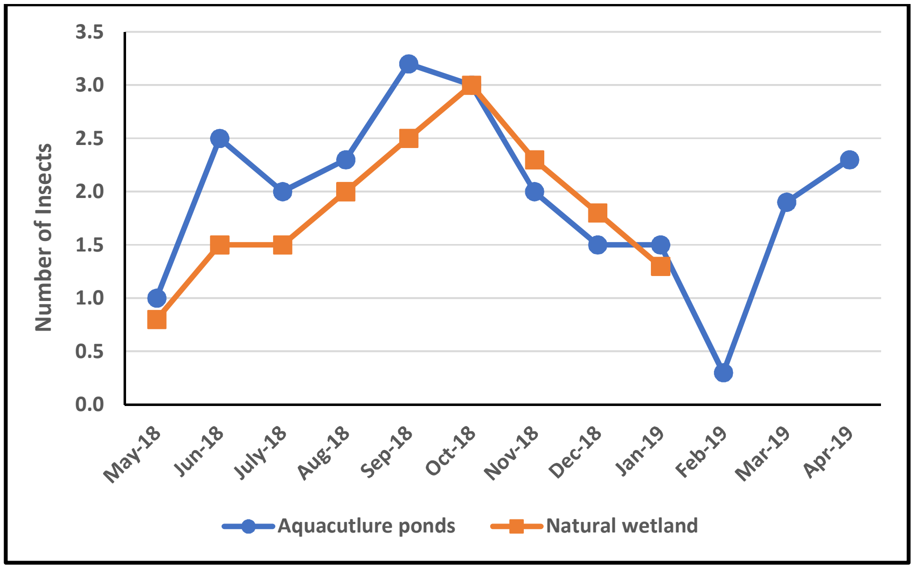


Figure 2.60. Year-round comparison of total diving beetle (*Laccophilus* sp.) count (numbers/3 square meter of water area) in aquaculture ponds under entrepreneurial fish production and in natural wetlands.

Benthos (chironomid larvae and tubifex) in wetlands and entrepreneurial fishponds

The average count (62.3/6 square inches pond bottom) of chironomid larvae in entrepreneurial fishponds was higher than that of tubifex (42.2/6 square inches bottom). The highest count of chironomid larvae was recorded in July in entrepreneurial fish ponds, on the other hand the highest count of tubifex was recorded in April (Figure 2.61). In the entrepreneurial fishpond system, the lowest count of both chironomid larvae and tubifex occurred during winter months (November, December and January). On the other hand, when the count of tubifex was highest in the pond system, the count of chironomid larvae was lowest. Overall, the present of tubifex and chironomid larvae was much greater than their presence in natural wetlands (Figure 2.61). Though both chironomid larvae and tubifex were present in considerably lower numbers in natural wetlands (App. Table 2.41 & 2.42), unlike the pond system, tubifex (1.7/6 square inches) was more dominant by number than the chironomid larvae (1.1/6 square inches).

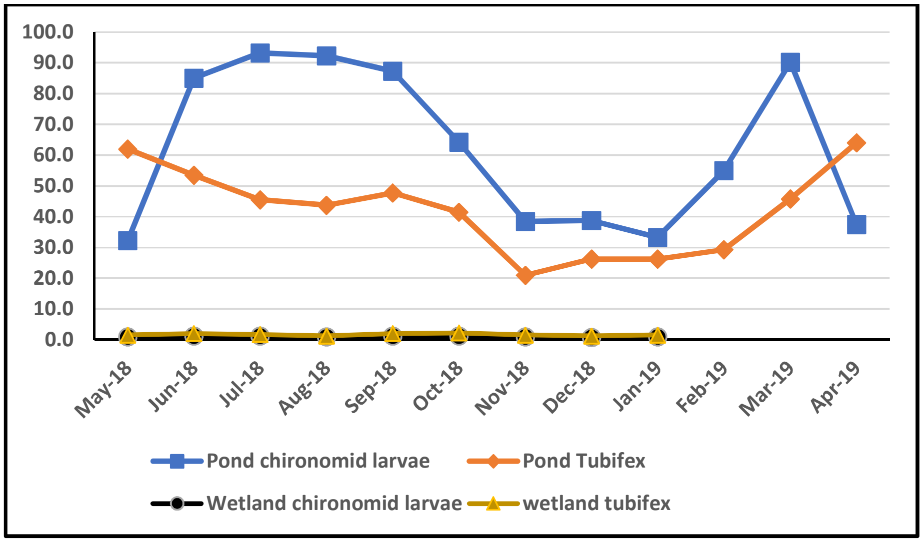


Figure 2.61. Year-round chironomid larvae and tubifex count in entrepreneurial fishponds and wetlands in the Barind Tract.

Haider *et al.* (2017) found Oligochaeta more dominant in terms of numbers ranging from 360.49 to 383.54/square meter, than the chironomid count which ranged from 107.41 to 125.10/square meter in a homestead pond in Dinajpur. The presence of benthos was attributed to the water quality of the waterbody. Chakma *et al.* (2015) recorded oligochaetes as the most dominant benthos in the ponds system in Noakhali and density was 888.89 and 1088.89 individuals/square meter in culture and household ponds respectively. Naser and Roy (2012) concluded after analyzing the gut content of chironomid larvae that feeding activity was lowest during winter months. This coincides with the current findings of a lower abundance of chironomid larvae during the winter months in this study. Gut content analysis of the chironomid larvae revealed (Naser and Roy, 2012) that detritus was the dominant food group, contributing 50 to 55%, followed by green algae (Chlorophyceae) and diatoms (Bacillariophyceae) collectively, contributing 8.22 to 12.86% of the total gut content. The greater availability of disintegrated feed debris and higher number of phytoplankton in entrepreneurial fishponds facilitated the greater abundance of chironomid larvae and tubifex in aquaculture ponds than in natural wetlands in present study. Nupur *et al.* (2013) found the highest concentration of macro zoobenthos in aquaculture ponds with a loamy soil bottom compared to the aquaculture ponds with a sandy loam and clay soil bottom. Of the macro zoobenthos, chironomidae (1422±4.88/m^2) was most dominant followed by oligochaeta (1200±4.25/m^2) in ponds with a loamy soil bottom. The highest density of macro zoobenthos was also recorded in water depths of 106.68cm compared to the concentrations found at other depths (60.96cm and 152.40cm) tested by Nupur *et al.* (2013).

Conclusion

Some environmentally sensitive practices like use of antibiotics for fish during winter season, where half of antibiotics are used as preventive option and withdrawal period is not maintained; use of harmful chemicals as fish toxicant, use of poultry litter as fish feed and use of pesticides for killing of aquatic insects and undesired fish species, are used at different degree and occasional use of steroids and digestive stimulants are present in entrepreneurial fisheries in the Barind Tract.

Despite of those harmful practices, water quality in entrepreneurial fisheries in the Barind Tract was more suitable for fish growth compared to the natural wetlands. Though the community structure of water insects, zooplankton and benthos in entrepreneurial fisheries was different than that of natural wetlands; counts of the aquatic invertebrates was higher in entrepreneurial fisheries and the diversity was similar to the natural wetlands. Pond bottom sediments had higher nutrient content than that of natural wetlands, but well within the limit. Use of pesticide in entrepreneurial fisheries is less intense than that of paddy fields.

CHAPTER THREE
IMPACTS OF ENTREPRENEURIAL FISHERIES ON AQUATIC ENVIRONMENT

Introduction

The activity cycle of commercial aquaculture in northwest Bangladesh begins with a complete wipeout of any existing fish that could not be removed live and water insects from the pond using various chemicals. This is followed with the stocking of a desired number of relatively large fish, usually Indian and Chinese major carps of 0.5kg to 1.5kg in size, in northwest Bangladesh. In the process of transferring the live fish from the nursery pond to the stocking pond, undesired small indigenous fish species, such as *Pseudambasis* sp., *Chanda* sp., *Glossogobius* sp. and *Puntius* sp., are also transferred unintentionally by the farmers into the stocking pond. Since these small indigenous species can breed in ponds within a few months, especially after the monsoon, they manage to multiply and populate the culture ponds of Indian major carps. Despite the disparity of size between these indigenous species and the large Indian and Chinese major carps (more than 1.5kg in size), farm owners view these small species as a challenge to the desired species which might face competition for food and space or at the very least become disturbed by these tiny fish. Some fish like *Chanda* sp. and *Pseudambasis* sp., which can be present in large numbers, feed on the scales of carps with jaws armed with curved and conical teeth, making the carp fish scales less glossy (Wahab, 2003). The damaged carp may experience hampered growth and will fetch a lower price in the market if sold. In addition, *Glossogobius* sp. is a voracious feeder and feeds on everything from decaying organic matter, protozoans, planktons to water insects, other fish and their eggs and larvae (Siddiqui *et al*., 2007). Thus *Glossogobius* sp. can create an environment of competition for feed supplied to the commercial aquaculture pond. Farmers engaged in entrepreneurial fish production in northwest Bangladesh use quinalphos to control these undesired small indigenous fish species.

With the development of commercial aquaculture, parasitic infestation of Indian and Chinese carp is also a common challenge that fish farmers need to deal with. Of the many parasites, *Ichthyophthirius* sp., *Trichodina* sp., *Dactylogyrus* sp., *Argulus* sp., *Lernaea* sp., are the common ones for Indian and Chinese carps in Bangladesh (Arthur and Ahmed 2002; Chandra 2006). To tackle the parasitic infestation problem in fish, emamectin benzoate is being used as parasiticides in entrepreneurial fisheries in northwest Bangladesh.

Aquatic insects are an integral part of any healthy natural aquatic ecosystems and often serve as an indicator of the health of the ecosystem (Pondinformer, 2019). Excess organic matter along with the presence of aquatic weeds increases the number of aquatic insects (Roysfarm, 2019). Culture of non-predatory carp species and intensive feeding by the fish make leftover feed available for aquatic insects. This coupled with an absence of predators and competitors for aquatic insects allows their population to flourish in commercial aquaculture ponds of northwest Bangladesh. Nasiruddin *et al*. (2014) reported a higher number of aquatic insects in the fishpond system as compared to a lake in Chattogram, Bangladesh. Tidwell *et al*. (1997) found macroinvertebrate densities to be significantly higher in fed and fertilized ponds than in unfed ponds. Aquatic insects are also very common in commercial carp aquaculture ponds of northwest Bangladesh due to intensive feeding and fertilizer use. Kashyap *et al*. (2013) recorded the presence of aquatic insects, many of which are detrimental to aquaculture, in various fishponds including the fish nursery, rearing and stocking ponds of northern and central states in India and. Marco *et al.* (1999) found that ponds with moderate aquatic vegetation had greater dragonfly species richness than ponds either devoid of vegetation or with extreme vegetation. The largest number of aquatic insects in aquaculture ponds was found during or after the rainy season (Yapo *et al.*, 2013; Kashyap *et al.*, 2013, Nasiruddin *et al.*, 2014). Many aquatic insects (dragonfly nymph, backswimmers, water scorpion, etc.) are harmful to juvenile fish due to their direct predation on fish fry and competition for food (Roysfarm, 2019; Gonzalez and Leal, 1995; Sano *et al.*, 2011 and Kashyap *et al.*, 2013).

Dragonfly nymph/larvae were reported as most effective common carp fry predator by Gonzalez and Leal (1995). Corbet (1980) described odonate (dragonfly) larvae as the exclusive predator in the aquatic environment. Marco *et al*. (1999) found that medium size dragonfly species were more abundant and potential predators of fish fry in aquaculture ponds of south-east Brazil. In addition, backswimmer (*Anisops* sp.) was found causing predation-related mortality of carp fry in Lao PDR by Sano *et al.* (2011). The larger size of the backswimmers meant an increased predation potential (Gonzalez and Leal, 1995). Backswimmers can prey on up to 46.7% of carp fish larvae within a 24-hour period in nursery ponds (Sano *et al.*, 2011). Water scorpions (*Nepa* sp. and *Ranatra* sp.) also can attack living insects and fish fry (Kashyap *et al*., 2013). The predation of fish fry by water insects was proportionate to the size ratio of water insects and fish fry; larger insects could capture larger fish fry (Gonzalez and Leal, 1995). Predation of fish fry by aquatic insects is primarily limited in nursery ponds and not present in culture ponds of adult fish.

Despite stocking and rearing larger fish (greater than 0.75kg of individual size) in commercial aquaculture ponds and having no predation risk by the insects, fish farmers in northwest Bangladesh consider aquatic insects, especially when they are present in large numbers, hazardous for their fish due to the perceived competition for food and disturbance from the aquatic insects. The use of pesticides in aquaculture in Bangladesh has also been reported by Chowdhury *et al.* (2012) and Shamsuzzaman and Biswas (2012). In fact, carp farmers of northwest Bangladesh were found (see chapter 2) to be using synthetic pyrethroid insecticides including cypermethrin, deltamethrin, abamectin and lambda-cyhalothrin in ponds in presence of fish once in a month during summer and monsoon months. Siegfried (1993) found aquatic insects to be more susceptible to pyrethroid insecticide doses on body weight basis than terrestrial insects.

Commercial pond aquaculture has an unrelenting economic pressure to produce fish in the most efficient yet inexpensive ways possible (Lennon *et al*., 1970). The use of fish toxicants as a management tool allows the commercial fish farmers of northwest Bangladesh to have greater control over the fish stock management through the elimination of undesired species, complete harvesting of all fish of the waterbody, and elimination and restart of the fish culture. Three different kinds of fish toxicants (rotenone, phostoxin tablets and fenpropathrin) are used (see chapter 2) in entrepreneurial fisheries of northwest Bangladesh.

Tilapia (*O. niloticus*) is the second most cultured species in the world after Carp and is often dubbed as the ‘aquatic chicken’ (WorldFish Center, 2015). Like many other parts of the world, tilapia is one of the single most cultured species in Bangladesh. The total production of tilapia has increased to 384,737 metric tons, 10.62% of total inland fish production in 2017-18 (DoF, 2018) from just 136,000 metric tons in 2012 (Hussain *et al*., 2014). Like other parts of the country, it is also commercially cultured in the Barind Tract though to a lesser extent. The production of tilapia in Bangladesh is highly profitable with less input cost and reduced risk (Rahman *et al.,* 2012). Helpful qualities such as the ability to take natural food from the pond, interest in supplementary feed, capacity to survive in adverse weather conditions, and high resistance to disease make tilapia favorable as one of the most cultured species throughout the world (Roysfarm, 2018). It is fast growing, hardy, environmentally friendly, and easy to grow by all sorts of fish farmers (WorldFish Center, 2015). The availability of sex inversed male tilapia fingerlings has played a significant role in the expansion of the commercial culture of tilapia by incorporating the advantage of a faster growth rate of the male tilapia over the female ones. This eliminates the unconditioned propagation ability that makes the population uncontrolled, incurring management difficulty and hindering desired production (Mair and Little, 1991). Male tilapia fingerlings are achieved by feeding the fish methyl testosterone in the juvenile stage.

Quinalphos

Quinalphos is a broad-spectrum organophosphate insecticide with contact and stomach action used against common pests such as aphids, caterpillars, mealybugs, mites, bollworms, leafhoppers and borers of various crops and plants like wheat, sugarcane, peanut, sorghum, cotton, and fiber-crops. It has been in widespread use since 1970 (IUPAC, 2019). The National Center for Biotechnology Information (NCBI) (2019) has categorized the toxicity of quinalphos as very toxic for aquatic life and often the effects last long. Despite being labeled as very toxic, it has a wide range when comes to the toxicity to fish. Even within the same family of fish, tolerance to quinalphos varies. For example, regarding quinalphos, the 96 hour LC_{50} value for Silver barb (*Barbonymus gonionotus*) is 4.70ppm (Mostakim *et al*., 2014), for *Labeo rohita* is 2.826ppm (Rathnamma and Nagaraju, 2013), for *Cyprinus carpio* is 2.75ppm (Padmanabha *et al*., 2016), for *Catla catla* is 2.91ppm (Rajput, 2012), for *Cirrhinus mrigala* is 0.128ppm (Nair *et al*., 2017) and for O*ncorhynchus mykiss* is 0.005ppm (IUPAC, 2019). Therefore, a given dose may be very toxic to one fish species while may not be harmful at all for another species. This phenomenon of quinalphos is exploited by the commercial fish farmers in northwest Bangladesh and quinalphos is being used as a selective poison to kill and wipeout the undesired fish species like *Chanda* sp., *Pseudambasis* sp. and *Glossogobius* sp.

Emamectin benzoate

One of the derivatives of abamectin, emamectin is a pesticide that stimulates the release of inhibitory neurotransmitters causing the paralysis and death of insects upon ingestion (Velde-Koerts 2014). Emamectin benzoate consists of a mixture of two chemicals with similar structures mixed at a minimum of 90% 4"-epi-methylamino-4"-deoxyavermectin B1a, and a maximum of 10% 4"-epi-methylamino-4"-deoxyaverrnectin Blb benzoate (Anderson *et al.*, 2009). Compared to the hydrochloride salt, the benzoate salt of emamectin has greater water solubility, better thermal stability and a broader insecticidal spectrum than avermectin

(Jansson and Dybas, 1998). Emamectin benzoate is being marketed currently under 136 different brand names in Bangladesh, mostly for use in controlling the hairy caterpillar, red spider mite and other mites, shoot and fruit borer, pod borer, bollworm, aphid, Jassid, termite, brown plant hopper in plants for crops, vegetables, fruits and cash crops like jute, tea and cotton (Bangladesh Crop Protection Association 2018).

The first known and public use of emamectin benzoate occurred in the USA in 1996-97 as a pesticide for control of a terrestrial pest. Soon after its use was extended to finfish aquaculture. Over the course of time emamectin benzoate in the brand name of Slice® has become an alternative to other pesticides, including ivermectin, dichlorvos, azamethiphos, hydrogen peroxide, cypermethrin, teflubenzuron and diflubenzuron to control sea lice internationally (Sanders and Swan 2013).Though in Bangladesh emamectin benzoate is still not sold in the market as a product to use in aquaculture, that does not prevent fish farmers from using it in commercial carp aquaculture for controlling ectoparasites, especially *Argulus* sp. and *Lernaea* sp., in northwest Bangladesh. While Slice® is registered to be used @50 µg/kg fish biomass with feed to be fed to the infected fish for 7 days consecutively (Sanders and Swan 2013), in Bangladesh emamectin benzoate is used in very large quantities compared to the feeding doses primarily as a water treating agent in infected fish ponds. Since emamectin benzoate is a broad-spectrum insecticide it is toxic to invertebrates at a low rate of application and has wide margin of safety for mammalians (Yen and Lin, 2004). However, the targeted ectoparasites are small and soft body crustaceans and the use of emamectin benzoate as a water treating agent may have impacts on non-targeted water insects, including many zooplankton that are from the same family of the parasites.

Cypermethrin and deltamethrin

Cypermethrin is a synthetic pyrethroid chemical first synthesized in 1974. It is similar to the natural pyrethrin in pyrethrum extract, has insecticidal properties, and is designed to have a greater durable/stable potency than the natural pyrethrins (WHO, 1989). Technical cypermethrin is mixture of eight different isomers with different physical and biological properties. Cypermethrin is light stable (ETN, 1996). Unlike cypermethrin, deltamethrin is a single pure chemical compound (Johnson *et al*., 2010), first described in 1974 (Elliot *et al*., 1974), but only registered as a pesticide by United States Environmental Protection Agency (USEPA) in 1994 (USEPA, 2010).

Both cypermethrin and deltamethrin are registered in Bangladesh for use in controlling aphid, jassid and bollworm on cotton; aphid on bean, potato, tomato, brinjal, mustard, and chili; aphid, *Helopeltis* and red spider mites on tea; whitefly, shoot and fruit borer on brinjal; diamond back moth on cabbage; leaf hopper on mango; flies on cucurbits; hairy caterpillars on jute; and for mosquito larvae and cockroach control (DAE, 2019). Both cypermethrin and deltamethrin are type-2 pyrethroids which function by blocking sodium channels and impacting the GABA-receptor's function in the nerve filaments of the insect (Roberts and Hudson, 1999).

Abamectin and lambda-cyhalothrin

Abamectin is made up of a mixture of at least 80% avermectin B_{1a}, (5-0-dimethyl avermectin A_{1a}) and at most 20% avermectin B_{1b} (5-0-demethyl-25-de(1-methylpropy1)-25-(1-methylethyl) avermectin A_{1a}) (USEPA, 2010). Abamectin is primarily used in livestock as a parasiticidal agent and in agriculture as an insecticide and acaricide (Campbell, 1989). Lambda-cyhalothrin is a synthetic pyrethroid insecticide for agricultural use registered by USEPA in 1988 (NPIC, 2001). Lambda-cyhalothrin is biologically more active than cyhalothrin, consists of one pair of enantiomeric pair of isomers and was first reported in 1984. It is not systemic but acts quickly upon contact and in the stomach (PPDB, 2019).

Rotenone

Historically rotenone has been used as a fish toxicant for centuries to catch food fish (Ling, 2003). Rotenone, a natural toxicant derived from leguminous plants mainly found in southeast Asia, Latin America and east Africa (Finlayson *et al.*, 2000), is highly toxic to fish and aquatic life, but significantly less toxic to birds and mammals, thus making it a favorable piscicide (Ling, 2003). Most of the fish species exhibit higher sensitivity to rotenone than most aquatic invertebrates (Durkin, 2008). Based on comprehensive ecological and human health risk assessment, the USEPA (2007) declared that rotenone is eligible only for piscicidal use. Ling (2003) described rotenone as the most environmentally benign piscicide available as a fisheries management tool. Rotenone is unstable in the presence of light, heat, oxygen and its persistence in natural water may last a few hours in the summer as it readily becomes absorbed by sediments and suspended particulate matter in the waterbody. Rotenone kills fish by affecting cellular aerobic respiration resulting in tissue anorexia (Ling, 2003). Due to the mechanism of killing, rotenone as a fish toxicant is more effective in higher temperatures (given that enough doses are applied) when oxygen demand for fish is high. However, in commercial aquaculture of northwest Bangladesh, it is used usually at the beginning or end of the culture cycle, and farmers use rotenone in the early hours of 3 to 5 am to facilitate the marketing of fish in the morning. Apart from application difficulty, due to the timing of use at night when temperatures are usually low, in order to kill the fish within 30 minutes farmers often need to use a higher quantity of rotenone, which may increase the cost. Also, to ensure the killing of fish species that are relatively hardy, such as catfish, *Channa* sp., and *Tilapia* sp., and those species with an auxiliary breathing apparatus (*Anabus* sp.) farmers would be required to use unusually high amounts of rotenone, which will again increase the cost significantly. Therefore, the choice of fish toxicant is made primarily based on economic consideration and secondarily on the efficacy of the toxicant and the convenience of use.

Aluminium phosphide (phostoxin tablets)

As an alternative to rotenone, like other parts of the country, most of the commercial fish farmers in northwest Bangladesh use aluminium phosphide tablets (phostoxin) as a fish toxicant. This is normally used as fumigation agent against grains-storage pests (Braid, 1994; Chowdhury *et al*., 2012; Rasul *et al*., 2017). According to Perschbacher and Sarkar (1989) phostoxin (aluminium phosphide) incurs the lowest concentration (0.25ppm) and lowest cost to attain 100% kill in 24 hours of a relatively hardy species, *Channa punctatus,* of many of the fish toxicants (sumithion, bleaching powder, dieldrin, phyphanon, rotenone, phostoxin and DDVP) used in aquaculture ponds at 27^0C temperature. The downside of phostoxin, in the way it is used in commercial aquaculture in north west Bangladesh, is that so as to use it at a concentration that makes it economically advantageous, farmers need to wait couple of hours or more in winter in the middle of the night for fish to die before netting. Typically netting is required to be done three times in between 3 am and 5 am in order to take the fish to the market in the early morning. In laboratory tanks, the detoxification period for rotenone and phostoxin was 6 days and 4 days respectively (Perschbacher and Sarkar, 1989), but commercial fish farmers in northwest Bangladesh experienced the opposite where 10 to 15 days was required (longer in winter than summer) for phostoxin detoxification of large earthen ponds. This is due to the suspended particulate matter in the water and organic content of the pond bottom retaining the phosphine gas from the phostoxin pills for longer in earthen pond on the other hand particulate organic matter in ponds absorb and detoxify rotenone quickly compared to phosphine gas (Ling, 2003). Rahman *et al.* (1992) noted that in earthen aquaculture pond aluminium phosphide toxicity lasts 10 to 15 days as opposed to 10 to 12 days for rotenone in Bangladesh. Farmers involved in commercial fisheries in northwest Bangladesh are continuously looking for an alternative to phostoxin (aluminium phosphide), due to the operational difficulties like broadcasting the phostoxin pills in the pond-a hazard to the broadcaster as well, then waiting a couple of hours in the middle of the night for the fish to die before netting, in addition to the long detoxification period that farmers have to wait before they can stock fish again in the pond.

Fenpropathrin

To eliminate the disadvantage of aluminium phosphide use, synthetic pyrethroid-fenpropathrin came into play. Fenpropathrin, a broad spectrum pyrethroid insecticide and acaricide, was first synthesized in 1971 by Suitomo Chemical Company, Ltd. and commercialized in the late 1980's (Kanawi *et al.*, 2013). According to ARNICA and AWHHE (2019) fenpropathrin is used to control a wide range of pests especially mites, aphids, mealybug, leafhopper, moths and leafrollers in fruits, vines, vegetables, cotton and other crops in the field and greenhouse. In Bangladesh, fenpropathrin is registered for the controlling of red mites in eggplant and tea (DAE, 2019). Valent USA incorporation (marketer of Danitol in Florida USA) categorized fenpropathrin as a 'restricted use pesticide' due to its extreme toxicity to fish and aquatic organisms (Valent, 2009). The 96-hour LC_{50} value of rainbow trout, grass carp and channel catfish are respectively 2.3 µg/liter, 3.59 µg/liter and 5.5 µg/liter respectively for fenpropathrin (Kanawi *et al.*, 2013). Due to its extreme toxicity to fish and relatively lower price it makes economically viable the use of a high-enough concentration to obtain a quick kill of within one hour. Commercial fish farmers in northwest Bangladesh have started using fenpropathrin (Danitol) as a fish toxicant to kill fish meant to be sold for food.

17- alfa methyl-testosterone

Almost all of the sex inversed male tilapia fingerlings are achieved through extensive feeding of the steroid androgen, 17 alfa-methyltestosterone @ 50 to 70mg per kg feed, in the juvenile stage of tilapia fry for about 20 to 30 days (Rouf *et al.*, 2008). There is another use of methyl testosterone at the growth stage of the tilapia, where it is fed at the relatively lower dose of 10mg per kg feed on a regular basis to enhance the growth of *O. niloticus.* Ahmad *et al.* (2002) found that 5ppm doses of 17 alfa-methyl testosterone when used as a growth promoter resulted in higher growth of *O. niloticus*. Hybrid tilapia fed with low doses of 17 α-ethynyltestosterone have led to an 11% additional weight-gain than weight-gain in fish in a control study in commercial polyculture pond conditions (Rothbard *et al*., 1988). A total of 825 private hatcheries produced a total of 2694.9 million juvenile tilapia seedlings in 2018 in Bangladesh (DoF, 2018).Din and Subasinghe (2017) reported that hormonal sex reversal is intensively practiced by the private hatcheries in Bangladesh to produce mono-sex male tilapia seedling. Therefore, it is safe to conclude that a large percentage of tilapia (*O. niloticus)* sold in the market in Bangladesh have either been exposed to the feeding of testosterone at the juvenile stage or at the juvenile stage and then at the growth stage at a lower rate.

In this study in northwest Bangladesh, a total of six different experiments were conducted with the following objectives:

1. To know the unintended consequences of the use of quinalphos in commercial aquaculture ponds on fish, zooplankton, aquatic insects and the benthos population.
2. To investigate the impact of the use of emamectin benzoate on zooplankton, aquatic insects and the benthos population in commercial aquaculture ponds.
3. To know the impact of the use of cypermethrin and deltamethrin on aquatic insects, zooplankton and the benthos population in commercial aquaculture ponds.
4. To observe the effect of the use of abamectin and lambda-cyhalothrin on aquatic insects, zooplankton and the benthos population in commercial aquaculture ponds.
5. To compare the ecological consequences of the use of fenpropathrin as a fish toxicant instead of traditional fish toxicants in commercial fish farming.
6. To determine whether sex inversed male tilapia have a higher level of blood testosterone than normal resulting from the feeding with testosterone at the juvenile stage; to monitor the change of blood testosterone level in tilapia during the feeding with methyl testosterone at a lower dose (10mg per kg feed) and to find out the duration it takes for the serum testosterone level to normalize.

The impacts of entrepreneurial fisheries on the socio-economic aspects of the life of the people near the fisheries sites will also be explored in this chapter. A final objective is to understand how the commercial fish production system will be impacted by the changing climatic conditions as predicted by climate change modeling as compared to paddy production systems.

Materials and Methods

All of the different experiments with the insecticides and fish toxicants were conducted at different times, in different ponds under commercial aquaculture, ranging in size from 1.5 to 3 acres. All ponds were in Hatgodagari area of Poba upazila in Rajshahi District. Farmers were instructed to inform the author just prior to using any of those insecticides or chemicals in their culture ponds. Accordingly, few farmers informed the author about the use of the chemicals and allowed the author to conduct the experiments while being present during the treatments.

The quinalphos experiment was conducted in three different ponds in the last week of November 2018, using the brand 'Deviqueen' 25 EC quinalphos marketed by The Limit Agroproducts Limited.

The emamectin benzoate experiment was conducted in three' ponds between March 2018 and April 2019, using the brands 'Wonder', 'Guilder' and 'Sharper'.

The cypermethrin experiment was conducted in three ponds during the first week of November 2018 using the brand 'Ripcord' 10 EC cypermethrin produced by BASF Bangladesh Limited and distributed by Padma Oil Company Limited.

The deltamethrin experiment was conducted three ponds during the first week of November 2018 using the brand 'Desis'2.5 EC deltamethrin marketed by Bayer Crop Science Limited Bangladesh.

The abamectin experiment was conducted in three ponds during the first week of February 2019 using the brand 'Likar'1.8 EC abamectin imported and marketed by Korbel International Limited.

The lambda-cyhalothrin experiment was conducted in farmers' ponds during the first week of February 2019 using the brand 'Fighter' 2.5 EC imported and distributed by ACI formulations Limited.

The total quantity of each of the pesticides, quinalphos as selective fish poisoning agent and cypermethrin and deltamethrin as insect killers, was diluted in a big aluminum pot and then broadcast manually over the entire body of water of the pond along the water's edges and as far as the farmer could throw towards the middle of the pond. The required quantity of abamectin and lambda cyhalothrin were poured into a big aluminium pot and mixed with about 5 to 7kg of sand by the farmers in order for the sand to become completely absorbed by the insecticides. Then the pesticide-soaked sand was broadcasted along the water's edges of the entire pond, and towards to middle as far as farmers were able to throw, approximately 5 meter inwards.

For the water treatment to kill fish parasites, the required amount of emamectin benzoate was diluted with water in a big aluminium pot and broadcast manually over the entire body of water of the pond along the water's edges and as far as farmers could throw towards the middle of the pond. In the feeding treatment, the required amount of emamectin benzoate was taken at @ 50 µg/kg fish biomass/day, ground as fine powder and diluted in half liter water. Then the solution was mixed with the required quantity of feed in a big aluminum pot so that the pallet feed soaked up the emamectin benzoate mixed water. The feed was administered to the pond using feeding tray to minimize the loss.

The farmers did not provide any supplementary feed to the fish on the day of pesticide (for quinalphos, cypermethrin, deltamethrin, abamectin, lambda-cyhalothrin) application and the following day. Based on the uses, the doses were back calculated using the following formula: Concentration (ppm) = total amount of active ingredient (in mg) / volume of the water (of the pond in liter). The sampling of zooplankton, aquatic insects, benthos, fish and water (for the quinalphos experiment only) was taken before the treatment and 1 day, 2 days, 5 days, 10 days, 15 days, 21 days and 28 days after the treatment.

Fenpropathrin treatment

The required amount of fenpropathrin ('Danitol' 10 EC imported and marketed by Setu Corporation Limited) was poured into a big aluminium pot and diluted with water. The mixture was then broadcast along the water's edges of the whole pond, and as far as it could be thrown (approximately 5 meters) towards the middle of the pond. The treatment took place on 4 April 2019 at 4.00 am in the aquaculture pond with a water area of two acres.

Rotenone treatment

The farmer took half of the rotenone powder ('aquanone powder' containing 9% rotenone, marketed by Agrovet Division of Square Pharmaceuticals Ltd.) and placed it in a big aluminium pot. A little bit of water was added to make a dough of rotenone powder such that balls can be formed. Then the balls of rotenone powder were thrown into the water towards the middle part of the pond. The other half of the rotenone was diluted with water in a big aluminium pot. The solution was then broadcast along the water's edges pond and towards the middle as far as can be thrown, approximately 5 meters. To avoid the rotenone powder from getting into his respiratory system, the farmer covered his nose and mouth with a towel at the time of dilution. The treatment took place on 12 May 2019 at 1.30 am in an aquaculture pond with a water area of one acre.

Phostoxin treatment

The farmer used a towel to cover his nose and mouth before he opened the can of phostoxin tablets ('Mimtox' containing 57% aluminium phosphide, imported and marketed by Mimpex Agrochemicals Ltd.) to avoid the phostoxin fumes from getting into his respiratory system. Then the tablets were thrown into the pond. Because of the tablet's large size, the farmer was able to throw those homogeneously throughout the pond including the middle part of the pond. The treatment took place on 24 May 2019 at 1.30 am in an aquaculture pond with a water area of four and half acres.

Sampling for fenpropathrin, rotenone and phostoxin treatments

Samples of zooplankton, water insects and benthos were collected the day before each treatment and then one day, two days, five days and 10 days after the treatment. Foreach of the treatments, sampling was done 3 times each day and was considered as three replications. After each of the treatments, the chemical concentration was back calculated based on the size of the water body, water depth and quantity of the fish toxicants or percentage of active ingredient used. Three replications of water samples from one-foot depth of each water body were taken before the treatment and after post treatment fish harvesting. Then turbidity for each sample was measured using the turbidity meter in the laboratory.

Sampling of water and fish for residue analysis

Sampling of water and fish for residue was done only for the quinalphos experiments. Fish and water samples were collected pretreatment and one day, two days, five days and 10 days after the application for testing the residue level of quinalphos. For each sampling approximately one to one and a half kg of fish (*Labeo rohita*) were caught using a cast net, put in a plastic bag and immediately transferred into the freezer. Water samples were collected from 18 inches below the surface of the water level, and at least from four different locations of the pond to total one liter. The water samples were put into non-transparent plastic bottle and immediately put into the freezer. Both the fish and water samples were coded with numbers and transferred to the 'Pesticide Analytical Laboratory' located in the Entomology Division of the Bangladesh Agricultural Research Institute (BARI). The Lab.

analyzed the samples for quinalphos residue using a GCMS-MS machine (level of detection of organophosphate insecticides ranged from 0.003 to 0.009ppm) and then delivered the report. Sampling of zooplankton, aquatic insects, benthos, soils were done as same as described in Chapter-2.

Tilapia testosterone experiment

Testosterone levels of regular adult female, adult male and sex inversed tilapia (*O. niloticus*) were determined. For obtaining regular male and female tilapia, a stock was identified in a homestead earthen pond in the Hatgodagari area of Poba upazila of Rajshahi district where a natural stock of tilapia was maintained at least for the last 5 years and no tilapia fingerling were released during that period. Therefore, it was considered free from any kind of hormonal feeding. Fish were caught with a cast net, the blood sample taken from the caudal vein of the live fish, and then by dissecting the fish, sex was determined by identifying the primary sexual organs (testis/ovary) under microscope for confirmation .The blood samples were then labeled as regular male or female tilapia. At the same time, the condition of the sex organs was documented. Similarly, live adult sex inversed tilapia was located and confirmed as sex inversed by the fish farmer to be used as the treated group. These were bought from the wholesale market located in Naodapara in Rajshahi city in several batches between May and August-2018 and blood samples were taken. After taking the blood samples, the fish were dissected for confirmation of their sex by observing under the microscope, and the condition of the sex organ was documented. For the normal, non-sex inversed population, 53 blood samples were collected from 53 different fish. For the sex inversed population, 50 blood samples were collected from 50 different fish. The size of the fish ranged from 200g to 500g each. For each fish 2 to 3ml blood sample was taken from caudal blood vessels using a 5ml disposable syringe.

After taking the blood sample, the needle was removed from the syringe. Then the blood from the syringe was transferred to a sterile red top glass blood tube without anticoagulant. Then the blood tubes with the blood sample were left for a half an hour without shaking at room temperature. The serum was separated following the standard serum separation protocol (Texas Department of State Health Services, 2018). After a half an hour, the blood tubes were put into a centrifuge machine to segregate the blood serum from the blood cells. The blood samples were centrifuged for 10 minutes @ 2000 rpm (Thermo Fisher Scientific, 2018).

After completion of the centrifuge process, the blood serum was taken from the top using a vacuum dropper and put into sterile Eppendorf tubes and put in the freezer immediately. Because the collection of blood samples took several months, the frozen blood serum samples were stored in the freezer at -80^{0}C in the Biochemistry Department of Rajshahi Medical College until used for testing the testosterone level. Due to the distance of the collection site from Rajshahi University and to avoid the transfer of live fish frequently during the experimental period, a mobile laboratory (consisting of a compound microscope, a small centrifuge machine, blood tubes, syringe, Eppendorf tubes, dissecting box, and ice box) for fish blood collection was set up near the sample collection site.

To determine the level of blood testosterone for adult tilapia fed with MT @ of 10mg per kg feed, regular male and female tilapia were put in 3 *hapa*s (net cages) set in the pond. Each *hapa* was 3 meters by 1 meter in size with 1-meter depth. Ten tilapia fish ranging from 200 to 300g in size consisting of both male and female were put in each of the *hapa*s. The experimental fish were collected from a pond that maintained a natural stock in the Hatgodagari area of Poba upazila in Rajshahi district and the *hapa*s were set in the same pond to hold the fish.

Feed preparation for testosterone experiment

For feed, 'AIT' pellet carp feed was used with a proximate composition of 27.26% protein, 7.20% lipid, 11.54% ash, 13.70% moisture, 5.90% fiber and 34.40% carbohydrate. To prepare the 15kg feed with hormone, first 150mg (at the rate of 10mg per kg feed) Methyl testosterone (marketed by 'Argenta' in Bangladesh) was diluted in 15ml of Lab. grade ethanol. Then tap water was added until the solution total was 250ml. Then the 250ml solution was sprayed over the 15kg feed and mixed so that the feed soaked up all the liquid containing 150mg methyl testosterone. Then the moist feed was air dried in the shed. Despite being air dried, the feed contained extra moisture, therefore, to avoid fungal growth, the feed was preserved in the refrigerator in a plastic bag.

Feeding of the fish in testosterone experiment

The prepared feed was fed to the fish in the *hapa* at the rate of 3% of fish body weight once a day for 30 days in the month of June. Feeding was maintained at the rate of 3% of body weight per day to ensure that all the feed is consumed by fish. The fish stock was maintained for another month with a normal diet (without testosterone).

Collection of blood samples in testosterone experiment

Blood samples were collected from 2 to 3 males and females every 5 days following the process mentioned above to monitor the serum testosterone level. After collecting the blood sample, the fishes were released into the *hapa* again. Because there were a limited number of fish, in this case fish were not dissected after the collection of the blood sample, rather the sex was identified by observing their genital opening. Fish from different *hapa*s were collected on a rotational basis for sampling to allow the fish to recover from the physical injury that incurred in the process of blood collection. Thus, over the period of one month, the *O. niloticus* fish were fed with methyl testosterone with feed and blood samples were collected to monitor the change in serum testosterone level. Blood samples continued to be collected for another month after the feeding of testosterone was stopped.

Determination of testosterone level

Serum testosterone levels were determined by ‘Das Plate Reader’, which was manufactured in Italy (http://www.dasitaly.com/en/prodotti/Plate%20Reader) and marketed by Bio-Trade International in Bangladesh. The testing was done using ‘AccuBind ELISA Microwells’ for Testosterone from ‘Monobind Inc.’, USA and followed the prescribed systems/procedure of the ELISA kit using the reagents supplied with the kit (Monobind Inc., 2018). This was conducted in the laboratory of Royal Hospital (pvt.) Ltd located in the Laxmipur area of Rajshahi city. Since the ELISA kit is customized for human use it can determine up to a maximum concentration of 12 ng/ml of testosterone. After testing the blood serum, for those samples where values were over 12 ng/ml, these were diluted with the diluent and tested again for determining the actual testosterone concentration.

Statistical analysis

Comparison of means test (t test for independent samples) was used to determine whether there is any difference between the testosterone level of regular tilapia and sex inversed tilapia of marketable size.

Focus group discussion

To know the socio-economic impact of entrepreneurial fisheries on the surrounding communities and the people involved in entrepreneurial fish production, three focus group discussion near three sampling clusters. Through the discussion, the interaction of different stakeholders of the supply chain was revealed along with its socio-economic impacts.

Results and Discussion

Impacts of use of quinalphos

The dose of quinalphos used in this experiment was 0.02ppm, which is a little less than 1/6th of the 96-hour LC_{50} value of *Cirrhinus mrigala* (LC_{50} value is 0.128ppm), the lowest 96 hour value for all of the Indian major carps. Within two days of using quinalphos, *Glossogobius* sp. were dead and floating on the pond water. Dead *Chanda* sp. was not found floating due to their body morphology, but later their death was confirmed by netting the water with fine meshed net. The farm owner confirmed that very few live *Chanda* sp. were caught with the fine meshed netting. The pond under commercial aquaculture where the study was conducted was 3 acres of water body in size with a depth of four feet. In this large volume of water 1200ml (25EC quinalphos) 'Deviqueen' was broadcast. Despite the natural movement of the fish, due to the sheer volume of the water the small quantity of quinalphos could not be mixed homogeneously. Therefore, there was always inconsistency in the density of quinalphos across the water body. This allowed some of the *Chanda* sp. Individuals, particularly those that are larger in size, to survive and avoid complete extinction. This means that every few months farmers need to repeat the treatment to keep control of *Chanda* sp. in their aquaculture ponds. This was verified by the farm owners.

Photograph 3.1. Dead *Glossogobius* sp. floating in the pond water treated with quinalphos (at 0.02mg per liter water).

Farmers also mentioned that usually *Glossogobius* sp. individuals are more sensitive to this treatment and almost all of the individuals would die. *Glossogobius* sp. would not show up again until it is unintentionally reintroduced with a new batch of desired species. Though the quinalphos concentration that was used in this experiment is less than one sixth of the 96 hour LC_{50} value of *Cirrhinus mrigala*, still in one replication few *C. mrigala* died due to the inconsistency of the distribution of the quinalphos in the pond water. However, in cases where *C. mrigala* have not died, they might have suffered some histopathological changes. Histopathological changes in fish have been identified in many other studies on other fish species when exposed in sublethal (one 10^{th} of the 96-hour LC_{50} concentration of quinalphos) concentration (Aswin, 2016).

The residue analysis for quinalphos showed that after 24 hours of the treatment the level of quinalphos in the water was below the level of detection range of the machine. In contrary, quinalphos became bioaccumulated in fish (*Labeo rohita*) and the level of residue after one day, two days and five days was 0.23ppm, 0.16ppm and 0.027ppm (App. Table 3.1). On the 10^{th} day after treatment, quinalphos residue was nil in both the fish and the water of the pond. This means that there are health hazards if the fish are sold in the market within five days of quinalphos treatment. MRL values for quinalphos in food animals are recorded as 0.01ppm by the European Commission (2019). Thinh *et al*. (2018) confirmed bioconcentration of quinalphos in fish muscle from a rice-fish experiment in Vietnam, where the half-life of quinalphos was 1.0 to 1.1 days for water and 1.3 to 1.9 days for *Cyprinus carpio*. The good news is that surveyed farm owners in northwest Bangladesh never harvest and sell fish to the market after using the pesticide. This is not because they are very concerned about public health, but simply for economic reasons. First of all, the pesticides are expensive, if the fish are to be harvested and sold then not to use the pesticide. Secondly, since almost all commercial farmers of northwest Bangladesh sell fish in a live condition, they understand that after pesticide treatments fish become weak and prone to death during transportation to the market due to the stress related to harvesting and transportation. This would cause the fish to decrease in value.

The effect of quinalphos bioconcentration in fish on other fish-eaters like heron, Indian cormorant, water snake (checkered keelback), monitor Lizard, who tend to live on these small species, was not possible to determine. Though these animals are abundant at the ponds where there is a presence of small fish, in this study when the dead *Glossogobius* sp. was floating at the top of the pondwater, no predators were preying on the dead fish. There is the assumption that the predators were able to detect the risk that these dead fish and the bioconcentration phenomena posed to them.

The quick degradation of quinalphos in the experimental pond water is justified by the quality parameters presented in App. Table 3.2. PPDB (2019) recorded the aqueous hydrolysis DT50 (days) as 39 days in 20^0C at pH 7, however the value reduced in both acidic and alkaline conditions. Goncalves *et al.* (2006) recorded the photolysis half-life of quinalphos in water samples ranged from 11.6 to 19 hours, while nitrate ion accelerated the photodegradation and dissolved organic carbon slowed the process. The application method of quinalphos in the pond where it is broadcasted on the pond water and the inconsistency of mixing with the water meant that a major portion of broadcast quinalphos remained on the water surface, exposed to bright sunlight and subjected photolysis. *Bacillus* and *Pseudomonas* sp. from aqueous streams were found to effectively biodegrade 80% of quinalphos within 17 days in Lab. experiments by Dhanjal *et al.* (2014). *Ochrobactrum* sp. often present in pesticide contaminated soil, can use quinalphos as a sole source of carbon and biodegrade it (Talwar *et al.*, 2014). Since the experimental pond is subject to the feeding of pallet feed, high supplies of agrochemicals and intensive management (Belton *et al.*, 2011), it is more likely that the presence of high nitrate ion and commonly occurring bacteria was available in the experimental pond (though not tested) and contributed to quick biodegradation of the quinalphos in the aqueous condition.

Zooplankton count showed that the zooplankton number reduced within one day of the treatment and reached the lowest point on day two of the treatment (Figure 3.1). After that the number started to increase and reached the previous level on day five and continued to be almost static.

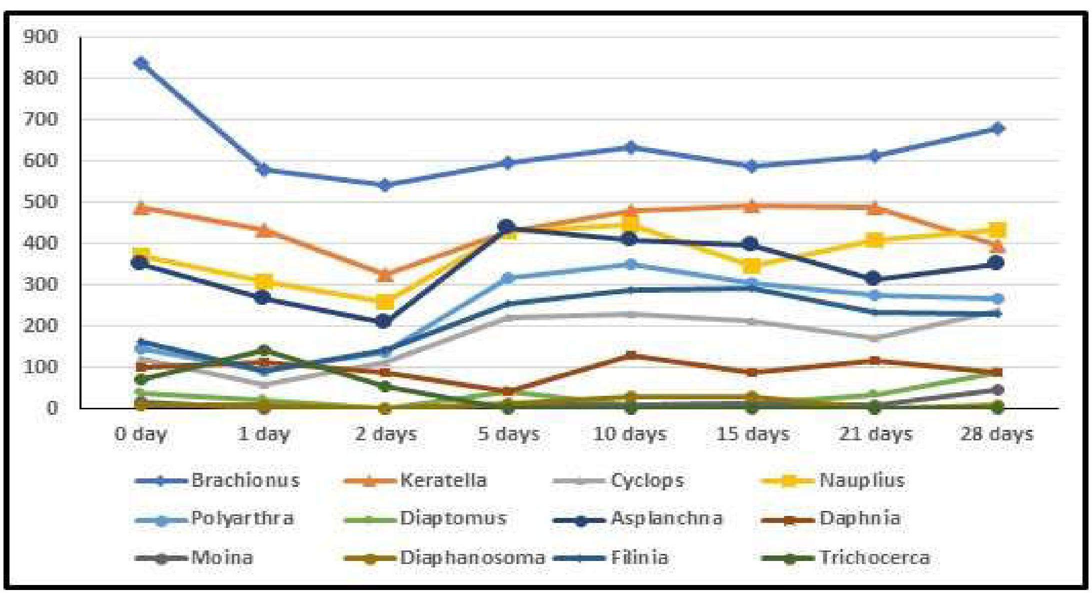


Figure 3.1. Zooplankton count (nos/liter) at various points of pond water treatment with quinalphos (at 0.02mg per liter water).

The relatively short half-life (App. Table 3.1) of quinalphos in water meant that the effect of quinalphos on zooplankton population showed up early, on days one and two, in the observation period. Then they were able to regenerate to the normal level. Gajbhiye *et al.* (2010) reported rapid degradation followed by short residual life of quinalphos in environmental conditions. The 48-hour EC_{50} of *Daphnia magna* for quinalphos was reported to be 0.00066ppm by IUPAC (2019). Despite the higher concentration (0.02ppm) the inconsistency in quinalphos dilution in the treatment ponds due to the large volume of water, in combination with the slow mobility of zooplanktons, were the reasons for the total zooplankton population not being completely eliminated.

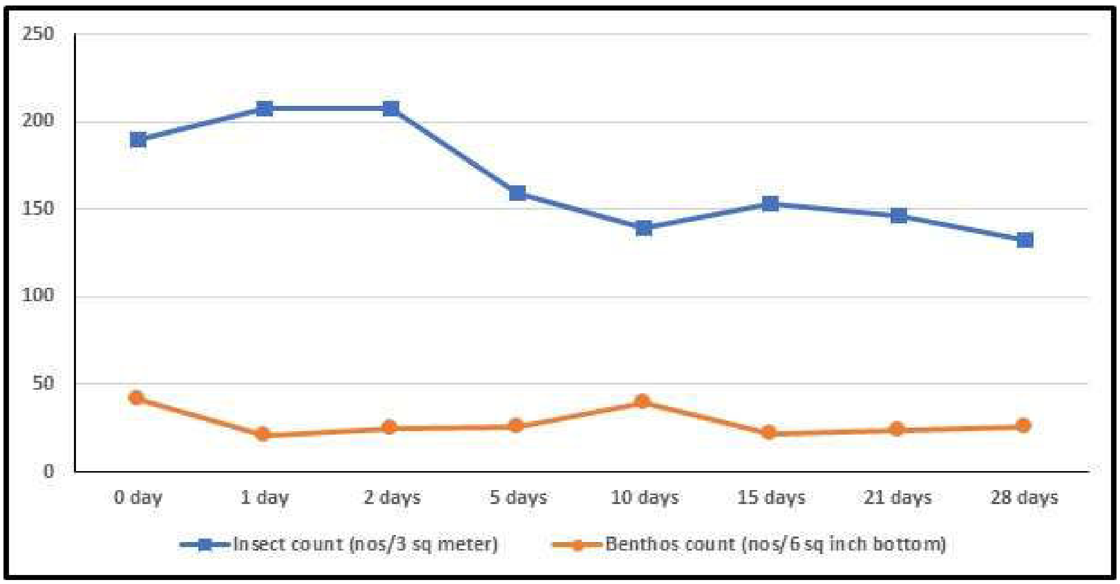


Figure 3.2. Aquatic insect and benthos count at various points of pond water treatment with quinalphos (at 0.02mg per liter water).

The aquatic insect count showed that around the 5th day after the treatment the numbers reduced and then from the 10th day onwards remained almost static (Figure 3.2). On the other hand, the benthos count showed a reduction in number one day after the treatment. After that a slight increase was observed and then the numbers leveled off until the end of the observation period at 28 days. A spike in number was observed on the 10th day. The author has no logical explanation for this spike except the possibility of sampling error.

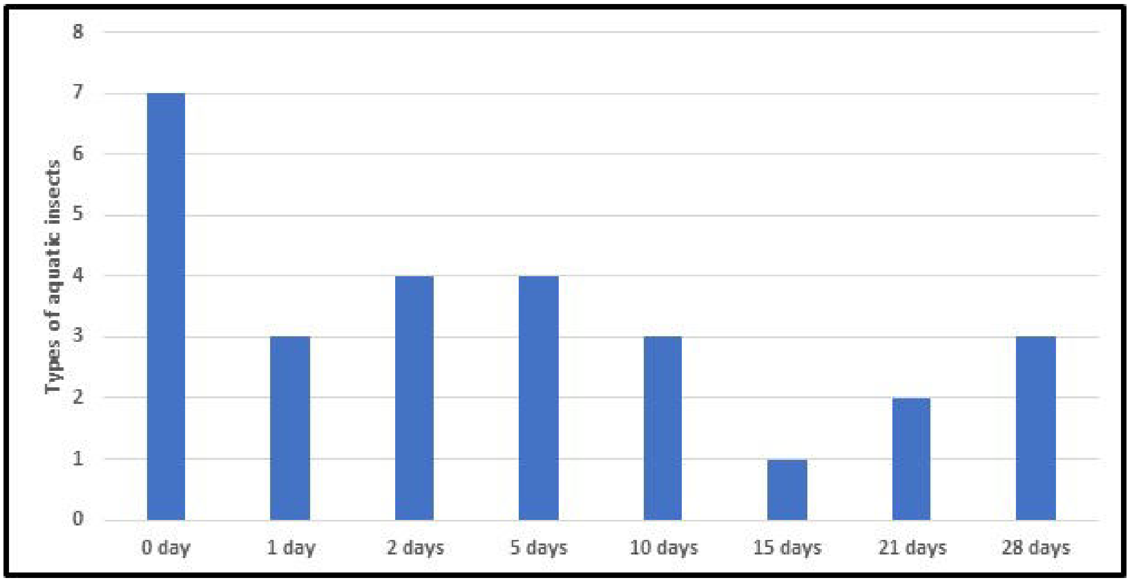


Figure 3.3. Water insect diversity at various points of pond water treatment with quinalphos (at 0.02mg per liter water).

The diversity of aquatic insects was more affected by the quinalphos treatment in pond water (Figure 3.3) than the total counts of insects (Figure 3.2). One day after the treatment the insect types were reduced to three as opposed to the seven types that were recorded at pre-treatment stage. Of all the aquatic insects, mayfly and *Gerris* sp. were not found after the fifth day, and *Ranatra* sp. and dragon fly did not show up after the 10^{th} day of the use of quinalphos. Water scavenger was missing in post treatment samplings except at the very end of the observation period. The number of backswimmer and boatman declined but recovered slowly. The insect diversity count did not recover to the pretreatment level until the end of the observation period. The non-recovery phenomenon may be attributed to the winter season as the treatment was done in the month of December. This may not be the primetime for many aquatic insects to reproduce in Bangladesh (Nasiruddin *et al*., 2014).

Impacts of use of emamectin benzoate

Emamectin benzoate was used @ 2µg/liter of pond water for water treatment and in feed use @ 50 µg/kg fish for seven days consecutively. For the treatment of emamectin benzoate in feed, no impact was observed in the general population of zooplankton in ponds; however, in the water treatment experiment, zooplankton count reduced significantly at 48 hours after treatment (Figure 3.4). Zooplankton count in water treatment declined 29.11% at 48 hours point compared to the pretreatment level (App. Table 3.6). The zooplankton count in the water treatment experiment become normal at five days and continued to increase until the 21^{st} day after treatment.

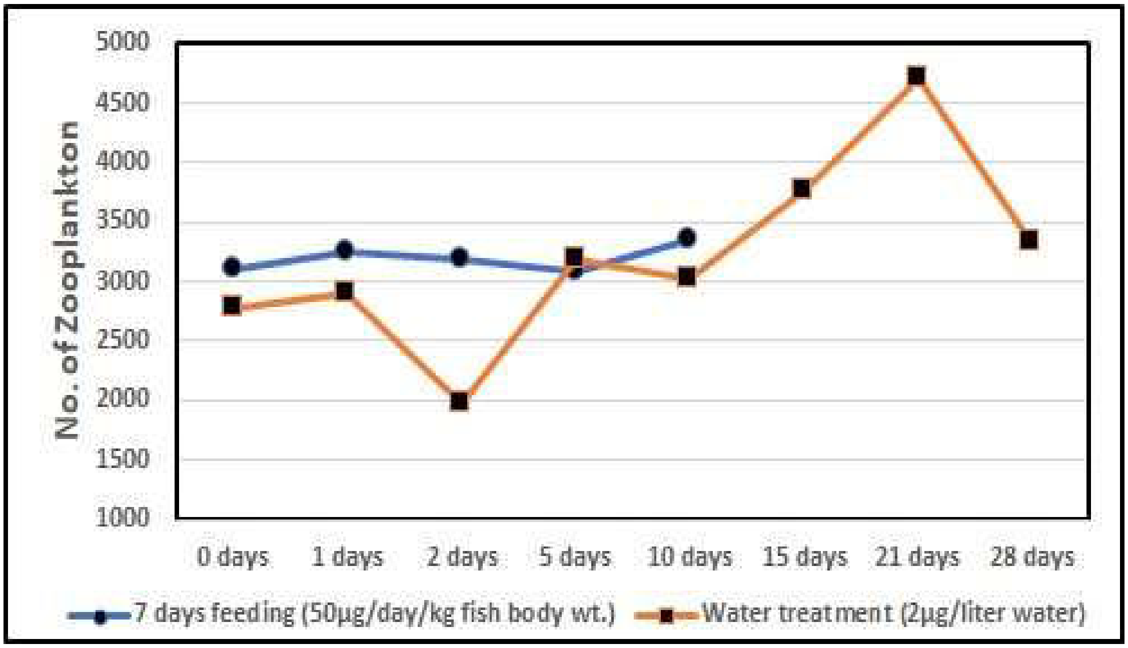


Figure 3.4. Total count (nos/liter water) of zooplankton at various points of emamectin benzoate treatment of fish in aquaculture ponds.

As emamectin benzoate treated feed is effectively consumed by fish, the amount of emamectin benzoate that gets into pond water through dilution and leaching are negligible in quantity and does not affect the non-targeted species like zooplankton, aquatic insects or benthos population. Willis and Ling (2003) pointed out that the use of emamectin benzoate in the form of infeed formulation in salmon aquaculture is less likely to affect the planktonic form of marine copepods.

Looking at the zooplankton composition during water treatment with emamectin benzoate reveals that the zooplankton numbers, other than *Cyclops* sp. and *Daphnia* sp., were reduced at 48 hours after treatment (Figure 3.5). It could be that these two types of zooplankton are less sensitive to emamectin benzoate or they thrived due to the change in the zooplankton composition when the treatment reduced the number of other types.

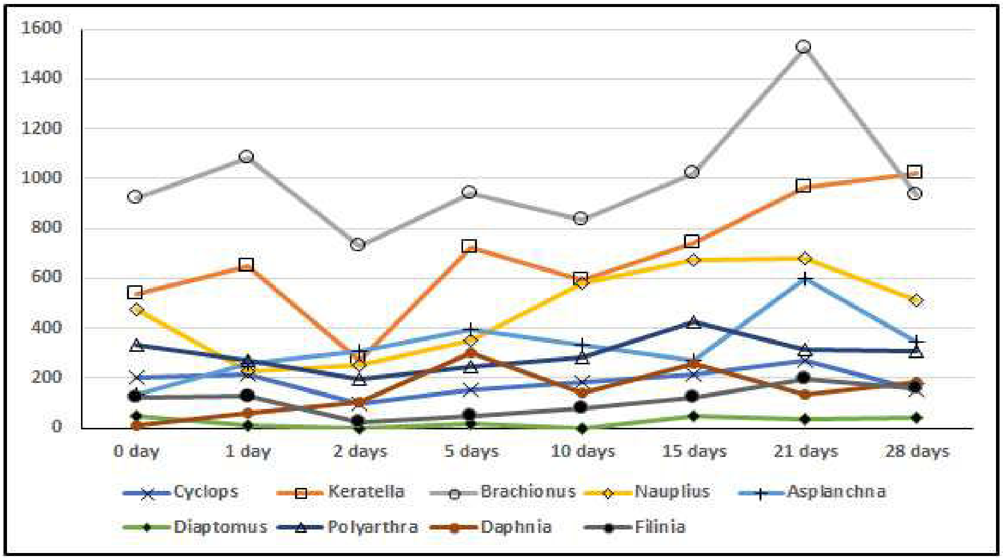


Figure 3.5. Zooplankton count (nos/liter) at various points of pond water treatment with emamectin benzoate @2µg/liter.

Emamectin benzoate is a systemic drug that goes into the fish body through the feeding treatment after which impacts the parasites feeding on the fish. In the water treatment, where the drug is diluted in water and broadcast over the pond water, it slowly becomes mixed in the water with the fish and parasites equally slowly affected. Due to the large volume of pond water, the mixing does not happen homogeneously, therefore there is always inconsistency where some places with a higher concentration and others a lower concentration of the drug in the pond water. Anderson *et al.* (2009) reported emamectin benzoate as 'very highly toxic' for *Daphnia magna*, with EC_{50} value reported as 1µg/liter. Static concentration of emamectin benzoate in this study @ 2µg/liter affected the zooplankton population (Figure 3.4 and 3.5); however, the inconsistency in drug concentration across the whole volume of the water body saved the slow-moving zooplankton population from being wiped out completely. On the other hand, fast moving fish and their parasites were in contact with enough of the highly concentrated drug that it resulted in the killing of the fish parasite population. Again, a 2µg/liter concentration of emamectin benzoate is safe for fish itself since its level of tolerance to this drug is higher; the LC_{50} for rainbow trout was reported to be 174 µg/liter by Anderson *et al.* (2009).

Like the zooplankton count, the aquatic insect count in the feeding experiment did not change over the course of the experiment (Figure 3.6). However, with the water treatment experiment, the insect count dramatically increased one day after the treatment (Figure 3.6). By the 10^{th} day, the insect count was similar to the pretreatment insect count and continued in the same way until the end of the observation period 28 days after treatment.

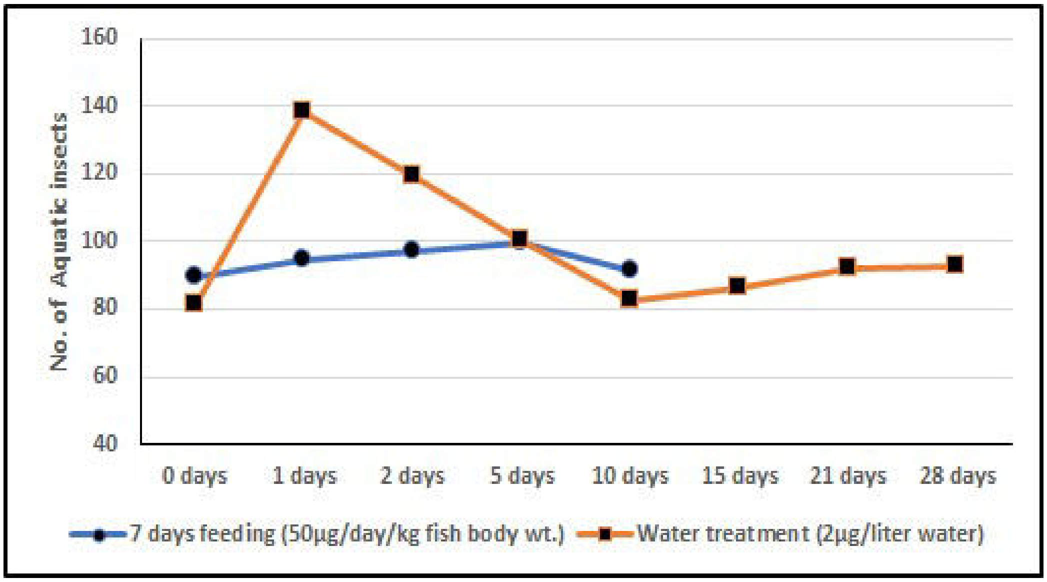


Figure 3.6. Total count (nos/3 sq meter) of aquatic insects at various points of emamectin benzoate treatment of fish in ponds.

The insect composition in the water treatment experiment revealed that backswimmer and boatman catchment increased significantly 24 hours after the treatment while counts for other insects after treatment went down or remained static (Figure 3.7). Since backswimmer and boatman are the dominant insect types in number, their increased catchment made the total insect count high at day one of the sampling. The insect count became static and normal from day 10 until the end of observation period 28 days after treatment.

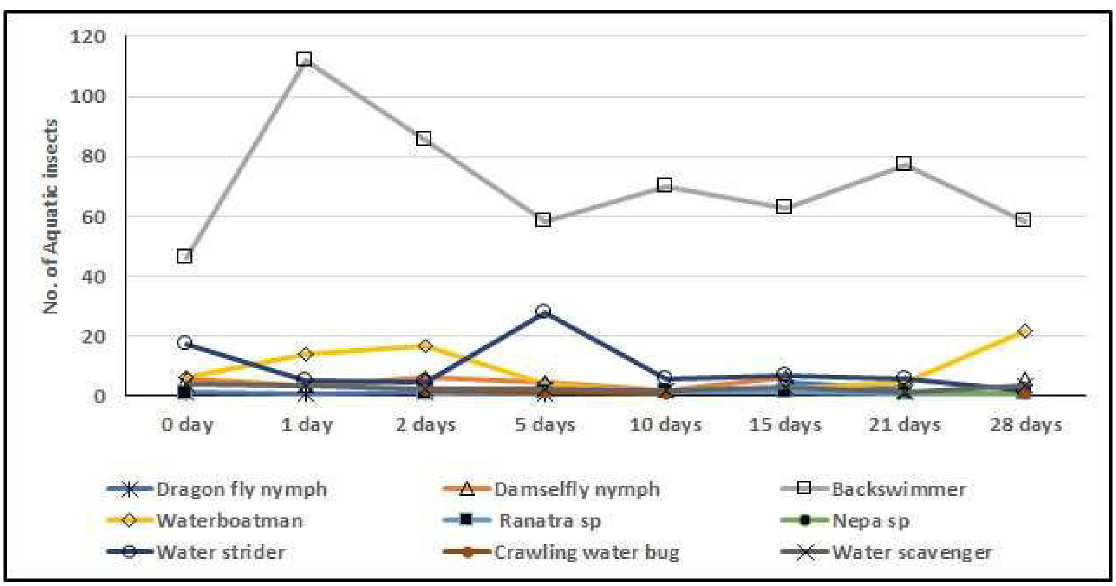


Figure 3.7. Insect count (nos/3 sq meter) in ponds when treated with emamectin benzoate @ 2µg/liter of pond water.

Opposite to the zooplankton response, the aquatic insect count increased at the 24-hour point after the water treatment. As an insecticide, emamectin benzoate is effective against soft-bodied terrestrial insects like hairy caterpillar, red spider mites and other mites, shoot and fruit borer, pod borer, bollworm, aphid, jassid, and termites, butut most of the aquatic insects are hard shelled, unlike zooplankton. Therefore, the doses used in the experiment with the intention to kill the anchor worm and *Argulus* in fish was not strong enough to kill the insects, especially the hard-shelled ones. It may have weakened the aquatic insects such that they were more easily caught during sampling, leading to the increase in count at the 24-hour mark.

The benthos count in the feeding experiment did not change during the observation period of ten days. On the other hand, the benthos population declined 58.57% after 24 hours (App. Table 3.8) of water treatment with emamectin benzoate @ 2µg/liter water (Figure 3.8).

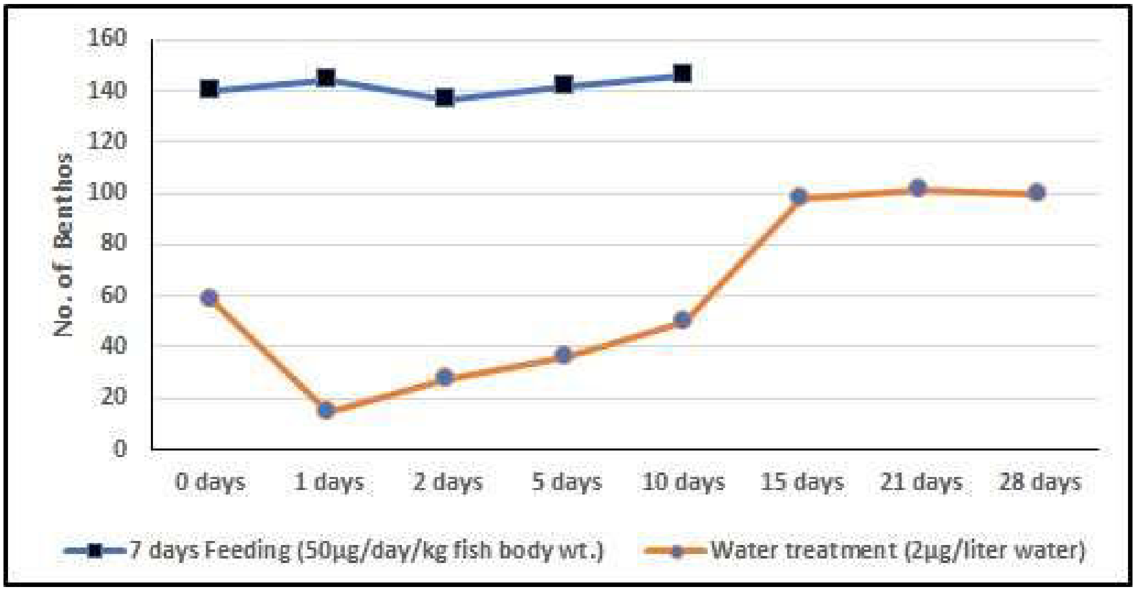


Figure 3.8. Total count (nos/6 square inches bottom) of benthos at various points of emamectin benzoate treatment of fish in aquaculture ponds.

The benthos population gradually increased after it hit the lowest number at 24 hours after treatment. After 15 days of treatment, the benthos count exceeded the pretreatment level and sustained that higher count until the end of the observation period 28 days after treatment.

The in-water treatment experiment revealed that the benthos composition was altered with the chironomid larvae dominating pretreatment, and tubifex dominating from day one post treatment until the end of the observation period (Figure 3.9).

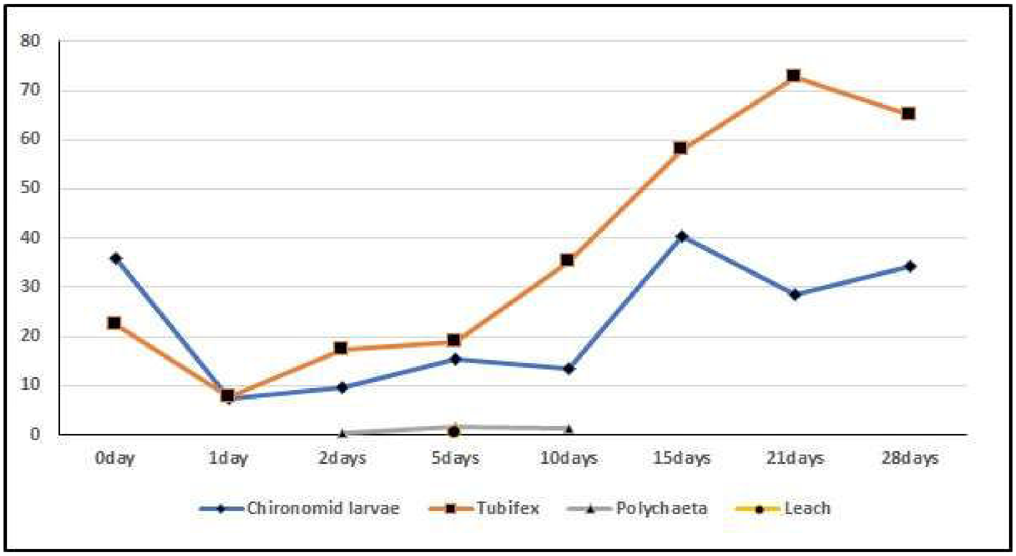


Figure 3.9. Benthos count (nos/6 sq inch bottom) after use of emamectin benzoate @ 2µg/liter water in aquaculture ponds.

The number of both chironomid larvae and tubifex declined severely at 24 hours after water treatment with emamectin benzoate. However, the number for both types started increasing after their lowest point and the increasing count continued until the observation period 28 days after treatment.

Though the benthos population was affected severely in the pond water treatment with emamectin benzoate, the dilution inconsistency of emamectin benzoate in the water body due to the large water volume meant there was an inconsistency in the deposition of the drug on the pond bottom, saving the benthos population from being wiped out completely. The half-life (DT_{50}) of [23-^{14}C]-emamectin B1a and B1b benzoate in sandy loam soil was 12 to 19 days for irradiated samples during a 30-day exposure to artificial sunlight at 25^{0}C (Velde-Koerts, 2014). In this study the benthos population returned to the pretreatment level around 15 days after the water treatment, consistent with the expected half-life of emamectin benzoate

Despite both methods of treatment being successful in controlling of targeted fish parasites, for a 2.3 acre water-body with a five feet water column and given its fish stock size of 2500kg, if the water treatment method is chosen over the feed treatment, it would require staggering 29 times more emamectin benzoate to go into the pond system for that particular waterbody. This is unnecessary and has unwanted environmental consequences on aquatic fauna. While the feeding method requires a small quantity of emamectin benzoate, it requires the hassle of mixing the drug with feed every time fish is fed for 7 days consecutively. The management or application difficulties, lack of knowledge, and cheaper cost of the emamectin benzoate pesticide is convincing the fish growers to keep using the environmentally harmful water treatment method over the feed treatment to control parasitic worms in Chinese and Indian major carp in northwest Bangladesh.

Impacts of uses of cypermethrin and deltamethrin

After the treatment, the doses of both cypermethrin and deltamethrin were back calculated based on the strength of the pesticide brand and water volume of the pond. It was found that for both cypermethrin and deltamethrin the doses were 5µg of active ingredient/liter of water. For cypermethrin the 96 hour LC_{50} value of silver carp was reported 0.917 µg/liter by Shaluei *et al*. (2012), for *Cirrhinus mrigala* it was reported 2.28 µg/liter by Veni and Veeraiah (2014), and for the common carp it was reported 7.5 µg/liter by Yhasmine (2013). The 24-h LC_{50} of *Labeo rohita* fingerling was 0.205 µg/liter (Tiwari *et al.*, 2012) and 0.225ppm (Das and Mukherjee, 2003) for cypermethrin. For deltamethrin, the 96-hour LC_{50} value for common carp was reported as 2.37 µg/liter at 30^0C temperature by Datta *et al.* (2003); for tilapia it was reported 15.47 µg/liter by Baoteng *et al.* (2006), and for *Labeo rohita* it was 0.38ppm reported by Suvetha *et al.* (2015).

Despite the doses of cypermethrin and deltamethrin in this study exceeding the acute toxicity concentration for some of the fish species that existed in the experimental pond, no fish mortality was recorded in the experimental ponds. It is recommended by test protocols that juvenile fish in their early life stages are to be used as the subject in toxicity testing This is based on the understanding that in the early life stages, fish are more susceptible to toxicants, hence having toxicity data on the most vulnerable life stage would give understanding of and protection during all life stages against toxic substances (Mohammed, 2013). All lethal concentration dose-determination experiments cited earlier were conducted in laboratory conditions, at room temperature and in tap water, using juveniles and fingerlings as the subject. The Lab. environment is different from the commercial aquaculture pond conditions in terms of temperature, turbidity, water hardness, pH, and light. During this study the fish that were in the study ponds were a minimum of 0.75kg in size (mostly adults) and were thus less susceptible to the cypermethrin and deltamethrin than at their juvenile stages (Mohammed, 2013) due to their larger body size (NPIC, 2010). Day (1991) also mentioned a lesser impact of pyrethroid pesticides in field conditions than the impact predicted by laboratory test data.

Datta *et al.* (2003) found that temperature, water hardness, and turbidity, i.e. the degree of suspended soil particles, profoundly impacted the toxicity of deltamethrin on common carp, where, even with the higher LC_{50} concentration, toxicity was the lowest at 30^0C temperature, lower in hard water as compared to soft water, and lower with the adsorption of deltamethrin in soil particles such as humus, clay, and organic carbon. Cypermethrin is also hydrophobic, meaning it has low water solubility (Jones, 1992) and strong absorption tendency into soil particles. Its hydrolysis is faster in basic solutions, photodegrades rapidly with half ranges from 8 to 16 hours, and experiences microbial degradation (ETN, 1996). Roberts and Hudson (1999) found that biodegradation of deltamethrin can be stalled due to its strong adsorption into particulate organic matter. Compared to other pyrethroids, deltamethrin has a higher potential of volatilization into the air from water. The average half-life is 2.5 days in a pH 9 solution. Microbial action, photolysis and hydrolysis also degrade deltamethrin (NPIC, 2010). High turbidity, pH, electrical conductivity, and favorable water temperatures of the treatment ponds (App. Table 3.9) have reduced the effect of both cypermethrin and deltamethrin to the level where it was nontoxic to the fish in the experimental ponds. Moreover, there were mixing inconsistency of both cypermethrin and deltamethrin due to the manual spray along the water's edge of the pond, leaving a large volume of water of the large treatment ponds with very little or almost no pesticide, such that fast moving fish could take refuge in water devoid of pesticides.

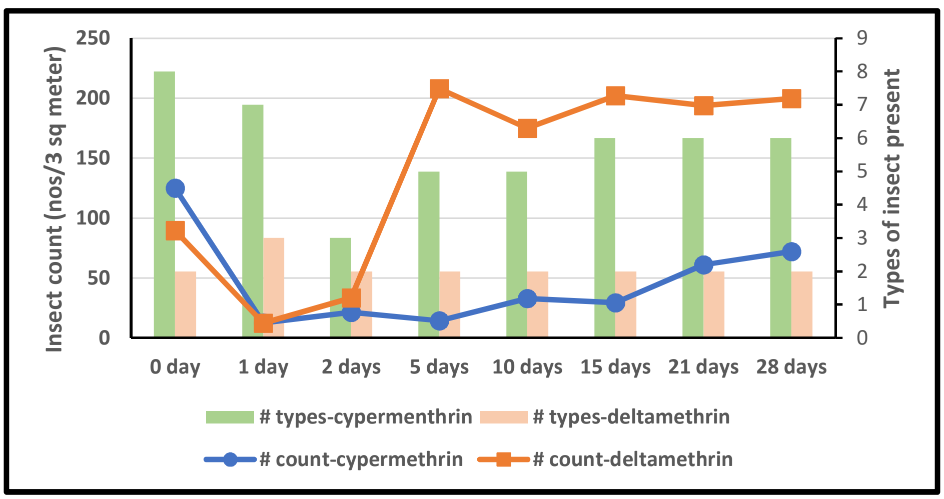


Figure 3.10. Total count of aquatic insects (nos/3 sq meter) and their diversity before and after use of cypermethrin and deltamethrin in commercial aquaculture ponds.

Within one day after the use of cypermethrin and deltamethrin, the total insect count went down 90 and 86% respectively for cypermethrin and deltamethrin (Figure 3.10). The total insect count for deltamethrin treatment went up sharply beyond the pretreatment level after day five of the treatment and continued to be almost at a static level until the end of the observation period. Fifty to 100% mortality of arthropods insects in the field ponds caused by pyrethroid insecticides and their recovery to the pretreatment level within 2 to 4 weeks of treatment was also recorded by Mulla *et al.* (1982). An increase of the insect count in cypermethrin treatment was much slower compared to the deltamethrin treatment and did not recover to the pretreatment level by the end of the observation period 28 days after treatment. Due to the higher turbidity, pH and conductivity, deltamethrin's toxicity was reduced (App. Table 3.9) sooner as compared to the cypermethrin treatment. These factors resulted in a return of total insect count to pretreatment levels earlier than cypermethrin treatment (Figure 3.10).

Insect diversity went down after the use of cypermethrin in aquaculture ponds and was lowest at day two post-treatment (Figure 3.10). The diversity gradually increased up to six types of insects at the end of the observation period of 28 days, while there were eight types of insect at the pretreatment stage.

The initial insect diversity was higher in the case of cypermethrin treatment as compared to the deltamethrin because the experiment was conducted in the month of November (at the end of monsoon) when generally insect abundance and diversity are higher on the Indian sub-continent (Kashyap *et al.*, 2013; Nasiruddin *et al.*, 2014). Insect diversity with the deltamethrin treatment was low at pretreatment levels, which was during the month of March, and remained the same after day two of the treatment. One day after deltamethrin treatment, the insect diversity increased, possibly because insects were weakened due to the drug and came under catchment, whereas in pretreatment sampling many escaped catchments.

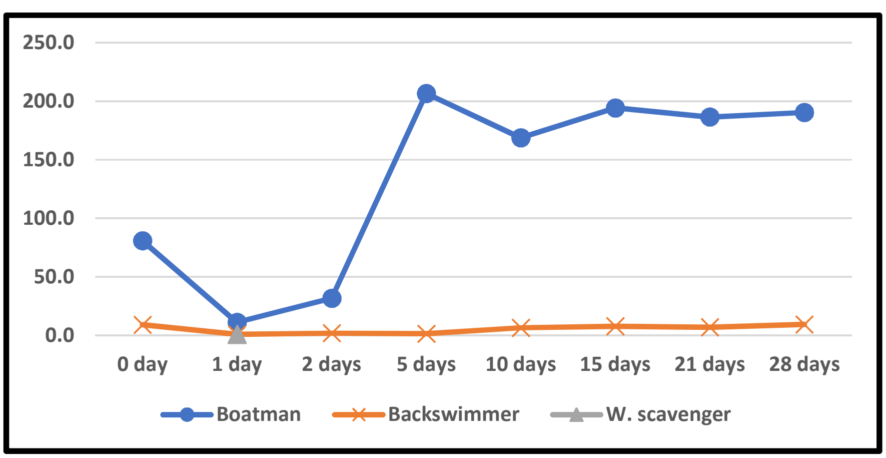


Figure 3.11. Aquatic insect count (nos/3 sq meter) at various points of deltamethrin treatment @ 5µg/liter of water in commercial aquaculture ponds.

One day after the use of deltamethrin, though the population of both boatman and backswimmer came down (Figure 3.11), the decline of backswimmer was steeper (92.59%) that that of the boatman (86.36%) population. The backswimmer population continued to be low until the 10th day after the treatment at which point it increased slowly. On the other hand, the boatman population sharply increased at day five after the treatment to levels beyond the pretreatment level and maintained those levels throughout the remaining sampling period. The drastic rise of boatman population pushed the total insect count higher from day five onwards after the deltamethrin treatment. Gutierrez (2016) found that the 72-hour LC_{50} values of *Buenoa tarsalis* and *Martarega bentoi* (backswimmers) to be 4.0 and 102.5 ng of active ingredient/liter for deltamethrin. Low insect diversity at just 2 major types, backswimmer's susceptibility to deltamethrin, and their relatively slow recovery, possibly gave boatman free space to thrive post treatment. The boatman population is considered less hazardous by the fish farmers compared to backswimmer in aquaculture ponds since backswimmers are predatory and boatman are non-predatory (Gonzalez and Leal, 1995).

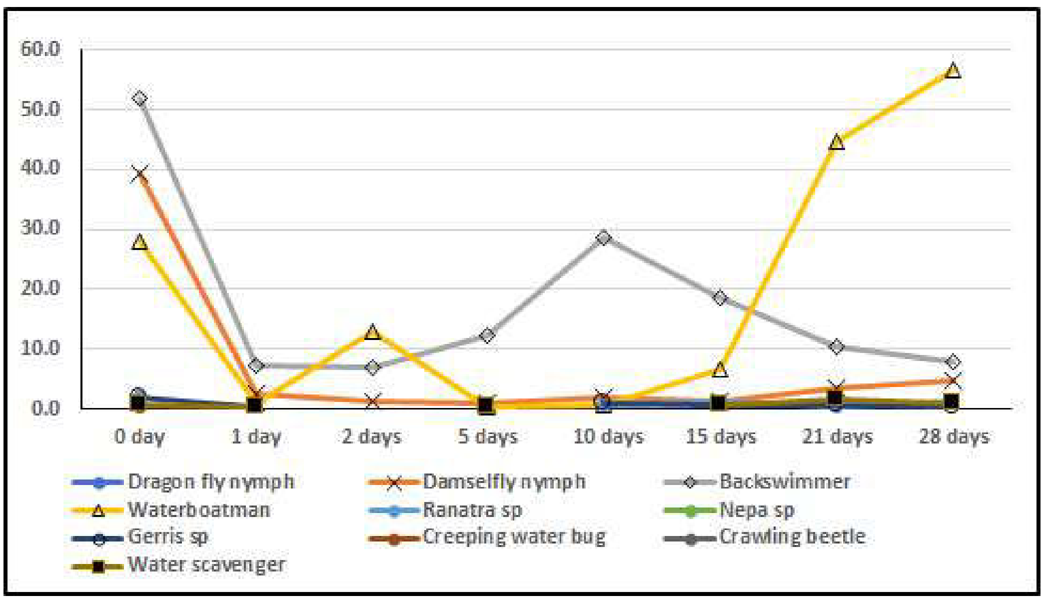


Figure 3.12. Insect count (nos/3 sq meter) at various points before and after the use of cypermethrin @ 5 µg (active ingredient) /liter in commercial aquaculture ponds.

Backswimmer, damselfly larvae and water boatman were the most dominant water insects in the pretreatment stage of cypermethrin experiment. The numbers of all of these insects were greatly affected with a decline of 93.22%, 85.90% and 96.42% respectively for damselfly nymph, backswimmer and boatman within 24 hours of the use of cypermethrin (Figure 3.12). The boatman count increased sharply 15 days after the treatment while backswimmer numbers started to decline from its high count at day ten after the treatment. All other types showed a slow recovery 15 days after the treatment, except *Nepa* sp., dragonfly nymph and creeping water beetle. *Nepa* sp. and dragonfly nymph were last found on day one after the treatment and did not show up for the remainder of the observation period. It can be deduced that the insects that take longer to reproduce were the most affected by the cypermethrin treatment. Saha and Kaviraj (2008) found the 96-hour LC_{50} value of *Rantra filiformis* is 0.06 µg/liter for cypermethrin. The 24-hour LD_{50} value for mayfly (Heptagenidae), damselfly (*Enellagma* and *Ishnura* sp.) and water scavenger beetle (*Hydrophilus* sp.) was 1.3, 1.4 and 8.3 µg/liter respectively for cypermethrin (Siegfried, 1993). Stephenson (1982) recorded a 24-hour LC_{50} value of adult *Corixa punctata* (water boatman) as ≥ 5 µg/liter for cypermethrin in the static test method in the laboratory. Since most of the insects prefer to live in shallow waters mostly at the surface and upper part of the water bodes and aquatic insects are seldom found at greater depths (Kashyap *et al.*, 2013), the manual spray of both cypermethrin and deltamethrin in this study that was done along the water's edge affected the water insects. However, the inconsistency in the mixing of the insecticides in the waterbody meant that some water insects were spared from being affected by the insecticides used and were able to reproduce in the later stages of the observation period.

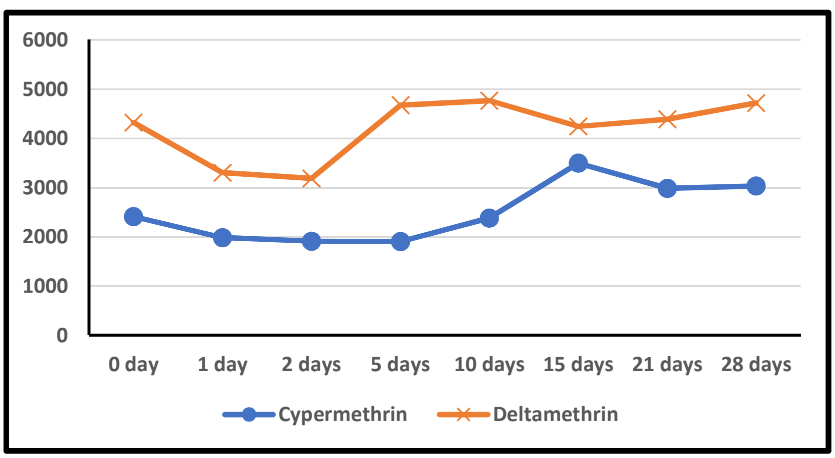


Figure 3.13. Zooplankton count (nos/liter) before and after the use of different insecticides in commercial aquaculture ponds.

The total zooplankton count came down to the lowest point after days two and five respectively for the deltamethrin and cypermethrin treatment (Figure 3.13). The decline of the zooplankton count was 26.33% at day two after the deltamethrin treatment, while 21.10% at day five after the cypermethrin treatment, compared to the pretreatment level. The zooplankton count exceeded the pretreatment level at days ten and five respectively for cypermethrin and deltamethrin treatment. The higher count of zooplankton for both treatments was then maintained until the end of the observation period 28 days after the treatment. The recovery of the zooplankton population was quicker and earlier in the deltamethrin treatment than in the cypermethrin treatment. Similar to the aquatic insects, the quicker recovery of the zooplankton population in deltamethrin treatment can be attributed to the water condition (higher turbidity, electrical conductivity and pH) of the treatment pond. Day (1991) found fenvalerate, deltamethrin and cyhalothrin toxicity for *Daphnia magna* decreased with the increase of dissolved organic carbon concentration in water. Compared to the aquatic insects, zooplankton were less affected by both the treatments, with a not as deep decline as the insects. (Figure-3.10 and 3.13). In addition to the water quality factor, the lower degree of predation from a lower number of aquatic insects may have triggered the quicker recovery of 5 days and 10 days for deltamethrin and cypermethrin treatments respectively for the zooplankton population.

Like the population count, zooplankton diversity was also not as affected by the cypermethrin and deltamethrin treatments. The count of *Asplanchna* sp., *Daphnia* sp. and *Filinia* sp. increased towards the end of the observation period 28 days after cypermethrin treatment; however, without any specific evidence it is hard to attribute that increased number to any specific factor. *Trichocerca* sp. is the only zooplankton that was found in small numbers in the pretreatment stage of cypermethrin experiment but was not found again in the post treatment observation period of 28 days. Lutnicka *et al.* (2014) recorded a 48-hour EC_{50} value for *Brachionus calycilforus* as 3.828 and 8.425ppm respectively for cypermethrin and deltamethrin. The 96-hour LC_{50} value of *Daphnia magna* was reported as 2 (1 to 5) µg/liter for cypermethrin in the static test method in the laboratory by Stephenson (1982) and 0.021 µg/liter for deltamethrin in the case of juvenile *D. magna* by Xiu *et al.* (1989). Beketov (2004) also reported similar toxicity (96-h LC_{50} value=0.0293 µg/liter) of deltamethrin for *D. magna*. Apart from the water quality factors, the mixing inconsistency of both cypermethrin and deltamethrin in the pond water along with its application along the water's edge left the majority of the zooplankton population not in contact with the pesticides and hence relatively unaffected.

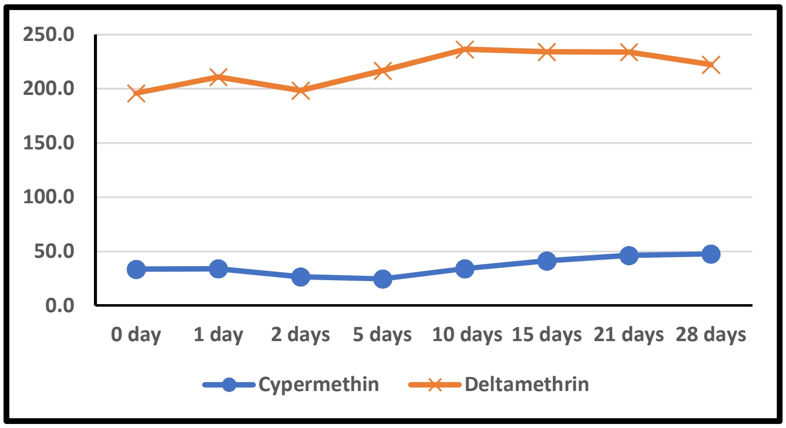


Figure 3.14. Benthos count (nos/6 sq inch bottom) before and after the use of different insecticides in ponds under commercial aquaculture.

The total benthos count showed insignificant change from their pretreatment levels in both the cypermethrin and deltamethrin treatments (Figure 3.14). The benthos count for both treatments remained mostly static until the end of the observation period 28 days after treatment. The higher organic carbon content in the sediment of the deltamethrin treatment ponds fostered a higher concentration of benthos population in the pretreatment stage as compared to the cypermethrin treatment ponds (App. Table 3.11).

Stephenson (1982) recorded a 24-hour LC_{50} value for *Chironomus thummi* as ≥ 5 µg/liter for cypermethrin in static test method in laboratory. Muir *et al.* (1985) reported lower bioavailability of pyrethroid pesticides including cypermethrin and deltamethrin in silt and clay sediments and water above those sediments. This was evidenced by a 5 to 15-fold higher bioaccumulation of pyrethroids by *Chironomus tenfans* larvae in sand sediments containing pyrethroids compared to than that of silt or clay sediments. High water turbidity (App. Table 3.9) of the experimental ponds indicates that the clay soil of the bottom, which is rich in organic carbon, has a very strong absorption capacity of cypermethrin and deltamethrin. This affected the bioavailability of the insecticides which left the benthos population unaffected in this study. Akerblom *et al.* (2008) confirmed a lower organic matter content of artificial sediments spiked with deltamethrin was highly toxic to *Chironomus riparius* larvae with the 28-day LC_{50} value at 11µg/kg sediments, while deltamethrin induced mortality @166 µg/kg was zero in natural sediments due to its high organic matter content.

Impacts of use of abamectin and lambda-cyhalothrin

After calculation it was found that abamectin and L-cyhalothrin was used @ 0.7 µg/liter and 0.5 µg/liter of pond water. No fish mortality was recorded in both treatments. Sanches *et al.* (2017) found the 48-hour LC_{50} value for adult zebra fish (*Danio rerio)* as 59 µg/liter for abamectin. The 96-hour LC_{50} values of rainbow trout, channel catfish and common carp (*Cyprinus carpio*) are respectively 3.6, 24 and 42 µg/liter (USEPA, 2019) for abamectin. Al-Khatani (2011) reported a sublethal concentration of abamectin for *Oreochromis niloticus* as 20 µg/liter.

However, for Lambda-cyhalothrin the 96-hour LC_{50} value of bluegill sunfish is 0.21 µg/liter, of rainbow trout 0.24 µg/liter (He *et al.*, 2008), of mirror carp (*C. carpio*) 0.5 µg/liter (Maund *et al.*, 1998), of juvenile tilapia (*O. niloticus*) is 2.901 µg/liter (Piner and Uner, 2012) and of *Labeo rohita* 2.1 µg/liter (Muthukumaravel *et al.*, 2013). In this study, both farmers had mostly Indian major carps with some *C. carpio* and silver carp in their ponds. The applied doses in this study were below the LC_{50} values mentioned earlier and hence no fish mortality was observed. The application method of the pesticides where it was broadcast along the water's edge and 7 meter towards the middle of the ponds, actually mixed the total amount of pesticides along the water's edge as opposed to in the middle, therefore the large portion of the water remain devoid of pesticides. The LC_{50} values were also calculated in laboratory testing, where the physico-chemical properties are different from the ponds, used in this study, particularly water turbidity and organic carbon content of the bottom sediment.

The water pH of both treatment ponds was around 8 (App. Table 3.14). Water turbidity was lower in the abamectin pond, but the organic carbon content of the bottom soil was higher. On the other hand, water turbidity was higher in the lambda-cyhalothrin treatment, but the organic carbon content was lower than the abamectin pond.

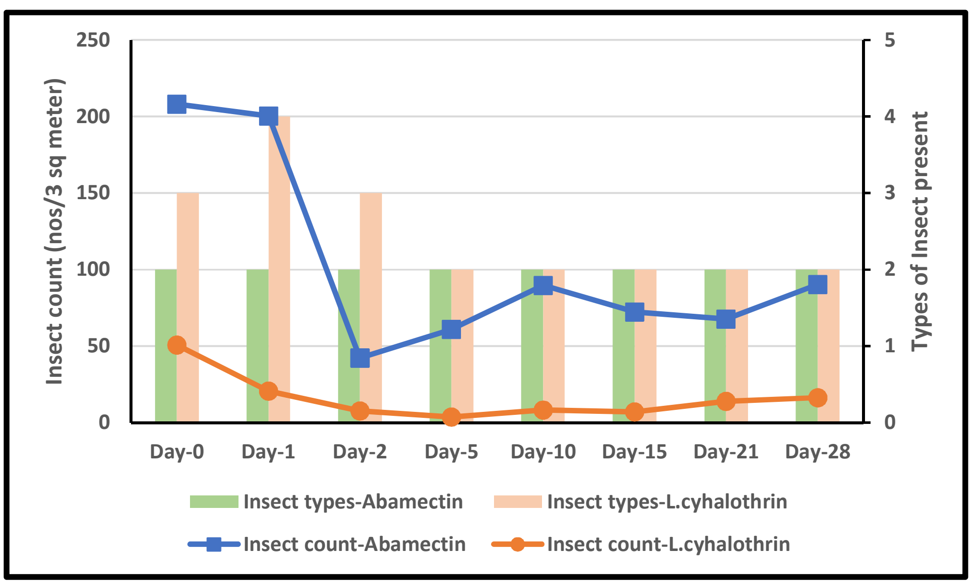


Figure 3.15. Total count of aquatic insects (nos/3 sq meter) and their diversity before and after the use of abamectin and lambda-cyhalothrin in commercial aquaculture ponds.

The aquatic insect count for the abamectin treatment was lowest with an 80% decline from the pretreatment level in day two of the treatment. On the other hand, in the lambda-cyhalothrin treatment, the lowest insect count was recorded on day five, showing a 93% decline from the initial count after the treatment (Figure 3.15). Aquatic invertebrates are more sensitive to lambda-cyhalothrin (Maund *et al.*, 1998) and abamectin (BPDB, 2019) than fish. The insect count from its lowest level for both treatments increased slowly but steadily until the end of the observation period but failed to reach the pretreatment level. Insect diversity in the abamectin treatment remained the same as the pretreatment level throughout the observation period. Insect diversity in the lambda-cyhalothrin treatment reduced to two from an initial three and did not reach the pretreatment level by the end of the observation period of 28 days.

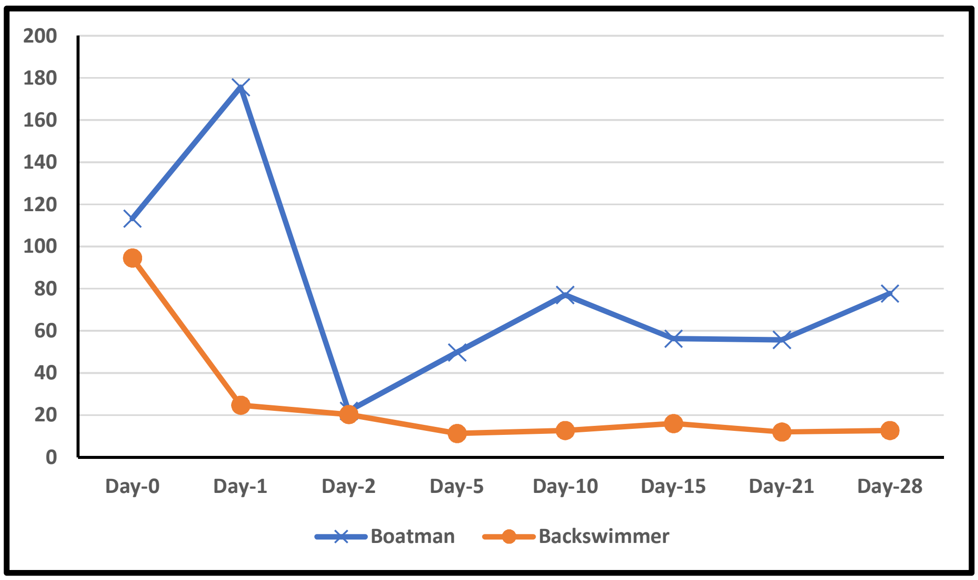


Figure 3.16. Aquatic insect count (nos/3 sq meter) at various points of abamectin treatment @ 0.7µg/liter of water in commercial aquaculture ponds.

In the abamectin treatment, the backswimmer population declined within the first day after the treatment while the boatman numbers increased in the first day after the treatment and then declined by 81% to its lowest point compared to the pretreatment level on day two after the treatment (Figure 3.16). The backswimmer population reached its lowest count with an 88% decline from the pretreatment level at day five after the abamectin treatment. From its post treatment lowest point, the boatman's increase in number was faster than the recovery of backswimmer, but the population count of both the insects failed to reach the pretreatment level by the end of the observation period 28 days after treatment. Ali *et al.* (1997) found a significant difference in Hemiptera nymph and coleoptera larval quantity pre and post treatment with abamectin @ 3.13 µg/liter spray on small man-made ponds, but with adults of the same insect population, the count was insignificant in between pre and post treatment even with 50 µg/liter of abamectin. Whereas in this study, a relatively lower dose (0.7 µg/liter) had successfully killed many aquatic insects, primarily due to the difference in application method where the pesticide was first mixed with sand and then broadcast across the pond, allowing better mixing across the water column and reduced photolysis. This is opposed to the application method used by Ali *et al.* (1997) of spraying a diluted mixture on the water surface which allowed greater photolysis and reduced mixing in the water column. The size difference of the water body between this study and the study done by Ali *et al.* (1997) also contributes to a difference in results. In this study, pesticides were broadcast on the water's edge of a 2-acre water area, leaving the vast middle part of the pond unattended and making the concentration much higher in the areas where it was broadcast. Twenty four (24) square meter pond was used for study by Ali *et al*. (1997). Also, water insects are more abundant along the water's edge where there is some vegetation (Marco *et al.*, 1999) and water depth is shallow (Kashyap *et al.*, 2013). That is where abamectin was applied in this study, contributing to the decline in aquatic insect numbers post treatment.

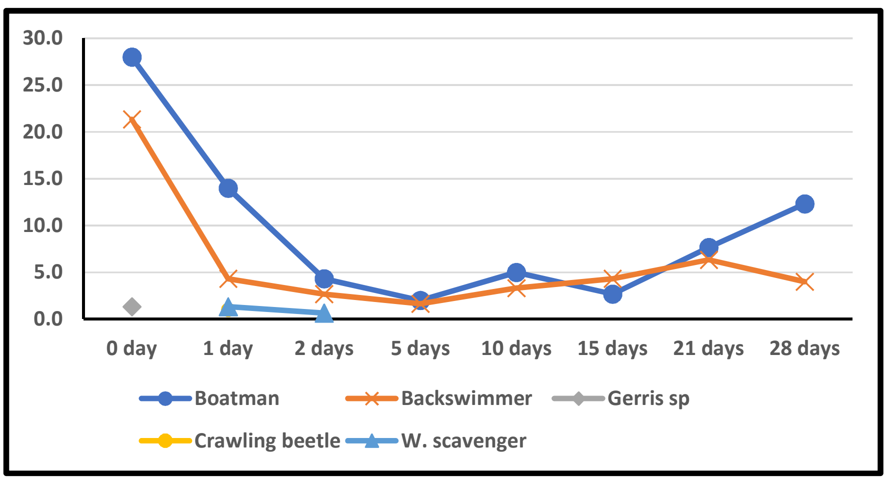


Figure 3.17. Aquatic insect count (nos/3 sq meter) at various points of lambda-cyhalothrin treatment @ 0.5µg/liter of water in commercial aquaculture ponds.

Of all of the insects, *Gerris* sp., crawling beetle, and water scavengers were most affected due to the lambda-cyhalothrin treatment (Figure 3.17). *Gerris* sp. was found in pretreatment but it was not found in the post treatment stage of lambda-cyhalothrin. Similarly, crawling beetle and water scavenger were found only after one day of the lambda-cyhalothrin treatment and not found from day two onwards. It is possible that they were only captured when they were weakened by lambda-cyhalothrin after the treatment. Boatman and backswimmer counts were the lowest with a 93% and 92% decline respectively from pretreatment level by day-five after the lambda-cyhalothrin treatment. Schroer *et al.* (2004) found that the 96-hour LC_{50} value of *Notonecta glauca* (backswimmer) was 16.4 ng/l, 34.6 ng/liter for *Caenis horaria* (mayfly) and the 48-hour LC_{50} value of *Sigara striata* (boatman) was 49.2 ng/liter for lambda-cyhalothrin in a short-term static laboratory test for toxicity. The effect of high water turbidity in the pond in this study was nullified by a similar application method of lambda-cyhalothrin as abamectin where water along the edges of the treatment pond received the total amount of lambda-cyhalothrin, resulting in an effective concentration of lambda-cyhalothrin along the water's edge that was much higher than the calculated average dose (0.5µg/liter) for the whole pond. This the treatment successfully killed most of the aquatic insects. However, due to the large volume of the pond water and inconsistency of mixing some insects were spared from being killed, in both treatments under this study.

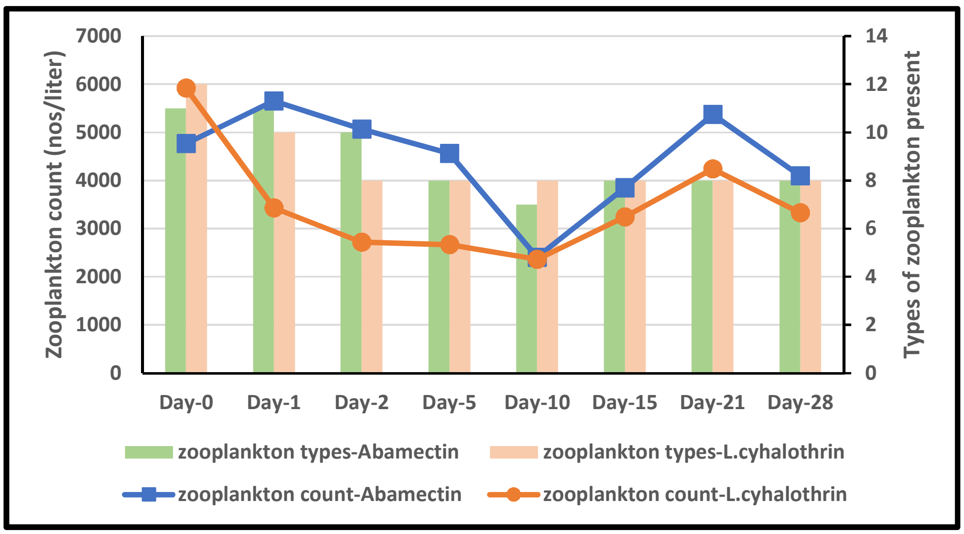


Figure 3.18. Zooplankton count (nos/liter) and diversity before and after use of abamectin and lambda-cyhalothrin in commercial aquaculture ponds.

The total zooplankton count for both abamectin and lambda-cyhalothrin treatment declined 49% and 60% respectively from the pretreatment level on day ten after the treatment (Figure 3.18). From its lowest point, the total count of zooplankton for both treatments increased. Zooplankton diversity also was the lowest, declined to 7 types from an initial 11 for abamectin, and to 8 from an initial 12 for lambda-cyhalothrin by day ten for both treatments (Figure 3.18). At the end of the observation period of 28 days, zooplankton diversity remained low compared to the initial diversity. The application method where the pesticide, in this case abamectin and lambda cyhalothrin, was mixed with sand and then broadcast along the water's edge of the pond , allowed a more homogeneous mixing of pesticides across the water column in the area of the pond where it was applied. The application method caused the sand particles to sink in the water column which in process washed the pesticide in water before the sand particle settled on the pond bottom. The spared of the chemical in the water column avoided the direct sunlight and reduced the chance of photolysis.

Ali and Baugh (2003) found that lambda-cyhalothrin is strongly absorbed primarily in the organic matter of soil, but not in silica. Despite the absorption issue, lambda-cyhalothrin is stable against hydrolysis at a pH below 8. Its photolysis half-life in water at pH 5 at 25^0C has been reported as 24.5 days and degradation half-life in aerobic soil requires 42.6 days (He *et al.*, 2008). Abamectin is also stable against aqueous hydrolysis, has a quick aqueous photolysis half-life of 1.5 days, a half-life in water sediment of 89 days, and possesses limited systemic activity as a pesticide (BPDB, 2019). Though abamectin absorbs strongly with suspended solids and soil surfaces, it is moderately persistent in the environment (USEPA, 2010). All of these characteristics of both abamectin and lambda-cyhalothrin along with their application method in this study has ensured its long endurance in aquaculture ponds under this study. Also, the application method ensured that a large portion of water in the middle part of the pond did not receive any pesticide. This spared the zooplankton in that part of the water body devoid of pesticides.

Of all the zooplankton types, zooplankton that were present relatively in lower numbers at pretreatment level namely *Daphnia* sp. *Moina sp*. and *Diaphanosoma* sp. were most affected in both treatments, in addition to *Diaptomus* sp. in lambda-cyhalothrin treatment, where all these species of zooplankton disappeared within two days of the treatment (App. Table 3.15). Wislocki *et al.* (1989) found that the 96-hour LC_{50} value of *D. magna* is 0.34 µg/liter for abamectin. The 48-hour LC_{50}value of *D. magna* is 0.5 to 2.9 µg/liter (Mokry and Hoagland, 1990) and *D. galeata* is 0.397 µg/liter for static exposure in lambda-cyhalothrin (Schroer *et al*., 2004). The count of *Filinia* sp. was nil on day ten after the abamectin treatment. *Cyclops* sp. declined 82% from the pretreatment level to its lowest level by day five of the abamectin treatment. *Brachionus* sp., *Keratella* sp., *Polyarthra* sp., *Asplanchna* sp., *Trichocerca* sp. and nauplius declined 29, 47, 30, 40, 58 and 52% respectively to reach their lowest level from their pretreatment level after 10 days of abamectin treatment. *Brachionus* sp., *Polyarthra* sp. and nauplius declined by 42, 40 and 56% respectively from their initial count to their lowest count on day two and *Filinia* sp. declined by 86% from its initial number on day five after the lambda-cyhalothrin treatment. *Cyclops* sp., *Trichocerca* sp., *Filinia* sp. and *Keratella* sp. declined 98, 95 86 and 63 % respectively from their pretreatment level to reach their lowest level on day 10 after the lambda-cyhalothrin treatment.

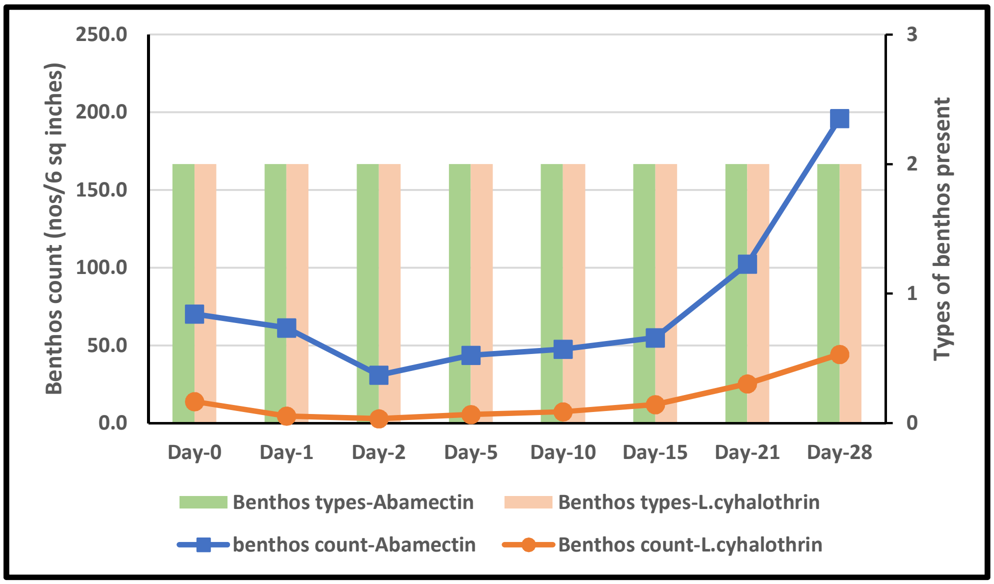


Figure 3.19. Benthos count (nos/6 sq inch bottom) before and after the use of abamectin and lambda-cyhalothrin in commercial aquaculture ponds.

The total benthos count was lowest with a 56% and 79% decline respectively from their pretreatment level on day two for abamectin and lambda-cyhalothrin treatment (Figure 3.19). Benthos diversity did not vary between pre and post treatment for both abamectin and lambda-cyhalothrin (Figure 3.19). A relatively lower mortality of benthos than the insect mortality of 93% in lambda-cyhalothrin treatment is consistent with the findings of Maund *et al.* (1998) that showed the risk of lambda-cyhalothrin toxicity to sediment dwelling organisms is lower than the organisms living in the water column in the aquatic system. The application method of both treatments where they were applied within a sand mixture into the pond ensured that suspended soil particles that had absorbed abamectin and lambda-cyhalothrin settled in the shallow zones of the pond where benthos sampling was done.

The count of chironomid larvae and tubifex declined 51% and 74% respectively to reach its lowest level after day two of abamectin treatment. On the other hand, the count of chironomid larvae and tubifex declined 95 and 78% from their pretreatment level to reach their lowest level after day two of lambda-cyhalothrin treatment (App. Table 3.16). The numbers of both types of benthos in both treatments kept increasing from their lowest points until the end of the observation period. Novelli *et al.* (2012) found the 96-hour LC_{50} value for *Chironomus xanthus* is 2.67 µg/liter for abamectin. The chronic 28-day NOEC of *Chironomus riparius* for lambda-cyhalothrin is 0.16 µg/liter of water (PPDB, 2019). The actual doses in the water's edge of the pond based on the part of the water area of the ponds under this study where the farmer broadcast the pesticides mixed with sand would be several times higher than the calculated average dose (0.7 and 0.5 µg/liter for abamectin and lambda-cyhalothrin respectively) for each of the pesticides for the pond's water area, causing the mortality of the benthos population as well.

Impacts of use of rotenone, aluminium phosphide and fenpropathrin

Based on back calculation, it was found that rotenone, aluminium phosphide and fenpropathrin were used at 0.272ppm, 0.61ppm and 0.065ppm respectively in the ponds under this study. Water temperature was around 30^0C for all ponds during the time of fish toxicants use.

Rotenone concentration of 0.272ppm was strong enough to kill and harvest all the carp species in this study but not strong enough to kill the predatory species *Channa punctatus*. Rotenone concentration of 2.5ppm is required to achieve 100% kill of *C. punctatus* within 24 hours (Perschbacher and Sarkar, 1989). Within one week after the harvest, the ownership of the pond where rotenone study was being conducted was changed and the new owner used phostoxin (aluminium phosphide @ 0.86ppm) to ensure the killing of all undesired fish. As a result, the *C. punctatus* that successfully survived the rotenone treatment were observed dead and floating on the water.

The 96-hour LC_{50} value of *Oncorhynchus mykiss* is recorded as 0.0097ppm for aluminium phosphide by IUPAC (2018), but Perschbacher and Sarkar (1989) required a concentration of 0.25ppm of aluminium phosphide to obtain 100% kill of *C. punctatus* within 24 hours. The doses exercised (0.61ppm) in this experiment were higher than the doses mentioned above and were effective to kill all of the Indian and Chinese carps.

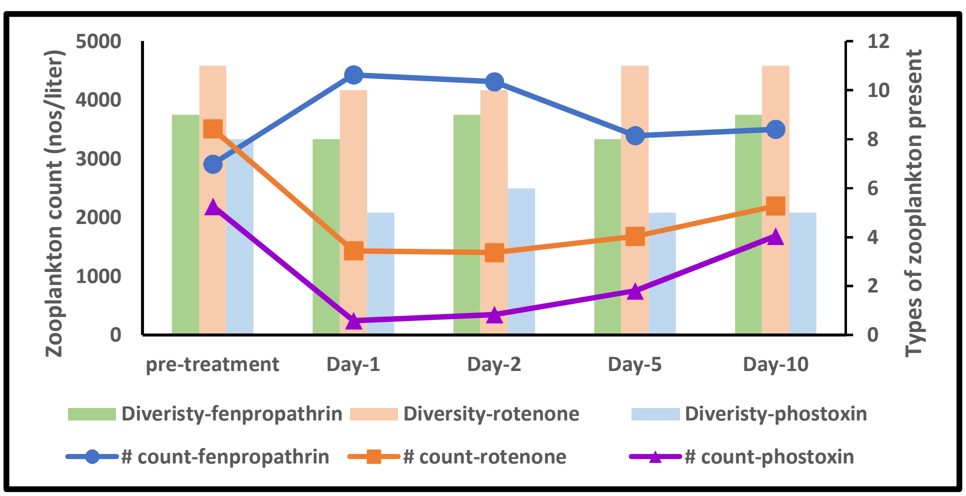


Figure 3.20. Total count (nos/liter) of zooplankton and diversity before and after use of different fish toxicants in commercial aquaculture ponds.

The zooplankton total count (nos/liter of water) increased 52% at the 24 hours point after the use of fenpropathrin @0.065ppm, which was not anticipated, while the zooplankton total count (nos/liter of water) declined by 59% and 89% respectively at the 24 hour point after the use of rotenone @0.272ppm and aluminium phosphide @ 0.61ppm (Figure 3.20). From days 2 to 10 after fenpropathrin treatment, the zooplankton count gradually declined, but after the rotenone and aluminium phosphide treatment, the zooplankton count gradually increased. Like the zooplankton count, zooplankton diversity, regarded as the types of zooplankton observed, was affected in the phostoxin treatment where the diversity count was reduced to five after 10 days of the treatment from an initial count of eight. Given that during the experimental period no fish feed was supplied, and there were no fish released, there was neither additional nutrient supply nor predation from fish, which let the zooplankton population grow and survive after the initial blow of fish toxicant use. This gradual increase in population was mostly determined by the existing nutrient supply and physico-chemical factors of the pond.

The 48-hour LC_{50} value of *Dhaphnia magna* for fenpropathrin is 0.53 ppb (PMEP, 1989) whereas for aluminium phosphide 48-hour EC_{50} value of *D. magna* is 0.37ppm (IUPAC, 2018). Given fenpropathrin's extreme toxicity to aquatic organisms (Valent, 2009; Chemwatch, 2012), the influx of zooplankton count in the fenpropathrin experiment was quite surprising. The explanation for this unusual zooplankton response can be found in the water turbidity data of the treatment pond.

In the fenpropathrin pretreatment stage, the water turbidity of the treatment pond was (App. Table 3.19) was higher than the other treatment ponds especially than rotenone pond, due to prior netting. Several times of netting immediately after the use of the fish toxicants to harvest fish increased the pond water turbidity two to three-fold than the pretreatment stage. Due to fenpropathrin's strong ability to be absorbed into the soil, it is very resistant to leaching and there is no risk of ground water contamination in normal circumstances (ARNICA and AWHHE, 2014). Fenpropathrin's nonpolar nature results in very low water solubility, causing it to be absorbed strongly to organic matter and soil in order to avoid contact with water Kanawi *et al.*, 2013). The high turbidity of the pre and post treatment pond water due to excessive netting of fish resulted in the binding of fenpropathrin with the soil particle at a greater degree and rate, as compared to the aluminium phosphide treatment. It is assumed that this made the fenpropathrin unavailable in the pond water in the short term and resulted in relatively quick detoxification of the water. This was tested by releasing a few fish into the pond on a test basis 4 days after the treatment. Day (1991) also observed a 20 to 80% reduction in pyrethroid induced mortality of *Daphnia magna* due to the presence of humic material. Due to the application method where the farmer broadcast fenpropathrin along the water's edge the large middle portion of the waterbody did not receive any fenpropathrin.

The 24-hour LC_{50} value of *Daphnia pulex* and *Diaptomus siciloides* for rotenone was <0.025ppm (Hamilton, 1941). Despite the use of higher doses of rotenone (0.272ppm) and aluminium phosphide (0.61ppm), the zooplankton population declined but did not get wiped out completely. Due to the large size of the pond (1 acre for rotenone treatment, 4.5 acres for aluminium phosphide treatment, and 2 acres for fenpropathrin treatment) and the sheer volume of water, the mixing of all the fish toxicants (rotenone, aluminium phosphide and fenpropathrin) in pond water was surely not homogeneous. Despite several times of netting for catching fish, the inconsistency in mixing left a blank space in the water body where some of the zooplanktons (due to their slow-moving nature) never encountered the fish toxicants. Fast moving fish encountered the toxicants and were killed.

The presence of heat, light and oxygen makes rotenone unstable and it also is easily absorbed by sediment and suspended soil particles in the water (Ling, 2003). Gilderhus *et al.* (1988) found rotenone loss to be 10 times faster at temperatures above 23^0C than at 1^0C in shallow ponds, and the half-life was generally less than 1 day in natural water at temperatures above 20^0C. Absorption of rotenone by the suspended soil particles in the water and the faster decay of rotenone helped the zooplankton population in the experiment to recover relatively faster than the zooplankton population in the aluminium phosphide treatment.

The zooplankton composition in the fenpropathrin experiment (App. Table 3.20) shows that the influx of *Brachionus* sp., *Keratella* sp. and *Asplanchna* sp. were the main reasons for the increase of the total zooplankton count after one day of fenpropathrin use. Zooplankton diversity (genus count) remained the same after fenpropathrin's use, although the genus composition was different. Of all zooplankton types, *Moina* sp. and *Diaptomus* sp., where were less abundant at pretreatment level, were most affected. These disappeared after the use of fenpropathrin and did not return until the end of the 10-day observation period. On the other hand, *Polyarthra* sp. and *Filinia* sp. were not found at pretreatment sampling but were present after one day and two days respectively with the use of fenpropathrin. It is most likely that they were present even in the pretreatment stage but due to their lower number they were not presence in the sampling.

In the aluminium phosphide experiment (App. Table 3.20), within the zooplankton composition *Moina* sp. and *Diphanosoma* sp. were affected the most. They disappeared after the treatment and did not return until the end of the observation period10 days after the treatment. *Brachionus* sp., *Cyclops* sp., nauplius, *Diaptomus* sp. and *Asplanchna* sp. declined sharply in number (92, 90, 82, 82 and 96% respectively) after one day of the aluminium phosphide treatment. The abundance of *Cyclops* sp., nauplius, *Diaptomus* sp. and *Asplanchna* sp. recovered to the pretreatment level by the end of the observation period but the *Brachionus* sp. count remained low.

For the zooplankton composition (App. Table 3.20) in the rotenone treatment, it was observed that *Moina* sp. disappeared completely one day after the treatment but was present again two days after of the treatment. *Brachionu*s sp., *Keratella* sp., *Cyclops* sp., nauplius, *Polyarthra* sp., *Diaptomus* sp., *Asplanchna* sp., *Daphnia* sp. and *Diaphanosoma* sp. declined 42, 87, 80, 40, 37, 75, 72, 55 and 85% respectively one day after rotenone's use. The zooplankton population showed continuous recovery within the observation period of 10 days.

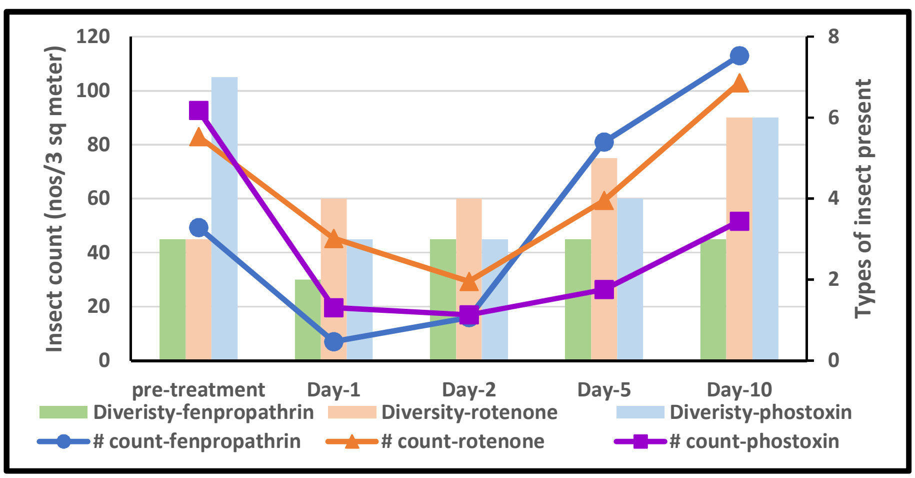


Figure 3.21. Total count (nos/3 sq meter) and diversity of aquatic insects before and after the use of different fish toxicants in commercial aquaculture ponds.

The total insect count in the fenpropathrin and aluminium phosphide treatment was the lowest one day after the treatment, declining 86 and 82% respectively from the pretreatment level. The lowest total insect count with a decline of 64% from the pretreatment level was found on day two after the rotenone treatment (Figure 3.21). Insect numbers reached back to the pretreatment level within five days of fenpropathrin treatment, compared to within 10 days in case of rotenone treatment. The recovery in insect count was slowest in the case of aluminium phosphide treatment and fastest in fenpropathrin treatment. In fact, the insect count did not reach to the pretreatment level for aluminium phosphide within the observation period of 10 days. Due to the inconsistent mixing in the water column, the quick decay of rotenone due to high water temperatures at around 35^0C, and the strong absorption of fenpropathrin by the suspended soil and humic particle in the pond water, the toxicity of rotenone and fenpropathrin was reduced more quickly than the toxicity of aluminium phosphide. Insect diversity (genus count) also came down from seven at pretreatment level to three one day after aluminium phosphide treatment. From day five of the aluminium phosphide treatment, the diversity started to increase. The diversity count reached to six on day 10 at the end of the observation period. Insect diversity increased after the rotenone treatment from three at the pretreatment level to six on the 10^{th} day after the treatment. Crawling beetles and tadpoles showed up towards the end of the observation period for the rotenone treatment but were not present at the pretreatment stage.

For the insect composition in the fenpropathrin experiment (App. Table 3.21), it was observed that boatman declined by 88% and backswimmer and *Gerris* sp. were absent one day after the fenpropathrin treatment. From day two after the fenpropathrin treatment, both the boatman and backswimmer count started to increase. The insect count at the end of the observation period was dominated by boatman, although backswimmer numbers increased slightly. *Gerris* sp. disappeared after the fenpropathrin treatment and never returned. As *Gerris* sp. is a fast-moving surface dweller and fenpropathrin was broadcast on the surface of the water, *Gerris* sp. became a victim of the toxicity of fenpropathrin before the pesticide became absorbed by the suspended soil and humic particle. *Nepa* sp. was another insect that was only found at day one after the treatment in a semi-dead condition and then was not present again afterwards. The timing of half-dead *Nepa*'s catch in the fenpropathrin treatment indicated that the bottom dwelling *Nepa* sp. was affected from the sediments which absorbed fenpropathrin from the water column and settled on the bottom. Insect diversity at the pretreatment (App. Table 3.21) and at the end of the observation period remained the same due to the appearance of crawling beetles from day two of the observation period. Given that this beetle can fly, it is not impossible for it to arrive from an adjacent pond, because during first two sampling (pretreatment and day one) it was not present.

Of all of the insects observed in aluminium phosphide treatment, the dragon fly nymph, which has a long reproductive cycle, was the most affected. It disappeared due to the treatment and did not appear again within the observation period (App. Table 3.21). Backswimmers also disappeared after the treatment but showed up again on 10th day after the aluminium phosphide treatment. Boatman numbers decreased drastically with an 81% decline the pretreatment level by the 2nd day after treatment. They slowly, along with *Gerris* sp. and damselfly, in the aluminium phosphide treatment. Water scavenger and crawling beetle were not significantly affected in the aluminium phosphide treatment and in fact their numbers slowly but steadily increased until the end of the observation period.

In the rotenone treatment (App. Table 3.21), the backswimmer numbers gradually declined to 77% from the pretreatment level up until day five after the treatment, then increased within the observation period of 10 days after treatment. During this time, they did not reach the pretreatment level. The 24-hour LC_{50} value of backswimmer for rotenone was recorded as 0.1ppm by Hamilton (1941). In contrast to backswimmer, the number of boatmen kept increasing after the treatment and reached the highest count at the end of the 10-day observation period. *Gerris* sp. was not affected with the numbers remaining low but steady throughout the observation period.

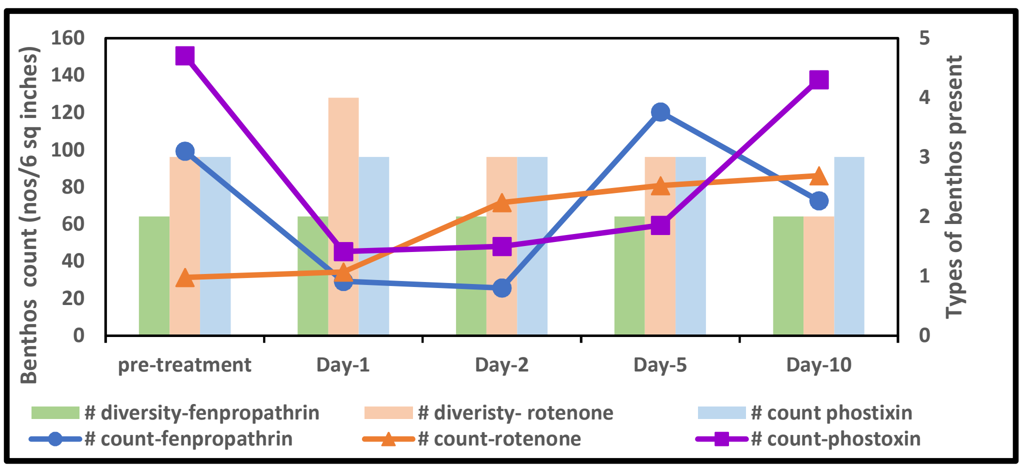


Figure 3.22. Total count (nos/6 sq inches) of benthos before and after the use of different fish toxicants in commercial aquaculture ponds.

Unlike the rotenone treatment (Figure 3.22), one day after the use of fenpropathrin and aluminium phosphide, the benthos count declined sharply (74% and 70% respectively), signifying the toxicity of those two toxicants to the benthos population. The benthos population count exceeded the pretreatment level within the first five days after the fenpropathrin treatment, but did not sustain the number, showing a decline at day 10 after the treatment. The recovery of the benthos population took the longest in the aluminium phosphide treatment. On the other hand, the benthos population in the rotenone treatment continuously kept increasing until the end of the observation period, indicating no negative impact of rotenone on the benthos population. The diversity count for benthos in the case of all three treatments was found not to be affected.

In the fenpropathrin treatment, (App. Table 3.22) both the chironomid larvae and tubifex count were in synchrony with each other at different sampling point. An influx of both chironomid larvae and tubifex (mostly young and small) were observed at day five after the treatment from their lowest count at day two of fenpropathrin treatment. The population count for all of the young chironomid and tubifex did not sustain due to the leftover toxicity of the pond sediments that absorbed the fenpropathrin. Hence many of them died, resulting in a lower population count for both chironomid larvae and tubifex (regular size) at day10 after the fenpropathrin treatment. The response of the benthos population to the fenpropathrin treatment are in alignment with ARNICA and AWHHE's (2014) characterization of fenpropathrin as moderately toxic to earthworms. Also, tiny snails showed up on the 5th day after the treatment and their numbers were seen to increase on day 10 of the observation period, while in the pretreatment stage they were not found. In commercial aquaculture ponds in northwest Bangladesh, farmers always rear some black carp along with other cultured carp species for the biological control of snails, but the absence of snail eating fish may have led to the presence of snails.

The leech was most affected in the aluminium phosphide treatment. It disappeared after the treatment and did not return within the observation period. The chironomid population in the aluminium phosphide treatment (App. Table 3.22) was lowest with a 96% decline from the pretreatment level on day two after the treatment. However, it was present again on day five after the treatment and exceeded the pretreatment level by day 10 of the observation period. On the other hand, the tubifex population declined to a lesser extent (47% decline on day one) than the chironomid larvae. It appeared to stabilize in the middle of the observation period but declined again towards the end to score the lowest count at day 10 after the aluminium phosphide treatment. This is contrast with chironomid larvae with showed an influx by day 10 (App. Table 3.22). Polychaetae numbers were very stable during the post treatment stage until the end of the observation period, although they were absent in the pretreatment level possibly due to sampling error. Like the fenpropathrin treatment (not shown in the App. Table 3.22) many tiny snails were observed on the 10th day after aluminium phosphide treatment. The absence of snail eating black carp allowed the snails to reproduce.

The benthos population composition in rotenone treatment showed that only the leech was impacted by the use of rotenone (App. Table 3.22). The 48-hour LC_{50} value of leech for rotenone was recorded as <0.1ppm by Hamilton (1941). The leech was found at pretreatment sampling and after one day of rotenone's use, but from days two to ten after the treatment, the leech was not found. The tubifex population count increased drastically on day two after the treatment and the high number continued until the end of the observation period. The count for chironomid larvae slowly increased towards the end of the observation period. from the pretreatment level with rotenone

Fish killed by farmers using fish toxicants in commercial fisheries in northwest Bangladesh are sold in the market for food. Therefore, there is always a concern about the food safety of the fish killed using a fish toxicant. For fenpropathrin the maximum residue level (MRL) value is not set for fish/meat by the European Commission of EU, but the fenpropathrin MRL for all other food items except citrus fruits is set at 0.01ppm (EC, 2019). The acceptable daily intake (ADI) level was set by the joint FAO/WHO meeting on pesticide residues (JMPR) as 0 to 0.03ppm body weight, and acute reference dose (ARfD) was also set as 0.03ppm (Shah and McGregor, 2012). Al-Makkawy and Madbouly (1999) found the bioconcentration factor of fenpropathrin for *Oreochromis nilotica* heads and flesh to be respectively 130 and 7 respectively at 3 days after the treatment @1µg fenpropathrin/liter of water. The high bioconcentration factor of 225 for fenpropathrin in fish (Giddings and Campasino, 2007) indicates that based on the doses of fenpropathrin (@ 65 µg/liter) used in this study this would mean a residue level of 14.625 mg fenpropathrin per kilogram of fish theoretically, which is much higher than the MRL value (0.01ppm) for fenpropathrin. Given the concentration of fenpropathrin in this study, if a child weighing 20kg consumes 100 g of such fish then the intake of fenpropathrin will be 0.07ppm, exceeding the ARfD value set by JMPR. However, given the high toxicity of fenpropathrin to fish, farmers could use lower doses than what was used in this experiment and still find fenpropathrin effective for killing fish especially in the winter since fenpropathrin is more toxic in cold water than warm water (Kanawi *et al.*, 2013). Using a lower dose would leave fish with lower residue level of fenpropathrin.

The MRL for rotenone is set as 0.01ppm for almost all food items by the European Commission of the EU (EC, 2019). BPDB (Bio-Pesticide Database) (2019) recorded a bioconcentration factor of 26 for rotenone than the ambient condition where ADI value was not available. A relatively lower bioconcentration factor for rotenone compared to fenpropathrin when used with fish and no set ADI value by the regulators indicates rotenone's relative safety as a fish toxicant even when fish is intended for human consumption. On the other hand, the bioconcentration factor for aluminium phosphide has been reported as low risk by IUPAC (2018). Although there is a set ADI of 0.019ppm and ARfD of 0.032ppm (IUPAC, 2018), given the low bioconcentration factor for fish, the use of aluminium phosphide as a fish toxicant for fish used for human consumption may mean fewer health consequences. Rahman *et al.* (1992) also determined that fish killed with the use of rotenone and aluminium phosphide are suitable for human consumption.

Perschbacher and Sarkar (1989) found aluminium phosphide at the required concentration of 0.25ppm to be the most inexpensive among the following fish toxicants: rotenone, sumithion, phyphanon, aluminium phosphide, DDVP and bleaching powder, where the cost of the organophosphorus pesticides () was deemed to be prohibitive for the commercial fish farmers' us. Fenpropathrin was not included in that study. The combination of fenpropathrin' s toxicity to fish and its low cost, similar to or slightly lower than aluminium phosphide per unit area of water body, has removed the economic barrier preventing the use of pyrethroids as fish toxicant. Moreover, it's using convenience allowing farmers to obtain a quick kill (within an hour after use) hence convenient regardless of ecological consequences and serious health concerns.

Significant factors such as ease of application, short duration of toxicity, no risk of future inhibition of the production potential of the pond, and low price dictate the farmers' choice of fish toxicants (Lennon *et al*., 1970). This means fenpropathrin is the top choice for use as a fish toxicant in northwest Bangladesh but rings an alarm for the fish consumer due to severe health concern. Therefore, it demands to be strongly regulated to be used as a fish toxicant for fish used for human consumption.

Impact of testosterone feeding on tilapia serum testosterone level

The average testosterone level in a male adult *O. niloticus* in a normal population was 7.3 ng/ml of blood serum, while in the female, the value was 1.95 ng/ml. As the distinct feature of the testosterone level for both males and females are high internal variability, the highest value of testosterone in the normal male population was 26.4 ng/ml, while the lowest was 0.6 ng/ml. For females in the normal population, the range was 0.01 to 10.3 ng/ml (App. Table 3.23).

The average testosterone level (4.87 ng/ml) of male individuals of sex inversed population was lower (though non-significant, P= 0.2648 at t test) than the normal population with high internal variability like the normal population (App. Table 3.23). Compared to only 45% in the normal population, a total of 68% of individual males in the sex inversed population was inclusive of many of the sex inversed phenotypic males which are genetically female, resulting in a higher number of males with poorly or less developed testis and lower level of testosterone production. The range of testosterone level varied from 0.09 to 36.8 ng/ml. The average testosterone level (1.33 ng/ml) of female individuals of sex inversed population was also lower (also non-significant, P=0.4041 at t test) than that of the normal population with high internal variability. The testosterone value for females in the sex inversed population ranged from 0.01 to 3.87 ng/ml. Wahbi and Shalaby (2010) reported significantly decreased plasma testosterone levels in six-month-old sex inversed males of *O. niloticus* compared to the control. While Khalil *et al.* (2011) found significantly different levels of serum testosterone in methyltestosterone treated males and the untreated control group of *O. niloticus*, testosterone levels were higher in treated males in most months during observation except for a couple of months where testosterone levels were several times higher in the untreated control group.

In this study, the sampling was done over the course of four months from May to August. The range of timing of the sampling could be the reason for the high level of variability of testosterone levels in the group. Month to month variation of plasma testosterone level in *O. mossambicus* has also been reported by Cornish (1998). Varying testosterone levels in *O. niloticus* in different months has also been reported by Khalil *et al.* (2011). The high variability of serum testosterone levels in this study may also be attributed to the size of the fish. Cornish (1993) found the inverse relationship between size and plasma testosterone level in *O. mossambicus*, meaning the larger specimen have a relatively lower plasma testosterone level.

Ideally, sex inversed populations are expected to have all male individuals; however, since the samples of this study were collected from the market, there was no control by the researcher of the sex inversion process. A low male percentage of 68% indicates that when sex inversion was done commercially, it was not done properly. Contrary to the normal population, the sex inversed population consisted of 68% males and 32% female, although 10% of the fish categorized as female had poorly developed ovaries that were not ready or suitable for breeding and thus, this part of the population was functionally serving as sterile individuals. Also, only 10% of the fish of the sex inversed population that were categorized as female had well developed ovaries. Unlike the normal population, a total of 30% of the fish (both male and female) in the sex inversed population had poorly developed testis and ovaries. This could be the result of an interaction between poor masculinization and the expression of inherent genetic characters. Macintosh *et al.* (1988) reported testicular degeneration in tilapia when fed with 17-alpha methyltestosterone at the rate of 60ppm of feed at the early developmental stage for sex reversal. A similar phenomenon was evident in the decreased Gonado Somatic Index (GSI) for both males and females treated with high methyl testosterone doses of 5 to 40ppm of feed (Ahmed *et al.,* 2002).

The positive correlation between serum testosterone level and the appearance of sex organs in a normal male (r value 0.72) was stronger than that for the males (r value 0.55) in sex inversed population. However, the correlation between serum testosterone level and the appearance of sex organs in females of the normal population was weaker (r value 0.36) than that for the females (r value 0.64) in the sex inversed population of *O. niloticus*.

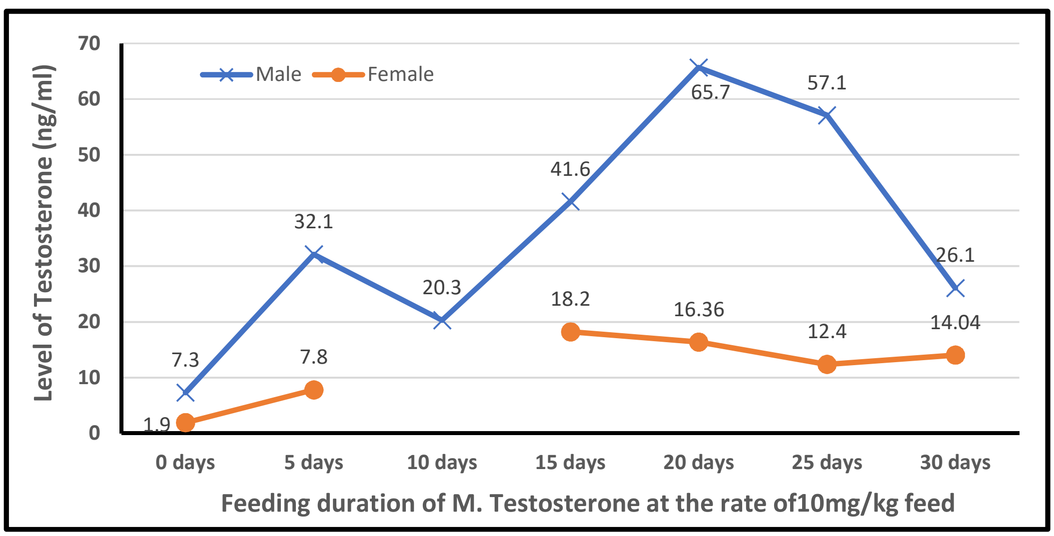


Figure 3.23. Testosterone level (ng/ml) in Adult *O. niloticus* serum when fed with methyltestosterone at the rate of 10mg per kg feed as a growth promoter.

The serum testosterone level of both male and female tilapia increased with the feeding of methyl testosterone as a growth promoter (Figure 3.23) when used at a lower dose (10ppm) compared to is the dose used (60ppm) in the sex reversal process. Since at each sampling different individuals were collected from the treatment pool on different days, the average level of serum testosterone was determined from different individuals. Therefore, it was not possible to determine the change pattern in testosterone in one individual fish. However, this graph gives a general idea of the increase of testosterone level in the treated population. During the feeding stage, the highest level of blood serum testosterone in male tilapia was 70.8 ng/ml on the 20^{th} day of feeding, compared to the highest value of 26.4 ng/ml found in males of the normal population.

Because the sex of sampled fish was confirmed by observing the difference in genital openings and the fish were not dissected for confirmation of sex after the collection of blood samples, it is possible there are errors in the identification of sexes. Males and females may have been labeled as the opposite, which would impact the value presented in the graphs, based on its grouping as male or female. Therefore, the overall trend of higher testosterone level is more important than the specific level of testosterone at any certain point of time in Figures 3.23 and 3.24.

While it was expected that the level of serum testosterone would increase when methyltestosterone was fed to the treated group, some unexpected behavior was observed. Generally, it is hard to get free ranging male and female tilapia to breed naturally when put in captivity or confinement. However, in this experiment though the previously free ranging adult male and female tilapia were put in confinement in the *hapa*, due to the low dose (10ppm) feeding of testosterone, the fish bred frequently. As a result, during each sampling many of the hatchlings had to be taken out of the treatment pool and out of the mouths of the females. This phenomenon continued even after the end of the feeding of the hormone. This unusual and increased mating activity may have positively influenced the serum testosterone level of the treated males and females. Significant increase of testosterone level in a normal male *O. niloticus* has been attributed to males paring with females during mating season by Khalil *et al.* (2011). On the other hand, Smith and Haley (1988) recorded high plasma testosterone in female mouthbrooders (*O. mossambicus*) in the latter half of the brooding period while testosterone level dropped at the end of brooding.

Serum testosterone levels in adult *O. niloticus* were high until the end of the observation period, one month after the feeding of methyltestosterone was stopped (Figure 3.24). There was no indication of a reduced serum testosterone level even at the end of the observation period one month after stopping the methyl testosterone feeding. The highest level of serum testosterone in females was 40.3 ng/ml, five days after stopping the feeding of methyltestosterone compared to the highest testosterone value of 10.3 ng/ml in females of the normal population. Since it takes several months for the plasma testosterone levels to come back to normal in sex inversed male tilapia (Wahbi and Shalaby, 2010; Rizkalla *et al.*, 2004; and Khalil *et al.*, 2011), it seems in the adult population where methyltestosterone is used as a growth promoter, the treatment resulted in a high serum testosterone level that will take several months to reach the normal level. However, this increase in testosterone level cannot be attributed only to the feeding of methyl testosterone as a growth promoter. External factors such as seasonality (Khalil *et al.*, 2011) and breeding cycle (Smith and Haley, 1988) have an effect on tilapia's testosterone level as well.

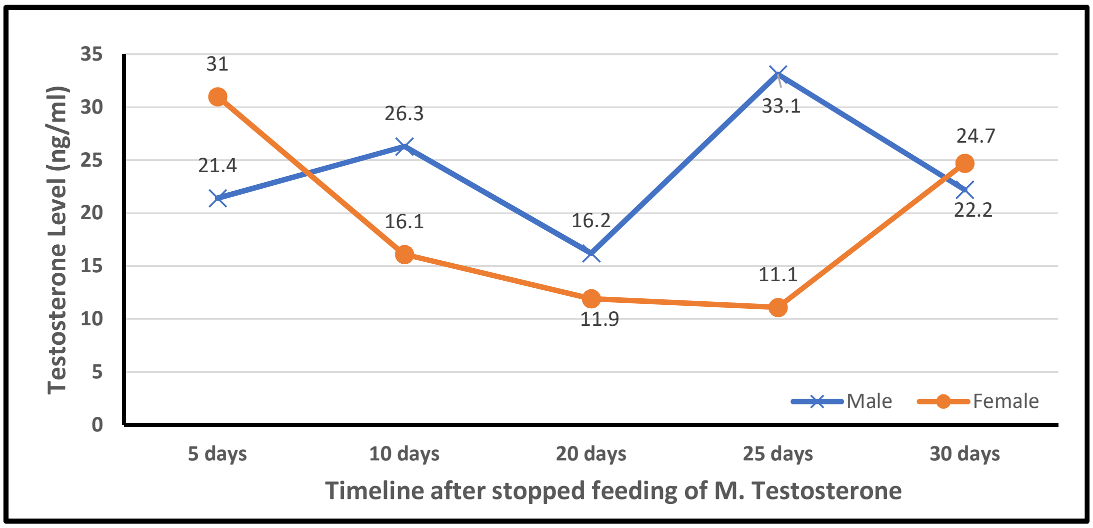


Figure 3.24. Serum testosterone levels over time in adult *O. niloticus* after the feeding of methyltestosterone was stopped.

Six to eight-month-old sex inversed tilapia were found to have serum testosterone levels similar to the normal population; therefore, there should be no difference between a normal tilapia and a sex inversed tilapia in terms of safety for human consumption in regards to serum testosterone level. On the other hand, when methyltestosterone was fed as a growth promoter in the growing stage of tilapia, the serum testosterone levels were higher even one month after the withdrawal period. Despite the rise of testosterone level, it cannot be said that using methyltestosterone as a growth promoter makes tilapia unsafe for human consumption even in the short term.

Socio-economic impacts of entrepreneurial fisheries

Backward linkage

There are many actors involved in backward linkage of entrepreneurial fisheries. Fish hatcheries (govt. and private) produce fish spawn and fry for almost all fish species that are under aquaculture. For this purpose, hatcheries manage to stock good quality brood fish. Hatcheries are the only source of fry for exotic fish (Chinese carps, perch) and catfish. There are harvesters and collectors of fish fry of mostly indigenous fish species including Indian major carps that gather these fish from natural breeding sources, such as rivers, in the breeding season and sell them to nursery farmers or fish fry/fingerling sellers/vendors who ultimately sell it to nursery fish farmers.

Nursery farmers release fish fry and raise to fingerling size in a purposefully built nursery pond or in a small homestead pond, then sell them to fish farmers who produce larger size fish seedling or to fish fingerling sellers who ultimately sell it to the producer of larger size fish seedlings. Fish fry / fingerling sellers/vendors are people who sell fish fry, from natural sources or from hatcheries or fish fingerlings collected from nursery farmers to the farmers who produce larger size fish seedlings.

Because entrepreneurial fisheries in the Barind Tract involve the stocking of larger size fish (usually 0.75kg or larger for Indian major carps and Chinese carps), there are groups of farmers who produce fish seedlings of that size. These farmers usually stock fish fry or fish fingerlings in their homestead ponds and then raise them to a larger seedling size for being used or stocked in ponds under entrepreneurial fish production. There are also traders who buy larger fish seedlings from farmers who produce these in their homestead ponds, and then transport and sell the fish seedlings to the entrepreneurial fishpond owners in a live condition for stocking.

There are the drivers of the transport vehicles including locally made three wheelers, mechanized rickshaw vans, and small trucks used for delivering seedlings and inputs supplies to the ponds under entrepreneurial fish production. Since entrepreneurial fish production is intensive fish aquaculture on a commercial basis, almost all ponds under entrepreneurial fish production receive pallet fish feed, which are produced by feed companies and sold by local traders/dealers.

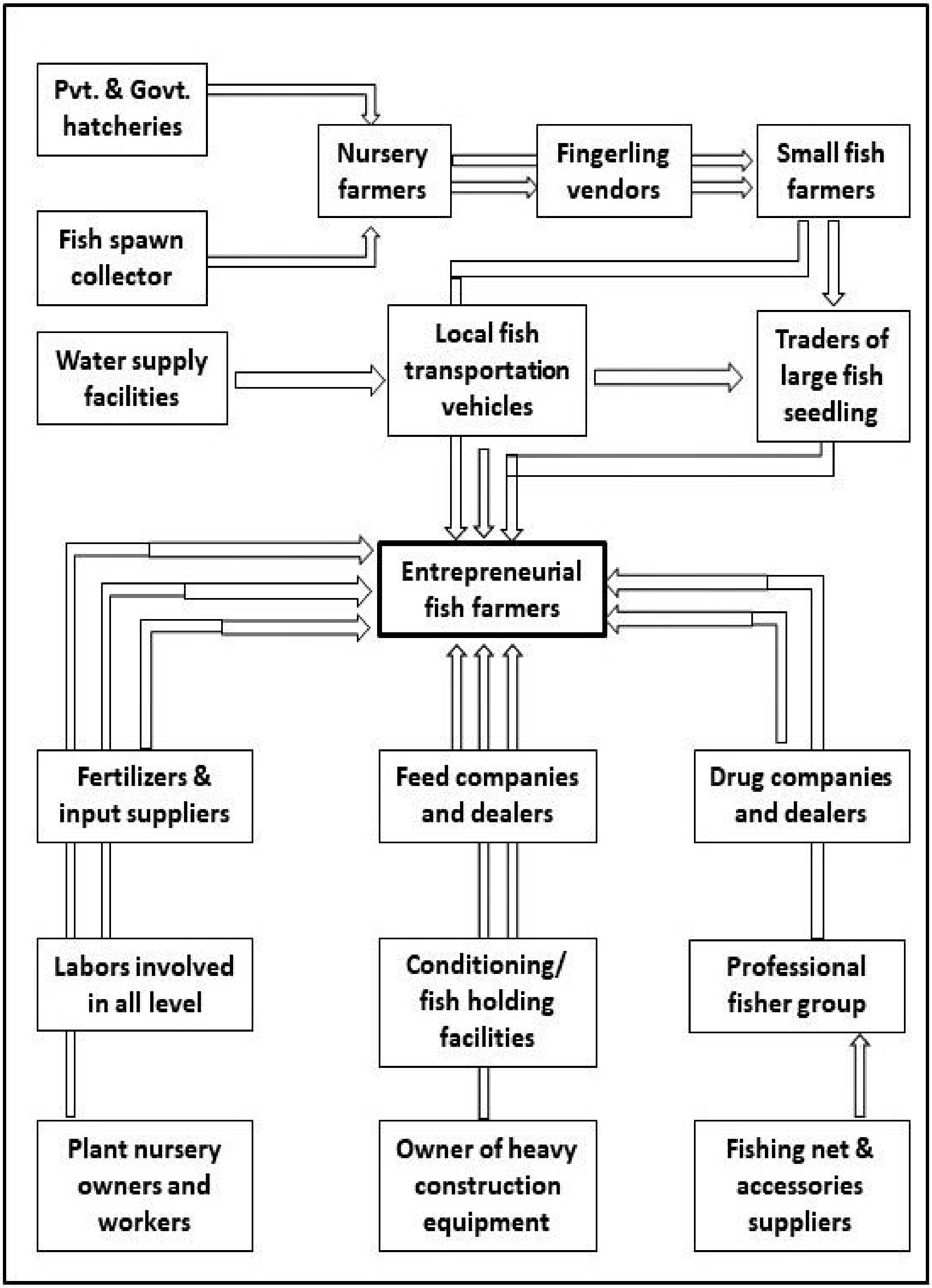


Figure 3.25. Supply chain diagram for entrepreneurial fisheries-backward linkage in the Barind Tract.

Like any intensive farming, in entrepreneurial fisheries many drugs, inputs and pesticides are used, which are produced, imported, packaged and/or distributed by drug companies and their dealers/sellers. Besides drugs, agricultural inputs like fertilizers and nutrient supplements are used in entrepreneurial fisheries and are sold by different local dealers and businesses. Ponds under entrepreneurial fish production are purposely built, and the excavation and construction of the large ponds are done by contractors who operate the heavy construction and earthmoving equipment.

Hired manual labor is used in loading, unloading, carrying of input supplies to the pond, applying these in ponds and guarding the ponds. There are professional fisher groups who catch fish from ponds under entrepreneurial fish production on a contractual basis. These can be groups of approximately ten people with one leader who owns fishing nets of various types. Depending on size of the pond the number of fishermen may vary in the group. There are holding facilities available by the roadside. The facilities, including submersible water pumps with a delivery to a concrete cistern, or sometimes square holding tanks with continues water flow, water inlet and outlet for keeping/conditioning the fish before taking them live over long distances to markets in cities such as Dhaka.

There are also facilities comprised of pumps and delivery pipes by the roadside to fill up the makeshift water tank in the backs of trucks with underground water. Professional fisher groups use the fishing nets heavily and periodically need to replace them. They are dependent on the sellers of fishing nets and their accessories. Almost all of the pond banks under entrepreneurial fish production are under intensive cultivation of fruits (papaya, guava and banana) and sometimes other vegetables. There are plant nursery owners and workers who produce the seedlings and sell these to be planted on the pond banks.

Forward linkage

There are also many actors involved in forward linkage of entrepreneurial fisheries. Transportation vehicles, such as local made three wheelers, mechanized rickshaw vans, and small and big trucks are used for the transportation of fish (usually live) to the wholesale market. There are the commission agents and their employees in the wholesale fish market who sell the fish on a commission basis to the fish traders. There are fish traders who buy fish from the wholesale market and sell it to consumers in retail markets. The common people who buy fish from retail markets for the purpose of consumption are consumers. In addition to the consumers, there are businesspeople and commission agents who buy the bananas, papayas, and other fruits grown on the pond bank from the fisheries entrepreneur, harvest them and take them to the wholesale or retail markets.

The entrepreneurial fisheries have a great impact on the livelihoods of the people of the nearby villages from clusters of ponds examined in this study. The type of work that many of the actors listed above do, is often intense work over short durations and at odd times, especially for the actors involved in the forward linkage. For example, the fish harvester group harvests the fish mainly between the hours of 3.00 am and 9.00 am in order to get the fish to the market so that it can reach to the consumer during the mid-morning. This results in a higher pay of up to 400% depending on the season for these workers as opposed to those involved in conventional agriculture work. The odd working time means that the people involved in this value chain also are from nearby villages so that they can travel to the ponds at the required time. These short working hours and higher pay is something that the value chain actors enjoy, given that they have more time to do their family work in addition. Also, this work is generally considered technical work and has gained a higher level of social acceptance as an upgraded livelihood option over agricultural labor or even traditional fishing in open waters.

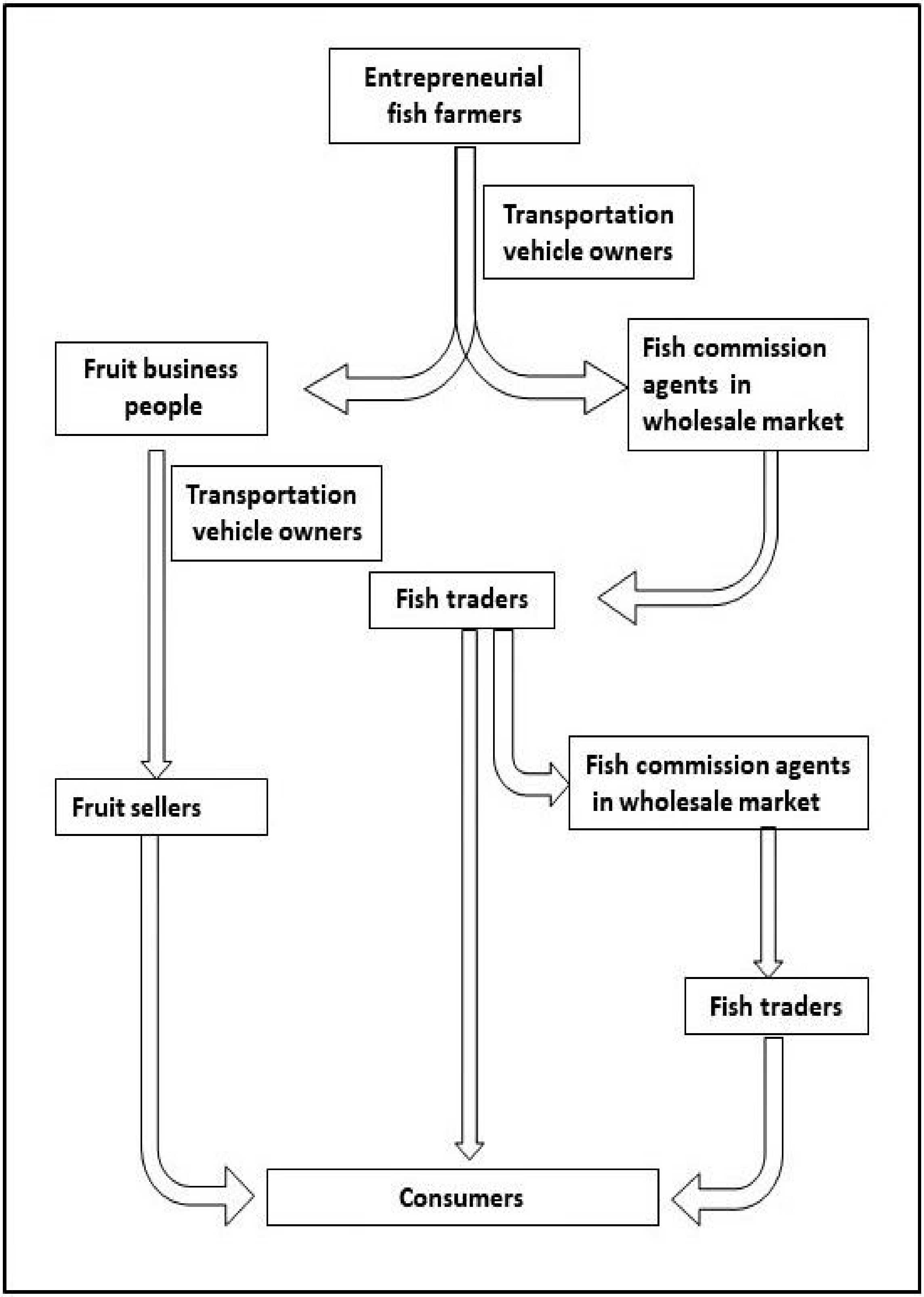
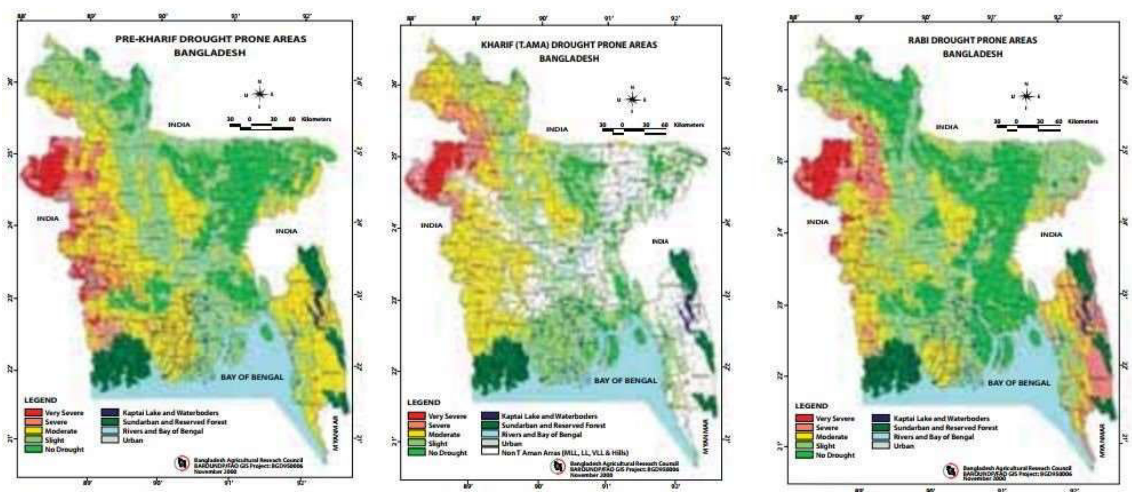


Figure 3.36. Supply chain diagram for entrepreneurial fisheries—forward linkage in the Barind Tract.

Climate change adaptation

Since climate change due to global warming is experienced in the present, it is not only something to planning for in the future but deserves that the current livelihood-means of both agriculture and aquaculture are looked at through the climate change adaptation lens. Given the climatic conditions of the Barind Tract, temperature and rainfall is the most dominant climatic feature at present and will continue to be in future, influencing the life and livelihoods of the people of the Barind Tract. Heim (2002) defined drought as a prolonged absence or marked deficiency of precipitation that results in water scarcity for a certain activity or group of people. IPCC (2007) defined agricultural drought as moisture deficiency at the root zones of the soil, or within the top first meter, that affects crops. Meteorological drought is defined as a prolonged deficiency of precipitation and hydrological drought as a lower than normal level of stream flow, lake and ground water.



Source: BARC, 2001

Figure 3.27. Drought zones in Bangladesh in various crop production seasons.

The Barind Tract has always been the most drought prone area of Bangladesh for all of the agricultural seasons (Figure 3.27). Of the most 20 severely affected upazila of Bangladesh, four are in Rajshahi, five are in Naogoan, and three are in Chapai Nawabganj (App. Table 3.26), based on data prior to 2000 (CDMP, 2013) districts under the Barind Tract. The rise of mean annual temperature, mean maximum temperature and mean minimum temperature between 1977 and 2008 in Bangladesh was respectively 0.016^{0}C/year, 0.02^{0}C/year and 0.012^{0}C/year (CDMP, 2013).

Based on the regional climate modeling under the A2 and B1 (App. Table 3.27) emission scenarios (IPCC, 2000), due to their appropriateness in the Bangladesh context, Hassan *et al.*

(2010) predicted a change in temperature and rainfall pattern in the Rajshahi division, located in the Barind Tract, along with other parts of the country. As per the modeling results (Hassan *et al.*, 2010) predicted a temperature rise in 2030 and 2050 under both the A2 and B1 scenarios in Rajshahi division. Though the temperature rise is predicted for around the year, the increase is more during the colder months when historically the rainfall is low (December-January-February), as compared to the other months of the year (App. Table 3.28).

Like the temperature increase, an increase in total precipitation is also predicted by Hassan *et al.* (2010) in both the years 2030 and 2050 under both the A2 and B1 emission scenarios. While increased precipitation might sound good for an area that has historically received lower rainfall than rest of the country, the pattern of precipitation increase does not give much hope. The wet months in Rajshahi division are predicted to be wetter and the dry months are predicted to be drier (App. Table 3.29). Rainfall during December-January-February months are predicted to decline considerably while the rainfall during other months of the year is predicted to increase.

The rainfall data of Rajshahi and Bogura weather stations from the year 2001 to February 2019 show that rainfall in the last eight years (2011 to 2018) during March-April-May months has already increased as compared to data for the same months from 2001 to 2010, while in the monsoon months precipitation declined slightly (App. Table 3.30). Based on the predicted change in temperature and precipitation in Rajshahi division, CDMP (2013) predicted a considerable reduction (0 to >40%) of paddy production in various upazila of Rajshahi districts (App. Table 3.31) along with many other parts of Bangladesh, unless otherwise managed.

Since entrepreneurial fisheries in Barind Tract primarily use precipitation as their major source of water including some surface runoff and use considerably less underground water, as an agriculture subsector it is more suited to the changed climatic conditions than the regular agriculture/paddy production. As entrepreneurial fishponds are large with high dikes, they will be able to accommodate a large volume of rainwater from the high intensity precipitation of the changed climatic conditions as predicted by regional climate modeling. In fact, entrepreneurial fisheries would benefit from higher rainfall, regardless of its pattern during the year.

Conclusion

The impact of pesticides on zooplanktons in entrepreneurial fisheries seemed temporary; usually zooplanktons were found recovering within a week of pesticide application. Pesticides had more lasting impact on aquatic insects, often up to a month, but aquatic insects from nearby ponds were always been able to populate the ponds where pesticides were used, thus the aquatic insects were able to manage their diversity count in entrepreneurial fisheries.

Entrepreneurial fishponds have been built in areas of seasonal flood plain and in paddy fields that means seasonal wetlands have been converted into perennial wetland in the form of fishponds. In addition, pond dikes that are supporting various kinds of plant growth, have ultimately created an ecosystem that comprises of highland (dike), perennial wetland and lots of shallow water zone that is very conducive for not only faunal (aquatic and terrestrial) but also floral diversity.

Supply chain in entrepreneurial fisheries is long and diverse. Also, the actors, even the daily basis workers engaged in supply chain of entrepreneurial fisheries, received higher remuneration per hour; takes pride and enjoys higher social dignity than those engaged in paddy production.

Overall, entrepreneurial fisheries have been providing more of an environmental service than the negative impact it is exerting due to some harmful practices. Entrepreneurial fisheries in the Barind Tract would be more environmentally friendly if the harmful practices are regulated.

CHAPTER FOUR

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APPENDICES

App. Table 1.1. Comparison of food intake (g/capita/day) found in different Household Income and Expenditure Surveys (HIES).

Food Intake (g/capita/day)	HIES-2005	HIES-2010	Growth %	HIES-2016	Growth %
Rice	439.64	416.01	-5.38	367.19	-11.74
Wheat	12.08	26.09	115.98	19.83	-23.99
Pulses	14.20	14.30	0.70	15.60	9.09
Vegetables	157.0	166.08	5.78	167.30	0.74
Fish	42.1	49.50	17.58	62.58	26.42
Meat	15.6	19.07	22.24	25.42	33.30
Egg	5.2	7.25	39.42	13.58	87.31
Milk & Milk product	32.4	33.72	4.07	27.31	-19.01
Fruit	32.5	44.70	37.53	35.78	-19.96

Source: BBS, 2019

App. Table 1.2. Comparison of pond aquaculture (area, production and ranking among all districts) between the Barind Tract districts and the top three aquaculture districts in Bangladesh.

Name of district	Area in Hectares					Production in Metric tonnes				
	2007-08	Ranking among all districts	2017-18	Ranking among all districts	Growth (%)	2007-08	Ranking among all districts	2017-18	Ranking among all districts	Growth (%)
Mymen-singh	8353	10	28620	1	242.6	28926	5	357816	1	1137.0
Comilla	13875	2	22464	2	61.9	44348	2	108262	3	144.1
Jessore	6797	17	16774	4	146.8	23301	12	118467	2	408.4
Bogura	9477	6	14188	5	49.7	25808	7	67092	4	160.0
Joypur-hat	3062	37	4635	31	51.4	9117	34	21678	25	137.8
Naogoan	10768	4	12750	8	18.4	24069	10	54470	7	126.3
Rajshahi	7729	13	12310	9	59.3	25576	9	54859	6	114.5

Source: DoF, 2009; DoF, 2018

App. Table 1.3. Comparison of species composition (weight in percentage) of pond production in the four Barind Tract districts in 2007-08 and 2017-18.

Species	Bogura		Joypurhat		Naogoan		Rajshahi	
	2007-08	2017-18	2007-08	2017-18	2007-08	2017-18	2007-08	2017-18
Labeo rohita	16.00	11.14	12.00	19.19	15.03	17.39	15.77	30.54
Catla catla	17.00	7.34	15.00	14.61	14.00	9.34	24.00	17.33
Cirrhinus mrigala	16.30	9.47	7.00	9.73	8.67	15.94	8.99	13.38
Labeo calbasu	0.00	1.83	0.00	0.00	0.45	2.06	0.30	3.73
Hypophthalmichthys molitrix	30.00	12.35	40.00	19.15	22.10	12.86	30.14	17.20
Ctenopharyngodon Idella	4.05	1.79	0.00	5.40	0.67	1.66	0.00	1.38
Cyprinus Carpio	0.73	3.57	13.00	4.85	15.00	4.03	0.00	7.86
Other exotic carp	0.00	1.71	1.00	0.80	1.50	2.23	7.62	1.15
Oreochromis niloticus/mossambicus	3.60	7.27	5.00	9.09	0.28	9.29	10.00	2.05
Pangasius hypophthalmus	5.00	31.96	2.00	14.05	19.30	22.62	1.18	1.70
C. batrachus/ H. fossilis	00	1.82	0.00	0.00	0.00	0.00	0.00	0.18
Koi	0.96	1.59	0.60	0.00	0.00	0.40	0.00	0.71
Others	6.36	8.16	4.40	3.13	3.00	2.18	2.00	2.79
Total	100	100	100	100	100	100	100	100

Source: DoF, 2009; DoF, 2018.

App. Table 1.4. Comparison of species composition (weight in percentage) of pond production in the three highest fish producing districts in 2017-18.

Species	Mymensingh		Jessore		Comilla	
	2007-08	2017-18	2007-08	2017-18	2007-08	2017-18
Labeo rohita	28.39	3.18	38.00	12.69	22.00	5.85
Catla catla	20.00	2.14	21.00	7.75	13.00	2.41
Cirrhinus mrigala	10.00	2.94	14.00	15.96	10.86	4.17
Labeo calbasu	0.00	0.71	0.00	2.55	0.00	0.44
Hypophthalmichthys molitrix	19.00	2.92	10.00	12.77	10.46	3.08
Ctenopharyngodon Idella	0.00	1.89	0.00	2.55	0.00	1.07
Cyprinus Carpio	2.65	1.07	3.39	3.83	0.52	0.75
Other exotic carp	0.00	2.58	0.00	0.00	0.00	0.00
Oreochromis niloticus/mossambicus	4.95	8.28	7.72	20.95	19.00	33.31
Pangasius hypophthalmus	4.00	52.93	2.07	15.19	7.58	38.41
C. batrachus / H. fossilis	0.00	4.34	0.00	0.00	0.00	2.36
Koi	3.79	6.94	1.79	0.00	3.22	3.15
Others	7.22	10.08	2.03	5.76	13.36	5.00
Total	100	100	100	100	100	100

Source: DoF, 2009; DoF, 2018.

App. Table 2.1. Use of construction equipment for pond construction under entrepreneurial fish production.

Machineries used	Duration of use (hour) /acre water area	Relative percentage
Excavator	131.5	48.8
Bulldozer	27.9	10.4
Dump trucks	109.9	40.8
Total	269.4	100

App. Table 2.2. Water use in ponds under entrepreneurial fish production in the Barind Tract.

Water source	Percentage of ponds	Duration (hour) of Deep tube well use /acre water area/year
Surface runoff/canal water	23.1	N/A
Deep tube well	69.2	31.2
Both sources	9.8	
No outside water used	17.5	N/A

App. Table: 2.3 Water use in paddy production in the Barind Tract.

Water source	Duration (hour) of Deep tube well use /acre water area/year
Deep tube well	74 hours

App. Table 2.4 Fish species composition in ponds under entrepreneurial fish production in the Barind Tract.

Fish Types	Fish species	% of culture ponds	Density/ acre water area	Relative density (%)
Indian Major carps	Rui (*Labeo rohita*)	100	533	56.9
	Catla (*Catla catla*)	100	100	10.7
	Mrigel (*Cirrhinus mrigala*)	100	187	20.0
Common carp	Common carp (*Cyprinus Carpio*)	84.6	30	3.2
Chinese carp	Silvercarp (*Hypophthalmichthys molitrix*)	100	29	3.1
	Grass carp (*Ctenopharyngodon idella*	100	13	1.4
	Black Carp (*Mylopharyngodon piceus)*	100	4	0.4
	Bighead carp (*Hypophthalmichthys nobilis)*	15.4	1	0.1
Predatory fish	Chithol (*Chitala chitala*)	61.5	3	0.3
	Pholi (Notopterus notopterus)	46.2	8	0.9
	Air (Sperata aor)	23.1	4	0.5
	Magur (*Clarias batrachus)*	6.3	26	2.7
Total for Mixed culture		N/A	938	100
Tilapia	Tilapia (*Oreochromis niloticus*)	4.3	15000	99*

*Mostly monoculture, but a negligible number of other species are used for ecological balance in the pond.

App. Table 2.5. Feed and feeding in ponds under entrepreneurial fish production in the Barind Tract.

SL#	Feed types	% of culture ponds received	Average quantity used (kg)/acre water area/year	Relative use percentage
1	Pallet feed	100	3753.4	75.6
2	Oilcake	46.2	651.2	13.1
3	Wheat bran	38.5	285.6	5.8
4	Rice bran	23.1	164.3	3.3
5	Corn	15.4	109.0	2.2
6	Biscuits	30.8	29.2	0.6
	Total	-	4963.5	100

App. Table 2.6. Feed additives used in entrepreneurial fisheries of the Barind Tract.

SL#	Types of feed additives	% of culture ponds received	Average quantity used (kg)/acre water area/year	Relative use percentage
1	Vitamin C	17.4	0.30	2.18
2	Multivitamin	38.5	8.90	64.63
3	Domperidone	23.1	1.10	7.99
4	Dicalcium Phosphate (DCP)	24.7	3.30	23.97
5	Probiotics	30.8	0.06	0.43
6	Non antibiotic growth promoter	15.3	0.11	0.80
Total		-	13.77	100

App. Table 2.7. Fertilizer uses in entrepreneurial fisheries in the Barind Tract.

Fertilizer Type	Name of fertilizer	% of culture ponds received fertilizer	Average quantity used (kg)/acre water area/year	Relative use (%) of the total
Organic	Poultry liter	38.5	1066.3	55.6
	Cow dung	30.8	280.9	14.6
Inorganic	Triple super phosphate	100	467.7	24.5
	Urea (46% Nitrogen)	76.9	88.8	4.6
	Muriate of potash	53.8	12.4	0.6
Grand Total		-	1918	100

App. Table 2.8. Fertilizer uses in paddy production in the Barind Tract.

Fertilizer Type	Name of fertilizer	% 0f farmers used	Average quantity used (kg)/acre water area/year	Relative use (%) of the total
Organic	Poultry litter	18.75	547.3	11.9
	Cow dung/compost	100	3674.6	79.7
	Subtotal	-		-
Inorganic	Triple super phosphate	100	98.3	2.1
	Urea (46% Nitrogen)	100	151.6	3.3
	Muriate of potash	100	80.1	1.7
	Gypsum	93.75	52.9	1.1
	Boron	50	2.5	0.1
	Zinc	56.25	2.6	0.1
	Subtotal	-		-
Total			4609.9	100

App. Table 2.9. Use of disinfectants in entrepreneurial fisheries in the Barind Tract.

SL#	Name of disinfectants	% of culture ponds received disinfectants	Average quantity used (kg)/acre water area/year	Relative use percentage
1	Lime	100	166.3	67.37
2	Zeolite	76.9	28.3	11.47
3	Table salt	61.5	51	20.67
4	Brominated salt containing products	30.8	0.3	0.12
5	N-alkyl dimethyl benzyl ammonium chloride containing products	46.2	0.4	0.15
6	Potassium permanganate	53.8	0.4	0.16
7	Malachite Green	7.7	0.04	0.02
8	Ammonia remover	30.8	0.1	0.04
	Total		246.82	100

App. Table 2.10. Use of fungicides in paddy production in the Barind Tract.

SL#	Fungicide types	% of paddy fields received	Average quantity (active ingredient) used (gram)/acre field /year	Relative use percentage
1	Azoxystobin (20%) + Difenoconazole (12.5%)	68.75	129.4	44.0
2	Difenoconazole	31.25	56.0	19.0
3	Propiconazole	81.25	96.4	32.8
4	Carbendazim	6.25	12.4	4.2
	Total	-	294.2	100

App. Table 2.11. Use of pesticides in entrepreneurial fisheries in the Barind Tract.

SL#	Pesticide types	% of culture ponds received disinfectants	Average quantity (active ingredient) used (gram)/acre water area/year	Relative use percentage
1	Cypermethrin	92.3	15.10	39.92
2	Deltamethrin	38.5	0.72	1.92
3	Abamectin	30.8	0.46	1.22
4	Lambda-cyhalothrin	23.1	0.64	1.69
5	Quinalphos	69.2	9.66	25.55
6	Fenitrothion	7.6	8.99	23.76
7	Emamectin benzoate	15.4	2.25	5.94
	Total	-	37.83	100

App. Table 2.12. Use of pesticides in paddy production in the Barind Tract.

SL#	Pesticide types	% of paddy fields received	Average quantity (active ingredient) used (gram)/acre area/year	Relative use percentage
1	Carbofuran	81.25	211.4	78.4
2	Thiamethoxam 20% + Chlorantraniliprole20%	62.5	53.7	19.9
3	Dimethoate	6.25	1.0	0.4
4	Lambda-cyhalothrin	31.25	0.4	0.2
5	Chlorpyriphos	18.75	3	1.1
	Total	-	269.6	100

App. Table 2.13. Use of herbicides on the pond banks under entrepreneurial fish production in the Barind Tract.

SL#	Herbicides	Type	% of culture pond's bank received	Average quantity (active ingredient) used (gram)/acre water area/year	Relative use percentage
1	Glyphosate	Nonselective systemic	61.5	376.38	52.0
2	2,4-Dichlorophenoxyacetic acid	Selective for broad leaves	23.1	304.8	42.2
3	paraquat	Post emergent	15.4	42.2	5.8
	Total		-	723.38	100

App. Table 2.14. Use of herbicide in paddy production in the Barind Tract.

SL#	Herbicides	type	% of paddy plots received	Average quantity (active ingredient) used (gram)/acre area/year	Relative use percentage
1	Bensulfuran methyl (4%) + Quinclorac (28%)	Pre-emergent	43.47	49.4	87.8
2	Pretilachlor	Pre-emergent	12.5	6.3	11.1
3	Pyrazosulfuran ethyl	Post-emergent	6.25	0.6	1.1
	Total		-	56.2	100

App. Table 2.15. Use of antibiotics in entrepreneurial fish production in the Barind Tract.

SL#	Antibiotic types	% of culture ponds received disinfectants	Average quantity (active ingredient) used (gram)/acre water area/year	Relative use percentage
1	Oxytetracycline	53.8	86.29	61.54
2	Doxycycline or (Doxycycline + Colistin)	15.4	5.61	4.01
3	Ciprofloxacin	61.5	28.07	20.02
4	Erythromycin thiocyanate +Sulfadiazine +Trimethoprim	7.6	20.22	14.43
	Total	-	140.20	100

App. Table 2.16 Use of other therapeutants in entrepreneurial fisheries in the Barind Tract.

SL#	Therapeutant types	% of culture pond received	Average quantity used (kg)/acre area/year	Relative use percentage
1	Sodium carbonate peroxyhydrate	68.4	1.2	16.37
2	Coper Sulfate	3.7	.0019	0.02
3	Tea seed cake	4.9	6.13	83.61
	Total	-	7.3319	100

App. Table 2.17. Use of fish toxicants in entrepreneurial fisheries in the Barind Tract.

SL#	Fish toxicants	% of pond received	Average usage frequency /year/pond	Average quantity used (active ingredient) kg /acre water area/year	Relative use percentage
1	Aluminium phosphide	69.2	0.92	1.06	73.79
2	Rotenone	38.5		0.17	11.99
3	Fenpropathrin	15.4		0.20	14.22
	Total	-	-	1.43	100

App. Table 2.18. Duration of vehicles/machineries used in entrepreneurial fisheries in the Barind Tract.

SL#	Source of use	% of pond used	Average use (hour) /acre /year	Relative use (%) of the total
1	Fish seedling transportation	100%	1.3	2.34
2	Fish transport to market	100%	47.8	86.13
3	Feed transportation	100%	4.8	8.65
4	Fertilizer transportation	100%	0.2	0.36
5	Manure transportation	53.9%	0.7	1.26
6	Medicine/ plant seedling transportation	69%	0.03	0.05
7	Agricultural product transportation	100%	1.1	1.98
	Total	-	55.5	100

App. Table 2.19. Duration of vehicles/machineries used in paddy production in the Barind Tract.

SL#	Source of use	% of farmers used	Average use (hour) /acre /year	Relative use (%) of the total
1	Plowing	100	4.47	24.44
2	Thrashing of the grains	56.25	6.59	36.10
3	Transportation	51	7.21	39.46
	Total	-	18.27	100

App. Table 2.20. Proximate composition of fish feed used in entrepreneurial fisheries in the Barind Tract.

Name of Fish Feed	% Moisture		% Lipid		% protein		% Ash		% Fiber		% Carbohydrate	
	Reference value (maximum)	Actual	Reference value (minimum)	Actual	Reference value (minimum)	Actual	Reference value (maximum)	Actual	Reference value (maximum)	Actual	Reference value (maximum)	Actual
Feed-1	12	13.77	6	6.90	26	25.38	18	16.88	8	6.56	32	30.51
Feed-2	11	12.11	6	5.40	24	16.32	17	18.20	6	8.40	-	39.57
Feed-3	12	14.51	4	6.05	24	21.79	-	12.88	7	7.80	-	36.97
Feed-4	11	13.05	5	7.30	22	27.88	-	15.43	9	5.88	-	30.46
Feed-5	12	13.70	5	7.20	23	27.26	21	11.54	9	5.90	40	34.40
Feed-6	10	13.92	6	6.70	22	25.22	10	13.31	4.5	6.66	25	34.19
Feed-7	12	14.05	5	6.38	22	24.30	21	9.72	9	6.70	-	38.85
Feed-8	12	13.80	3	6.26	22	24.16	21	15.69	10	6.90	40	33.19
Feed-9	11	11.41	6	6.10	26	16.09	-	21.06	-	7.60	-	37.74
Feed-10	11	13.20	5	6.33	24	25.24	11	15.45	9	6.80	-	32.98
Feed-11	11	12.82	5.3	6.20	26	25.60	21	12.59	9	6.98	32	35.81
Feed-12	11	11.12	6	5.80	26	14.40	-	29.14	11	7.80	-	31.74
Feed-13	11	13.57	6	5.7	26	16.40	-	21.99	-	8.40	-	33.94
Feed-14	12	14.39	6	5.2	24	16.37	20	22.56	10	8.52	-	32.96

App. Table 2.21. Physical appearance of feed pellet.

Name of Feed	Maximum Pellet length in mm	Average Pellet length for 25 pellets in mm	Pellet diameter in mm
Feed-1	18	8.5	2.9
Feed-2	53	22.2	4.0
Feed-3	14	10.0	3.0
Feed-4	16	6.1	2.7
Feed-5	20	10.4	3.1
Feed-6	18	10.5	3.2
Feed-7	19	11.7	3.2
Feed-8	25	14.3	2.7
Feed-9	23	7.9	4.0
Feed-10	24	11.8	3.5
Feed-11	16	9.1	3.0
Feed-12	25	10.6	3.5
Feed-13	26	14.5	3.0
Feed-14	12	8.3	4.5

App. Table 2.22. Raw materials/ingredients used in feed formulations.

Feed	Corn	Rice polish	Soybean meal	Fish meal	Meat and bone meal	Mustard Oilcake	Lime- stone/DCP	Salts	Vitamins & minerals	Wheat floor	Wheat bran	Oil	Pellet binders	Full fat soybean	Shrimp meal	Animal protein	De oil rice bran	Amino Acids	Enzymes & preservatives
Feed-3	√	√	√	-	-	√	-	-	√	√	-	-	-	√		√	-	√	-
Feed-5	√	√	√	√	-	√	√	√	√	-	-	-	-	-	-	-	-	-	-
Feed-6	-	√	√	√	√	√	-	-	√	√	-	-	-	√	√	-	-	-	-
Feed-7	√	√	√	√	√	√	-	√	√	√	-	√	√	-	-	-	-	-	-
Feed-8	√	√	√	-	√	√	√	√	√	√	√	√	-	-	-	-	√	√	√
Feed-11	√	√	√	√	√	√	-	√	√	√	-	√	√	-	-	-	-	-	-

App. Table 2.23. Time it took (duration)for the feed pellet to become dissolved in a Shaker.

Name of Feed	Time (minutes) to become dissolved @75rpm in shaker	Time (minutes) to become dissolved @150 rpm in shaker
Feed-1	10	8
Feed-2	180	125
Feed-3	186	128
Feed-4	24	18
Feed-5	171	107
Feed-6	18	14
Feed-7	41	31
Feed-8	37	15
Feed-9	13	5
Feed-10	56	21
Feed-11	35	18
Feed-12	47	21
Feed-13	73	44
Feed-14	24	15

App. Table 2.24. Heavy metal content in fish feed used in entrepreneurial fisheries in the Barind Tract.

Feed	Pb (mg/kg)	Cr (mg/kg)	Cd (mg/kg)
Feed-1	0.000000	13.980170	0.293382
Feed-2	0.000000	6.945635	0.272437
Feed-3	0.475665	0.946476	0.390725
Feed-4	1.665291	0.713392	0.313041
Feed-5	0.000000	0.865241	0.525931
Feed-6	0.406329	1.784947	0.333771
Feed-7	0.000000	0.554271	0.154186
Feed-8	0.487632	1.149417	0.024879
Feed-9	0.000000	0.705510	0.425412
Feed-10	0.000000	0.658170	0.309000
Feed-11	0.000000	0.536334	0.290829
Feed-12	0.000000	1.734413	0.531514
Feed-13	0.000000	0.837819	0.463989
Feed-14	0.000000	10.118160	2.842560

App. Table 2.25. Heavy metal content in chemical fertilizers available in the market in the Barind Tract.

Fertilizer	Lead (Pb)	Chromium (Cr)	Cadmium (Cd)
	mg/kg	mg/kg	mg/kg
TSP-1	0.132 ± 0.187	13.8 ± 0.2	3.8 ± 0.32
TSP-2	0.264 ± 0.373	13.5 ± 0.6	2.8 ± 0.06
TSP-3	0.396 ± 0.187	11.6 ± 0.3	1.5 ± 0.25
TSP-4	0.396 ± 0.187	18.2 ± 0.8	5.0 ± 0.32
TSP-5	Below detection range	12.0 ± 0.9	3.1 ± 0.04
MOP-1	Below detection range	249.0	0.258
MOP-2	0.264	25.1	0.204
MOP-3	0.264	5.4	0.474

App. Table 2.26. Macronutrient content and pH of soil from different sources.

Soil Type /source	pH	Organic carbon	Nitrogen	Potassium	Calcium	Magnesium	Phosphorus	Sulfur
		%	%	m. mole /100g soil	m. mole /100g soil	m. mole /100g soil	µg/g soil	µg/g soil
Pond bottom (2-5 years under entrepreneurial fish production)	7.80 ± 0.08	2.74 ± 0.50	0.27 ± 0.04	0.90 ± 0.18	38.48 ± 3.58	5.18 ± 1.94	81.12 ± 31.02	79.61 ± 25.12
Pond bank (without agriculture)	7.70 ± 0.2	1.56 ± 0.02	0.16 ± 0.02	0.68 ± 0.13	38.80 ± 4.84	6.56 ± 2.37	6.70 ± 2.31	3.77 ± 2.39
Pond bank (under intensive Agriculture)	7.90 ± 0.24	1.99 ± 0.58	0.20 ± 0.06	0.86 ± 0.49	36.64 ± 5.20	5.46 ± 1.76	15.87 ± 10.21	1.99 ± 0.07
Beel (perineal, static water)	7.55 ± 0.35	2.70 ± 0.82	0.27 ± 0.08	0.70± 0.22	23.16± 9.40	4.22 ± 0.60	19.40 ± 7.36	43.42 ± 31.79
Canal in *Beel* (flowing water)	7.45 ± 0.28	0.97 ± 0.04	0.10 ± 0.01	0.37 ± 0.04	12.36 ± 1.60	4.44 ± 0.21	18.04 ± 1.36	49.55 ± 20.92

App. Table 2.27. Micronutrient content of soil from different sources

Soil Type /source	Boron	Zinc	Copper	Iron	Manganese
	µg/g soil	µg/g soil	µg/g soil	µg/g soil	µg/g soil
Pond bottom (2-5 years under entrepreneurial fish production)	0.41 ± 0.27	1.38 ± 0.44	5.25 ± 0.99	147.32 ± 18.51	28.73 ± 10.53
Pond bank (without agriculture)	0.29 ± 0.14	1.00 ± 1.09	3.11 ± 0.13	35.90 ± 4.48	18.20 ± 15.83
Pond bank (under intensive Agriculture)	0.45 ± 0.07	0.90± 0.38	2.18 ± 0.35	27.42 ± 4.87	12.74 ± 5.69
Beel (perineal, static water)	0.50 ± 0.14	1.12 ± 1.02	4.39 ± 0.94	213.53 ± 134.51	34.90 ± 15.74
Canal in *Beel* (flowing water)	0.23 ± 0.13	0.80 ± 0.32	1.89 ± 0.49	95.60 ± 30.41	12.95 ± 4.88

App. Table 2.28. Heavy metal concentration in soil from different sources.

Soil Type /source	Lead (Pb)	Chromium (Cr)	Cadmium (Cd)
	mg/kg soil	mg/kg soil	mg/kg soil
Pond bottom (2-5 years under entrepreneurial fish production)	8.54 ± 7.50	2.79 ± 0.74	0.32 ± 0.08
Pond bank (without agriculture)	1.83 ± 1.63	2.88 ± 0.60	0.30 ± 0.33
Pond bank (under intensive Agriculture)	5.16 ± 6.68	2.31 ± 0.35	0.15 ± 0.15
Beel (perineal, static water)	5.22 ± 4.31	2.88 ± 0.53	0.36 ± 0.17
Canal in *Beel* (flowing water)	below detection range	1.94 ± 0.08	0.45 ± 0.63

App. Table 2.29. Reference table for heavy metals in freshwater sediments.

Standard	Lead (mg/kg)	Chromium (mg/kg)	Cadmium (mg/kg)
LEL (Lowest Effect Level)	31	26	0.6
TEC (Threshold Effect Concentration)	35.8	43.4	0.99
PEC (Probable Effect Concentration)	128	111	4.9
SEL (Severe Effect Level)	250	110	10

Source: NOAA (US department of Commerce), 2008

App. Table 2.30. Year-round comparison of water pH in aquaculture ponds under entrepreneurial fish production and natural wetlands in the Barind Tract.

Treatments	Replication	May -18	Jun -18	Jul -18	Aug -18	Sep -18	Oct -18	Nov -18	Dec -18	Jan -19	Feb -19	Mar -19	Apr -19
Aquaculture ponds	R-1	7.36	7.37	7.20	7.44	7.89	7.35	7.74	7.80	7.83	7.87	7.32	7.33
	R-2	7.40	7.35	7.22	7.41	7.38	7.33	7.41	7.58	7.61	7.72	7.40	7.21
	R-3	7.56	7.51	7.35	7.62	8.26	7.39	7.68	7.80	8.29	8.04	7.92	7.71
	R-4	7.55	7.91	7.45	7.67	8.22	7.54	7.67	7.71	8.09	7.88	8.30	7.62
	Average	7.47	7.54	7.31	7.54	7.94	7.40	7.63	7.72	7.96	7.88	7.74	7.47
Natural wetlands	R-1	7.15	6.80	6.86	6.90	7.19	6.81	7.32	7.61	7.41	7.16	-	-
	R-2	7.48	7.15	6.74	7.28	6.99	7.28	6.95	7.37	7.63	7.85	-	-
	R-3	7.12	7.55	6.70	7.01	6.60	6.07	7.43	7.71	7.23	6.87	-	-
	R-4	7.35	7.11	6.75	6.83	6.73	6.44	7.27	7.67	7.68	7.47	-	-
	Average	7.28	7.15	6.76	7.01	6.88	6.65	7.24	7.59	7.49	7.34	-	-

App. Table 2.31. Year-round comparison of Secchi disk readings (Inches) in aquaculture ponds under entrepreneurial fish production and natural wetlands in the Barind Tract.

Treatments	Replication	May -18	Jun -18	Jul -18	Aug -18	Sep -18	Oct -18	Nov -18	Dec -18	Jan -19	Feb -19	Mar -19	Apr -19
Aquaculture ponds	R-1	12.0	11.0	9.0	10.5	12.0	14.0	22.0	15.0	14.0	14.0	8.5	9.0
	R-2	12.0	12.0	10.0	12.0	14.0	14.0	11.0	11.5	10.0	10.0	9.0	14.0
	R-3	10.0	10.5	10.5	11.5	15.0	16.0	14.0	11.0	10.5	11.0	8.5	8.0
	R-4	14.0	11.5	10.0	12.5	13.5	16.0	15.0	11.0	13.0	11.5	11.0	16.0
	Average	12.0	11.3	9.9	11.6	13.6	15.0	15.5	12.1	11.9	11.6	9.3	11.8
Natural wetlands	R-1	27.0	19.0	22.0	46.0	18.0	60.0	50.0	30.0	35.0	40.0	-	-
	R-2	20.0	27.0	27.0	39.0	32.0	35.0	54.0	42.0	26.0	28.0	-	-
	R-3	19.0	31.0	31.0	42.0	37.0	48.0	34.0	50.0	35.0	32.0	-	-
	R-4	19.0	24.0	25.0	13.0	15.5	13.0	15.0	13.0	16.0	15.0	-	-
	Average	21.3	25.3	26.3	35.0	25.6	39.0	38.3	33.8	28.0	28.8	-	-

App. Table 2.32. Year-round comparison of Electrical conductivity (µS) of water in aquaculture ponds under entrepreneurial fish production and natural wetlands in the Barind Tract.

Treatments	Replication	May -18	Jun -18	Jul -18	Aug -18	Sep -18	Oct -18	Nov -18	Dec -18	Jan -19	Feb -19	Mar -19	Apr -19
Aquaculture ponds	R-1	383.0	399.0	396.0	388.0	349.0	363.0	330.0	329.0	266.0	362.0	399.0	374.0
	R-2	406.0	392.0	463.0	391.0	345.0	332.0	310.0	309.0	279.0	268.0	365.0	490.0
	R-3	462.0	441.0	423.0	408.0	324.0	383.0	371.0	374.0	320.0	388.0	544.0	515.0
	R-4	561.0	530.0	526.0	470.0	438.0	400.0	359.0	333.0	388.0	464.0	733.0	655.0
	Average	453.0	440.5	452.0	414.3	364.0	369.5	342.5	336.3	313.3	370.5	510.3	508.5
Natural wetlands	R-1	535.0	485.0	563.0	362.0	284.0	442.0	584.0	626.0	536.0	417.0	-	-
	R-2	315.0	252.0	214.0	207.0	195.0	341.0	225.0	249.0	248.0	358.0	-	-
	R-3	157.0	115.0	99.0	73.0	56.0	93.0	87.0	97.0	138.0	146.0	-	-
	R-4	225.0	141.0	156.0	158.0	203.0	124.0	183.0	210.0	198.0	219.0	-	-
	Average	308.0	248.3	258.0	200.0	184.5	250.0	269.8	295.5	280.0	285.0	-	-

App. Table 2.33. Year-round comparison of total dissolved solids (ppm) in water in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Treatments	Replications	May -18	Jun -18	Jul -18	Aug -18	Sep -18	Oct -18	Nov -18	Dec -18	Jan -19	Feb -19	Mar -19	Apr -19
Aquaculture ponds	R-1	191.5	199.5	198.0	194.0	174.5	181.5	165.0	164.5	133.0	181.0	199.5	187.0
	R-2	203.0	196.0	231.5	195.5	172.5	166.0	155.0	154.5	139.5	134.0	182.5	245.0
	R-3	231.0	220.5	211.5	204.0	162.0	191.5	185.5	187.0	160.0	194.0	272.0	257.5
	R-4	280.5	265.0	263.0	235.0	219.0	200.0	179.5	166.5	194.0	232.0	366.5	327.5
	Average	226.5	220.3	226.0	207.1	182.0	184.8	171.3	168.1	156.6	185.3	255.1	254.3
Natural wetlands	R-1	267.5	242.5	281.5	181.0	142.0	221.0	292.0	313.0	268.0	208.5	-	-
	R-2	157.5	126.0	107.0	103.5	97.5	170.5	112.5	124.5	124.0	179.0	-	-
	R-3	78.5	57.5	49.5	36.5	28.0	46.5	43.5	48.5	69.0	73.0	-	-
	R-4	112.5	70.5	78.0	79.0	101.5	62.0	91.5	105.0	99.0	109.5	-	-
	Average	154.0	124.1	129.0	100.0	92.3	125.0	134.9	147.8	140.0	142.5	-	-

App. Table 2.34. Year-round comparison of water turbidity (ntu) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Treatments	Replication	May -18	Jun -18	Jul -18	Aug -18	Sep -18	Oct -18	Nov -18	Dec -18	Jan -19	Feb -19	Mar -19	Apr -19
Aquaculture ponds	R-1	13.7	11.9	13.4	28.5	22.2	17.9	12.6	26.4	27.3	14.0	28.7	32.5
	R-2	9.6	16.8	16.4	23.3	29.8	18.7	28.1	47.6	46.0	45.9	25.8	21.1
	R-3	22.8	22.1	11.3	15.7	19.9	12.1	24.2	39.7	41.6	33.5	36.4	45.7
	R-4	14.4	12.1	9.3	17.2	20.8	12.4	23.2	35.0	32.4	23.6	20.1	23.1
	Average	15.1	15.7	12.6	21.2	23.2	15.3	22.0	37.2	36.8	29.2	27.7	30.6
Natural wetlands	R-1	1.0	0.4	0.0	3.8	12.5	7.1	3.4	7.7	7.2	5.4	-	-
	R-2	3.1	1.9	0.5	10.0	11.5	10.7	4.4	4.6	4.9	7.8	-	-
	R-3	1.6	0.2	0.0	4.8	11.2	10.0	4.1	3.8	5.9	4.7	-	-
	R-4	0.3	2.2	0.4	23.0	25.8	23.2	22.4	33.4	25.5	12.4	-	-
	Average	1.5	1.2	0.2	10.4	15.2	12.8	8.6	12.4	10.9	7.6	-	-

App. Table 2.35. Year-round zooplankton count (number/liter water) in aquaculture ponds under entrepreneurial fish production in the Barind Tract.

Zooplankton	May -18	Jun -18	Jul -18	Aug -18	Sep -18	Oct -18	Nov -18	Dec -18	Jan -19	Feb -19	Mar -19	Apr -19
Cyclops sp.	197 ±119	103 ±26	225 ±77	423 ±31	264 ±160	275 ±285	148 ±140	328 ±220	231 ±188	177 ±266	543 ±117	94 ±44
Filinia sp.	118 ±40	186 ±85	284 ±168	430 ±65	115	22	199 ±116	199 ±163	572 ±92	170 ±120	189 ±154	170 ±88
Diaphanosoma sp.	22	45 ±1	46	53 ±53	55 ±36	23	-	-	-	-	171 ±217	
Keratella sp.	337 ±87	377 ±121	735 ±110	790 ±149	341 ±138	429 ±126	393 ±380	583 ±202	1276 ±316	550 ±222	964 ±191	808 ±699
Brachionus sp.	1043 ±148	772 ±154	1062 ±278	1093 ±225	551 ±202	502 ±227	274 ±254	707 ±297	1100 ±197	873 ±376	935 ±186	1007 ±213
Nauplius	435 ±253	415 ±57	582 ±92	672 ±92	375 ±173	359 ±170	204 ±72	451 ±111	690 ±177	429 ±56	805 ±170	326 ±228
Asplanchna sp.	229 ±119	595 ±338	500 ±190	614 ±55	572 ±653	276 ±139	69 ±1	400 ±305	742 ±148	411 ±226	557 ±282	278 ±70
Diaptomus sp.	34 ±17	24	40 ±29	151 ±24	23 ±0	69 ±27	30 ±13	54 ±45	127 ±125	115	345 ±128	24 ±1
Trichocerca sp.	-	67 ±64	132 ±74	-	39 ±14	61 ±35	-	70 ±69	554 ±154	183 ±103	136 ±31	-
Polyarthra sp.	346 ±186	405 ±44	226 ±136	663 ±160	240 ±158	162 ±94	51 ±35	201 ±87	515 ±150	487 ±210	443 ±97	306 ±178
Daphnia sp.	93 ±39	67 ±45	64 ±55	186 ±83	70 ±58	30 ±14	21	48 ±37	44	-	66 ±41	85 ±13
Moina sp.	-	-	35 ±17	45 ±38	-	-	-	-	-	-	-	-
Total	2738 ±500	2964 ±96	3779 ±638	5095 ±701	2522 ±546	2151 ±274	1261 ±513	2904 ±906	5786 ±1482	3217 ±1045	5076 ±1217	3041 ±492

App. Table 2.36. Year-round zooplankton count (number/liter water) in natural wetlands in the Barind Tract.

Zooplankton	May -18	Jun -18	Jul -18	Aug -18	Sep -18	Oct -18	Nov -18	Dec -18	Jan -19	Feb -19	Mar -19	Apr -19
Cyclops sp.	74 ±55	111 ±60	59 ±36	288 ±27	94 ±51	105 ±117	124 ±46	151 ±30	103 ±46	-	-	-
Filinia sp.	-	81 ±16	115	191 ±56	74 ±91	-	28 ±13	96 ±83	186 ±102	-	-	-
Diaphanosoma sp.	202	-	-	62 ±28	-	-	45			-	-	-
Keratella sp.	194 ±231	178 ±114	133 ±85	400 23±	78 ±62	56 ±17	164 ±114	618 ±605	585 ±181	-	-	-
Brachionus sp.	489 ±389	244 ±186	240 ±110	516 ±59	192 ±103	37 ±26	70 ±73	439 ±165	474 ±207	-	-	-
Nauplius	283 ±48	477 ±389	427 ±272	419 ±106	402 ±203	175 ±121	311 ±142	403 ±163	314 ±129	-	-	-
Asplanchna sp.	139 ±9	174 ±39	64 ±39	373 ±62	218 ±258	89 ±4	71 ±71	129 ±97	246 ±82	-	-	-
Diaptomus sp.	123 ±112	175 ±115	20	189 ±112	214 ±167	198 ±125	22 ±1	44 ±22	35 ±10	-	-	-
Trichocerca sp.	-	110 ±97	46	-	21 ±1	-	22 ±2	45 ±0	110 ±41	-	-	-
Polyarthra sp.	213 ±127	102 ±58	51 ±36	320 ±44	21 ±1	44	-	44 ±22	210 ±86	-	-	-
Daphnia sp.	47 ±43	46	-	42 ±18	23	-	22 ±1	30 ±13	128 ±107	-	-	-
Moina sp.	-	23	-	-	-	-	22	-	-	-	-	-
Total	1397 ±564	1486 ±313	955 ±180	2721 ±405	1260 ±376	536 ±132	763 ±170	1910 ±991	2281 ±875	-	-	-

App. Table 2.37. Annual average count (number/liter) of different zooplankton in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Treatments	Replications	*Cyclops* sp.	*Filinia* sp.	*Diaphanosoma* sp.	*Keratella* sp.	*Brachionus* sp.	Nauplius	*Asplanchna* sp.	*Diaptomus* sp.	*Trichocerca* sp.	*Polyarthra* sp.	*Daphnia* sp.	*Moina* sp.	Total
Aquaculture ponds	R-1	258	305	50	651	903	593	501	104	208	342	80	23	3741
	R-2	263	227	146	667	836	403	455	115	178	378	95	68	3390
	R-3	145	243	30	562	869	446	350	79	144	346	66		3098
	R-4	360	223	44	647	697	471	475	114	200	283	76	24	3282
	Average	257 ±88	250 ±38	67 ±53	632 ±47	826 ±90	478 ±82	445 ±66	103 ±17	183 ±28	337 ±39	79 ±12	38 ±26	3378 ±270
	Percent	7.61	7.4	1.98	18.71	24.45	14.15	13.17	3.05	5.42	9.98	2.34	1.13	100
	Ranking	6	7	11	2	1	3	4	9	8	5	10	12	
Natural wetlands	R-1	127	70	43	335	377	174	195	43	93	133	69		1337
	R-2	128	144		240	282	405	166	167	61	123	32		1392
	R-3	147	160	142	189	248	500	158	158	48	133	36		1553
	R-4	110	149	45	386	351	348	226	91	58	169	75	23	1632
	Average	128 ±15	131 ±41	77 ±57	287 ±89	315 ±60	357 ±137	186 ±31	115 ±59	65 ±20	139 ±20	53 ±22	23 ±0	1479 ±138
	Percent	8.66	8.85	5.21	19.41	21.29	24.14	12.57	7.78	4.39	9.4	3.58	1.55	100
	Ranking	7	6	9	3	2	1	4	8	10	5	11	12	

App. Table 2.38. Year-round aquatic insects count (number/3 square meter water) in aquaculture ponds under entrepreneurial fish production in the Barind Tract.

Aquatic insects	May -18	Jun -18	Jul -18	Aug -18	Sep -18	Oct -18	Nov -18	Dec -18	Jan -19	Feb -19	Mar -19	Apr -19
Dragon fly nymph	-	0.5 ±0.6	0.5 ±0.6	1.5 ±1.3	2.0 ±1.4	2.0 ±2.4	1.0 ±1.4	0.5 ±0.6	0.5 ±0.6	0.3 ±0.5	0.3 ±0.5	-
Damselfly nymph	3.8 ±1.7	4.5 ±1.7	6.0 ±1.8	12.0 ±3.2	20.0 ±6.5	30.5 ±16.5	37.3 ±10.4	18.0 ±5.1	14.0 ±4.5	4.8 ±1.7	0.5 ±0.6	0.5 ±0.6
Mayfly nymph	0.3 ±0.5	0.5 ±0.6	0.8 ±0.5	1.5 ±1.3	8.8 ±5.6	12.5 ±9.3	6.8 ±3.5	2.0 ±2.2	1.3 ±2.5	0.8 ±0.5	-	-
Backswimmer	40.5 ±12.5	30.8 ±8.8	35.0 ±10.0	36.3 ±9.9	36.8 ±10.7	37.8 ±14.5	49.3 ±17.4	24.0 ±12.6	27.8 ±7.4	33.3 ±10.2	29.0 ±15.9	16.8 ±6.8
Water boatman	38.8 ±13.1	49.3 ±12.8	40.3 ±7.6	35.8 ±4.6	25.0 ±5.0	18.3 ±6.2	34.0 ±18.8	34.3 ±14.8	31.3 ±8.7	36.8 ±6.1	60.8 ±12.6	54.3 ±13.3
Water scorpion *Ranatra* sp.	0.3 ±0.5	0.3 ±0.5	0.8 ±0.5	1.3 ±1.0	1.0 ±0.8	0.8 ±1.0	2.3 ±0.5	0.8 ±1.0	0.3 ±0.5	-	-	-
Water scorpion *Nepa* sp.	-	0.3 ±0.5	0.3 ±0.6	0.5 ±0.6	0.8 ±1.0	1.8 ±2.4	0.3 ±0.5	0.3 ±0.5	-	-	-	0.3 ±0.5
Water strider *Gerris* sp.	0.5 ±0.6	0.5 ±0.6	0.8 ±0.5	1.0 ±1.2	0.8 ±1.0	5.3 ±3.9	1.5 ±2.4	0.8 ±1.0	1.0 ±1.4	0.5 ±0.6	1.0 ±0.0	0.8 ±1.0
Creeping water bug/Giant water bug	-	-	0.3 ±0.5	0.5 ±0.6	1.3 ±1.0	1.0 ±1.4	0.5 ±0.6	0.3 ±0.5	-	-	-	-
Water scavenger	0.5 ±0.6	0.3 ±0.5	1.5 ±1.3	1.8 ±0.5	3.3 ±1.0	3.8 ±1.7	0.8 ±1.0	0.5 ±0.6	0.3 ±0.5	0.5 ±0.6	1.3 ±1.3	2.0 ±2.4
Diving beetle *Laccophilus* sp.	1.0 ±0.8	2.5 ±1.0	2.0 ±0.5	2.3 ±0.6	3.2 ±1.2	3.0 ±1.0	2.0 ±1.4	1.5 ±2.4	1.5 ±1.3	0.3 ±0.5	1.8 ±0.5	1.3 ±2.5
Total	84.8 ±22.3	89.3 ±19.2	88.1 ±6.8	94.3 ±10.9	102.7 ±15.6	116.5 ±23.6	135.5 ±17.9	82.8 ±6.8	77.8 ±8.9	77.1 ±10.0	94.7 ±26.8	76.8 ±16.1

App. Table 2.39 Year-round aquatic insects count (number/3 square meter water) in natural wetlands in the Barind Tract.

Aquatic insects	May -18	Jun -18	Jul -18	Aug -18	Sep -18	Oct -18	Nov -18	Dec -18	Jan -19	Feb -19	Mar -19	Apr -19
Dragon fly nymph	0.8 ±1.0	1.0 ±1.2	1.0 ±1.4	1.3 ±1.5	1.8 ±1.5	4.8 ±4.1	2.3 ±1.5	1.3 ±1.3	1.3 ±1.5	-	-	-
Damselfly nymph	1.3 ±1.5	1.5 ±1.9	1.3 ±1.5	1.8 ±2.1	2.3 ±1.5	3.0 ±1.8	5.5 ±2.4	2.5 ±1.3	2.0 ±1.4	-	-	-
Mayfly nymph	0.5 ±0.6	1.5 ±1.3	0.5 ±0.6	0.8 ±1.0	1.3 ±1.3	2.0 ±1.8	3.3 ±1.3	0.5 ±0.6	1.8 ±0.5	-	-	-
Backswimmer	5.0 ±1.8	5.5 ±1.3	7.3 ±1.7	6.3 ±1.0	7.0 ±1.6	7.3 ±1.5	6.3 ±1.5	6.8 ±2.6	5.0 ±1.4	-	-	-
Water boatman	4.8 ±1.7	4.5 ±1.3	4.8 ±1.7	3.3 ±1.3	6.0 ±1.8	7.8 ±1.0	6.5 ±1.3	6.8 ±1.3	7.5 ±0.6	-	-	-
Water scorpion *Ranatra* sp.	0.5 ±0.6	0.8 ±1.0	1.5 ±1.3	1.5 ±1.7	1.0 ±1.4	1.8 ±2.1	2.0 ±1.8	4.7 ±4.6	1.3 ±1.9	-	-	-
Water scorpion *Nepa* sp.	0.5 ±0.6	1.0 ±0.0	0.8 ±0.5	0.8 ±1.0	1.5 ±1.3	1.5 ±0.6	1.5 ±1.3	0.5 ±0.6	0.5 ±0.6	-	-	-
Water strider *Gerris* sp.	3.0 ±1.4	2.8 ±1.0	2.8 ±1.0	1.3 ±1.0	2.0 ±0.8	2.8 ±0.5	2.8 ±0.5	2.0 ±0.8	3.0 ±0.8	-	-	-
Creeping water bug/ Giant water bug	0.8 ±1.0	0.8 ±1.0	1.3 ±1.5	1.8 ±2.1	1.3 ±1.5	2.0 ±2.4	1.8 ±2.1	1.3 ±1.5	1.0 ±1.4	-	-	-
Water scavenger	0.8 ±1.0	1.0 ±1.4	0.8 ±1.0	1.5 ±1.3	1.5 ±1.7	2.3 ±2.6	1.8 ±2.4	1.3 ±1.5	0.5 ±0.6	-	-	-
Diving beetle *Laccophilus* sp.	0.8 ±0.5	1.5 ±0.6	1.5 ±1.3	2.0 ±2.4	2.5 ±1.3	3.0 ±1.8	2.3 ±1.3	1.8 ±2.1	1.3 ±1.3			
Total	18.6 ±6.2	21.8 ±7.9	23.3 ±7.4	22.0 ±11.6	28.0 ±10.3	38.0 ±11.2	35.8 ±12.3	28.1 ±4.6	25.1 ±4.6	-	-	-

App. Table 2.40. Annual average aquatic insect count (number/3 square meter water area) in aquaculture ponds under entrepreneurial fish production and in natural wetlands in the Barind Tract.

Treatments	Replications	Dragon fly nymph	Damselfly nymph	Mayfly nymph	Backswimmer	Water boatman	Water scorpion *Ranatra* sp.	Water scorpion *Nepa* sp.	Water strider *Gerris* sp.	Creeping water bug	Water scavenger	Diving beetle	Total
Aquaculture ponds	R-1	0.6	12.8	4.4	34.3	41.3	0.8	0.8	1.4	0.3	1.0	1.2	99.1
	R-2	0.6	12.7	3.5	34.1	37.8	0.3	0.1	1.8	0.3	1.4	2.4	95.0
	R-3	0.7	13.9	1.9	30.3	38.6	0.8	0.3	0.6	0.6	1.8	1.7	91.1
	R-4	0.8	11.2	1.8	33.3	35.1	0.7	0.3	1.0	0.1	1.3	2.1	87.6
	Average	0.7	12.6	2.9	33.0	38.2	0.6	0.4	1.2	0.3	1.4	1.9	93.2
	Percentage	0.7	13.6	3.1	35.4	41.0	0.7	0.4	1.3	0.3	1.5	2.0	100.0
	Ranking	8	3	4	2	1	8	9	7	10	6	5	-
Natural wetlands	R-1	2.4	3.6	1.7	6.4	6.0	2.4	1.3	2.8	2.6	2.9	3.4	35.5
	R-2	2.8	3.9	2.3	5.4	6.0	2.3	0.9	2.9	2.7	2.0	2.8	34.0
	R-3	1.2	1.0	0.7	6.0	5.6	1.2	0.8	2.2	0.0	0.0	0.0	18.7
	R-4	0.3	0.9	0.7	7.1	5.4	0.1	0.8	2.0	0.0	0.1	1.1	18.5
	Average	1.7	2.3	1.3	6.3	5.8	1.5	0.9	2.5	1.3	1.3	1.8	26.7
	Percentage	6.4	8.7	5.0	23.4	21.6	5.7	3.5	9.3	4.9	4.7	6.8	100.0
	Ranking	6	4	8	1	2	7	11	3	9	10	5	-

App. Table. 2.41. Year-round benthos (chironomid larvae and tubifex) count (number/6 square inches) in ponds under entrepreneurial fish production in the Barind Tract.

Benthos	May-18	Jun-18	Jul-18	Aug-18	Sep-18	Oct-18	Nov-18	Dec-18	Jan-19	Feb-19	Mar-19	Apr-19
Chironomid larvae	32.3 ±6.8	85.0 ±36.7	93.3 ±36.2	92.3 ±32.4	87.3 ±42.9	64.3 ±17.3	38.5 ±6.9	38.8 ±5.6	33.3 ±5.3	55.0 ±10.6	90.3 ±26.3	37.5 ±7.0
Tubifex	62.0 ±10.6	53.5 ±8.1	45.5 ±5.4	43.8 ±5.6	47.8 ±2.5	41.5 ±3.4	21.0 ±5.0	26.3 ±4.4	26.3 ±5.4	29.3 ±5.6	45.8 ±6.9	64.0 ±7.3
Total	94.3 ±12.9	138.5 ±30.9	138.8 ±35.2	136.0 ±29.9	135.0 ±43.4	105.8 ±18.4	59.5 ±9.3	65.0 ±1.8	59.5 ±6.6	84.3 ±16.1	136.0 ±26.2	101.5 ±6.0

App. Table. 2.42. Year-round benthos (chironomid larvae and tubifex) count (number/6 square inches) in natural wetlands in the Barind Tract.

Benthos	May-18	Jun-18	Jul-18	Aug-18	Sep-18	Oct-18	Nov-18	Dec-18	Jan-19
Chironomid larvae	1.0 ±0.8	1.3 ±1.0	1.3 ±1.0	1.0 ±0.8	1.3 ±0.5	1.3 ±1.0	1.0 ±0.0	0.8 ±0.5	1.0 ±0.0
Tubifex	1.5 ±1.3	2.0 ±1.4	1.8 ±1.0	1.3 ±1.0	2.0 ±0.5	2.3 ±1.5	1.5 ±2.4	1.3 ±1.5	1.5 ±1.3
Total	2.5 ±1.9	3.3 ±2.2	3.0 ±1.6	2.3 ±1.5	3.3 ±1.3	3.5 ±1.7	2.5 ±2.4	2.0 ±1.8	2.5 ±1.3

App. Table 3.1. Quinalphos residue in water and fish on different days after the pond water treatment with quinalphos (at 0.02 mg/liter water).

Duration of exposure	Sample	Level of residue (mg/kg)
Pretreatment	Water	Not detected
	Fish	Not detected
After 1 day	Water	Not detected
	Fish	0.23
After 2 days	Water	Not detected
	Fish	0.16
After 5 days	Water	Not detected
	Fish	0.027
After 10 days	Water	Not detected
	Fish	Not detected

App. Table 3.2. Water quality parameters of the experimental ponds.

Treatment pond	Water pH	Water temp (^{0}C)	Water turbidity (ntu)	Water electrical conductivity (µS/cm)	Organic carbon content of the pond bottom sludge (%)
quinalphos	7.7±0.72	23.2±0.15	21.1±6.52	378±56.2	2.6±0.48

App. Table 3.3. Zooplankton count (nos/liter) on different days after pond water treatment with quinalphos.

Zooplankton	0 day	1 day	2 days	5 days	10 days	15 days	21 days	28 days
Brachionus sp.	838 ± 421	578 ± 187	541 ± 297	596 ± 362	634 ± 284	590 ± 294	611 ± 313	679 ± 316
Keratella sp.	490 ± 354	433 ± 192	325 ± 117	430 ± 296	479 ± 300	491 ± 243	488 ± 189	395 ± 315
Cyclops sp.	122 ± 125	60 ± 68	111 ± 86	220 ± 217	231 ± 211	212 ± 143	173 ± 224	239 ± 149
Nauplius	373 ±250	311 ± 65	257 ± 80	432 ± 203	444 ±175	345 ± 134	407 ± 217	433 ± 135
Polyarthra sp.	147 ± 77	92 ± 94	139 ± 110	318 ± 327	352 ± 318	305 ± 296	276 ± 270	268 ± 237
Diaptomus sp.	38 ± 35	23 ± 23	-	44 ± 42	7 ± 12	15 ± 12	36 ± 44	89 ± 59
Asplanchna sp.	351 ± 376	267 ± 87	208 ± 238	436 ± 134	409 ± 144	397 ± 216	312 ± 196	351 ± 194
Daphnia sp.	100 ± 36	112 ± 136	89 ± 94	43 ± 20	129 ± 132	87 ± 87	116 ± 103	89 ± 98
Moina sp.	16 ± 27	-	-	7 ± 12	7 ± 12	14 ± 24	7 ± 12	44 ± 44
Diaphanosoma sp.	8 ± 13	7 ± 12	-	14 ± 24	29 ± 12	28 ± 49		7 ± 12
Filinia sp.	163 ± 146	91 ± 22	141 ± 110	254 ± 243	289 ± 213	294 ± 187	233 ± 134	231 ± 127
Trichocerca sp.	70 ± 102	142 ± 227	53 ± 46	-	-	-	-	-
Total	2716 ±1602	2116 ±870	1863 ±1042	2794 ±1795	3009 ±1707	2777 ±1553	2658 ±1565	2827 ±1633

App. Table 3.4. Aquatic insect and benthos count on different days after pond water treatment with quinalphos.

Category	0 day	1 day	2 days	5 days	10 days	15 days	21 days	28 days
Insect count (number/3 sq meter)	189.7 ± 63.3	207.3 ± 35.8	207.7 ± 34.2	158.7 ±92.8	139.0 ± 37.5	153.7 ± 36.8	146.7 ± 32.1	132.3 ± 7.2
Benthos count (number/6 sq inch bottom)	41.7 ± 7.6	21.0 ± 4.0	25.0 ± 7.2	25.7 ± 18.9	39.7 ± 22.5	22.0 ± 11.4	23.3 ± 3.2	25.7 ± 10.7

App. Table 3.5. Water insect count and diversity on different days after pond water treatment with quinalphos.

Insects	0 day	1 day	2 days	5 days	10 days	15 days	21 days	28 days
Boatman	1.0 ± 1.7	2.7 ± 2.1	2.0 ± 1.0	1.0 ± 1.0	-	-	1.3 ± 0.6	5.3 ± 2.1
Backswimmer	182.7 ± 61.7	202.3 ± 33.6	204.7 ± 35.1	156.7 ± 92.5	138.0 ± 36.5	153.7 ± 36.8	145.3 ± 31.6	126.3 ± 7.6
Ranatra sp.	0.3 ± 0.6	-	-	-	0.3 ± 0.6	-	-	-
Dragon fly	0.3 ± 0.6	-	-	0.3 ± 0.6	0.7 ± 0.6	-	-	-
May fly	1.0 ± 1.0	-	0.3 ± 0.6	-	-	-	-	-
Gerris sp.	3.0 ± 2.0	2.3 ± 0.6	0.7 ± 0.6	0.7 ± 1.2	-	-	-	-
Water scavenger	1.3 ± 1.5	-	-	-	-	-	-	0.7 ± 0.6

App. Table 3.6. Zooplankton count in aquaculture ponds after use of emamectin benzoate as feeding and water treating agent respectively.

Treatment	Zooplankton count/liter	Pre-treatment	Day-1	Day-2	Day-5	Dat-10	Day-15	Day-21	Day-28
Feeding of emamectin benzoate	*Cyclops* sp.	252 ±166	186 ±58	111 ±93	226 ±130	124 ±78	-	-	-
	Keratella sp.	712 ±282	867 ±128	634 ±85	638 ±41	750 ±93	-	-	-
	Brachionus sp.	695 ±62	786 ±98	693 ±164	580 ±145	756 ±67	-	-	-
	Nauplius	466 ±129	499 ±209	396 ±102	430 ±29	464 ±106	-	-	-
	Asplanchna sp.	466 ±195	452 ±26	512 ±254	433 ±160	474 ±40	-	-	-
	Diaptomus sp.	53 ±34	7 ±13	23 ±39	14 ±24	69 ±68	-	-	-
	Polyarthra sp.	283 ±179	334 ±163	363 ±33	451 ±83	457 ±88	-	-	-
	Daphnia sp.	23 ±39	61 ±34	211 ±58	73 ±126	68 ±118	-	-	-
	Filinia sp.	145 ±233	47 ±48	242 ±31	245 ±263	178 ±166	-	-	-
	Total	3098 ±904	3241 ±542	3189 ±114	3092 ±970	3342 ±659	-	-	-
Emamectin benzoate as water treating agent	*Cyclops* sp.	205 ±186	212 ±66	96 ±35	154 ±186	186 ±200	213 ±12	268 ±172	149 ±0
	Keratella sp.	536 ±67	649 ±149	272 ±85	722 ±113	593 ±32	740 ±275	964 ±294	1018 ±746
	Brachionus sp.	920 ±43	1086 ±233	727 ±83	942 ±262	835 ±181	1020 ±287	1521 ±426	932 ±79
	Nauplius	475 ±154	224 ±17	252 ±14	353 ±13	577 ±236	674 ±9	682 ±171	514 ±152
	Asplanchna sp.	133 ±157	259 ±133	305 ±174	397 ±21	334 ±21	267 ±251	600 ±194	343 ±152
	Diaptomus sp.	45 ±30	11 ±16	-	19 ±26	-	45 ±63	36 ±51	42 ±60
	Polyarthra sp.	331 ±152	271 ±83	193 ±4.9	248 ±26	284 ±340	426 ±57	315 ±139	309 ±317
	Daphnia sp.	11 ±15	59 ±83	100 ±7	300 ±127	142 ±170	256 ±202	131 ±151	182 ±227
	Filinia sp.	122 ±172	129 ±183	23 ±33	45 ±64	79 ±112	124 ±176	195 ±275	160 ±226
	Total	2779 ±70	2901 ±200	1970 ±342	3182 ±417	3033 ±325	3768 ±838	4714 ±943	3651 ±755

App. Table 3.7. Aquatic insect count in aquaculture ponds after use of emamectin benzoate as feeding and water treating agents respectively (corresponding Figure 3.7 and 3.8).

Treatment	Insect sp.	Pre-treatment	Day-1	Day-2	Day-5	Day-10	Day-15	Day-21	Day-28
Feeding of emamectin benzoate	Dragon fly	2.7 ±2.5	3.5 ±4.9	-	-	0.5 ±0.7	-	-	-
	Damselfly	36.7 ±13.4	38.0 ±9.9	25.0 ±26.9	29.5 ±10.6	47.0 ±7.1	-	-	-
	Backswimmer	22.0 ±6.0	32.5 ±4.9	37.0 ±22.6	41.0 ±14.1	21.5 ±3.5	-	-	-
	Water boatman	5.3 ±3.1	3.0 ±0.0	4.0 ±1.4	14.0 ±15.6	4.0 ±1.4	-	-	-
	Ranatra sp.	0.7 ±1.2	2.5 ±3.5	2.0 ±0.0	2.0 ±0.0	4.0 ±4.2	-	-	-
	Nepa sp.	2.3 ±2.5	1.5 ±2.1	0.5 ±0.7	-	-	-	-	-
	Gerris sp.	6.0 ±4.4	2.5 ±0.7	23.5 ±31.8	10.0 ±4.2	4.5 ±2.1	-	-	-
	Sphaerodema sp.	1.3 ±1.5	3.0 ±4.2	0.5 ±0.7	-	-	-	-	-
	Crawling water bug	8.0 ±9.5	5.0 ±4.2	3.5 ±0.7	1.0 ±0.0	5.0 ±1.4	-	-	-
	Water scavenger	4.3 ±1.5	3.0 ±1.4	1.0 ±0.0	2.0 ±1.4	5.0 ±2.8	-	-	-
	Total	89.3 ±4.5	94.5 ±24.7	97 ±26.9	99.5 ±14.8	91.5 ±10.6	-	-	-
Emamectin benzoate as water treating agent	Dragon fly	1.5 ±2.1	0.5 ±0.7	1.0 ±1.4	0.5 ±0.7	-	4.5 ±2.1	2.5 ±0.7	
	Damselfly	5.5 ±4.9	3.5 ±0.7	6.5 ±3.5	4.5 ±2.1	2.0 ±1.4	6.0 ±1.4	-	5.5 ±0.7
	Backswimmer	46.0 ±9.9	112.0 ±38.2	85.5 ±23.3	58.5 ±9.2	70.0 ±9.9	62.5 ±7.8	77.0 ±5.7	58.5 ±16.3
	Water boatman	6.0 ±4.2	14.0 ±4.2	17.0 ±0.0	4.0 ±1.4	1.5 ±0.7	2.5 ±0.7	4.5 ±2.1	21.5 ±6.4
	Ranatra sp.	0.5 ±0.7		0.5 ±0.7	1.0 ±1.4	1.0 ±0.0	1.0 ±0.0	0.5 ±0.7	0.5 ±0.7
	Nepa sp.	-	-	-	-	-	-	1.0 ±0.0	0.5 ±0.7
	Gerris sp.	17.5 ±20.7	5 ±1.4	4.5 ±0.7	28.0 ±25.5	5.5 ±2.1	7.0 ±1.4	5.5 ±2.1	2.0 ±1.4
	Sphaerodema sp.	-	-	-	-	-	-	-	-
	Crawling water bug	-	-	2.0 ±2.8	1.5 ±2.1	0.5 ±0.7	-	-	0.5 ±0.7
	Water scavenger	4.0 ±0.0	3.5 ±2.1	2.5 ±2.1	2.5 ±2.1	2.0 ±1.4	3.0 ±0.0	1.0 ±1.4	3.5 ±0.7
	Total	81.0 ±22.6	138.5 ±41.7	119.5 ±14.8	100.5 ±20.5	82.5 ±12.0	86.5 ±6.4	92.0 ±5.7	92.5 ±9.2

App. Table 3.8. Benthos count in aquaculture ponds after use of emamectin benzoate as feeding and water treating agent respectively (corresponding Figure 3.9 and 3.10).

Treatment	Benthos count/liter	Pre-treatment	Day-1	Day-2	Day-5	Dat-10	Day-15	Day-21	Day-28
Feeding of emamectin benzoate	*Chironomid larvae*	86.3 ±8.5	90.6 ±7.8	84.6 ±7.5	88.0 ±3.6	86.6 ±10.0	-	-	-
	Tubefex	54.0 ±6.2	53.3 ±8.1	52.0 ±6.3	53.3 ±7.1	59.0 ±9.2	-	-	-
	Total	140.3 ±11.5	144 ±15.9	136.6 ±13.7	141.3 ±9.2	145.6 ±10.7	-	-	-
Emamectin benzoate as water treating agent	*Chironomid larvae*	36.0 ±3.0	7.3 ±4.0	9.6 ±3.1	15.3 ±7.8	13.3 ±0.6	40.33 ±10.2	28.6 ±3.8	34.3 ±3.5
	Tubefex	22.3 ±2.5	7.6 ±2.1	17.3 ±2.5	19.0 ±5.0	35.3 ±4.0	58.0 ±10.5	72.6 ±5.1	65.0 ±8.5
	Polychaeta	-	-	0.3 ±0.6	1.6 ±0.6	1.3 ±1.5	-	-	-
	Leech	-	-	-	0.3 ±0.6	-	-	-	-
	Total	58.3 ±3.1	15.0 ±6.1	27.3 ±4.7	36.3 ±5.7	50.0 ±5.6	98.3 ±19.3	101.3 ±7.4	99.3 ±5.0

App. Table 3.9. Some important water quality parameters in cypermethrin and deltamethrin treated experimental ponds.

Treatment ponds	Water pH	Water temp (^{0}C)	Water turbidity (ntu)	Water electrical conductivity (µS/cm)	Organic carbon content of the pond bottom sludge (%)
cypermethrin	7.41	23.1	28.05	310	2.19
deltamethrin	7.70	30.4	43.19	516	3.40

App. Table 3.10. Zooplankton count (nos/liter water) before and after use of cypermethrin and deltamethrin treatment in commercial aquaculture ponds.

Insecticide	Benthos	Day-0	Day-1	Day-2	Day-5	Day-10	Day-15	Day-21	Day-28
Cypermethrin	*Brachionus* sp.	995 ±269	751 ±104	803 ±32	546 ±32	653 ±183	907 ±48	756 ±12	727 ±71
	Keratella sp.	333 ±204	391 ±140	428 ±21	510 ±56	602 ±224	1037 ±177	727 ±53	679 ±78
	Cyclops sp.	177 ±59	69 ±23	14 ±12	60 ±26	140 ±46	150 ±89	241 ±118	145 ±36
	Nauplius	622 ±183	232 ±39	184 ±67	249 ±54	248 ±95	339 ±92	326 ±58	435 ±27
	Polyarthra sp.	148 ±181	263 ±117	198 ±110	53 ±58	159 ±112	228 ±159	214 ±67	392 ±93
	Diaptomus sp.	7 ±12	7 ±13	-	22 ±22	7 ±13	23 ±23	14 ±12	23 ±23
	Asplanchna sp.	7 ±12	147 ±103	89 ±16	175 ±55	323 ±158	445 ±116	473 ±70	321 ±32
	Daphnia sp.	14 ±25	15 ±27	52 ±14	83 ±11	27 ±16	86 ±17	53 ±32	53 ±36
	Moina sp.	7 ±12	-	-	7 ±12	-	-	-	-
	Filinia sp.	125 ±126	108 ±81	141 ±104	195 ±87	220 ±63	282 ±92	183 ±34	260 ±75
	Trichocerca sp.	14 ±25	-	-	-	-	-	-	-
	Total	2412 ±473	1987 ±492	1910 ±107	1903 ±95	2383 ±765	3500 ±502	2989 ±238	3038 ±130
Deltamethrin	*Brachionus* sp.	936 ±80	718 ±8	591 ±32	1628 ±161	1133 ±220	1040 ±179	1123 ±117	1151 ±96
	Keratella sp.	720 ±34	520 ±73	432 ±42	570 ±108	1321 ±61	1125 ±183	1004 ±67	1159 ±53
	Cyclops sp.	263 ±104	86 ±34	98 ±66	192 ±41	121 ±47	210 ±61	217 ±33	172 ±45
	Nauplius	632 ±86	505 ±35	439 ±81	529 ±126	534 ±67	516 ±84	531 ±100	598 ±44
	Polyarthra sp.	714 ±60	725 ±51	912 ±94	721 ±62	647 ±181	59 ±164	654 ±61	646 ±58
	Diaptomus sp.	-	-	7 ±12	64 ±14	8 ±13	7 ±13	-	7 ±13
	Asplanchna sp.	650 ±58	552 ±70	577 ±31	810 ±69	794 ±32	664 ±79	733 ±88	772 ±61
	Daphnia sp.	143 ±109	148 ±56	75 ±71	144 ±22	129 ±75	69 ±46	69 ±83	125 ±11
	Moina sp.	-	-	-	-	8 ±13	-	-	-
	Diphanosoma sp.	15 ±13	7 ±13	37 ±34	-	-	-	-	-
	Filinia sp.	182 ±158	23 ±39	15 ±26	16 ±28	15 ±27	-	23 ±41	39 ±26
	Trichocerca sp.	65 ±113	16 ±27	-	-	56 ±50	15 ±13	31 ±56	46 ±22
	Total	4324 ±285	3302 ±200	3185 ±88	4676 ±323	4768 ±650	4243 ±633	4388 ±308	4718 ±127

App. Table 3.11. Benthos count (nos/6sq inches of the pond bottom) before and after use of cypermethrin and deltamethrin treatment in commercial aquaculture ponds.

Insecticide	Benthos	Pre-treatment	Day-1	Day-2	Day-5	Day-10	Day-15	Day-21	Day-28
cypermethrin	Chironomid larvae	27.0 ±7.0	29.3 ±13.0	18.7 ±18.0	20.7 ±7.0	31.0 ±28.0	34.3 ±16.0	38.3 ±7.0	38.7 ±8.0
	Tubifex	6.0 ±1.0	3.7 ±1.0	8.0 ±4.0	3.0 ±1.0	3.0 ±2.0	6.0 ±3.0	7.7 ±4.0	8.3 ±2.0
	Leech	0.7 ±0.6	1.0 ±1.0	-	1.0 ±1.0	-	1.0 ±1.0	0.3 ±0.6	0.7 ±1.2
	Total	33.7 ±6.8	34.0 ±13.1	26.7 ±15.0	24.7 ±6.7	34.0 ±29.5	41.3 ±19.1	46.3 ±10.7	47.7 ±10.4
deltamethrin	Chironomid larvae	151.3 ±25.0	174.3 ±12.0	165 ±25.0	171.3 ±25.0	186.3 ±8.0	193.3 ±9.0	190 ±17	179.3 ±12.0
	Tubifex	44.7 ±8.0	36.7 ±5.5	33.3 ±6.0	45.3 ±7.4	50.0 ±2.6	41.0 ±9.6	44.0 ±7.9	43.0 ±4.6
	Total	196.0 ±30.8	211.0 ±8.5	198.3 ±31.0	216.7 ±32.7	236.3 ±7.6	234.3 ±18.3	234.0 ±12.1	222.3 ±16.5

App. Table 3.12. Aquatic insect count (nos/3 sq meter) on different days after deltamethrin treatment @ 5µg/liter of water in commercial aquaculture pond.

Aquatic insects	0 day	1 day	2 days	5 days	10 days	15 days	21 days	28 days
Boatman	80.7 ±57.7	11.0 ±2.0	31.7 ±21.2	206.7 ±72.4	168.7 ±28.9	194.3 ±12.1	186.7 ±47.9	190.3 ±20.1
Backswimmer	9.0 ±2.0	0.7 ±0.6	1.7 ±1.5	1.3 ±1.5	6.3 ±5.7	7.7 ±8.6	7.0 ±5.3	9.3 ±7.1
W. scavenger	-	0.7 ±1.2	-	-	-	-	-	-
Total	89.7 ±55.8	12.3 ±1.5	33.3 ±21.4	208.0 ±71.4	175.0 ±34.1	202.0 ±19.2	193.7 ±43.4	199.7 ±24.8

App. Table 3.13. Insect count (nos/3 sq meter) on different days before and after use of cypermethrin @ 5 µg (active ingredient) /liter in commercial aquaculture ponds.

Aquatic insects	0 day	1 day	2 days	5 days	10 days	15 days	21 days	28 days
Dragon fly nymph	-	0.7 ±1.2	-	-	-	-	-	-
Damselfly nymph	39.3 ±11.7	2.7 ±1.5	1.3 ±1.5	1.0 ±0.0	2.0 ±2.6	1.3 ±1.5	3.3 ±1.5	4.7 ±5.7
Backswimmer	52.0 ±20.2	7.3 ±3.2	7.0 ±1.0	12.3 ±9.0	28.7 ±15.0	18.7 ±5.7	10.3 ±2.1	8.0 ±4.6
Water boatman	28.0 ±17.7	1.0 ±1.7	13.0 ±13.0	0.3 ±0.6	0.7 ±1.2	6.7 ±5.7	44.7 ±22.3	56.7 ±15.8
Ranatra sp.	2.3 ±0.6	-	-	-	0.7 ±1.2	1.3 ±2.3	0.3 ±0.6	1.3 ±0.6
Nepa sp.	-	0.3 ±0.6	-	-	-	-	-	-
Gerris sp.	2.0 ±2.6	0.3 ±0.6	-	-	1.0 ±0.0	0.7 ±0.6	0.7 ±1.2	0.3 ±0.6
Creeping water bug	0.3 ±0.6	-	-	-	-	-	-	-
Crawling beetle	0.3 ±0.6	-	-	0.3 ±0.6	-	-	-	-
Water scavenger	0.7 ±0.6	0.3 ±0.6	-	0.3 ±0.6	-	0.7 ±1.2	1.7 ±0.6	1.0 ±1.0
Total	125 ±15.4	12.7 ±2.3	21.3 ±11.2	14.3 ±9.0	33 ±14.7	29.3 ±11.0	61.0 ±25.5	72.0 ±14.8

App. Table 3.14. Physico-chemical parameters of abamectin and lambda cyhalothrin treated experimental ponds before application of pesticides.

Treatment pond	Water Temperature (^{0}C)	Water pH	Water turbidity (ntu)	Electrical conductivity of water (µS/cm)	Organic Carbon content of bottom soil (%)
Abamectin pond	21	7.87	13.97	362	3.40
L-cyhalothrin pond	21	8.04	33.47	388	2.53

App. Table 3.15. Zooplankton count (nos/liter water) before and after use of abamectin and lambda-cyhalothrin treatment in commercial aquaculture ponds.

Insecticide	zooplankton	Day-0	Day-1	Day-2	Day-5	Day-10	Day-15	Day-21	Day-28
Abamectin @ 0.7µg/l	*Brachionus* sp.	1039 ±157	1107 ±44	1035 ±157	1393 ±704	736 ±38	1153 ±235	1908 ±473	998 ±475
	Keratella sp.	1025 ±187	986 ±58	1016 ±48	910 ±318	545 ±149	630 ±182	781 ±216	611 ±253
	Cyclops sp.	149 ±186	417 ±71	292 ±20	27 ±25	47 ±40	88 ±110	140 ±133	70 ±102
	Nauplius	661 ±79	758 ±35	655 ±102	489 ±234	316 ±120	401 ±133	606 ±125	487 ±202
	Polyarthra sp.	538 ±70	545 ±11	453 ±83	382 ±218	375 ±117	589 ±130	591 ±96	510 ±127
	Asplanchna sp.	553 ±66	811 ±21	788 ±113	729 ±465	331 ±49	710 ±226	693 ±225	611 ±277
	Daphnia sp.	15±13	37±26	7±13	-	-	-	-	-
	Moina sp.	99±58	15±13	-	-	-	-	-	-
	Diaphanosoma sp.	75±72	60±34	29±33	-	-	-	-	-
	Filinia sp.	477 ±62	545 ±57	473 ±100	162 ±161	-	100 ±130	311 ±212	370 ±204
	Trichocerca sp.	137 ±113	373 ±265	318 ±135	468 ±299	58 ±53	175 ±81	394 ±179	440 ±167
	Total	4768 ±771	5654 ±408	5065 ±647	4559 ±2372	2408 ±187	3846 ±911	5373 ±1398	4097 ±1372
Lambda-cyhalothrin @ 0.5µg/l	*Brachionus* sp.	971 ±114	682 ±90	559 ±49	734 ±55	641 ±80	829 ±25	895 ±164	810 ±80
	Keratella sp.	967 ±53	535 ±103	451±49	482 ±67	355 ±126	577 ±59	754 ±73	530 ±119
	Cyclops sp.	400 ±162	53 ±12	29±33	47 ±23	7 ±13	45 ±61	121 ±47	91 ±22
	Nauplius	759 ±75	403 ±58	337 ±30	354 ±74	363 ±57	471 ±42	602 ±58	527 ±123
	Polyarthra sp.	640 ±116	418 ±31	382 ±49	392 ±159	416 ±34	541 ±36	671 ±34	490 ±120
	Diaptomus sp.	7±13	-	-	-	-	-	-	-
	Asplanchna sp.	916 ±87	519 ±68	426 ±129	475 ±90	375 ±104	450 ±57	693 ±77	549 ±60
	Daphnia sp.	18±16	30±12	-	-	-	-	-	-
	Moina sp.	10±17	7±13	-	-	-	-	-	-
	Diaphanosoma sp.	7±13	-	-	-	-	-	-	-
	Filinia sp.	682 ±143	441 ±23	298 ±51	92 ±100	177 ±36	203 ±74	235 ±103	221 ±107
	Trichocerca sp.	547 ±71	339 ±60	233 ±114	92 ±60	29 ±33	128 ±72	273 ±99	114 ±2
	Total	5923 ±582	3429 ±236	2716 ±473	2669 ±522	2363 ±175	3245 ±264	4243 ±275	3333 ±347

App. Table 3.16. Benthos count (nos/6sq inches of the pond bottom) before and after use of abamectin and lambda-cyhalothrin in commercial aquaculture ponds.

Insecticide	Aquatic insects	Pre treatment	Day-1	Day-2	Day-5	Day-10	Day-15	Day-21	Day-28
abamectin @ 0.7µg/l	Chironomid larvae	54.7 ±12.9	51.7 ±11	27 ±10.4	37.3 ±6.1	38 ±7.5	31.5 ±9.3	80.3 ±26.6	176.7 ±23.2
	Tubifex	15.7 ±2.1	9.7 ±3.1	4.0 ±1.0	6.3 ±2.5	9.7 ±7.4	23.7 ±2.1	22 ±5.6	19.3 ±2.5
	Total	70.3 ±11.9	61.3 ±13.4	31.0 ±11.3	43.7 ±8.1	47.7 ±14.8	55.0 ±9.5	102.3 ±31.4	196.0 ±25.4
lambda-cyhalothrin @ 0.5µg/l	Chironomid larvae	6.3 ±1.5	0.3 ±0.6	1.3 ±0.6	1.0 ±1.7	2.3 ±2.5	7.3 ±3.2	15.3 ±8.0	29.7 ±7.4
	Tubifex	7.7 ±6.7	4.3 ±1.5	1.7 ±0.6	4.7 ±1.5	5.0 ±1.0	4.7 ±2.1	10 ±2.6	14.7 ±1.5
	Total	14.0 ±6.0	4.7 ±1.2	3.0 ±0.0	5.7 ±3.1	7.3 ±3.5	12.0 ±3.0	25.3 ±7.8	44.3 ±6.1

App. Table 3.17. Aquatic insect count (nos/3 sq meter) on different days after abamectin treatment @ 0.7µg/liter of water in commercial aquaculture pond.

Aquatic insects	Day-0	Day-1	Day-2	Day-5	Day-10	Day-15	Day-21	Day-28
Boatman	113.3 ±30.9	175.7 ±56.4	22.0 ±14.7	49.7 ±13.9	77.0 ±44.0	56.3 ±31.0	55.7 ±17.8	77.7 ±18.2
Backswimmer	94.7 ±73.2	24.7 ±20.1	20.3 ±7.6	11.3 ±4.7	12.7 ±12.9	16.0 ±11.3	12.0 ±5.6	12.7 ±6.1
Total	208.0 ±91.4	200.3 ±62.0	42.3 ±22.0	61.0 ±17.0	89.7 ±55.2	72.3 ±21.5	67.7 ±22.0	90.3 ±12.1

App. Table 3.18. Aquatic insect count (nos/3 sq meter) on different days after lambda-cyhalothrin treatment @ 0.5µg/liter of water in commercial aquaculture pond.

Aquatic insects	0 day	1 day	2 days	5 days	10 days	15 days	21 days	28 days
Boatman	28.0 ±16.1	14.0 ±19.9	4.3 ±5.9	2.0 ±1.7	5.0 ±2.0	2.7 ±1.2	7.7 ±13.3	12.3 ±13.6
Backswimmer	21.3 ±22.2	4.3 ±4.5	2.7 ±2.3	1.7 ±1.5	3.3 ±2.5	4.3 ±4.9	6.3 ±2.1	4.0 ±1.0
Gerris sp.	1.3 ±1.5	-	-	-	-	-	-	-
Crawling beetle	-	1.0 ±1.7	-	-	-	-	-	-
Water scavenger	-	1.3 ±2.3	0.7 ±1.2	-	-	-	-	-
Total	50.7 ±36.3	20.7 ±22.7	7.7 ±2.9	3.7 ±1.2	8.3 ±3.8	7.0 ±4.6	14.0 ±11.3	16.3 ±14.5

App. Table 3.19. Water turbidity of treatment ponds before and after treatment with various fish toxicants (and netting in between).

Sl.	Treatment ponds	Pretreatment turbidity (ntu)	Post treatment (after 3 times netting) turbidity (ntu)
1	Rotenone treatment pond	21.31	62.18
2	Aluminium phosphide (phostoxin)- pond	27.74	65.44
3	Fenpropathrin (Danitol) treatment pond	34.57	83.56

App. Table 3.20. Zooplankton count (nos/liter) before and after use of fenpropathrin, phostoxin and rotenone in commercial aquaculture pond.

Toxicant	Zooplankton	Pretreatment	Day-1	Day-2	Day-5	Day-10
Fenpropathrin @0.065 mg/liter	*Brachionus* sp.	892 ± 34	1654±122	1512±301	1135±217	1201 ± 225
	Keratella sp.	822 ±138	1236 ± 19	913 ± 219	748 ± 80	622 ± 110
	Cyclops sp.	148 ± 64	97 ± 45	15 ± 25	7 ± 13	71 ± 21
	Nauplius	375 ± 100	435 ± 55	346 ± 50	333 ± 95	383 ± 39
	Polyarthra sp.	-	88 ± 92	361 ± 116	315 ± 86	264 ± 74
	Diaptomus sp.	7 ± 12	-	-	-	-
	Asplanchna sp.	356 ± 55	523 ± 206	660 ± 168	484 ± 150	535 ± 158
	Daphnia sp.	75 ± 48	16 ± 28	15 ± 27	-	24 ± 25
	Moina sp.	13 ± 23	-	-	-	-
	Filinia sp.	-	-	278 ± 126	173 ± 47	299 ± 265
	Trichocerca sp.	219 ± 149	377 ± 118	208 ± 132	196 ± 86	101 ± 54
	Total	2908±404	4427±607	3311±675	3393±609	3502±822
Aluminium phosphide @0.61 mg/liter	*Brachionus* sp.	651 ± 72	54 ± 13	35 ± 25	40 ± 37	111 ± 88
	Cyclops sp.	634 ± 102	61 ± 21	86 ± 22	174 ± 121	625 ± 94
	Nauplius	566 ± 77	102 ± 40	193 ± 86	380 ± 242	586 ± 29
	Polyarthra sp.	-	-	-	24 ± 42	-
	Diaptomus sp.	109 ± 44	20 ± 20	14 ± 25	-	208 ± 119
	Asplanchna sp.	159 ± 29	7 ± 12	7 ± 12	134 ± 93	152 ± 49
	Daphnia sp.	36 ± 32	-	14 ± 12	-	-
	Moina sp.	21 ± 21	-	-	-	-
	Diaphanosoma sp.	14 ± 13	-	-	-	-
	Total	2191±57	244±51	349±122	752±521	1682±280
Rotenone @ 0.272mg/liter	*Brachionus* sp.	584 ± 91	337 ± 270	414 ± 138	481 ± 59	566 ± 110
	Keratella sp.	151 ± 262	20 ± 35	-	45 ± 23	63 ± 28
	Cyclops sp.	428 ± 78	87 ± 65	105 ± 76	255 ± 38	346 ± 107
	Nauplius	1191±280	715 ± 680	704 ± 283	549 ± 37	520 ± 87
	Polyarthra sp.	138 ± 240	88 ± 152	-	15 ± 13	19 ± 19
	Diaptomus sp.	270 ± 77	67 ± 100	13 ± 23	92 ± 24	156 ± 57
	Asplanchna sp.	269 ± 119	74 ± 94	83 ± 63	155 ± 39	178 ± 91
	Daphnia sp.	15 ± 26	7 ± 12	13 ± 12	22 ± 22	97 ± 59
	Moina sp.	343 ± 141	-	7 ± 12	29 ± 34	155 ± 85
	Diaphanosoma sp.	89 ± 154	13 ± 23	21 ± 36	22 ± 22	81 ± 21
	Filinia sp.	31 ± 54	27 ± 46	27 ± 32	15 ± 25	13 ± 11
	Total	3510±402	1434±1040	1405±471	1679±141	2194±449

App. Table 3.21. Aquatic insect count (nos/3 sq meter) before and after use of fenpropathrin, phostoxin and rotenone in commercial aquaculture pond.

Toxicant	Aquatic insects	Pretreatment	Day-1	Day-2	Day-5	Day-10
Fenpropathrin @0.065 mg/liter	Boatman	46.3±22	6.7±8.3	5.3±3.2	68.7±52.4	98±114
	Backswimmer	2.7±0.6	-	0.7±1.2	1.0±1,7	6.3±4
	Gerris sp.	0.3±0.6	-	-	-	-
	Nepa sp.	-	0.3±0.6	-	-	-
	Crawling beetle	-	-	10±2.6	7.4±8.7	8.7±4.7
	Total	49.3±22.1	7.0±8.9	16.0±6.2	81.0±56.4	113.0±123.0
Aluminium phosphide @0.61 mg/liter	Boatman	59.3±17.2	15±10.1	11.0±6.0	12.3±6.1	21±9.2
	Backswimmer	27.3±19	-	-	-	5.0±3.6
	Gerris sp.	0.7±0.6	-	-	-	0.7±0.6
	Water scavenger	0.3±0.6	1.0±1.0	1.3±1.5	4.7±2.1	4.0±2.6
	Crawling beetle	3.0±3.0	3.7±2.1	4.7±1.2	6.7±2.1	12±5.6
	Dragon fly	1.7±1.2	-	-	-	-
	Damselfly	0.3±0.6	-	-	2.7±1.5	9.0±5.0
	Total	92.7±31.4	19.7±12.1	17.0±6.6	26.3±8.7	51.7±13.7
Rotenone @ 0.272mg/liter	Boatman	5.7±3.1	8.0±6.6	9.7±4.0	36±9.6	59.0±18.5
	Backswimmer	76.7±79.2	36.7±8.4	19.0±10.4	17.3±8.5	32.3±8.0
	Gerris	0.7±0.6	0.3±0.6	0.3±0.6	0.7±0.6	0.3±0.6
	Water scavenger	-	0.3±0.6	0.3±0.6	0.3±0.6	0.3±0.6
	Crawling beetle	-	-	-	5.0±2.0	8.0±2.6
	Tadpole	-	-	-	-	2.7±0.6
	Total	83.0±82.6	45.3±9.7	29.3±13.9	59.3±17.0	103.0±29.1

App. Table 3.22. Benthos count (nos/6 sq inches of the pond bottom) before and after use of fenpropathrin, phostoxin and rotenone in commercial aquaculture pond (corresponding Figure 3.23).

Toxicant	Benthos	Pretreatment	Day-1	Day-2	Day-5	Day-10
Fenpropathrin @0.065 mg/liter	Chironomid larvae	34.3±3.5	3.3±0.6	0.7±0.6	41±6.6	16.7±4.5
	Tubifex	65.0±8.5	26±17	25±10.6	79.3±3.5	56±11.5
	Total	99.3±5.0	29.3±16.5	25.7±10.8	120.3±9.0	72.7±13.9
Aluminium phosphide @0.61 mg/liter	Chironomid larvae	70.7±7.4	3.3±1.5	2.7±0.6	4.3±4.5	124.0±100
	Tubifex	78.7±8	41.3±11.7	44.7±17.2	54±9.8	12.7±4
	Leech	1.3±0.6	-	-	-	-
	Polychaeta	-	0.7±0.6	0.7±1.2	1.0±1.0	1.0±1.0
	Total	150.7±14.4	45.3±10.4	48.0±15.7	59.3±11.7	137.7±104.2
Rotenone @ 0.272mg/liter	Chironomid larvae	13.3±1.5	18.3±8.1	13.3±9	17.7±3.5	22.7±4.2
	Tubifex	14.3±1.5	15.0±4.0	57.3±10.6	62.7±8.5	63.3±12.5
	Leech	3.7±1.2	0.3±0.6	-	-	-
	Polychaeta	-	0.7±1.2	1.0±0	0.3±0.6	-
	Total	31.3±2.5	34.3±6.0	71.7±6.0	80.7±12.1	86.0±9.6

App. Table 3.23. Testosterone level (ng/ml) of adult males and females in normal and sex inversed populations of *O. niloticus*.

Population Category	Sex	Number of Individuals	Testosterone Level (ng/ml)	Range of Value	Score for Appearance of Sex Organ
Normal Population	Male	7 (13.2% of total)	16.7±7.3	8.2-26.4	6
	Male	14 (26.4% of total)	3.9± 3.7	0.9-10.1	5
	Male	3 (5.66% of total)	0.8±0.4	0.6-1.2	3
	Combined	24 (45.3% of total)	7.3±7.8	0.6-26.4	
	Female	22 (41.5% of total	2.5± 2.9	0.04-10.3	5
	Female	7 (13% of total)	0.16±0.36	0.01-1.04	3
	Combined	29 (54.7% of total)	1.95±2.8	0.01-10.3	
Sex Inversed Population	Male	6 (12% of total)	19.4±11.6	4.35-36.8	6
	Male	14 (28% of total)	2.4±1.16	0.66-4.13	5
	Male	4 (8% of total)	2.85±1.04	2.22-4.42	4
	Male	10 (20% of total)	0.37±0.22	0.09-0.75	2
	Combined	34 (68% of total)	4.87±8.29	0.09-36.8	
	Female	5 (10% of total)	2.34±1.18	1.42-3.87	5
	Female	6 (12% of total)	1.38±0.83	0.25-2.69	3
	Female	5 (10% of total)	0.26±0.39	0.01-0.95	1
	Combined	16 (32% of total)	1.33±1.17	0.01-3.87	

Note: 6 means very well developed, 5 means well developed, 4 means one testis/ovary well developed, 3 means less developed, 2 means poorly developed, 1 means very poorly developed and functionally not ready to breed.

App. Table 3.24. Testosterone levels (ng/ml) in adult *O. niloticus* serum when fed with methyltestosterone at the rate of 10 mg/kg feed as growth promoter.

Sex	0 days	5 days	10 days	15 days	20 days	25 days	30 days
Male	7.3±7.8	32.1±18.4	20.3±9.1	41.6±40.4	65.7±7.2	57.1±0.0	26.1±5.0
Female	1.9±2.7	7.8±0.0	-	18.2±0.0	16.4±3.3	12.4±0.7	14.04±0.0

App. Table 3.25. Serum testosterone levels in adult *O. niloticus* after the feeding of methyltestosterone was stopped.

Sex	5 days	10 days	20 days	25 days	30 days
Male	21.4±2.9	26.3±2.9	16.2±9.6	33.1±24.2	22.2±12.3
Female	31.4±14.1	16.1±3.0	11.9±3.9	11.1±0.0	24.7±7.8

App. Table 3.26. List of most drought vulnerable upazillas (drought hotspots) in Bangladesh based on data prior to the year 2000.

District	**Upazilla**	**Severity Ranking**
Rajshahi	Tanore	2
Rajshahi	Godagari	12
Rajshahi	Shah Makhdum	15
Rajshahi	Durgapur	20
Naogoan	Niamatpur	1
Naogoan	Porsha	4
Naogoan	Sapahar	5
Naogoan	Patnitola	8
Naogoan	Mohadebpur	13
Nawabganj	Nachole	3
Nawabganj	Shibganj	9
Nawabganj	Gomostapur	14
Joypurhat	Panchbibi	10

Source: CDMP II, 2013

App. Table 3.27. Emission scenarios as described by IPCC.

Category	Scenario
A2	"The A2 storyline and scenario family describes a very heterogeneous world. The underlying theme is self-reliance and preservation of local identities. Fertility patterns across regions converge very slowly, which results in continuously increasing global population. Economic development is primarily regionally oriented and per capita economic growth and technological change are more fragmented and slower than in other storylines."
B1	"The Bl storyline and scenario family describes a convergent world with the same global population that peaks in mid-century and declines thereafter, as in the Al storyline, but with rapid changes in economic structures toward a service and information economy, with reductions in material intensity, and the introduction of clean and resource-efficient technologies. The emphasis is on global solutions to economic, social, and environmental sustainability, including improved equity, but without additional climate initiatives."

Source: IPCC, 2000; pp70

App. Table 3.28. Mean temperature Change (^{0}C) scenario in Rajshahi division.

Scenario	2030	2050	2030	2050	2030	2050	2030	2050	2030	2050
	Dec-Jan-Feb		Mar-Apr-May		Jun-Jul-Aug		Sep-Oct-Nov		Annual	
A2	1.25	2.14	0.77	1.47	0.41	0.84	0.64	1.16	0.76	1.39
B1	1.44	2.47	0.96	2.07	0.26	1.07	0.64	1.46	0.82	1.75

Source: Hassan *et al*., 2010

App. Table 3.29. Precipitation Change (%) scenario in Rajshahi division.

Scenario	2030	2050	2030	2050	2030	2050	2030	2050	2030	2050
	Dec-Jan-Feb		Mar-Apr-May		Jun-Jul-Aug		Sep-Oct-Nov		Annual	
A2	-19.96	-27.88	28.20	38.25	9.68	14.02	2.46	6.62	6.55	10.62
B1	-47.72	-50.25	22.92	7.55	13.86	14.34	2.39	8.68	8.02	10.30

Source: Hassan *et al*., 2010

App. Table 3.30. Comparison of average monthly rainfall (mm) in Rajshahi and Bogura by decade over the last 19 years.

Weather station	Time frame	December-January-February	March-April-May	June-July-August	September-October-November
Bogura	2001-2010	5.3±2.6	94.4±74.5	318.1±21.4	144.7±123.4
	2011-2019	10.7±9.7	117.3±107.3	268.0±18.3	115.0±117.4
Rajshahi	2001-2010	5.5±1.0	71.3±67.2	248.3±39.0	121.0±111.2
	2011-2019	8.8±4.5	96.1±68.9	230.1±40.3	97.2±83.3

Source: BMD, 2019

App. Table 3.31. Change in yield percentage (over the base year of 1979-2008) for selected upazilas (drought hotspots) of Rajshahi district in A2 and B1 scenario for year 2030 and 2050 for Aman (BR 11) and Boro (BR 14) paddy.

Upazilla	Scenario	Rice variety	Yield decrease	Rice variety	Yield decrease
Bagha	A2 (year 2030)	Aman (BR-11)	<10%	Boro (BR-14)	<10%
Bagha	A2 (year 2050)	Aman (BR-11)	0%	Boro (BR-14)	<10%
Bagha	B1 (year 2030)	Aman (BR-11)	<10%	Boro (BR-14)	<10%
Bagha	B1 (year 2050)	Aman (BR-11)	<10%	Boro (BR-14)	<10%
Baghmara	A2 (year 2030)	Aman (BR-11)	0%	Boro (BR-14)	20-30%
Baghmara	A2 (year 2050)	Aman (BR-11)	0%	Boro (BR-14)	20-30%
Baghmara	B1 (year 2030)	Aman (BR-11)	0%	Boro (BR-14)	20-30%
Baghmara	B1 (year 2050)	Aman (BR-11)	0%	Boro (BR-14)	>40%
Mohonpur	A2 (year 2030)	Aman (BR-11)	<10%	Boro (BR-14)	>40%
Mohonpur	A2 (year 2050)	Aman (BR-11)	<10%	Boro (BR-14)	>40%
Mohonpur	B1 (year 2030)	Aman (BR-11)	<10%	Boro (BR-14)	>40%
Mohonpur	B1 (year 2050)	Aman (BR-11)	0%	Boro (BR-14)	>40%
Poba	A2 (year 2030)	Aman (BR-11)	0%	Boro (BR-14)	0%
Poba	A2 (year 2050)	Aman (BR-11)	0%	Boro (BR-14)	>40%
Poba	B1 (year 2030)	Aman (BR-11)	0%	Boro (BR-14)	>40%
Poba	B1 (year 2050)	Aman (BR-11)	0%	Boro (BR-14)	>40%
Puthia	A2 (year 2030)	Aman (BR-11)	<10%	Boro (BR-14)	30-40%
Puthia	A2 (year 2050)	Aman (BR-11)	<10%	Boro (BR-14)	30-40%
Puthia	B1 (year 2030)	Aman (BR-11)	0%	Boro (BR-14)	20-30%
Puthia	B1 (year 2050)	Aman (BR-11)	0%	Boro (BR-14)	20-30%
Tanore	A2 (year 2030)	Aman (BR-11)	<10%	Boro (BR-14)	0%
Tanore	A2 (year 2050)	Aman (BR-11)	<10%	Boro (BR-14)	0%
Tanore	B1 (year 2030)	Aman (BR-11)	<10%	Boro (BR-14)	0%
Tanore	B1 (year 2050)	Aman (BR-11)	0%	Boro (BR-14)	0%

Source: CDMP II, 2013

Questionnaire-1: Survey Form for Fisheries Entrepreneurs

Name of the entrepreneur: Date:

Number of total cultivated ponds:

Area of total cultivated pond-----------Bigha

What is the size of smallest pond------bigha, size of largest pond------bigha.

How many years have you been involved in commercial fisheries: ------- years

What are the fish species you are currently cultivating? At what density per unit water area?

Answer specific to one pond: ----------size

Making of the pond:
What are the machines you have used for digging the pond?

Machines	# Nos	# hours operated	Amount paid	
Excavator				
Dozer				
Dump Trucks				

Water:
What are the sources of water for your pond?
How many hours/how many cards (prepaid cards for water) you have used for watering the pond last year: -------------------hours
How much money did you spend in total for watering the pond from deep tube well last year? (including *samity* expense)

Inputs:
What are the fertilizers in what quantity you have used in that pond last year?

Type (organic/inorganic)	Name of fertilizer	Quantity (kg)

Are there any season when fertilizers are used more or less? What are those seasons? Why the seasonal variation?

What are the other chemicals inputs (lime, zeolite, tea seed cake etc.) that you have used in the pond last year?

Name of input/chemicals	# of application	Quantity used in each application (kg)	Total quantity (kg)

Are there any season when those inputs are used more or less? What are those seasons? Why the seasonal variation?

Use of pesticide:

Do you use any pesticide in the pond? Yes/NO

In which situation/for what reason did you use the pesticide in the pond?

Please describe the type/name of pesticide and quantity at which they were used in last year:

Type/Name of pesticide	# of application	Quantity in each application (kg/ liter)

Is there any season when pesticides are used more or less? What are those seasons? Why the seasonal variation?

Are there any precautions that were used regarding pesticide use: yes/No

IF yes, what precautions were used?

Are you familiar with the term 'withdrawal period' for pesticide? If yes, would you please explain?

Did you maintain any sort of withdrawal period for pesticide used in your pond? Yes/No, if yes what is the period?

Use of medicine:

Are there any medicines that you have used in that pond over the period of last year?
If yes what were the medicines used in your ponds?

Name of medicine	# of application	Duration of each application (days)	quantity used in each application (gram)

In which situation did you use those medicine?

Is there any specific season when medicines are used more or less? What are those seasons? Why the seasonal variation?

Species diversity:

What are the fish species you are currently cultivating?
Species ratio:

Other than the cultured species what are the other fish species do you see in your pond?

Which one is in highest density?

Which one do occur most frequently?

What would be the total quantity (kg) of these undesired species that have occurred over the last year in the pond?

What are the birds’ species that you see in your ponds?

What are the reptile species that you see in your ponds?

Feed:
What is the feed/feed ingredients that you feed your fish?
Feeding rate?

Feeding frequency?

What is the total amount of feed that you have used over the last year in that pond?

Do you always use same feed in your pond? Why/why not?

Why did you choose the feed that you are feeding or fed in the past? What are the factors influencing the choice of feed?

What is the total release amount for fish in your pond? How many trips of what vehicle required for seedling transportation? How long for each trip?

What is the total catch from that pond? How many trips of what vehicle required for fish marketing? How long for each trip?

Do you wipeout the entire fish community from the pond each year? if yes

Why do you do this?

What chemical at what doses do you use in this activity?

Additional crops:

Do you grow crops/vegetables/fruits on the bank of the pond? Yes/No

What crops did you grow on the back of that pond last year?

Did you use any herbicides in you pond bank? If yes, then please give details as below.

Name of input/chemicals	# of application	Quantity used in each application (kg)	Total quantity (kg)

Overall:
Do you see any environmental implications occurring from your overall operation of entrepreneurial fisheries? Yes/No, if yes what are those:

Thank You very much for your valuable time and the information you shared.

Questionnaire-2: Survey Form for Rice Farmers

Name of farmer: Phone number:

Size of a specific plot under cultivation of rice: -----Decimal

All following answer must be specific about that plot of land

Last *Boro* season:

Land cultivation:

Type of equipment's	Tractor	Power tiller	Manual plowing
Total duration of use (hours)			

Fertilization:

Fertilizer	TSP	Urea	Potash	Gypsum	Boron	zinc	Cow-dung/compost	Poultry liter
Basal dose (kg)								
Top dressing (kg)								

Irrigation:

Pumps	Pump specification (hp/ kW)	Frequency	Total duration of use (hours)
Deep tube well			
Diesel pump			
Electric pump			
Tube wells			

Pesticide used:

Brand name of pesticide/fungicide	Frequency	Total quantity used (gm or ml)

Harvesting:

Types of harvester	Pump specification (hp)	Total duration of use (hours)
Manual	X	X
Diesel engine harvester		

Thrashing:

Types of Thrasher	Pump specification (hp)	Total duration of use (hours)
Manual	X	X
Diesel engine thrasher		

Transportation: For Rice and Straw

Mode of transportation	Pump specification (hp/ kW)	Total duration of use (hours)
Manual (manual van)	X	X
Auto/battery van/battery rickshaw	X	
Trolley		
Tractor trailer		

Last *Aman* season

Land cultivation:

Type of equipment's	Tractor	Power tiller	Manual plowing
Total duration of use (hours)			

Fertilization:

Fertilizer	TSP	Urea	Potash	Gypsum	Boron	zinc	Poultry liter	Cow-dung/compost
Basal dose (kg)								
Top dressing (kg)								

Irrigation:

Pumps	Pump specification (hp/ kW)	Frequency	Total duration of use (hours)
Deep tube well			
Diesel pump			
Electric pump			
Tube wells			

Pesticide used:

Brand name of pesticide/fungicide	Frequency	Total quantity used (gm or ml)

Harvesting:

Types of harvester	Pump specification (hp)	Total duration of use (hours)
Manual	X	
Diesel engine harvester		

Thrashing:

Types of thrasher	Pump specification (hp)	Total duration of use (hours)
Manual	X	X
Diesel engine thrasher		

Transportation: For Rice and Straw

Mode of transportation	Pump specification (hp/ kW)	Total duration of use (hours)
Manual (manual van)	X	X
Auto/battery van/battery rickshaw	X	
Trolley		
Tractor trailer		

Thank You

List of Publication

Rahman MM and Islam MN, 2019. Usages and impacts of quinalphos in commercial aquaculture in Rajshahi, Bangladesh. *Rajshahi University Journal of Environmental Science*, **8**: 71-78.

Rahman MM and Islam MN. 2019. Effects of abamectin and lambda-cyhalothrin on aquatic invertebrates in two aquaculture ponds. *Journal of Life and Earth Science*, **14**: 19-25.

Rahman MM and Islam MN. 2019. Impacts of cypermethrin and deltamethrin's use on aquatic invertebrates in commercial aquaculture ponds. *Bangladesh Journal of Fisheries*, **31(2)**: 211-220.

Rahman MM, Sarker MSA, Islam MN and Hoque N. 2019. Consequences of use of fenpropathrin compared to other fish toxicants in commercial aquaculture. *Asian Australasian Journal of Food Safety and Security*, **3(1)**: 27-37.

Rahman MM and Islam MN. 2018. Practice of using emamectin benzoate and its environmental impacts on aquaculture of northwest Bangladesh. *Journal of Life and Earth Science*, **13**: 1-5.

Rahman MM and Islam MN. 2018. Serum testosterone level of hormone fed Tilapia (Oreochromis niloticus) in northwest Bangladesh. *Rajshahi University Journal of Environmental Science*, **7**: 85-92.

Usages and Impacts of Quinalphos in Commercial Aquaculture in Rajshahi, Bangladesh

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Abstract

Fish farmers involved in commercial aquaculture in northwest Bangladesh is striving for ruthless efficiency of the system. In this process they have been selective poisoning the undesired species (!) by using quinalphos pesticides while keeping their desired species (Indian and Chinese major carps) seemingly unaffected. The study was conducted to know the impact of the use of quinalphos on zooplankton, aquatic insects and benthos population in commercial aquaculture ponds. To know the bioaccumulation phenomena, fish were collected pre and post-treatment and analyzed for residual content of quinalphos. Zooplankton, aquatic insects and benthos sample were collected and counted at pretreatment and after 1 day, 2 days, 5 days, 10 days, 15 days, 21 days and 28 days after the treatment. Quinalphos was found to be bioaccumulated in fish till 10 days after the use. Zooplankton were found to be impacted for short duration and were able to regenerate within 5 days of the use of quinalphos. Aquatic insects and benthos were also affected but the loss of insect diversity was observed till the end of the observation period.

Keywords: Quinalphos; Selective poisoning; Aquaculture; Bioaccumulation; Zooplankton; Aquatic insects.

1. Introduction

The activity of commercial aquaculture begins with complete wipeout of existing batch of left-over fish (that didn't come under catchment as live) and water insects from the pond using various chemicals (like starting with a clean sheet), which is followed by stocking of desired number of relatively large fish (Indian major carps of 0.5 kg to 1.5 kg in size)in northwest Bangladesh compared to the other parts of the country. In the process of transferring the live fish from the nursery pond to the stocking pond, undesired (!) small indigenous fish species like *Pseudambasis* sp., *Chanda* sp., *Glossogobius* sp. and *Puntius* sp. etc. also gettransferred unintentionally by the farmersinto the stocking pond. Since these small indigenous species can breed in ponds, within few months (especially after monsoon) they manage to multiply and populate the culture ponds of Indian major carps. Despite of the disparity of the size between these indigenous species and large Indian and Chinese major carps (more than 1.5 kg in size) in commercial ponds, farm owners view these small species as a challenge to the desired species who might face competition for food and space or at least get disturbed by these tiny fish. Some fish like *Chanda* sp. and *Pseudambasis* sp.(which usually present in large number) does keep biting and feeding on scales of carps, especially silver carps with their jaws that is armed with curved and conical teeth (Wahab, 2003) and thus make the carp fish scale less glossy, will reduce the price in the market if sold, apart from the risk of hampering the growth.On the other hand, *Glossogobius* sp. is a voracious feeder and almost feeds on everything namely decaying organic matter, protozoans, planktons, water insects, other fish and their eggs and larvae (Siddiqui *etal.*, 2007). Thus *Glossogobius* sp. can create competition for feed supplied to the commercial aquaculture pond.

Quinalphos is a broad-spectrum organophosphate insecticide with contact and stomach action used against common pests like aphids, caterpillars, mealybugs, mites, bollworms, leafhoppers and borers of various crops and plants like wheat, sugarcane, peanut, sorghum, cotton, fiber-crops etc. has been in widespread use since 1970 (IUPAC, 2019). National Center for Biotechnology Information (NCBI) (2019) has categorized the toxicity of quinalphos as 'very toxic to aquatic life' and 'very toxic to aquatic life with long lasting effects'. Despite being labeledas very toxic, it has a wide range when comes to the toxicity to fish, that means for some fish tolerance level of quinalphos is way higher than others even within the same family of fish. For example, regarding quinalphos, 96 hour LC_{50} value of silver barb (*Barbonymus gonionotus*) is 4.70 mg/l (Mostakim*et al.*, 2014), of *Labeorohita* is 2.826 mg/l (Rathnamma and Nagaraju, 2013), of *Cyprinuscarpio* is 2.75 mg/l (Padmanabha *et al.*, 2016), of Catlacatla is 2.91mg/l (Rajput, 2012), of *Cirrhinus mrigala* is 0.128mg/l (Nair *etal.*, 2017) and of

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J. Life Earth Sci., Vol. 14: 19-25, 2019
http://banglajol.info.index.php/JLES

ISSN 1990-4827

EFFECTS OF ABAMECTIN AND LAMBDA-CYHALOTHRIN ON AQUATIC INVERTEBRATES IN TWO AQUACULTURE PONDS

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Abstract: A study was conducted to know the impact of abamectin and lambda-cyhalothrin on aquatic insect, zooplankton and benthos populations in commercial aquaculture ponds. Accordingly, the ponds under commercial aquaculture located in Poba upazila of Rajshahi, Bangladesh were selected that had similar and commonly accepted management practices. Abamectin and lambda-cyhalothrin were used at 0.7 and 0.5μg/liter of pond water. Water and soil samples from the pond bottom was collected for analysis. Sampling for aquatic insects, zooplankton and benthos was done before treatments and on days 1, 2, 5, 10, 15, 21 and 28 following the treatments. No fish mortality was recorded in either of these treatments. A decline of 80%, 49% and 56% respectively in water insect, zooplankton and benthos count were found in abamectin treatment. Treatment of lambda-cyhalothrin caused 93%, 60% and 79% decline respectively in water insect, zooplankton and benthos numbers. Along with the decline in number, diversity loss for aquatic insects and zooplankton was also observed in both treatments. Diversity count did not recover to the pretreatment level within the observation period of 28 days.

Keywords: Lambda-cyhalothrin, Abamectin, Aquatic-insect, Zooplankton, Benthos, Aquaculture

সারাংশঃ বাণিজ্যিক মাছ চাষের পুকুরে জলজ কীটপতঙ্গ, জুপ্ল্যাংটন ও পানির নিচে মাটিতে বসবাসকারী পোকার উপর এ্যাবামেকটিন ও ল্যাম্বডাসাইহ্যালোথ্রিনের প্রভাব জানার জন্য রাজশাহীর পবা উপজেলায় বাণিজ্যিক মাছ চাষের পুকুরে গবেষণাটি করা হয়। এ্যাবামেকটিন ও ল্যাম্বডাসাইহ্যালোথ্রিন যথাক্রমে ০.৭ মাইক্রোগ্রাম/লিটার ও ০.৫ মাইক্রোগ্রাম/লিটার হারে পুকুরে ব্যবহার হয়েছিল। গবেষণা পুকুরের পানি ও মাটি সংগ্রহ করে গবেষণাগারে পরীক্ষা করা হয়। বাণিজ্যিক মাছ চাষের পুকুরে এ্যাবামেকটিন ও ল্যাম্বডাসাইহ্যালোথ্রিন ব্যবহারের পূর্বে ও ব্যবহারের ১, ২, ৫, ১০, ১৫, ২১, ২৮ দিন পরে জলজ কীটপতঙ্গ, জুপ্ল্যাংটন ও পানির নিচে মাটিতে বসবাসকারী পোকার নমুনা সংগ্রহ করে পরীক্ষা করা হয়। কীটনাশক ব্যবহারের ফলে মাছ মৃত্যুর কোন ঘটনা ঘটেনি। পুকুরে এ্যাবামেকটিন ব্যবহারের ফলে জলজ কীটপতঙ্গ, জুপ্ল্যাংটন ও পানির নিচে মাটিতে বসবাসকারী পোকার সংখ্যা যথাক্রমে ৮০%, ৪৯%, ৫৬% হ্রাস পেয়েছিল। অপর পক্ষে পুকুরে ল্যাম্বডাসাইহ্যালোথ্রিন ব্যবহারের ফলে জলজ কীটপতঙ্গ, জুপ্ল্যাংটন ও পানির নিচে মাটিতে বসবাসকারী পোকার সংখ্যা যথাক্রমে ৯৩%, ৬০% এবং ৭৯% হ্রাস পেয়েছিল। উভয় কীটনাশকের ক্ষেত্রে কীটপতঙ্গ, জুপ্ল্যাংটন ও পানির নিচে মাটিতে বসবাসকারী পোকার সংখ্যা হ্রাসের সাথে সাথে তাদের বৈচিত্র্য হারানোর ঘটনাও পরিলক্ষিত হয় এবং ২৮ দিন পর্যবেক্ষন কালের মধ্যে বৈচিত্র্য সংখ্যা পরীক্ষন পূর্ববর্তী অবস্থায় ফিরে যায়নি।

Introduction

Aquatic insects are an integral part of any healthy natural aquatic ecosystems and often serve as an indicator of the health of an ecosystem (Pondinformer, 2019). Excess organic matter along with the presence of aquatic weeds increases the number of aquatic insects (Roysfarm, 2019). Culture of non-predatory carp species and intensive feeding of the fish make leftover feed available for aquatic insects in the absence of predators and competitors. This allows the aquatic insect population to flourish in commercial aquaculture ponds of northwest Bangladesh. Insect abundance (count) was found to be higher in pond systems than lakes (Nasiruddin *et al.* 2014). Marco *et al.* (1999) found that ponds with moderate aquatic vegetation had greater dragonfly species richness than ponds either devoid of vegetation or with extreme vegetation. Highest abundance of aquatic insects in aquaculture ponds was found during or after rainy season (Yapo *et al.* 2013; Kashyap *et al.* 2013; Nasiruddin *et al.* 2014). Many aquatic insects (dragonfly nymph, backswimmers, water scorpion, etc.) are harmful to juvenile fish due to their direct predation of fish fry and competition for food (Roysfarm 2019; Gonzalez and Leal 1995; Sano *et al.* 2011 and Kashyap *et al.* 2013). Despite having no predation risk from aquatic insects due to rearing of larger than 0.75 kg fish in commercial aquaculture ponds; aquatic insects when present in large numbers are considered hazardous by the fish farmers in northwest Bangladesh due to the disturbances that the aquatic insects cause to fish and potential competition for food and space (Rahman and Islam 2019). Though there is lack of proof on how much harm these regular aquatic insects (not parasites) may cause to large fish in aquaculture ponds; use of pesticides in aquaculture is common in Bangladesh, as documented by Hossain *et al.* (2008), Chowdhury *et al.* (2012), Shamsuzzaman and Biswas (2012), Rahman *et al.* (2015), Chowdhury *et al.* (2015) and Hossain *et al.* (2018). To control the aquatic insect population, farmers in northwest Bangladesh use various insecticides in aquaculture ponds on a regular basis (once in a month) during summer and monsoon months (Rahman and Islam 2019). Abamectin is primarily used in livestock as a parasiticidal agent and in agriculture as an insecticide and acaricide (Campbell 1989). Lambda-cyhalothrin is a synthetic pyrethroid insecticide for agricultural use registered by USEPA in 1988 (NPIC 2001). The study was conducted to know the effect of abamectin and lambda-cyhalothrin on aquatic insect, zooplankton and benthos populations when used in commercial aquaculture ponds.

Materials and Methods

The study was conducted in aquaculture ponds in the Hatgodagari area of Poba upazilla in Rajshahi district. Two different farmers were identified who were engaged in commercial aquaculture, rearing Indian major carps and Chinese carps with size of one to two kilogram each, and intended to use the insecticides abamectin and lambda-cyhalothrin in their ponds. Accordingly, the farmers were informed and they allowed the author to conduct the study. Abamectin ('Likar'1.8 EC imported and marketed by Korbel International Ltd.) and lambda-cyhalothrin ('Fighter' 2.5 EC imported and distributed by ACI Formulations Ltd.) were used at the rate of 0.7 and 0.5 µg active ingredient/liter water respectively in two different ponds with 2 acres and 1.5 acres of water area on February 01, 2019. The farmers did not provide any supplementary feed to the fish on the day of pesticide application and the following day. Sampling for aquatic insects, zooplankton and benthos was done the day before the application of abamectin and lambda-cyhalothrin and subsequently on days 1, 2, 5, 10, 15, 21, and 28 following the treatment. Before the treatment, water samples from both the ponds were collected one foot below the surface to measure water pH, turbidity, electrical conductivity and total dissolve solid in the water quality testing lab of the Institute of Environmental Science (IES) at Rajshahi University. Soil samples from the bottom of the ponds were also collected, dried and analyzed for organic carbon content in the Soil Resource Development Institute (SRDI), Rajshahi.

Application method of the drug

The required amount of insecticide was poured into large aluminum pot and mixed with 5 to 7 kg of sand, in such that the sand became soaked with the pesticide. Then the pesticide-soaked sand was broadcasted along the edges of the water of each pond and towards to middle (approximately 5-7-meter inwards), as far as the farmers were able to throw.

Sampling of aquatic insects

A fine meshed (0.25 mm mesh size) net of one square meter in size fitted into a bamboo frame was used for the sampling of aquatic insects. The net was towed for a 3-meter distance along the water's edge of the pond. Then the collected insects were transferred into a plastic container with some water and 10 ml of formalin was added to preserve the insects. The process was repeated three times during each sampling. The collected insects were taken into the lab, where the insects were put into petri dishes and identified and counted for each type.

Sampling of zooplankton

A cone shaped plankton net (specification- 200 US with 75 to 85 microns mesh size) with one tapering end was used for the zooplankton sampling. The tapering end was fitted with a collection bottle. The other end was fitted with a circular metal frame. The zooplankton net with the open end (fitted with metal frame) was towed through the water of the pond (one foot below the surface) for a certain distance so that water could get in through the open end of the net and zooplankton could get caught and be deposited in the collection bottle at the other end. Water volume sieved through the plankton net was measured as water volume in liter = 'π (3.14)' multiplied by the 'square radius of the plankton net metal frame in meters' multiplied by 'the net's traversed distance in meters' multiplied by 1000. Collected zooplankton with water (approximately 95 ml in volume) was taken in a plastic bottle and 5 ml formalin was added to preserve the zooplankton. The process was repeated three times to collect three samples and represents three replications for each treatment in each sampling day. The collected samples were taken into the lab, measured for total volume in milliliter. This amount (volume) of zooplankton represents the total amount of water sieved through the plankton net. The zooplankton were observed and counted using a Sedgewick rafter cell counter (Welch 1948) and microscope. Then the count of each kind of zooplankton was converted into zooplankton density per liter of pond water as per the following formula:

Zooplankton density/liter pond water = (total count of zooplankton in 10 cells of Sedgewick rafter cell counter X 100 X total volume (in ml) of zooplankton sample)/ Total volume of water (in liter) that passed through the plankton net.

Sampling of benthos

A metal scoop with a six square inch opening was used to take the soil sample from the bottom of the pond at one feet depth from the surface of the water. During each sampling for each treatment, three such samples were taken to be considered as three replications. The collected soil samples were transferred to a plastic container and taken into the lab. The soil samples were washed under running water using a fine meshed sieve to remove all of the soil particle. The left-over benthos population from the sieve was transferred into a petri dish with some water and observed under bright light to identify the type and count.

Results

Water pH of both treatment ponds was around 8 (Table 1). Water turbidity value was lower in the abamectin-treated pond, but the organic carbon content of the bottom soil was higher. On the other hand, water turbidity was higher in lambda-cyhalothrin treatment, but the organic carbon content was lower than the abamectin pond.

Table 1. Physiochemical properties of the study environment before application of pesticides.

Treatment pond	Water Temperature (°C)	Water pH	Water turbidity (ntu)	Electrical conductivity (µS/cm)	Organic Carbon content of bottom soil (%)
Abamectin pond	21	7.87	13.97	362	3.40
L-cyhalothrin pond	21	8.04	33.47	388	2.53

Impacts on aquatic insects

Aquatic insect count for abamectin treatment was found to be lowest (80% decline from the pretreatment level) on the second day after the treatment. On the other hand, for lambda-cyhalothrin treatment, the lowest insect count was found to be on day five (93% decline from the initial count) after the treatment (Figure 1). Insect count from its lowest level for both treatments kept increasing slowly but steadily till the end of the observation period but failed to reach the pretreatment level. Insect diversity in abamectin treatment remained the same as the pretreatment level during the observation period. Insect diversity for lambda-cyhalothrin treatment reduced to two types of insects from an initial three and did not reach the pretreatment level by the end of the observation period of 28 days.

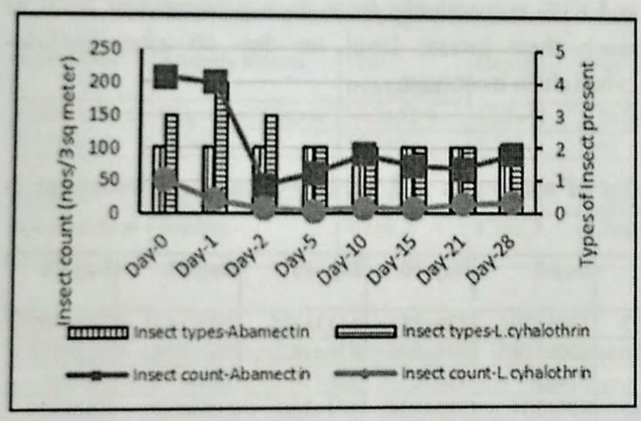

Fig. 1 Total count of aquatic insects (nos/3 sq meters) and their diversity before and after use of abamectin and lambda-cyhalothrin in commercial aquaculture ponds.

In the abamectin treated ponds, backswimmer populations declined within the first day post- treatment. Boatman numbers increased in the first day post-treatment and then declined to its lowest point (81% declined compared to pretreatment level) on day two after the treatment (Figure 2). Backswimmer populations fell to its lowest count (88% declined from pretreatment level) in day five after the abamectin treatment. From its post treatment lowest point, boatman's recovery (increase in number) was faster than the recovery of backswimmer, but the count of both insects failed to reach the pretreatment level at the end of the observation period of 28 days after the treatment.

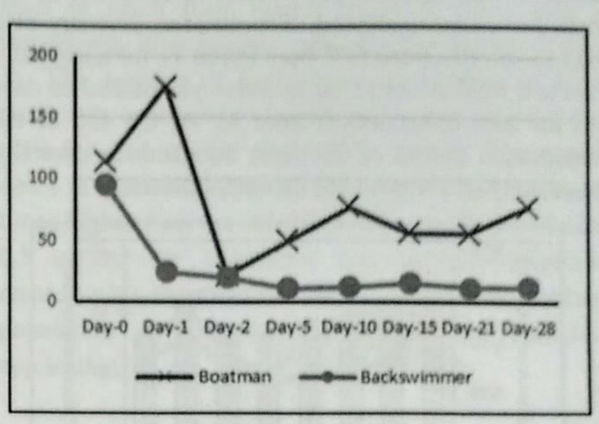

Fig. 2 Aquatic insect count (nos/3 sq meter) at various points of abamectin treatment at 0.7µg/liter of water in commercial aquaculture pond.

Of all the insects, *Gerris* sp., crawling beetles and water scavengers were most affected by the lambda-cyhalothrin treatment (Figure 3). *Gerris* sp. was found in pretreatment but it was not found again after treating the pond with lambda-cyhalothrin. Similarly, the crawling beetle and water scavenger were found only day one and two following lambda-cyhalothrin treatment. The fact that crawling beetle and scavenger were not found in pretreatment sampling and only found in fewer number at day one and two following the lambda-cyhalothrin treatment but not after, proved that those insects were weakened by lambda-cyhalothrin and were caught. Boatman and backswimmer count found to be the lowest (93 and 92% decline respectively from pretreatment level) on day-five after the lambda-cyhalothrin treatment.

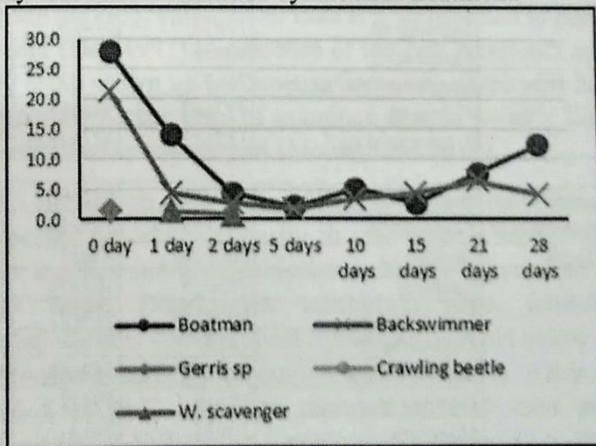

Fig. 3 Aquatic insect count (nos/3 sq meter) at various points after lambda-cyhalothrin treatment at 0.5µg/liter of water in commercial aquaculture pond.

Impacts on zooplankton

The total zooplankton count for both abamectin and lambda-cyhalothrin treatment was found to be lowest (49% and 60% decline from the pretreatment respectively) on day-10 after the treatment (Figure 4). From its lowest point total count of zooplankton for both treatments increased. Zooplankton diversity also was lowest (declined to 7 from initial 11 for abamectin, and to 8 from initial 12 for lambda-cyhalothrin) on day-10 for both treatments (Figure 4). At the end of the observation period of 28 days, zooplankton diversity remained low compared to the initial diversity.

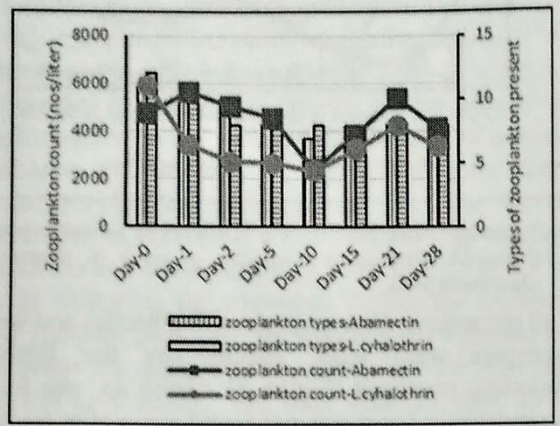

Fig. 4 Zooplankton count (nos/liter) and diversity before and after use of abamectin and lambda-cyhalothrin in commercial aquaculture ponds.

Of all the zooplankton types, the zooplankton that were present in relatively lower numbers the pretreatment stage, namely *Daphnia* sp., *Moina* sp., and *Diaphanosoma* sp. were most affected in both treatments. *Diaptomus* sp. was additionally impacted in the lambda-cyhalothrin treatment, where all zooplankton disappeared within 2 days of the treatment (Table 2). In this study, the count of *Filinia* sp. was nil on day 10 after the abamectin treatment. *Cyclops* sp. declined 82% from the pretreatment level to its lowest level on day 5 of abamectin treatment. *Brachionus* sp., *Keratella* sp., *Polyarthra* sp., *Asplanchna* sp., *Trichocerca* sp. and nauplius declined 29%, 47%, 30%, 40%, 58% and 52% respectively to reach their lowest level from their pretreatment level 10 days post abamectin treatment. *Brachionus* sp., *Polyarthra* sp. and nauplius declined to their lowest count (42%, 40% and 56% respectively) on day-2 and *Filinia* sp. declined 86% from its initial level on day-5 after lambda-cyhalothrin treatment. *Cyclops* sp., *Trichocerca* sp., *Filinia* sp. and *Keratella* sp. declined 98%, 95%, 86%, and 63% respectively from their pretreatment level to reach their lowest level on day 10 after lambda-cyhalothrin treatment

Table-2. Zooplankton count (nos/liter water) before and after of abamectin and lambda-cyhalothrin treatment in commercial aquaculture ponds.

Insecticide	zooplankton	Pre treatment	Day-1	Day-2	Day-5	Day-10	Day-15	Day-21	Day-28
Abamectin at 0.7µg/l	*Brachionus* sp.	1039±157	1107±44	1035±157	1393±704	736±38	1153±235	1908±473	998±475
	Keratella sp.	1025±187	986±58	1016±48	910±318	545±149	630±182	781±216	611±253
	Cyclops sp.	149±186	417±71	292±20	27±25	47±40	88±110	140±133	70±102
	Nauplius	661±79	758±35	655±102	489±234	316±120	401±133	606±125	487±202
	Polyarthra sp.	538±70	545±11	453±83	382±218	375±117	589±130	591±96	510±127
	Asplanchna sp.	553±66	811±21	788±113	729±465	331±49	710±226	693±225	611±277
	Daphnia sp.	15±13	37±26	7±13	-	-	-	-	-
	Moina sp.	99±58	15±13	-	-	-	-	-	-
	Diaphanosoma sp.	75±72	60±34	29±33	-	-	-	-	-
	Filinia sp.	477±62	545±57	473±100	162±161	-	100±130	311±212	370±204
	Trichocerca sp.	137±113	373±265	318±135	468±299	58±53	175±81	394±179	440±167
lambda-cyhalothrin at 0.5µg/l	*Brachionus* sp.	971±114	682±90	559±49	734±55	641±80	829±25	895±164	810±80
	Keratella sp.	967±53	535±103	451±49	482±67	355±126	577±59	754±73	530±119
	Cyclops sp.	400±162	53±12	29±33	47±23	7±13	45±61	121±47	91±22
	Nauplius	759±75	403±58	337±30	354±74	363±57	471±42	602±58	527±123
	Polyarthra sp.	640±116	418±31	382±49	392±159	416±34	541±36	671±34	490±120
	Diaptomus sp.	7±13	-	-	-	-	-	-	-
	Asplanchna sp.	916±87	519±68	426±129	475±90	375±104	450±57	693±77	549±60
	Daphnia sp.	18±16	30±12	-	-	-	-	-	-
	Moina sp.	10±17	7±13	-	-	-	-	-	-
	Diaphanosoma sp.	7±13	-	-	-	-	-	-	-
	Filinia sp.	682±143	441±23	298±51	92±100	177±36	203±74	235±103	221±107
	Trichocerca sp.	547±71	339±60	233±114	92±60	29±33	128±72	273±99	114±2

Impacts on benthos

Total benthos count was found to be lowest with a 56% and 79% decline from pretreatment levels on day two after abamectin and lambda-cyhalothrin treatment respectively (Figure 5). Benthos diversity did not vary between pre and post treatment for both abamectin and lambda-cyhalothrin (Figure 5).

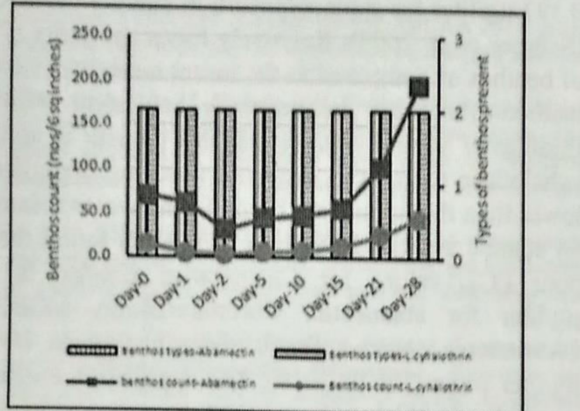

Fig. 5 Benthos count (nos/6 sq inch pond-bottom) before and after use of abamectin and lambda-cyhalothrin in commercial aquaculture ponds.

The count of chironomid larvae and tubifex declined 51% and 74% respectively to reach their lowest levels on day two post abamectin treatment. On the other hand, count of chironomid larvae and tubifex declined 95% and 78% respectively from their pretreatment level to reach their lowest levels at day-1 and 2 respectively after lambda-cyhalothrin treatment (Table 3). The number of both types of benthos in both treatments continued to increase from that lowest point until the end of the observation period. The actual doses in the water edges of the pond would be several times higher than the calculated average dose (0.7 and 0.5 µg/liter for abamectin and lambda-cyhalothrin respectively), therefore caused the mortality of benthos population at that low concentration of pesticide application.

Table 3. Benthos count (nos/6sq inches pond- bottom) before and after use of abamectin and lambda-cyhalothrin in commercial aquaculture ponds.

Insecticide	Aquatic insects	Pre treatment	Day-1	Day-2	Day-5	Day-10	Day-15	Day-21	Day-28
Abamectin at 0.7µg/l	Chironomid larvae	54.7±12.9	51.7±11	27±10.4	37.3±6.1	38±7.5	31.5±9.3	80.3±26.6	176.7±23.2
	Tubifex	15.7±2.1	9.7±3.1	4±1	6.3±2.5	9.7±7.4	23.7±2.1	22±5.6	19.3±2.5
lambda-cyhalothrin at 0.5µg/l	Chironomid larvae	6.3±1.5	0.3±0.6	1.3±0.6	1.0±1.7	2.3±2.5	7.3±3.2	15.3±8.0	29.7±7.4
	Tubifex	7.7±6.7	4.3±1.5	1.7±0.6	4.7±1.5	5.0±1.0	4.7±2.1	10±2.6	14.7±1.5

Discussion

No supplementary feed was supplied to the treatment ponds during and after one day of the treatment, prevented any direct ingestions of pesticides that would have consumed by fish along with supplied supplementary feed (if given). Sanches *et al.* (2017) found 48-hour LC_{50} value of adult zebra fish (*Danio rerio)* as 59 µg/liter for abamectin. 96-hour LC_{50} value of rainbow trout, channel catfish and common carp (*Cyprinus carpio*) are respectively 3.6, 24 and 42 µg/liter (USEPA 2019). Al-Kahtani (2011) reported sub lethal concentration of abamectin for *Oreochromis niloticus* as 20 µg/liter. But for Lambda-cyhalothrin 96-hour LC_{50} value of bluegill sunfish is 0.21 µg/liter, of rainbow trout is 0.24 µg/liter (He *et al.* 2008), of mirror carp (*C. carpio*) is 0.5 µg/liter (Maund *et al.* 1998), of juvenile tilapia (*O. niloticus*) is 2.901 µg/liter (Piner and Uner 2012) and of *Labeo rohita* is 2.1 µg/liter (Muthukumaravel *et al.* 2013). The applied doses in this study were below the LC_{50} values mentioned earlier and hence no fish mortality was observed. Since LC_{50} values are generally calculated in laboratory testing using juvenile specimens, where the toxicity deterrents like suspended solids and presence of organic carbon and presence of sunlight is usually very low hence make the LC_{50} value lower than if it were tested in pond water (Day 1991) in presence of toxicity deterrents and by using grown up specimens. Grown up specimens has generally higher level of tolerance against toxicity than their juvenile counterpart (Mohammed 2013).

Ali and Baugh (2003) found that lambda-cyhalothrin is strongly adsorbed primarily in soil organic matter but not in silica and this adsorption is mostly irreversible to the water. Despite the adsorption issue, lambda-cyhalothrin is stable against hydrolysis at a pH below 8. Photolysis half-life of lambda-cyhalothrin in water at pH 5 at 25^0C has been reported as 24.5 days and degradation half-life in aerobic soil requires 42.6 days by He *et al.* (2008). Abamectin is also stable against aqueous hydrolysis but has a quick aqueous photolysis half-life of 1.5 days, a half-life in water sediment of 89 days and possesses limited systemic activity as a pesticide (BPDB 2019). Though abamectin is absorbed strongly into suspended solids and soil surfaces, it is moderately persistent in the environment (USEPA 2010). Abamectin pond had relatively lower turbidity (Table 1) means lower quantity of suspended solids, resulted in lower absorption of abamectin. Since abamectin got mixed along the water column, chance of photolysis of abamectin got reduced. All these characteristics of both abamectin and lambda-cyhalothrin along with their application method in this study has ensured its long endurance in aquaculture ponds under this study.

Aquatic invertebrates are more sensitive to lambda-cyhalothrin (Maund *et al.* 1998) and abamectin (BPDB 2019) than fish. Ali *et al.* (1997) found significant difference in Hemiptera nymph and Coleoptera larval quantity in between pre and post treatment of abamectin at 3.13 µg/liter spray on small man-made ponds, but with adults of same insect, the population count was insignificant in between pre and post treatment even with 50 µg/liter of abamectin. Whereas in this study, relatively lower dose (0.7 µg/liter) had successfully eliminated most aquatic insects, primarily due to the difference in application method and water area size. Mixing the pesticide with sand and then broadcasting it across the water surface allowed better mixing across the water column and reduced photolysis, as opposed to the direct spray method on the water surface that allowed photolysis and reduced mixing in the water column in the study by Ali *et al.* (1997). In this study, the two-acre pond size meant that vast amounts of the central part of the pond were left untouched by the pesticide application, but the pesticide was more concentrated in the areas it did reach. Whereas in the study by Ali *et al.* (1997) the 24 square meter pond size combined with the application method reduced the impact of the pesticide. Also, water insects are more abundant along the water edges where there is more vegetation (Marco *et al.* 1999) and water depth is shallower (Kashyap *et al.* 2013), where abamectin was applied in this study.

Schroer *et.al.* (2004) found that the 96-hour LC_{50} value of *Notonecta glauca* (backswimmer) is 16.4 ng/l and *Caenis horaria* (may fly) is 34.6 ng/liter and the 48-hour LC_{50} value of *Sigara striata* (boatman) is 49.2 ng/liter for lambda-cyhalothrin in a short-term static laboratory test for toxicity. The effect of higher water turbidity of L-cyhalothrin treatment pond was nullified by the application method resulted in practically higher concentration along the water edges than the average doses calculated for the whole pond.

Wislocki *et al.* (1989) found the 96-hour LC_{50} value of *D. magna* is 0.34 µg/liter for abamectin The48-hour LC_{50} value of *Daphnia magna* is 0.5 to 2.9 µg/liter (Mokry and Hoagland 1990) and of *Daphnia galeata* is 0.397 µg/liter for static exposure in lambda-cyhalothrin (Schroer *et al.* 2004). Relatively lower mortality (79%) of benthos as compared to the insect mortality (93%) in lambda-cyhalothrin treatment is consistent with the findings of Maund *et al.* (1998) that the risk of lambda-cyhalothrin toxicity to sediment dwelling organisms is lower than the organisms living in the water column in an aquatic system. Novelli *et al.* (2012) found the 96-hour LC_{50} values for *Chironomus xanthus* is 2.67 µg/liter for abamectin. Chronic 28-day NOEC of *Chironomus riparius* for lambda-cyhalothrin is 0.16 µg/liter water (PPDB 2019). The application method of pesticides in this study resulted in part of the ponds (water edges) where it was mixed well at practically higher density than the average density (benthos sampling was done in shallow zone) and vacuum spots in the middle portion of the ponds affected the total count of benthos at greater degree than the total count of zooplankton. Though some of the zooplankton genera were well affected, however based on total count, zooplankton was least affected compared to the aquatic insects and benthos, due to their own distribution pattern in the ponds and application method of the pesticides.

Conclusion

Application method, physicochemical parameters of pond, doses of abamectin and lambda-cyhalothrin are critical in order to eliminate aquatic insects without killing the fish of the pond. Use of both abamectin and lambda-cyhalothrin at 0.7 µg/liter and 0.5 µg/liter respectively was highly successful in eliminating not only lots of aquatic insects but also zooplankton and benthos population. However, it deserves serious consideration by the farmers in the first place whether there is enough justification for using pesticides for controlling the aquatic insects, especially in commercial fish culture ponds.

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Impacts of cypermethrin and deltamethrin's use on aquatic invertebrates in commercial aquaculture ponds

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Abstract. Agricultural pyrethroid insecticides especially cypermethrin and deltamethrin are being used regularly for the control of aquatic insects in commercial carp aquaculture ponds in northwest Bangladesh. Thisstudy was conducted in a farmer's ponds under commercial aquaculture to know the impact of cypermethrin and deltamethrin on aquatic invertebrates (insects, zooplanktons and benthos). Commercial aquaculture ponds were treated with cypermethrin and deltamethrin @ 5 μg (active ingredient)/liter pond water in presence of fish. Required amount of each of the insecticides were diluted with water in big aluminium pots and broadcasted over the pond water. Water quality parameters of the experimental ponds were measured at pretreatment stage. Sampling of aquatic invertebrateswasdone pretreatment and after 1 day, 2 days, 5 days, 10 days, 15 days, 21days and 28 days of the application of insecticides. No fish mortality was recorded after the treatment. Aquatic insect population was found to be most affected;number count declined89.87% and 86.24% for cypermethrin and deltamethrin respectively within one day after treatment. The insect count recovery began after day 10 and day 5 of cypermethrin and deltamethr in treatment respectively. Loss of insect diversity was observed in cypermethrin treatment and diversity count did not recover within observation period of 28 days.Zooplankton populationwas relatively less impacted (21.10% and 26.33% declined respectively in cypermethrin and deltamethrin treatment). Benthos population count and diversity was not affected, possibly due to the high organic carbon content and clay soil of the pond bottom.
Key words: Pyrethroids, Cypermethrin, Deltamethrin, Aquatic invertebrates

Introduction

Aquatic insects are a common part of any aquatic ecosystem. Nasiruddin *et al.* (2014) reported higher abundance (number) of aquatic insects in pond system compared to the lake in Chattogram, Bangladesh. Tidwell *et al.* (1997) found macro-invertebrate densities to be significantly higher in fed and fertilized ponds than in unfed ponds. Aquatic insects are also common in commercial carp aquaculture ponds of northwest Bangladesh due to intensive feeding and fertilization. Kashyap*et al.* (2013) recorded the presence of aquatic insects in various fishponds including nursery, rearing and stocking ponds of northern and central states of India, many of which were detrimental to aquaculture.Dragonfly nymph/larvae were found to be a most effective common carp fry predator by Gonzalez and Leal (1995). Corbet (1980) described odonate (dragonfly) larvae as the exclusive predator in the aquatic environment. Marco *et al.* (1999) found that medium size dragonfly species were more abundant and potential predators of fish fry in aquaculture ponds of south-east Brazil. Additionally, backswimmer (*Anisops* sp.)was found to be causing predation-related mortality of carp fry in Lao PDR by Sano *et al.* (2011). Increased size of backswimmers increased their predation potential (Gonzalez and Leal 1995) and can prey onup to 46.7% carp fish larvae within 24 hours in nursery ponds (Sano *et al.*2011). Water scorpions (*Nepa* sp. and *Ranatra* sp.) also attack living insects and fish fry (Kashyap*et al.*2013). Predation of fish fry by water insects was found to be proportionate to the size ratio of water insects and fish fry:larger insects could capture larger fish fry (Gonzalez and Leal

1995). Predation of fish fry by aquatic insects is primarily limited in nursery ponds but and not in culture ponds of table fish.

Despite having no predation risk, commercial carp farmers of northwest Bangladesh consider aquatic insects hazardousfor table fish in aquaculture ponds due to the potential competition of supplied feed for fish and disturbances posed to fish by large number of aquatic insects. Therefore,carp farmers of northwest Bangladesh were found to be using pyrethroid insecticides (cypermethrin and deltamethrin) once in a monthat low concentrations in aquaculture ponds to keep the aquatic insect population under control.This study was conducted to understand the impact of cypermethrin and deltamethrin's use on aquatic insect, zooplankton and benthos populations of ponds in northwest Bangladesh as practiced by the farmers.

Materials and Methods

The study was conducted in ponds under commercial aquaculture in Hatgodagari area of Poba Upazilla of Rajshahi district. Farmers were asked to inform the author before using pesticides in their ponds so that the author could conduct the study. Accordingly, a farmer informed the author prior to using cypermethrin (brand name Ripcord, BASF Bangladesh ltd., distributed byPadma Oil Company limited) and deltamethrin (brand name Desis, marketed by Bayer Crop Science limited Bangladesh) in commercial aquaculture ponds measured over 2 acres in size (water area) respectively in November 2018 andMarch 2019 @5μg (active ingredient)/liter pond water for both insecticides.Before the use of the insecticides water quality parameters of the treatment ponds were measured. Soil samples from the bottom of both experimental ponds were collected at pretreatment stage, sundried, grounded, and tested for organic carbon content by the Soil Resource Development Institute (SRDI) laboratory in Rajshahi. Required quantity of both insecticides were diluted with water in a large aluminium pots and manually broadcasted along the water's edge of the whole pond. The farmer made sure that no supplementary feed was supplied to the fish on the treatment day and the following day. The sampling of zooplankton, aquatic insects and benthos was done before the treatment and on days 1, 2, 5, 10, 15, 21, 28 following the cypermethrin and deltamethrin treatment.

***Sampling of aquatic insects*:** A one square meter fine meshed net fitted in a bamboo frame was used for samplingof aquatic insects. The net was towed in the water along the edges of the pond for a three-meter distance. Then the accumulated insects from the net were transferred to a plastic containercontaining water and 10 ml of formalinwas added. The sampling process was repeated three times to be considered as three replications for sampling of both treatments.

***Sampling of zooplankton*:** A funnel shaped plankton net with specificationsof75 to 85 microns mesh sizewas fitted with a collecting bottle on the tapering end anda round metal frame on the open end.During each sampling the plankton net was towed for a 13-meter distance in the water, one foot below the surface. Then the sample was transferred to a plastic bottle and 5 ml formalin was added. The collected samples were transferred to the lab, where the total volume was measured, representing the amount of zooplankton per volume of water the net was traversed through and calculated using following formula: Water volume in liter= 'л (3.14)' multiplied by the'square radius of the plankton net metal frame in meters' multiplied by the'distance the net traversed through in meters' multiplied by1000. Zooplankton were identified and counted using a Sedgewick rafter cell counter (Welch 1948) and microscope.

***Sampling of benthos*:** A metal scoop with a 38.7 square centimeter opening was used to collect the soil samples from the bottom of the pond at 30-centimeter depth from the surface of the water. The process was repeated three times during each sampling to be considered as three replications for both treatments. In the lab, the collected soil samples were put under running tap water on a fine meshed sieve, through which all soil particle was washed away and the separated benthos samples were collected.

Results

Water pH in both treatmentswas found to be around 7.5. Water turbidity was higher (43.19 ntu) in the deltamethrin treated pond than in cypermethrin treated pond (28.05 ntu). Electrical conductivity was also higher in deltamethrin treatment (516 μS/cm) than in cypermethrin treatment (310 μS/cm). Organic carbon content of the pond bottom soil was 2.19 and 3.40% (dry weight basis) respectively for cypermethrin and deltamethrin treatment (Table I).

Table I. Some important water quality parameters in experimental ponds

Treatment ponds	Water pH	Water temp (^{0}C)	Water turbidity (ntu)	Water electrical conductivity (μS/cm)	Organic carbon content of the pond bottom sludge (%)
Cypermethrin	7.41	23.1	28.05	310	2.19
Deltamethrin	7.70	30.4	43.19	516	3.40

Within one day after use of cypermethrin and deltamethrin, the total insect count went down (90 and 86% respectively for cypermethrin and deltamethrin) for both treatments to reach to their lowest point of the study period (Fig. 1). The total insect count for deltamethrin treatment went up sharply above the pretreatment levelfive days after the treatment and continued to beat a near static level until the end of the observation period. Increase of the insect-count in cypermethrin treatment was much slower compared to the deltamethrin treatment and did notrecover to pretreatment levelswithin the observation period. Insect diversity went down after use of cypermethrin in the aquaculture pond. Insect diversity was lowest at day-2 after the use of cypermethrin (Fig. 1). The diversity gradually increased up to six types of insects at the end of the observation period of 28 days, while eight types of insect were recorded at pretreatment stage.

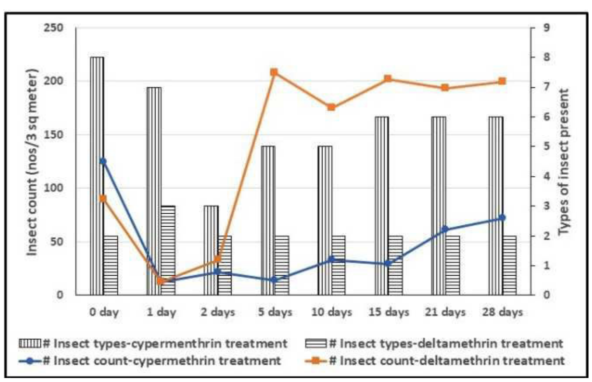

Fig. 1. Total count of aquatic insects (nos/3 sq meter) and their diversity before and after use ofcypermethrin and deltamethrin in commercial aquaculture ponds.

One day after the use of deltamethrin, the population of both boatmen and backswimmer declined with the decline of backswimmer deeper (92.59%) that that of boatman (86.36%) population. The backswimmer population continued to remain at a low level until the 10th day after the treatment and then increased slowly. On the contrary boatman population sharply increased at day 5 after the treatment above the pretreatment level and maintained that density until the end of the observation period of 28 days (Fig. 2).

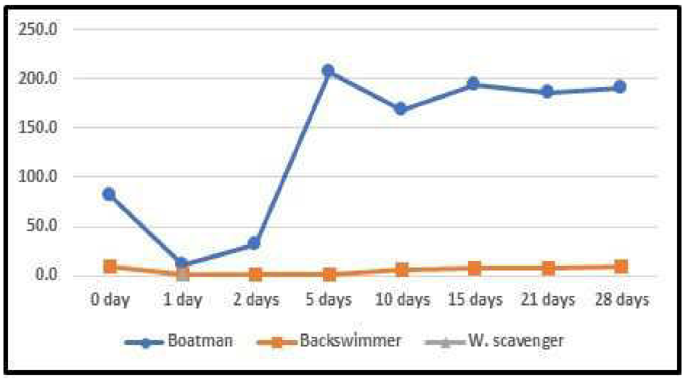

Fig. 2. Aquatic insect count (nos/3 sq meter) at various points of deltamethrin treatment @ 5μg/liter of water in commercial aquaculture pond.

Backswimmer, damselfly nymph and water boatman were the most dominant water insects in the pretreatment stage of the cypermethrin application. A decline of count by 93.22%, 85.9% and 96.42% was recorded respectively for damselfly nymph, backswimmer and boatman within 24 hours of the use of cypermethrin (Fig. 3). Boatmen count increased sharply at 15 days after the treatment while the backswimmer population first rose and then declined at day10 after the treatment. All other types of insects except *Nepa* sp., dragonfly nymph and creeping water beetle showed slow recovery 15 days post treatment. *Nepa* sp. and dragonfly nymph was observed on day1 after the cypermethrin treatment and did not return until the end of observation period.

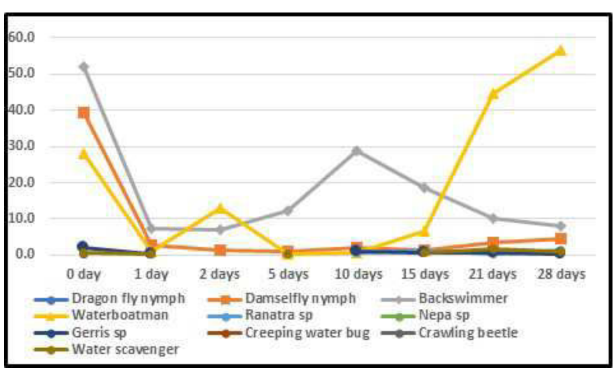

Fig. 3. Insect count (nos/3 sq meter) at various points before and after use of cypermethrin @5 μg (active ingredient) /liter in commercial aquaculture ponds.

The decline of the zooplankton count was 26.33% on day 2 after the deltamethrin treatment and 21.10% on day 5 after the cypermethrin treatment, reaching their lowest level compared to the pretreatment level (Fig. 4).Recovery of the zooplankton population occurred earlier and more rapidly in deltamethrin treatment than in cypermethrin treatment. Zooplankton count exceeded the pretreatment level at day 5 and day 10 respectively afterdeltamethrin and cypermethrin treatment. The higher count of zooplankton for both treatments (after recovering from their lowest point) was maintained until the end of the observation period on28thdayafter the treatment.

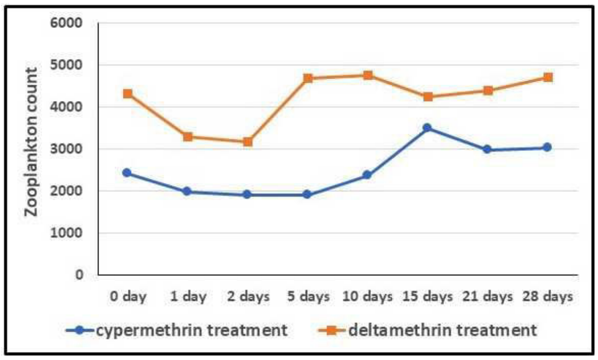

Fig. 4. Zooplankton count (nos/liter) before and after use of cypermethrin and deltamethrin in commercial aquaculture pond.

Zooplankton diversity showed little change in both the cypermethrin and deltamethrin treatments (Table II). The count of *Asplanchna* sp., *Daphnia* sp. and *Filinia* sp. increased towards the end of the observation period of 28 days after cypermethrin treatment, however without any specific evidence it is hard to attribute that increase to any specific factor. *Trichocerca* sp. is the only zooplankton that was found in small numbers inthe pretreatment stage of cypermethrin experiment but not found again during thepost treatment observation period of 28 days.

Total benthos counts for both cypermethrin and deltamethrin treatmentchanged little from their pretreatment level andalmost remain static till the end of the observation period of 28 days after treatment (Fig. 5). Like the benthos count, benthos diversity was not affected by both treatments (Table III).

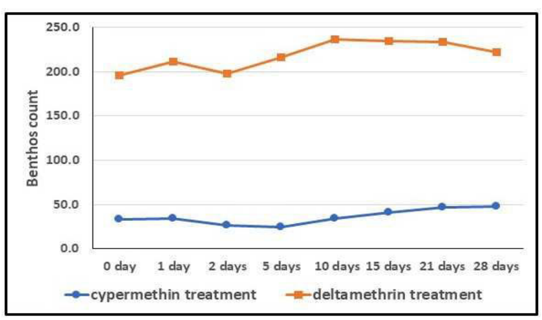

Fig. 5. Benthos count (nos/38.7 sq. cm. pond-bottom) before and after use of different insecticide in ponds under commercial aquaculture.

Table II. Zooplankton count (nos/liter water) before and after of cypermethrin and deltamethrin treatment in commercial aquaculture ponds

Insecticide	Benthos	Pre-treatment	Day-1	Day-2	Day-5	Day-10	Day-15	Day-21	Day-28
Cypermethrin	*Brachionus* sp.	995±269	751±104	803±32	546±32	653±183	907±48	756±12	727±71
	Keratella sp.	333±204	391±140	428±21	510±56	602±224	1037±177	727±53	679±78
	Cyclops sp.	177±59	69±23	14±12	60±26	140±46	150±89	241±118	145±36
	Nauplius	622±183	232±39	184±67	249±54	248±95	339±92	326±58	435±27
	Polyarthra sp.	148±181	263±117	198±110	53±58	159±112	228±159	214±67	392±93
	Diaptomus sp.	7±12	7±13	-	22±22	7±13	23±23	14±12	23±23
	Asplanchna sp.	7±12	147±103	89±16	175±55	323±158	445±116	473±70	321±32
	Daphnia sp.	14±25	15±27	52±14	83±11	27±16	86±17	53±32	53±36
	Moina sp.	7±12	-	-	7±12	-	-	-	-
	Filinia sp.	125±126	108±81	141±104	195±87	220±63	282±92	183±34	260±75
	Trichocerca sp.	14±25	-	-	-	-	-	-	-
Deltamethrin	*Brachionus* sp.	936±80	718±8	591±32	1628±161	1133±220	1040±179	1123±117	1151±96
	Keratella sp.	720±34	520±73	432±42	570±108	1321±61	1125±183	1004±67	1159±53
	Cyclops sp.	263±104	86±34	98±66	192±41	121±47	210±61	217±33	172±45
	Nauplius	632±86	505±35	439±81	529±126	534±67	516±84	531±100	598±44
	Polyarthra sp.	714±60	725±51	912±94	721±62	647±181	59±164	654±61	646±58
	Diaptomus sp.	-	-	7±12	64±14	8±13	7±13	-	7±13
	Asplanchna sp.	650±58	552±70	577±31	810±69	794±32	664±79	733±88	772±61
	Daphnia sp.	143±109	148±56	75±71	144±22	129±75	69±46	69±83	125±11
	Moina sp.	-	-	-	-	8±13	-	-	-
	Diphanosoma sp.	15±13	7±13	37±34	-	-	-	-	-
	Filinia sp.	182±158	23±39	15±26	16±28	15±27	-	23±41	39±26
	Trichocerca sp.	65±113	16±27	-	-	56±50	15±13	31±56	46±22

Table III. Benthos count (nos/38.7 square meter bottom) before and after of cypermethrin and deltamethrin treatment in commercial aquaculture ponds

Insecticide	Benthos	Pre-treatment	Day-1	Day-2	Day-5	Day-10	Day-15	Day-21	Day-28
Cypermethrin	Chironomid larvae	27.0±7	29.3±13	18.7±18	20.7±7	31.0±28	34.3±16	38.3±7	38.7±8
	Tubifex	6.0±1	3.7±1	8.0±4	3.0±1	3.0±2	6.0±3	7.7±4	8.3±2
	Leech	0.7±0.6	1.0±1.0		1.0±1.0		1.0±1.0	0.3±0.6	0.7±1.2
Deltamethrin	Chironomid larvae	151.3±25	174.3±12	165±25	171.3±25	186.3±8	193.3±9	190±17	179.3±12
	Tubifex	44.7±8.0	36.7±5.5	33.3±6.0	45.3±7.4	50.0±2.6	41.0±9.6	44.0±7.9	43.0±4.6

Discussion

No fish mortality was recorded during this study, despite of the doses (5 μg/liter)in this study exceeding the acute toxicity concentration for silver carp (Shaluei *et al.* 2012), *Cirrhinus mrigala* (Veni and Veeraiah 2014) and *Labeo rohita* (Tiwari *et al.* 2012, Das and Mukherjee, 2003) regarding cypermethrin; common carp (Datta *et al.* 2003) and *Labeo rohita* (Suvetha *et al.* 2015) regarding deltamethrin. During this study the fish that were in the ponds were a minimum of 0.75 kg in size (mostly adults) and were less susceptible to the cypermethrin and deltamethrin than at their juvenile stages (Mohammed2013) due to their larger body size (NPIC 2010). All of lethal concentration dose-determination experiments cited above, were conducted in laboratory conditions (room temperature and tap water) using small individuals (juveniles and fingerling) as subject, which is different than the culture conditions (temperature, turbidity, water hardness, pH, light etc.) of commercial aquaculture ponds used in this study. Day (1991) also mentioned a lesser impact of pyrethroid pesticides in field conditions than the impact predicted by laboratory test data.

Datta *et al.* (2003) found that temperature, water hardness and turbidity profoundly impacted the toxicity of deltamethrin on common carp, where toxicity was lowest (higher LC_{50} concentration) at 30^0C temperature, in hard water as compared to soft water, and in the presence of soil particles (humus, clay, organic carbon) where deltamethrin was absorbed by the soil. Cypermethrin is also hydrophobic, having low water solubility (Jones 1992) and strong absorption tendencies into soil particle. Hydrolysis is faster in a basic solution and the pesticide photodegrades rapidly with half ranges from 8 to 16 hours, with microbial degradation also possible (ETN 1996). Roberts and Hudson (1999) found that biodegradation of deltamethrin can be stalled due to its strong absorption by particulate organic matter. Compared to other pyrethroids deltamethrin has a potential of volatilization to the air from water, with an average half-life found to be 2.5 days in pH 9 solution. Microbial action, photolysis and hydrolysis also degrades deltamethrin (NPIC 2010). High turbidity, pH, electrical conductivity and favorable water temperature (Table I) of the treatment ponds have reduced the effect of both cypermethrin and deltamethrin to the level where it was nontoxic to the fish in the experimental pond.

Total insect count bounced back earlier (Fig. 1) in the deltamethrin treatment primarily due to the higher water turbidity, pH and conductivity that reduced the deltamethrin's toxicity (Table I) sooner than that of cypermethrin. Mulla*et al.* (1982) found 50 to 100% mortality of arthropod insects in field ponds caused by pyrethroid insecticides and 2 to 4 weeks after the treatment were required for their recovery of arthropod insects to the pretreatment level.In this study, the initial insect diversity was higher in the of cypermethrin treatment comparedto the deltamethrin pond. This is due to the cypermethrin experiment being conducted in the month of November, at the end of monsoon when generally insect abundance and diversity are higher (Kashyap *et al.* 2013,Nasiruddin*et al.* 2014) in Indian sub-continent. Insect diversity of deltamethrin treatment was low at pretreatment level asthe study was conducted during the month of March and remained the same after day2 of the treatment. After the deltamethrin treatment, the insect diversity increased. It ispossible that this is due tomore species being caught post-treatmentbecause of weak swimming due to the drug than thepretreatment stage when insects were likely stronger and evaded catchment.

The drastic rise of boatman population made the total insect count higher for deltamethrin treatment from day5 onwards after the treatment (Figs. 1 & 2). Gutierrez (2016) found that the 72-hour LC_{50} values of *Buenoatar salis* and *Martarega bentoi* (backswimmers) to be 4.0 and 102.5 ng (active ingredient) /liter respectively for deltamethrin. A low insect diversity of two typesplus backswimmers' susceptibility to deltamethrin and their relative slow recovery, possibly gave boatman free space to thrive after the deltamethrin treatment. Boatman population is considered less hazardous by the fish farmers compared to backswimmer in aquaculture ponds since backswimmers are predatory to fish fry and boatman are non-predatory (Gonzalez and Leal 1995).

It seems the insects that take longer to reproduce were the most affected by cypermethrin treatment. Saha and Kaviraj (2008) found the 96-hours LC_{50} value of *Rantrafiliformis* is 0.06μg/liter for cypermethrin. The 24-hour LD_{50} value of mayfly (Heptagenidae), damselfly (*Enellagma* and *Ishnura* sp.) and water scavenger beetle (*Hydrophilus* sp.) was found to be 1.3, 1.4 and 8.3μg/liter respectively for cypermethrin (Siegfried 1993). Stephenson (1982) recorded the 24-hour LC_{50} value of adult *Corixapunctata* (water boatman) as $\geq$ 5μg/liter for cypermethrin in a static test method in the laboratory. Aquatic insects are seldom found at greater water depths, rather prefer to live in shallow waters towards the surface and upper part of the water bodies (Kashyap *et al.* 2013).The manual spray of both cypermethrin and deltamethrin in this study was done along the edges of the water of the pond, henceheavily affecting the water insects.

Compared to the aquatic insects, zooplanktons were less affected; population decline was not as deep as in the insect populationin both treatments (Figs. 1 and 4). Though *Daphnia magna* is more susceptible against deltamethrin (Xiu *et al.* 1989 and Beketov 2004) than cypermethrin (Stephenson 1982) but in this experiment it was found to be less affected in both treatments. Day (1991) found fenvalerate, deltamethrin and cyhalothrin toxicity to *Daphnia magna* decreased with the increase of dissolved organic carbon concentration in water. Quicker recovery of the zooplankton population in deltamethrin treatment can be attributed to the water quality (higher turbidity, electrical conductivity and pH) of the treatment pond. In addition to the water quality factor, the lower degree of predation from lower number of aquatic insects may have triggered the quicker recovery (5 days and 10 days for deltamethrin and cypermethrin) of zooplankton population in post treatment condition.

Due to the higher organic carbon content in the sediment (Table I), the deltamethrin treated pond fostered higher concentrations of benthos population (pretreatment stage) compared to the cypermethrin treatment-pond (Table III). Though Stephenson (1982) recorded a 24-hour LC_{50} value of *Chironomus thummi* as $\geq$ 5 μg/liter for cypermethrin in a static test method in the laboratory,high water turbidity (Table I) of the experimental ponds indicate the clay soil of the bottom which is rich in organic carbon has a strong absorption capacity of cypermethrin and deltamethrin, affecting the bioavailability of the insecticides; left the benthos population unaffected in this study. Akerblom *et al.* (2008) confirmed lower organic matter content of artificial sediments that was spiked with deltamethrin was highly toxic to *Chironomus riparius* larvae (the 28-day LC_{50} value was 11μg/kg sediments), while deltamethrin induced (@166μg/kg) mortality was zero in natural sediment due to its high organic matter content. Muir et al. (1985) reported lower bioavailability of pyrethroid pesticides, including cypermethrin and deltamethrin, in silt and clay sediments and water above those sediments. This is evidenced by a 5 to 15-fold higher bioaccumulation of pyrethroids by *Chironomus tenfans* larvae in sand sediments containing pyrethroids as compared to that of silt or clay sediments.Moreover, there was mixing inconsistency of both cypermethrin and deltamethrin due to the large volume of water of the experimental ponds and the manual spray method along the water edges, leaving the large portion of the pond water in the middle with very little or almost no pesticide. This left enough space for fast moving fish to take refuge in water devoid of pesticides and spared some aquatic insects and zooplanktons to reproduce within the observation period.

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(Manuscript received 29 August 2019)

Asian Australas. J. Food Saf. Secur. 2019, 3 (1), 27-37

Asian-Australasian Journal of Food Safety and Security
ISSN 2523-1073 (Print) 2523-2983(Online)
www.ebupress.com/journal/aajfss

Article

Consequences of use of fenpropathrin compared to other fish toxicants in commercial aquaculture

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Received: 16 April 2019/Accepted: 18 May 2019/ Published: 30 May 2019

Abstract: Use of fish toxicants is an important management tool in inland commercial aquaculture. In entrepreneurial fishery in northwest Bangladesh where pond ownership (using rights due to lease) changes frequently (every few years) use of fish toxicants is very routine and more crucial. Along with some traditional fish toxicants (rotenone and aluminium phosphide), unconventional and insecticides like fenpropathrin (not approved for aquaculture use) are being used by fish farm owners in northwest Bangladesh. The study was conducted to understand the consequences of use of fenpropathrin compared to other traditional fish toxicants in commercial aquaculture for harvesting of food fish. Of all the toxicants, fenpropathrin' s impact was lowest on zooplankton and aquatic insect population, while rotenone had the lowest impact on benthos population in terms of killing and quick recovery time for the population, primarily due to the high turbidity (suspended soil particle) of the pond water (under this study) by which both fenpropathrin and rotenone got affected. Aluminium phosphide found to be more damaging in terms of killing and relatively longer recovery time for zooplankton, aquatic insect and benthos population. Using convenience, quick killing, cheaper price, short duration of toxicity and no potential long-term damage of the waterbody contributes positively for fenpropathrin as fish toxicant except the severe potential public health concern from eating of fish killed by fenpropathrin due to very high bioconcentration factor of fenpropathrin; hence, demands regulation of fenpropathrin' s use as fish toxicants for food fish.

Keywords: fenpropathrin; fish toxicant; aquaculture; zooplankton

1. Introduction

Commercial pond aquaculture has its unrelenting pressure of economic necessity to produce fish in most efficiently but inexpensively as possible (Lennon *et al*., 1970). Use fish toxicants as a management tool allows the commercial fish farmers of northwest Bangladesh to have greater control over the fish stock management (elimination of undesired species, complete harvesting of all fish of the waterbody, elimination and restart of fish culture etc.). Rotenone, a natural toxicant derived from leguminous plants mainly found in southeast Asia, Latin America and east Africa (Finlayson *et al*., 2000) is highly toxic to fish and aquatic life but significantly less toxic to birds and mammals made it favorable as piscicide, hence used historically as most environmentally benign pesticide (Ling, 2003). Most of the fish species exhibit higher sensitivity to rotenone than most of the aquatic invertebrates (Durkin, 2008). Based on the comprehensive ecological and human health risk assessment US EPA (2007) declared that rotenone is eligible only for piscicidal use. Apart from rotenone's using difficulty, to ensure killing of fish species that are relatively hardy (catfish, *Channa* sp., *Tilapia* sp., *Anabus* sp etc.) may require use of unusually high amount of rotenone, which increases the cost significantly.

As an alternative to rotenone, like other parts of the country most of the commercial fish farmers in northwest Bangladesh use aluminium phosphide tablets (phostoxin) as fish toxicant; a chemical normally used as fumigation agent against grains-storage pests (Braid, 1994; Chowdhury *et al*., 2012; Rasul *et al*., 2017). According to Perschbacher and Sarkar (1989) phostoxin (aluminium phosphide) incurred lowest concentration (0.25 ppm) and lowest cost to attain 100% kill in 24 hours of relatively hardy species, *Channa punctatus* of many fish toxicants (sumithion, bleaching powder, dieldrin, phyphanon, rotenone, phostoxin and DDVP) used in aquaculture ponds at 27^0C temperature. Rahman *et al.* (1992) noted that in earthen aquaculture pond aluminium phosphide toxicity lasts 10 to 15 days as opposed to 10 to 12 days for rotenone in Bangladesh. Farmers involved in commercial fishery in northwest Bangladesh are continuously looking for alternative of phostoxin (aluminium phosphide), due to the operational difficulties like broadcasting of phostoxin pills in the pond, which is hazardous to the broadcaster as well and then waiting for couple of hours at least after the application in the middle of the night to get the fish killed before netting, in addition to the long detoxification period.

In these circumstances, synthetic pyrethroid, fenpropathrin came into play. Fenpropathrin, is a broad spectrum pyrethroid insecticide and acaricide, first synthesized in 1971 by Suitomo chemical company ltd and commercialized in late 1980's (Kanawi *et al.*, 2013). In Bangladesh fenpropathrin is registered for controlling of red mites in eggplant and tea (DAE, 2019). Valent USA incorporation (marketer of Danitol in Florida USA) categorized fenpropathrin as 'restricted use pesticide' due to its toxicity to fish and aquatic organisms (Valent, 2009). Due to its convenience of use, extreme toxicity to fish and relatively lower price that makes the use of high-enough concentration economically viable to obtain quick kill (within an hour) of fish; commercial fish farmers in northwest Bangladesh have started using fenpropathrin (Danitol) as fish toxicant to kill food fish. The study was conducted to compare the ecological consequences of fenpropathrin' s use as fish toxicant compared to the traditional fish toxicants in commercial fish farming in northwest Bangladesh.

2. Materials and Methods

For this study 3 different farmers were identified in Parila union of Poba upazilla of Rajshahi district who intended to use different fish toxicants namely fenpropathrin (Danitol), rotenone and aluminium phosphide (phostoxin) in commercial aquaculture ponds. Accordingly, the author was present with the farmers at the time of treatment. Different farmers used the treatment in different times in between March and May of 2019.

2.1. Fenpropathrin application

The total amount of ('Danitol' 10 EC imported marketed by Setu corporation ltd) fenpropathrin were poured into a big aluminium pot and diluted with water. Then the dilution was broadcasted along the water edges of the whole pond, and as far as it can be thrown (approximately 5 meters) towards the middle of the pond. The treatment took place on 4 April 2019, 4.00 am in aquaculture pond with water area of two acres.

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2.2. Rotenone application

Farmer took half of the rotenone powder ('Aquanone powder' containing 9% rotenone, marketed by agrovet division of square pharmaceuticals ltd.) in a big aluminium pot, little bit of water was added to make dough of rotenone powder in such condition that balls can be made using the dough. Then the balls of rotenone powder were thrown into the water mostly in the middle part of the waterbody. The rest half of the rotenone was diluted in a big aluminium pot with water, then the dilution was broadcasted along the water edges of the pond and as far as it can be thrown (approximately 5 meters) towards the middle. To avoid the rotenone powder from getting into his respiratory system, farmer covered his nose and mouth with a towel at the time of dilution. The treatment took place on 12 May 2019, 1.30 am in aquaculture pond with water area of one acre.

2.3. Phostoxin application

Farmer used a towel to cover his nose and mouth before he opened the can of phostoxin tablets ('Mimtox' containing 57% aluminium phosphide, imported and marketed by Mimpex agrochemicals ltd.) to avoid the phostoxin fumes from getting into his respiratory system. Then the tablets were thrown into the pond. Because of the large size of the tablet's farmer was able to through those homogeneously throughout the pond including the middle part of the pond. The treatment took place on 24 May 2019, 1.30 am in aquaculture pond with water area of four and half acres.

From each treatment, samples of zooplankton, water insects and benthos were collected at day before (previous day) and after 1 day, 2 days, 5 days and 10 days of the treatment. From each of the treatment, each day sampling was done 3 times and was considered as 3 replications. After each of the treatment, chemical concentration was back calculated based on the size of the water body, water depth and quantity of the fish toxicants (percentage of active ingredient) used. Water samples (3 replications) from one-foot depth of each water body was taken before the treatment and after completion of (3 times) netting in post treatment stage. Then turbidity for each sample was measured using turbidity meter in the lab.

2.4. Zooplankton sampling

A cone shape plankton net (specification- 200 US with 75 to 85 microns mesh size) was used for zooplankton sampling. The wide end of the net was fitted with a round metal frame and the tapering end was fitted with a collection bottle. Holding the meatal frame, the net was towed for certain distance in the pond water, one foot below the surface. Then the collected samples from the collection bottle were taken into a plastic bottle, added with 5% formalin as preservative. The process was performed three times for each sampling and were considered as three replications. The collected samples represented the total amount of water passed through the plankton net, calculated by using following formula: Water volume in liter = ‘л (3.14)’ multiplied with ‘square radius of the plankton net metal frame in meter’ multiplied with ‘distance the net was traversed through in meter’ multiplied with 1000. The zooplankton sample was transferred to the lab. Using a compound microscope and Sedge-wick Rafter cell counter (Welch 1948), zooplankton type and density was counted and expressed in numbers/liter of pond water.

2.5. Sampling of aquatic insects

A square shape fine meshed net bag fitted with a bamboo frame (1 meter by 1 meter) at the open end was used for collecting aquatic insect sample. The net was towed in the water along the edge of the pond for 3-meter distance. The collected insects were transferred in a plastic jar with 5% formalin solution. The process was performed three times for each sampling and was considered as three replications. Then the samples were taken to the lab where the insects were identified and counted.

2.6. Sampling of benthos

For collection of benthos sample, a metal scoop (2.76 inches diameter and 1.5 inches depth) was used to collect the mud from the pond bottom at 18 inches depth from the surface. A total of three scoops of mud was collected from 3 different locations of the pond to constitute three replications. The mud samples were then transferred into separate plastic bags and carried to the lab. The mud samples were then washed under running water on a fine meshed sieve. The benthos separated from the mud, then was transferred into a Petri dish from the sieve. Some tap water was added into the petri dish and the benthos was observed and counted under bright light.

3. Results and Discussion

Based on the calculation, rotenone, aluminium phosphide and fenpropathrin were found to be used at 0.272 mg/liter, 0.61 mg/liter and 0.065mg/liter respectively. Water temperature was around 30^0C for all ponds during the time of fish toxicants use.

Rotenone concentration of 0.272 ppm was good enough to kill and harvest all the carp species in this study but not good enough to kill the predatory species like *Channa punctatus*. Rotenone concentration of 2.5 ppm is required to achieve 100% kill of *C. punctatus* within 24 hours (Perschbacher and Sarkar, 1989). Within one week after the harvest the ownership of the pond (where rotenone study was conducted) got changed, and new owner used phostoxin (aluminium phosphide @ 0.86 ppm) to ensure killing of all weed fish. As a result, many *C. punctatus* was observed dead and floating on the water, that successfully survived the rotenone treatment.

96-hour LC_{50} value of *Oncorhynchus mykiss* is recorded as 0.0097 mg/liter for aluminium phosphide by IUPAC (2018), but Perschbacher and Sarkar (1989) required a concentration of 0.25 ppm of aluminium phosphide to obtain 100% kill of *C. punctatus* within 24 hours. The doses exercised (0.61 mg/liter) in this experiment were higher than the doses mentioned above and was effective to kill all the fish (Indian and Chinese carps).

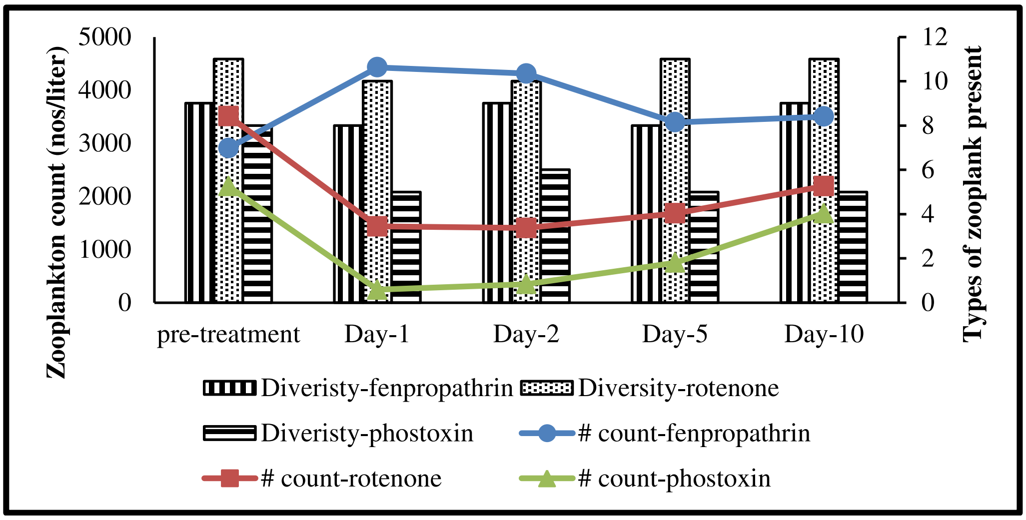

Figure 1. Total count (nos/liter) of zooplankton and diversity before and after use of different fish toxicants in commercial aquaculture ponds.

Zooplankton total count (nos/liter water) increased 52% at 24 hours point (was not anticipated!) after use of fenpropathrin @0.065mg/liter, while the zooplankton total count (nos/liter water) declined by 59% and 89% respectively at 24 hours point after use of rotenone @0.272 mg/liter and aluminium phosphide @ 0.61 mg/liter (Figure 1). From day 2 to 10 days point after fenpropathrin treatment zooplankton count gradually declined, but for rotenone and aluminium phosphide treatment zooplankton count gradually kept increasing. Like the zooplankton count, zooplankton diversity (types of zooplankton available) was affected in phostoxin treatment where diversity count reduced to five after 10 days of the treatment from initial count of eight. Given that during the experimental period no fish feed was supplied, and there was no fish released, there was neither additional nutrient supply nor predation from fish, let the zooplankton population grow and survive (after the initial blow of fish toxicant use), which was mostly determined by the existing nutrient supply and physico-chemical factors of the pond.

48-hours LC_{50} value of *Dhaphnia magna* for fenpropathrin is 0.53 ppb (PMEP, 1989) whereas for aluminium phosphide 48-hour EC_{50} value of *D. magna* is 0.37 ppm (IUPAC, 2018). Given fenpropathrin's extreme toxicity to aquatic organisms (Valent, 2009; Chemwatch, 2012), the influx of zooplankton count in fenpropathrin experiment was quite surprising. The explanation for this unusual zooplankton response lays in the water turbidity data of the treatment pond.

Table 1. Water turbidity of treatment ponds before and after treatment (and netting in between) with various fish toxicant.

Sl. No.	Treatment ponds	Pretreatment turbidity (ntu)	Post treatment (after 3 times netting) turbidity (ntu)
1	Rotenone treatment pond	21.31	62.18
2	Aluminium phosphide (phostoxin)- pond	27.74	65.44
3	Fenpropathrin (Danitol) treatment pond	34.57	83.56

In the pretreatment stage the water turbidity of fenpropathrin treatment (due to prior netting) pond (Table 1) was higher than the other treatment ponds especially than rotenone pond. Several times of netting immediately after the use of the fish toxicants to harvest fish increased the pond water turbidity in between two to three-fold of the pretreatment stage. Due to fenpropathrin' s strong absorbance by soil, it is very resistant to leaching and there is no risk of ground water contamination in normal circumstances (ARNICA and AWHHE, 2014). Fenpropathrin' s nonpolar nature resulted in very low water solubility causes it to be sorbed strongly to organic matter and soil to avoid contact with water (Kanawi *et al.*, 2013); highest turbidity of pre and post treatment (after netting) pond water have resulted in binding of fenpropathrin with the soil particle at a greater degree and more quickly, has surely made it unavailable in the pond water in short term and result in relatively quick detoxification of water (tested by releasing few fish on test basis after 4 days of treatment) compared to aluminium phosphide treatment. Day (1991) also observed 20 to 80% reduction in pyrethroid induced mortality of *Daphnia magna* due to the presence of humic material. Due to the application method (farmer broadcast fenpropathrin along the water edges) of fenpropathrin large middle portion of the waterbody didn't received any fenpropathrin.

24-hour LC_{50} value of *Daphnia pulex* and *Diaptomus siciloides* for rotenone was <0.025 ppm (Hamilton, 1941). Despite the use of higher doses of rotenone (@0.272 ppm) and aluminium phosphide (0.61 ppm) zooplankton population declined but not got wiped out. Due to the large size of the pond (1 acre for rotenone treatment, and 4.5 acres for aluminium phosphide treatment, 2 acres for fenpropathrin treatment) and sheer volume of water the mixing of all the fish toxicants (rotenone, aluminium phosphide and fenpropathrin) in pond water was surely not homogeneous. Despite of several times of netting for catching fish, the inconsistency in mixing left blank space in the water body where some of the zooplanktons (due to their slow-moving nature) never encountered the fish toxicants. Whereas fast moving fish encountered the toxicants and was killed.

Presence of heat, light and oxygen makes rotenone unstable; rotenone also gets absorbed by sediment and suspended soil particle in water (Ling, 2003). Gilderhus *et al.* (1988) found rotenone loss to be 10 times faster at temperatures above 23^0 C than 1^0 C in shallow ponds, and half-life was generally less than 1 day in natural water at temperatures above 20^0 C. Absorption of rotenone by the suspended soil particle in water and faster decay of rotenone, helped recover the zooplankton population in the experiment relatively faster than that of aluminium phosphide treatment.

Table 2. Zooplankton count (nos/liter) before and after use of fenpropathrin, phostoxin and rotenone in commercial aquaculture pond.

Toxicant	Zooplankton	Pretreatment	Day-1	Day-2	Day-5	Day-10
Fenpropathrin @0.065 mg/liter	*Brachionus* sp.	892 ± 34	1654±122	1512±301	1135±217	1201 ± 225
	Keratella sp.	822 ±138	1236 ± 19	913 ± 219	748 ± 80	622 ± 110
	Cyclops sp.	148 ± 64	97 ± 45	15 ± 25	7 ± 13	71 ± 21
	Nauplius	375 ± 100	435 ± 55	346 ± 50	333 ± 95	383 ± 39
	Polyarthra sp.	-	88 ± 92	361 ± 116	315 ± 86	264 ± 74
	Diaptomus sp.	7 ± 12	-	-	-	-
	Asplanchna sp.	356 ± 55	523 ± 206	660 ± 168	484 ± 150	535 ± 158
	Daphnia sp.	75 ± 48	16 ± 28	15 ± 27	-	24 ± 25
	Moina sp.	13 ± 23	-	-	-	-
	Filinia sp.	-	-	278 ± 126	173 ± 47	299 ± 265
	Trichocerca sp.	219 ± 149	377 ± 118	208 ± 132	196 ± 86	101 ± 54
Aluminium phosphide @0.61 mg/liter	*Brachionus* sp.	651 ± 72	54 ± 13	35 ± 25	40 ± 37	111 ± 88
	Cyclops sp.	634 ± 102	61 ± 21	86 ± 22	174 ± 121	625 ± 94
	Nauplius	566 ± 77	102 ± 40	193 ± 86	380 ± 242	586 ± 29
	Polyarthra sp.	-	-	-	24 ± 42	-
	Diaptomus sp.	109 ± 44	20 ± 20	14 ± 25	-	208 ± 119
	Asplanchna sp.	159 ± 29	7 ± 12	7 ± 12	134 ± 93	152 ± 49
	Daphnia sp.	36 ± 32	-	14 ± 12	-	-
	Moina sp.	21 ± 21	-	-	-	-
	Diaphanosoma sp.	14 ± 13	-	-	-	-
Rotenone @ 0.272mg/liter	*Brachionus* sp.	584 ± 91	337 ± 270	414 ± 138	481 ± 59	566 ± 110
	Keratella sp.	151 ± 262	20 ± 35	-	45 ± 23	63 ± 28
	Cyclops sp.	428 ± 78	87 ± 65	105 ± 76	255 ± 38	346 ± 107
	Nauplius	1191±280	715 ± 680	704 ± 283	549 ± 37	520 ± 87
	Polyarthra sp.	138 ± 240	88 ± 152	-	15 ± 13	19 ± 19
	Diaptomus sp.	270 ± 77	67 ± 100	13 ± 23	92 ± 24	156 ± 57
	Asplanchna sp.	269 ± 119	74 ± 94	83 ± 63	155 ± 39	178 ± 91
	Daphnia sp.	15 ± 26	7 ± 12	13 ± 12	22 ± 22	97 ± 59
	Moina sp.	343 ± 141	-	7 ± 12	29 ± 34	155 ± 85
	Diaphanosoma sp.	89 ± 154	13 ± 23	21 ± 36	22 ± 22	81 ± 21
	Filinia sp.	31 ± 54	27 ± 46	27 ± 32	15 ± 25	13 ± 11

Zooplankton composition in fenpropathrin experiment (Table 2) shows that the influx of *Brachionus* sp., *Keratella* sp. and *Asplanchna* sp. were the main reason of increasing the total zooplankton count after one day of fenpropathrin use. Zooplankton diversity (genus count) remain same after fenpropathrin's use, though the genus composition was different. Of all zooplankton types *Moina* sp. and *Diaptomus* sp. (less abundant at pretreatment level) were most affected, disappeared after the use of fenpropathrin and didn't show up till the end of the observation (10 day) period. On the other hand, *Polyarthra* sp. and *Filinia* sp. were not found at pretreatment sampling but showed up after 1 day and 2 days respectively of the use of fenpropathrin. It is most likely that they were present even in pretreatment stage but due to their lower number didn't show up at sampling.

Zooplankton composition in aluminium phosphide experiment (Table 2) showed that *Moina* sp. and *Diphanosoma* sp. were affected most; disappeared after the treatment and didn't showed up till the end of the observation period of 10 days after the treatment. *Brachionus* sp., *Cyclops* sp., nauplius, *Diaptomus* sp. and *Asplanchna* sp. declined sharply (92, 90, 82, 82 and 96% respectively) in number after one day of aluminium phosphide treatment. Abundance of *Cyclops* sp., nauplius, *Diaptomus* sp. and *Asplanchna* sp. recovered to the pretreatment level by the end of the observation period but *Brachionus* sp. count remained low.

Zooplankton composition (Table 2) in rotenone treatment showed that *Moina* sp. disappeared completely after 1 day of the treatment but showed up again after 2 days of the treatment. *Brachionu*s sp., *Keratella* sp., *Cyclops* sp., nauplius, *Polyarthra* sp., *Diaptomus* sp., *Asplanchna* sp., *Daphnia* sp. and *Diaphanosoma* sp. declined 42, 87, 80, 40, 37, 75, 72, 55 and 85% respectively after one day of rotenone's use. Zooplankton population showed continuous recovery within the observation period of 10 days.

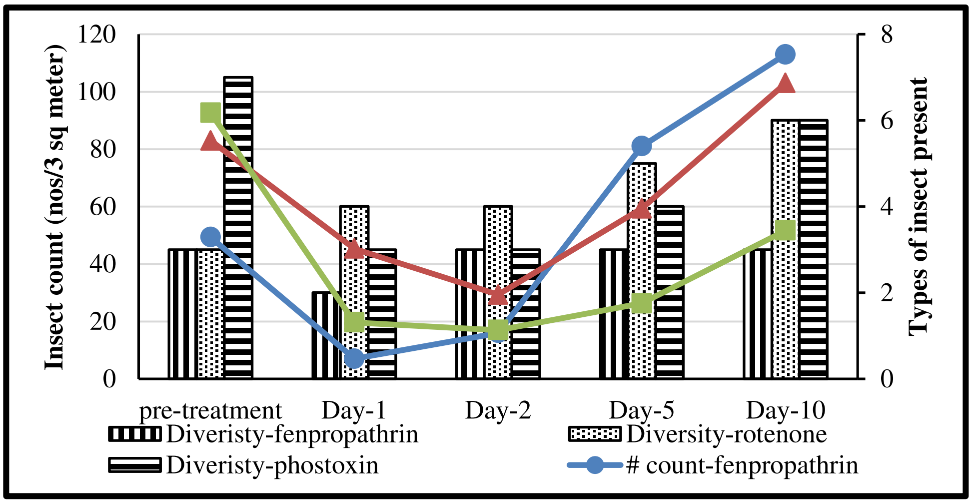

Figure 2. Total count (nos/3 sq meter) and diversity of aquatic insects before and after use of different fish toxicants in commercial aquaculture pond.

Total insect count in fenpropathrin and aluminium phosphide treatment was found to be lowest after one day of the treatment, declined 86 and 82% respectively from the pretreatment level (Figure 2). Lowest total insect count (a decline of 64% of pretreatment level) for rotenone treatment found to be on day-2 after the treatment. Insect number reached back to the pretreatment level within 5 days of fenpropathrin treatment, compared to within 10 days in case of rotenone treatment. The recovery in insect count is slowest in case of aluminium phosphide treatment and fastest in fenpropathrin treatment. In fact, insect count didn't reach to the pretreatment level for aluminium phosphide within the observation period of 10 days. In addition to inconsistency of mixing, quick decay of rotenone due to high environmental temp (around 35^0C) and strong absorption of fenpropathrin by the suspended soil and humic particle of the pond water reduced the endurance of toxicity of rotenone and fenpropathrin quicker than the toxicity of aluminium phosphide. Insect diversity (genus count) also got down from seven at pretreatment level to three after one day of aluminium phosphide treatment. From day-5 of the aluminium phosphide treatment the diversity started to increase, and the diversity count reached to six on day 10 at the end of the observation period. Insect diversity increased after the rotenone treatment from three at pretreatment level to six on day 10 after the treatment. Crawling beetles and tadpoles showed up towards the end of the observation period of rotenone treatment, which were not available at pretreatment stage.

Table 3. Aquatic insect count (nos/3 sq meter) before and after use of fenpropathrin, phostoxin and rotenone in commercial aquaculture pond.

Toxicant	Aquatic insects	Pretreatment	Day-1	Day-2	Day-5	Day-10
Fenpropathrin @0.065 mg/liter	Boatman	46.3±22	6.7±8.3	5.3±3.2	68.7±52.4	98±114
	Backswimmer	2.7±0.6	-	0.7±1.2	1.0±1,7	6.3±4
	Gerris sp.	0.3±0.6	-	-	-	-
	Nepa sp.	-	0.3±0.6	-	-	-
	Crawling beetle	-	-	10±2.6	7.4±8.7	8.7±4.7
Aluminium phosphide @0.61 mg/liter	Boatman	59.3±17.2	15±10.1	11.0±6	12.3±6.1	21±9.2
	Backswimmer	27.3±19	-	-	-	5±3.6
	Gerris sp.	0.7±0.6	-	-	-	0.7±0.6
	Water scavenger	0.3±0.6	1.0±1.0	1.3±1.5	4.7±2.1	4.0±2.6
	Crawling beetle	3.0±3.0	3.7±2.1	4.7±1.2	6.7±2.1	12±5.6
	Dragon fly	1.7±1.2	-	-	-	-
	Damselfly	0.3±0.6	-	-	2.7±1.5	9.0±5.0
Rotenone @ 0.272mg/liter	Boatman	5.7±3.1	8.0±6.6	9.7±4.0	36±9.6	59.0±18.5
	Backswimmer	76.7±79.2	36.7±8.4	19.0±10.4	17.3±8.5	32.3±8.0
	Gerris	0.7±0.6	0.3±0.6	0.3±0.6	0.7±0.6	0.3±0.6
	Water scavenger	-	0.3±0.6	0.3±0.6	0.3±0.6	0.3±0.6
	Crawling beetle	-	-	-	5.0±2.0	8.0±2.6
	Tadpole	-	-	-	-	2.7±0.6

Insect composition in fenpropathrin experiment (Table 3) showed that boatman declined 88%, backswimmer and *Gerris* sp. were absent after one day of fenpropathrin treatment. From day two of the fenpropathrin treatment, both boatman and backswimmer count started to increase. Insect count at the end of the observation period was dominated by boatman, though backswimmer number got some bump as well. Of the insects *Gerris* sp. got disappeared after the fenpropathrin treatment and never showed up. *Gerris* sp. is fast moving a surface dweller and fenpropathrin was broadcasted on the water, made the fast-moving surface dweller *Gerris* sp. became victim of toxicity fenpropathrin before it got absorbed by the suspended soil and humic particle. *Nepa* sp. was another insect that was only found at Day-1 of the treatment in semi-dead condition, but it never showed up again afterwards. The timing of half-dead *Nepa*' s catch in fenpropathrin treatment indicated the bottom dwelling *Nepa* sp. got affected from the sediments which absorbed fenpropathrin from being suspended in the water column during the experiment that got settled on the bottom. Insect diversity at the pretreatment (Table 3) and at the end of the observation period remain same due to the appearance of crawling beetles from day-2 of the observation period. Given, this beetle can fly it is not impossible for it to arrive from side by side ponds, because during first two sampling (pretreatment and day 1) it was not found.

Of the insects in aluminium phosphide treatment, dragon fly nymph (which has long reproductive cycle) was most affected, got disappeared due to the treatment and did not appear again within the observation period (Table 3). Backswimmers also got disappeared after the treatment but showed up again on 10^{th} day after the aluminium phosphide treatment. Boatman number got drastically down (81% decline at day 2 from the pretreatment level) but slowly recovered like *Gerris* sp. and damselfly larvae in the aluminium phosphide treatment. Water scavenger and crawling beetle were almost not affected in aluminium phosphide treatment and in fact their number slowly but steadily got increased till the end of observation period.

In rotenone treatment (Table 3) backswimmer number gradually declined (77% of the pretreatment level) after the treatment till 5 days then increased but not reached the pretreatment level within the observation period (10 days after treatment). 24-hour LC_{50} value of backswimmer for rotenone was recorded as 0.1 mg/liter by Hamilton (1941). Opposite to the backswimmer, number of boatmen kept increasing after the treatment and reached the highest count at the end of observation period of 10 days. *Gerris* sp. was not affected, the number remain low but steady all along the observation period.

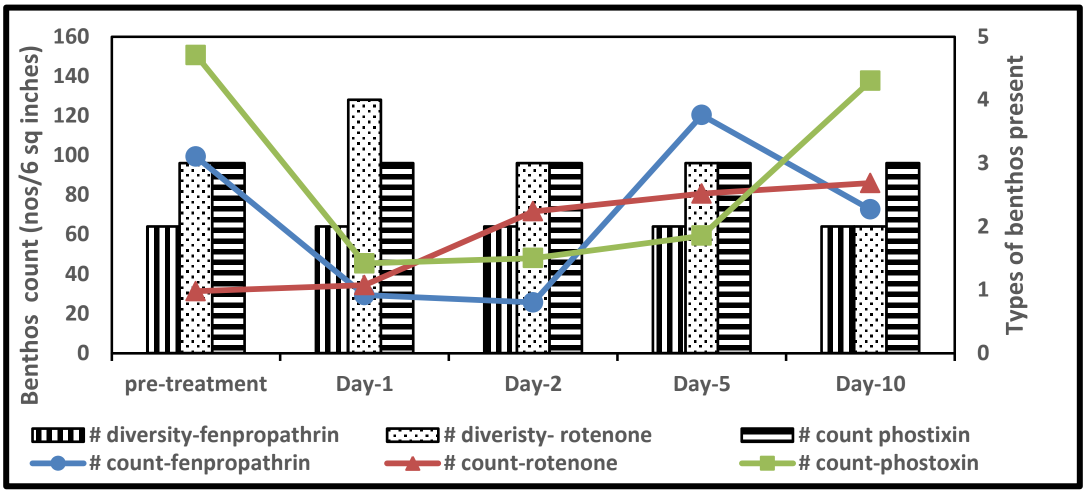

Figure 3. Total count (nos/6 sq inches) of benthos before and after use of different fish toxicants in commercial aquaculture ponds.

Unlike the rotenone treatment (Figure 3) one day after use of fenpropathrin and aluminium phosphide the benthos count declined (74 and 70% respectively) sharply, signifies the toxicity of those two toxicants to benthos population. Benthos population count exceeded the pretreatment level within 5 days of fenpropathrin treatment but didn't sustain the number and declined a bit at day-10 after the treatment. The recovery of benthos population took longest in aluminium phosphide treatment. On the other hand, benthos population in rotenone treatment continuously kept increasing till the end of the observation period indicates no negative impact of rotenone on benthos population. Diversity count for benthos in case of all three treatments found to be not affected.

Table 4. Benthos count (nos/6 sq inch bottom) before and after use of fenpropathrin, phostoxin and rotenone in commercial aquaculture pond.

Toxicant	Benthos	Pre treatment	Day-1	Day-2	Day-5	Day-10
Fenpropathrin @0.065 mg/liter	Chironomid larvae	34.3±3.5	3.3±0.6	0.7±0.6	41±6.6	16.7±4.5
	Tubifex	65.0±8.5	26±17	25±10.6	79.3±3.5	56±11.5
Aluminium phosphide @0.61 mg/liter	Chironomid larvae	70.7±7.4	3.3±1.5	2.7±0.6	4.3±4.5	124.0±100
	Tubifex	78.7±8	41.3±11.7	44.7±17.2	54±9.8	12.7±4
	Leech	1.3±0.6	-	-	-	-
	Polychaeta	-	0.7±0.6	0.7±1.2	1.0±1.0	1.0±1.0
Rotenone @ 0.272mg/liter	Chironomid larvae	13.3±1.5	18.3±8.1	13.3±9	17.7±3.5	22.7±4.2
	Tubifex	14.3±1.5	15.0±4.0	57.3±10.6	62.7±8.5	63.3±12.5
	Leech	3.7±1.2	0.3±0.6	-	-	-
	Polychaeta	-	0.7±1.2	1.0±0	0.3±0.6	-

In fenpropathrin treatment (Table 4) both the chironomid larvae and tubifex number found to be in synchrony with each other at different samplings. An influx of both chironomid larvae and tubifex (mostly young and small) were observed at day five after the treatment from their lowest count at day-two of fenpropathrin treatment. Population count for all those young individuals (for both chironomid and tubifex) didn't sustain due to the left-over toxicity of the pond sediments that absorbed the fenpropathrin and hence many of them died resulted in lower population count for both chironomid larvae and tubifex (regular size) at day-10 of fenpropathrin treatment. The response of benthos population in fenpropathrin treatment are in alignment with ARNICA and AWHHE (2014) characterization of fenpropathrin as moderately toxic to earthworms. Also, tiny snails showed up from the day-5 after the treatment (not shown in Table 4) and their number increased on day 10 of the observation period, while in the pretreatment stage they were not found. In commercial aquaculture

ponds in northwest Bangladesh farmers always rear some black carp along with other cultured carp species, for biological control of snails, but the absence of snail eater fish (back carp) may have led the production of snails.
Leech was most affected in aluminium phosphide treatment, disappeared after the treatment and didn't come back again within the observation period. Chironomid population in aluminium phosphide treatment found (Table 4) to be lowest (96% decline of the pretreatment level) on day-2 after the treatment but swung back after day-5 of the treatment and exceeded the pretreatment level at day-10 of the observation period. On the other hand, tubifex population declined to a lesser extent (47% decline on day one) than chironomid larvae; seemed stabilized in the middle but declined again towards the end to score the lowest count at day-10 after aluminium phosphide treatment, opposite to the influx of chironomid larvae at that point of time (Table 4). Polychaetae number found to be very stable at post treatment stage till the end of the observation period, though it was absent in pretreatment level (most probably to the sampling error). Like the fenpropathrin treatment (not shown in the Table 4) lot of tiny snails were observed at the day-10 after aluminium phosphide treatment. Absence of snail eater black carp has allowed snails to reproduce.
Benthos population composition in rotenone treatment showed (Table 4) that none other than leech was impacted by the use of rotenone. 48-hour LC_{50} value of leech for rotenone was recorded as <0.1mg/l by Hamilton (1941). Leech was found at pretreatment sampling and after one day of rotenone's use. But from the day-two to 10 days after the treatment leech was not found. Tubifex population count found to be increased drastically on day-2 after the treatment and the high number continued to be there till the end of the observation period. Count for chironomid larvae slowly increased in rotenone treatment over the pretreatment level towards the end of the observation period.

3.1. Health impact

Fish killed using fish toxicant in commercial fishery by the farmers in northwest Bangladesh are sold in the market as food fish. Therefore, there is always a concern about the food safety of the fish killed by using fish toxicant. For fenpropathrin maximum residue level (MRL) value is not set for fish/meat by the European Commission of EU. But fenpropathrin MRL for all food items except citrus fruits are set to be 0.01mg/kg (EC, 2019). Acceptable daily intake (ADI) level was set by joint FAO/WHO meeting on pesticide residues (JMPR) as 0 to 0.03mg/kg body weight, and acute reference dose (ARfD) was also set as 0.03mg/kg (Shah and McGregor, 2012). Al-Makkawy and Madbouly (1999) found bioconcentration factor of fenpropathrin for *Tilapia nilotica* heads and flesh to be respectively 130 and 7 respectively at 3 days of the treatment @1µg fenpropathrin/liter water. Given the high bioconcentration factor 225 for fenpropathrin in fish (Giddings and Campasino, 2007) indicates that based on the doses of fenpropathrin (@ 65 µg/liter) used in this study would leave 14.625 mg fenpropathrin per kilogram of fish theoretically, which is way higher than the MRL value (0.01 mg/kg) for fenpropathrin. Given the concentration of fenpropathrin in this study, if a child weighted 20 kg consumes 100 g of such fish then the intake of fenpropathrin will be 0.07 mg/kg exceeding the ARfD value set by JMPR. But given the high toxicity of fenpropathrin to fish if lower doses (than the doses used in this experiment) are used as fish toxicant, could be still effective in killing fish especially in winter since fenpropathrin is more toxic in cold water than warm water (Kanawi *et al.*, 2013) and may leave fish with lower concentration of fenpropathrin.
MRL for rotenone is set as 0.01mg/kg for almost all food items by the European Commission of EU (EC 2019). BPDB (Bio-Pesticide Database) (2019) recorded bioconcentration factor for rotenone as 26 than the ambient condition where ADI value was not available. Relatively lower bioconcentration factor of rotenone compared to fenpropathrin for fish and no set ADI value by the regulators indicates rotenone's relative safety as fish toxicants even for food fish. On the other hand, bioconcentration factor for aluminium phosphide has been reported as low risk by IUPAC (2018). Though there is set ADI 0.019 mg/kg and ARfD 0.032 mg/kg (IUPAC, 2018), but given to low bioconcentration factor in fish, use of aluminium phosphide as fish toxicant for food fish and consumption of those may not have any health consequences. Rahman *et al.* (1992) also described fish killed with the use of rotenone and aluminium phosphide are suitable for human consumption.

3.2. Economics

Perschbacher and Sarkar (1989) found aluminium phosphide to be most inexpensive (given the required concentration of 0.25 ppm) among following fish toxicants: rotenone, sumithion, phyphanon, aluminium phosphide, DDVP and bleaching powder, where cost of organophosphorus pesticides (fenpropathrin was not included in the study) were deemed to be prohibitive for the commercial fish farmers to be used as fish toxicants. Combination of fenpropathrin' s toxicity to fish and low cost (similar or bit lower than even aluminium phosphide per unit area of water body) has made that economic barrier (that had prevented other pyrethroid' s use as fish toxicant) obsolete in current situation. Moreover, it has allowed farmers to obtain quick kill (within an hour after use) hence convenient, regardless of ecological consequences and serious health concern.

4. Conclusions

Significant factors such as ease of application, short duration of toxicity, no risk of future inhibition of production potential of the pond and low price, that dictates farmers choice of fish toxicants (Lennon *et al.*, 1970) are all in favor of fenpropathrin' s use in killing of food fish in northwest Bangladesh but rings the alarm for fish consumer due to severe health concern; hence it demands to be strongly regulated to be used as fish toxicants for food fish.

Conflict of interest

None to declare.

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J. Life Earth Sci. Vol. 13: 1-5, 2018
http://banglajol.info.index.php/JLES
ISSN 1990-4827

PRACTICE OF USING EMAMECTIN BENZOATE AND ITS ENVIORNMENTAL IMPACTS ON AQUACULTURE OF NORTHWEST BANGLADESH

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Abstract: Parasitic infestation of carp fish is a major challenge for commercial fish farmers of northwest Bangladesh. Commercial fish farmers of this region are using emamectin benzoate (a plant pesticide) for water treatment of parasite infested fish ponds. The study was conducted to know the practice of using emamectin benzoate (water treatment at the rate of 2µg/L pond water and feed at the rate of 50µg/kg fish for seven consecutive days) impact on zooplanktons, aquatic insects and benthos population in aquaculture ponds. Regular sampling of zooplanktons, aquatic insects and benthos were done and counted at pretreatment and after 1, 2, 5, 10, 15, 21 and 28 days of the treatment for both methods of treatment. Though both the feeding and water treatment with emamectin benzoate was found to be equally successful in killing the target fish parasites, however, the unintended impact of the water treatment was found to be huge on zooplanktons and benthos population. Aquatic insects were also found to be affected partially. Moreover, choice of application method creates a great difference (water treatment requires many times more) in amount of total emamectin benzoate required for the treatment and ultimately used in the water body.

Key words: Environmental impacts, Emamectin benzoate, Fish parasites, Aquaculture

সারাংশ: কার্প জাতীয় মাছে পরজীবীর আক্রমণ বাংলাদেশের উত্তরপশ্চিম অঞ্চলের বাণিজ্যিক মাছ চাষীদের জন্য এক বড় বাধা। এই অঞ্চলের বাণিজ্যিক মাছ চাষীরা মাছের পরজীবীর আক্রমণ প্রতিরোধকল্পে এমামেকটিন বেনজয়েট (একটি উদ্ভিদ আপদনাশক) মাছ চাষের পুকুরের পানিতে ব্যবহার করছে। মাছের খামারের পানিতে এমামেকটিক বেনজয়েট ব্যবহারের ফলে জুপ্ল্যাংক্টন, পানির পোকা ও পানির নিচের মাটিতে বসবাসকারী পোকার উপর কি প্রভাব পড়ে তা জানার জন্য এই গবেষণাটি করা হয়। খাবারের সাথে এবং পানিতে এমামেকটিন বেনজয়েট ব্যবহারের আগে এবং ব্যবহারের ১, ২, ৫, ১০, ১৫, ২১ ও ২৮ দিন পর জুপ্লাংক্টন, পানির পোকা ও পানির নিচে মাটিতে বসবাসকারী পোকার নমুনা সংগ্রহ করা হয় এবং গবেষণাগারে এদের আলাদা করে গণনা করা হয়। মাছের পরজীবী দমনে উভয় পদ্ধতি (এমামেকটিন বেনজয়েট খাওয়ানো ও পানিতে ব্যবহার করা) যথেষ্ট পরিমাণ কার্যকর হলেও, জুপ্লাংক্টন ও পানির নিচে মাটিতে বসবাসকারী পোকার উপর এমামেকটিন বেনজয়েট পানিতে ব্যবহারের ব্যাপক ঋণাত্বক প্রভাব রয়েছে। পানির পোকার উপরেও এর প্রভাব দৃশ্যমান। এছাড়াও ব্যবহার পদ্ধতি নির্বাচনের ক্ষেত্রে 'পানিতে ব্যবহার' নির্বাচন করা হলে একটি নির্দিষ্ট পুকুরে অনেক বেশী পরিমাণ এমামেকটিন বেনজয়েট ব্যবহারের প্রয়োজন হয়।

Introduction

Commercial aquaculture involving Indian major carps in northwest Bangladesh is a recent phenomenon (last 7 to 12 years) that has gained momentum and popularity as such that a lot of the wetland, paddy fields, and *beels* or low-lying and seasonally submerged lands are being purposefully converted into large fish ponds for commercial fish (primarily Indian major carps) production. With the development of commercial aquaculture, parasitic infestation of fish (Indian and Chinese carps) is also very common challenge that the fish farmers need to deal with. Of many parasites, *Ichthyophthirius* sp., *Trichodina* sp., *Dactylogyrus* sp., *Argulus* sp. and *Lernaea* sp. are the common ones for Indian and Chinese carps in Bangladesh (Arthur and Ahmed 2002; Chandra 2006).

One of the derivatives of Abamectin, Emamectin is a pesticide that stimulates the release of inhibitory neurotransmitter causing the paralysis and death of insects upon ingestion (Velde-Koerts 2014). Emamectin benzoate consists of a mixture of two chemicals with similar structure mixed @ minimum of 90% 4"-epi-methylamino-4"-deoxyavermectinB1a, and a maximum of 10%4"-epi-methylamino-4"-deoxyaverrnectin Blb benzoate (Anderson *et al.* 2009). Compared to the hydrochloride salt the benzoate salt of Emamectin has greater water solubility, better thermal stability and has broader insecticidal spectrum than avermectin (Jansson and Dybas 1998). Excluding the combined preparations, just Emamectin benzoate are being marketed currently under 136 different brand names in Bangladesh, mostly for being used in controlling of hairy caterpillar, red spider mites, other mites, shoot and fruit borers, pod borers, bollworms, aphids, termites and brown plant hoppers in plants ranging from crops to vegetables, fruits and cash crops like jute, tea and cotton (Bangladesh Crop Protection Association 2018).

First known and public use of emamectin benzoate occurred in the USA in 1996-97 as pesticide for control of terrestrial pest, soon after that it got its use extended to finfish Aquaculture. Over the course of time emamectin benzoate in the form of Slice® has become alternative of pesticides like ivermectin, dichlorvos, azamethiphos, hydrogen peroxide, cypermethrin, teflubenzuron and diflubenzuron in control of sea lice internationally (Sanders and Swan 2013). Emamectin benzoate is still not sold in the market as a product to use in aquaculture. But this does not prevent fish farmers from using the emamectin benzoate (plant pesticide brands) in commercial carp aquaculture for controlling ectoparasites especially *Argulus* sp. and *Lernaea* sp. in northwest Bangladesh. While Slice® is registered to be used at the rate of 50 µg/kg fish biomass with feed to be fed to the infected fish for consecutively 7 days (Sanders and Swan

2013), in Bangladesh emamectin benzoate is used (very large quantity compared to the feeding doses) primarily as water treating agent in infected fish ponds. Since emamectin benzoate is a broad-spectrum insecticide, very toxic to the invertebrates with very low rate of application and has wide margin of safety for mammalians (Yen and Lin 2004) and targeted ectoparasites are small and soft body crustaceans, therefore use of emamectin benzoate as water treating agent may have impacts on non-target water insects including many zooplanktons that are from the same family of the parasites. Therefore, the study was conducted to investigate the impact of emamectin benzoate using practices on zooplanktons, aquatic insects and benthos population in commercial aquaculture ponds of northwest Bangladesh.

Materials and Methods

The study was conducted in commercial fishponds (each 2 to 3 acres in size) in Rajshahi district in between March 2018 to April 2019. Since the purpose of the study was to assess the impact of emamectin benzoate resulting from farmers' uses in commercial fishponds, farmers were requested to inform the researcher prior to start of the treatment in their ponds. Before the application of the treatment, zooplankton and aquatic insect samples were collected that served as baseline of the study. After the application of treatment by the farmers, the doses of emamectin benzoate was back calculated based on the percent of the active ingredient (emamectin benzoate) in the used product, and water volume by measuring the area and water depth of the water body. This has been repeated with three different fish farmers. Three different brands of emamectin benzoate (*Wonder*, *Guilder* and *Sharper*) were used by three different farmers. All the products contained 5% emamectin benzoate as active ingredient with 95% carrier material, came as 10g aluminum foil pack. In the feeding experiment *Wonder* was used as a source of emamectin benzoate.

Doses were calculated using the following formula: Doses in µg/L = (% of active ingredient) × (total weight of pesticide converted into µg) ÷ [Area of water body (square metre) × water depth (metre) × 1000]. Sampling for zooplankton, aquatic insects and benthos were done before treatment (designated as 0 day and served as baseline) and after 1, 2, 5, 10, 15, 21 and 28 days post treatments.

At the same time, separate farmers who intended to use emamectin benzoate were advised to feed emamectin benzoate at the rate of 50µg/kg fish biomass/day in the pond consecutively for 7 days (MSD Animal Health 2010). To calculate the fish biomass, average fish weight was multiplied with the total number of fish stocked in the pond. Based on the fish biomass ration size (quantity of feed) was determined at 2% of body weight/day. Ration size was determined a bit low than normal to ensure all the feed are consumed immediately by fish so that the medicine (emamectin benzoate) mixed with the fish was consumed and loss was minimized. The ration was divided into two portions to be fed in the morning and in the afternoon. For mixing and binding the emamectin benzoate with feed, required amount of the commercial product was taken, grounded as fine powder, and diluted in half liter water. Then the solution was mixed with the required quantity of feed in a big aluminum pot so that the pallet feed socked all the emamectin benzoate mixed water. The feed was provided to the pond using feeding tray to minimize the loss. Zooplankton, aquatic insects and benthos samples were collected before the beginning of the experiment and after 1, 2, 5, and 10 days post treatments.

Sampling of zooplanktons

Plankton net made of silk bolting cloth (specification-200 US with 75 to 85 microns mesh size) were used for sampling of zooplankton. To minimize variations, sampling was done at certain place of the water body in between 10.30 am to 11.30 am of the day during sample collection. Certain length of the water body was marked for traversing the plankton net. A collecting bottle with valve was attached at the tapering end of the plankton net. The plankton net was traversed to the designated length one foot below the surface of the water during every sampling. After traversing the net, planktons were taken into a plastic bottle by opening the valve of the collecting bottle of the net. This amount now represents the total water volume that is sieved through the plankton net. Immediately after collection of samples formalin was added @ 5ml for each 100 ml sample for fixation of the zooplanktons. The zooplanktons were identified and counted using Sedgewick-Rafter cell counter (Welch 1948) by observing under microscope. Water volume that traversed through the rounded net was calculated by using following formula: Volume of water (L) = л (3.14) × square of radius (square metre) of the rounded net × length of water column traversed by the net (metre) × 1000.

Sampling of aquatic insects

A square net, 1 square metre in size made up of silk bottling cloth (specification- 35 US with 500µ mesh size), was used for insect sampling. Using this net 3 m horizontal distance in water was sieved through towards the edge of the pond to catch the aquatic insects. After harvesting, the insects were transferred into plastic bottles with some water. Formalin was added in the bottles for fixation of the insects. Later on, the insects were identified and counted in the laboratory.

Sampling of benthos

A metal scoop (2.75 inch diameter and 1.5 inch maximum depth) was used to collect the soil samples containing benthos population from pond bottom. The mud was then transferred in plastic bag. It was taken to the lab and sieved under running water using a fine mesh sieve, where all the dirt was washed, and all the benthos insects were collected in the sieve. Then the insects were transferred in large Petri dishes with some water. Then all types of the benthos insects were separated and counted under light.

Results

In feeding treatment of emamectin benzoate no impact was observed in general population of zooplankton in ponds; but in water treatment experiment zooplankton count reduced significantly at 48 hours after treatment (Fig. 1). The zooplankton count in water treatment experiment become normal at 5 days point and continued to increase till the 21st day after treatment.

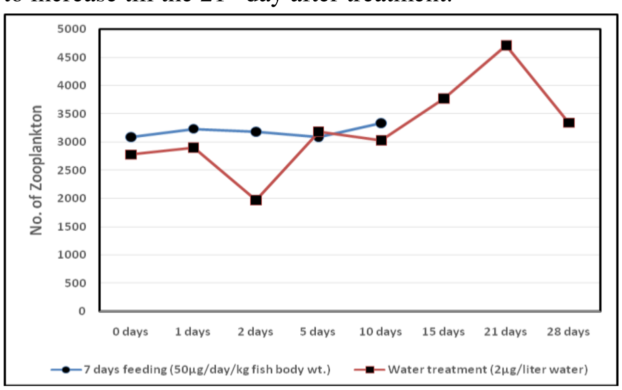

Fig. 1 Total count (n/L water) of zooplankton at various points of emamectin benzoate treatment of fish in aquaculture ponds.

Looking at the zooplankton composition during water treatment with emamectin benzoate reveals that all zooplankton number other than *Cyclops* sp. and *Daphnia* sp. were reduced at 48 hours after treatment (Fig. 2). These two types of zooplankton could be less sensitive to emamectin benzoate or they thrived due to the change in the zooplankton composition due to the reduced number of other types.

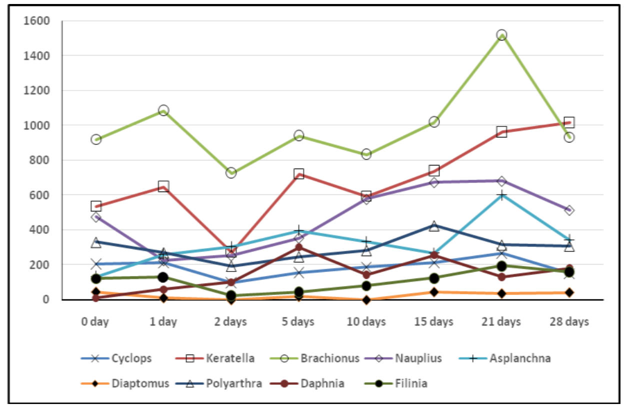

Fig. 2 Zooplankton counts (n/L) at various points of pond water treatment with emamectin benzoate @2µg/L

Like the zooplankton count, aquatic insect count in feeding experiment did not change over the course of the experiment (Fig. 3). However, in water-treatment experiment, insect count dramatically increased after one day of treatment. On the 10th day the insect count was found to be similar to the pre-treatment insect count and continued the same way till the end of the observation period of 28 days after treatment.

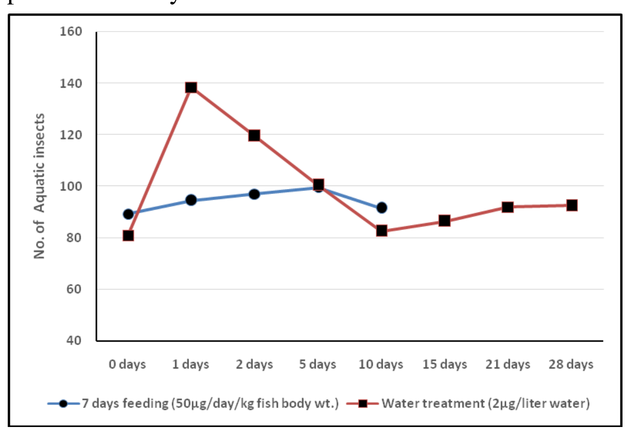

Fig. 3 Total count (n/3 sq. metre) of aquatic insects at various points of emamectin benzoate treatment of fish in ponds.

Insect composition in water treatment experiment reveals that backswimmer and boatman catchment increased significantly 24 hours after the treatment while counts for other insects after treatment went down or remain static (Fig. 4). Since backswimmer and boatman are the dominant insect types in number, their increased catchment has made the total insect-count high at day-1 sampling. The insect count became static and normal from day 10 till the end of the observation period of 28 days after treatment.

Benthos count in the feeding experiment did not change during the observation period of 10 days. On the other hand, benthos population found to be declined significantly after 24 hours of water treatment with emamectin benzoate @ 2µg/L water (Fig. 5).

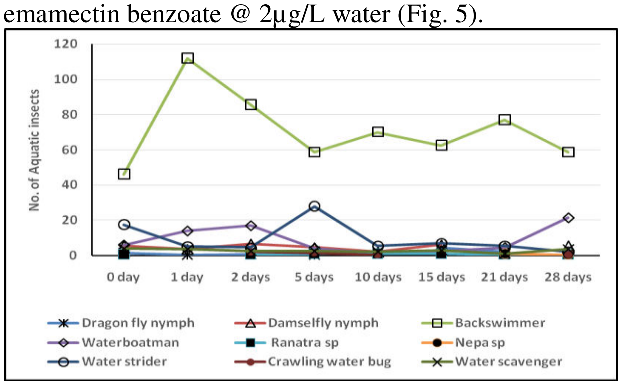

Fig. 4 Insect counts (n/3 sq metre) in ponds when treated with emamectin benzoate @ 2µg/L of pond water.

Benthos population gradually kept increasing after it declined to the lowest number at the 24th hours after treatment. After 15 days, benthos count was found to exceed the pre-treatment level and sustained that higher count till the end of the observation period at 28 days after treatment.

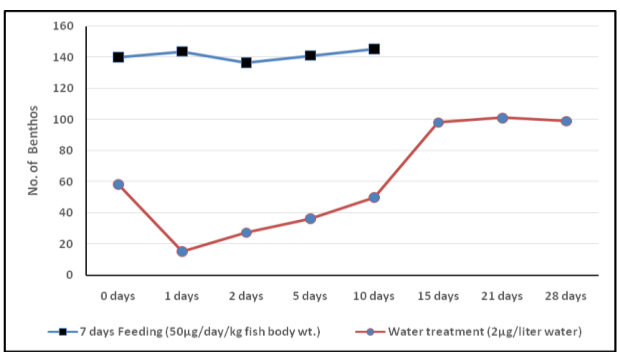

Fig. 5 Total count (n/6 sq inch bottom) of benthos at various points of emamectin benzoate treatment of fish in aquaculture ponds.

Benthos composition in water treatment experiment revealed that the chironomid larvae were the dominant type in pre-treatment condition. However, the dominancy got altered after treatment and *Tubifex* became the dominant type till the end of the observation period (Fig. 6).

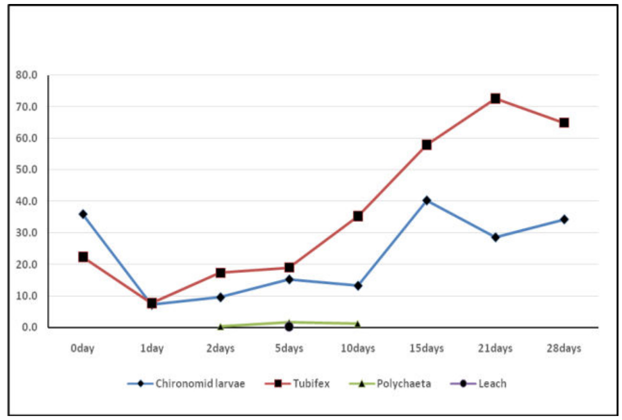

Fig. 6 Benthos count (n/6 sq. inch bottom) after use of emamectin benzoate @ 2µg/L water in aquaculture ponds.

The number of both chironomid larvae and *Tubifex* declined severely after the 24th hour of the water treatment with emamectin benzoate. But the number for both types started increasing after their lowest point and the increasing count continued till the observation period at 28th day after treatment.

Discussion

Use of emamectin benzoate in feed makes it mostly consumable by the fishes and the amount gets into the system through dilution and leaching in negligible quantity that does not affect the non-targeted species like zooplanktons, aquatic insects or benthos populations. Willis and Ling (2003) pointed that the use of emamectin benzoate in the form of feed formulation in salmon aquaculture was less likely to affect the planktonic form of marine copepods.

Through feeding treatments, emamectin benzoate, being a systemic drug, goes into the fish body from which the fish parasites affected by the drug. But in water treatments, the drug is diluted and broadcasted over pond water slowly and gets mixed up in the water, thus slowly gets in touch with the fish and the parasites as well. Due to the large volume of pond water, the mixing never happens homogeneously. Therefore, there are always inconsistencies, for example, some places with higher densities and other places with lower densities of the drug. Anderson *et al.* (2009) reported emamectin benzoate as 'very highly toxic' for *Daphnia magna*, where flow through EC_{50} value was recorded as 1µg/L. Static concentration of emamectin benzoate in this study @ 2µg/L affected the zooplankton population (Figs. 1 and 2), however, the inconsistency in drug concentration across the whole volume of the water body saved the slow-moving zooplankton population from being wiped out completely. On the other hand, fast mover fish and their parasites got in enough contact with the high-density drug containing water (due to the movement of fish) resulted in killing of the fish parasite population. Again 2µg/L concentration of emamectin benzoate is safe for fish itself since its level of tolerance to this drug is higher; LC_{50} for rainbow trout was reported to be 174 µg/L by Anderson *et al.* (2009).

In contrast to the zooplankton response, aquatic insect counts increased at the 24^{th} hour point after the water treatment. This could be because, as an insecticide emamectin benzoate is effective against soft bodied terrestrial insects like hairy caterpillar, red spider mites, other mites, shoot and fruit borer, pod borer, bollworm, aphid and termites. But most of the aquatic insects are hard shelled unlike zooplanktons, therefore the doses used in the experiment (to kill the anchor worm and *Argulus* in fish) was not strong enough to kill the insects. It may have weakened the aquatic insects that ultimately have got them caught in higher numbers during sampling.

Though the benthos population was affected severely in pond water treatment with emamectin benzoate, however, the dilution inconsistency of emamectin benzoate in water body (due to the large water volume) similarly leave the inconsistency in deposition of the drug in the pond bottom, saving the benthos population from being wiped out completely. The half-life (DT_{50}) of [23-^{14}C]-emamectin B1a and B1b benzoate in sandy loam soil found to be 12 to 19 days for irradiated samples during a 30-day exposure to artificial sunlight at 25^0C (Velde-Koerts 2014). In this study the benthos population got back to the level of pretreatment around 15 days mark after the water treatment.

Conclusion

Despite both methods of treatment being successful in controlling the target fish parasites; for a 2.3 acre water body with 5 feet water-column and given its fish stock size of 2500 kg, if water-treatment method is chosen over feed treatment, it would require staggering 29 times more emamectin benzoate to go into the system (which is unnecessary) for that particular water body, and definitely has unwanted environmental consequences on the aquatic fauna. While feeding method requires very little quantity of emamectin benzoate, however, it requires the hassle of mixing the drug with feed every time fish is fed consecutively for 7 days. The management or application difficulties, lack of knowledge and cheaper cost of the emamectin benzoate pesticide is eluding the fish growers into keep using the environmentally harmful water treatment method over feed treatment for control of parasitic worms in Chinese and Indian major carps in northwest Bangladesh.

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Manuscript received on 24.04.2019 and revised on 23.05.2019.

Serum Testosterone Level of Hormone Fed Tilapia (*Oreochromis niloticus*) in Northwest Bangladesh

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Abstract

Tilapia (*Oreochromis niloticus*) is one of the most cultured species in Bangladesh. For many practical reasons, almost all tilapia fry used in commercial tilapia culture are sex inversed male obtained by feeding of 17-alpha methyltestosterone. Sometimes methyltestosterone is also fed as growth promoter during growth stage of tilapia. The study was conducted to compare the serum testosterone level of sex inversed tilapia (marketable size) with the normal tilapia population and observe the effect of methyltestosterone feeding as growth promoter on serum testosterone level of adult tilapia. For the first objective, blood samples from both sex-inversed and normal tilapia were collected and serum testosterone level were determined using ELISA test kit. For the second objective, normal adult male and female tilapia were fed with feed (at the rate of 3% of body weight daily) containing 10 mg/kg methyltestosterone over the period of one month and blood serum testosterone level were determined at various point during feeding and up to another month after stopped feeding of the hormone. In this study, no significant difference in serum testosterone level was found in between adult sex inversed and normal tilapia (for both male and female). However, serum testosterone level was found to be several times higher than the normal population when methyltestosterone was fed as growth promoter and high serum testosterone level continued up to the end of the experiment (after one month of stopped feeding). Feeding methyltestosterone as growth promoter also triggered higher rate of reproduction of tilapia in cages.

Keywords: Tilapia, Serum testosterone, Methyl testosterone and Sex inversed

1. Introduction

Tilapia (*O. niloticus*) is the second most cultured species in the world after Carp and is often dubbed as 'aquatic chicken' (World Fish Center, 2015). Like many other parts of the world, tilapia is one of the single most cultured species in Bangladesh. Total production of tilapia has increased to 384,737 metric ton, 10.62 percent of total inland fish production in 2017-18 (Do F, 2018) from just 136,000 metric ton in 2012 (Hussain *et al*., 2014). Like the other parts of the country it is also commercially cultured in Barind tract as well. The production of tilapia in Bangladesh is good with high profitability along with less input cost and reduced risk (Rahman *et al.*, 2012). Helpful qualities like ability to take natural food from the pond, good interest in supplementary feed, surviving capacity in adverse weather condition and high disease resistance capacity made it favorable as one of the most cultured species throughout the world (Roysfarm, 2018). It is fast growing, hardy, environment friendly and easy to grow by all sort of fish farmers (World Fish Center, 2015). Availability of sex inversed male tilapia fingerling has played a significant role in expansion of commercial culture of tilapia by incorporating the advantage of faster growth rate of the male tilapia over the female ones and by eliminating the unconditioned propagation ability that makes the population uncontrolled, incurring management difficulty and hindering desired production (Mair and Little, 1991). Almost all the sex inversed male tilapia fingerlings are achieved through extensive feeding of steroid androgen, 17 alfa-methyltestosterone @ 50 to 70 mg/kg feed, in juvenile stage of tilapia fry for about 20 to 30 days (Rouf *et al*., 2008).

There is other use of methyl testosterone at the growth stage of the tilapia, where it is fed at relatively lower dose (at 10 mg/kg feed) compared to the sex reversal dose, with feed on regular basis to enhance the growth of *O. niloticus*. Ahmad *et al.* (2002) found that 5 mg/kg doses of 17 alfa-methyl testosterone when used as growth promoter resulted in higher growth of *O. niloticus*. Hybrid tilapia fed with low doses of 17 α-ethynyltestosterone

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have led in 11% additional weight gain over control in commercial polyculture pond conditions (Rothbard *et al.*, 1988). A total of 825 private hatcheries produced a total of 2694.9 million juvenile tilapia seedlings in 2018 in Bangladesh (DoF, 2018). On the other hand, Din and Subasinghe (2017) reported that hormonal sex reversal is intensively practiced by the private hatcheries in Bangladesh to produce mono-sex male tilapia seedling. Therefore, it is safe to conclude that large percentage of all tilapia (*O. niloticus)* sold in the market in Bangladesh have either been exposed to the feeding of testosterone at juvenile stage or in both at juvenile stage and growth stage at lower rate. The experiment was conducted to determine whether sex inversed male tilapia has higher rate of blood testosterone level than normal resulting from feeding of testosterone at juvenile stage. At the same time part of the experiment was done to monitor the change of blood testosterone level in tilapia while feeding of methyl testosterone was done at lower dose (10 mg/kg feed) in growth stage and observed the duration to get normalized the serum testosterone level.

2. Materials and Methods

For the first objective testosterone levels of regular adult female, adult male and sex inversed tilapia (*O. niloticus*) were determined. For getting regular male and female tilapia, a stock was identified in a homestead earthen pond in Hatgodagari area of Pobaupazilla of Rajshahi district where natural stock of tilapia was maintained at least for last 5 years and no tilapia fingerling was released during that period. Therefore, it was considered as free from any kinds of hormonal feeding. Fish were caught with cast net; the blood sample were taken from the caudal vain of live fish, and then by dissecting the fish, sex was determined by observing (often under microscope for confirmation) the primary sexual organs (testis/ovary) to label the blood sample as regular male or female tilapia. At the same time the condition of the sex organs was documented. Similarly, live adult sex inversed (confirmed by the fish farmer) tilapia (treated group) were bought from the wholesale market located in Naodapara of Rajshahi city in several batches in between May to August-2018 and blood samples were taken. After taking the blood sample, the fishes were dissected for confirmation of their sex (often by observing under microscope) and condition of the sex organ was documented. For normal population, 53 blood samples were collected from 53 different fish. For sex inversed population 50 blood samples were collected from 50 different fish as well. The size of the fish ranged from 200 g to 500 g each. For each fish 2 to 3 ml blood sample was taken from caudal blood vessel using 5 ml disposable syringe.

After taking the blood sample the needle was removed from the syringe. Then the blood from the syringe was transferred to the sterile red top blood tube (glass tube) without anticoagulant. Then the blood tubes with blood sample were left for half an hour without shaking in room temperature. The serum was separated following the standard serum separation protocol (Texas Department of State health services, 2018). After half an hour the blood tubes were put in a centrifuge machine to centrifuge for segregating the blood serum from the blood cells. The blood samples were centrifuge for 10 minutes @ 2000 rmp (Thermo Fisher Scientific, 2018).

After completion of the centrifuge, the blood serum from the top were taken using vacuum dropper and put it in sterile Eppendorf tubes and put in freezer immediately. Because the collection of blood samples took several months, the frozen blood serum samples were stored in freezer at -80^{0}C in Biochemistry Department of Rajshahi Medical College until used for testing the testosterone level. Due to the distance of the collection site from Rajshahi University, to avoid the transfer of live fish frequently during the experimental period, a mobile laboratory (consisting of a compound microscope, a small centrifuge machine, blood tubes, syringe, Eppendorf tubes, dissecting box, ice box etc.) for fish blood collection was set near the sample collection site.

To determine the level of blood testosterone at adult stage of tilapia when fed with MT @ of 10 mg/kg feed, regular male and female tilapia were put in 3 *hapa*s (net cages) set in the pond. Each *hapa* was 3 meters by 1 meter in size with 1-meter depth. 10 tilapia fish ranging from 200 to 300 g in size consisting of both male and female were put in each of the *hapa*s. The experimental fish were collected from a pond that maintained a natural stock in Hatgodagari area of Pobaupazila of Rajshahi district and the *hapa*s were set in the same pond to put the fish inside.

2.1. Feed Preparation

For feeding 'AIT' pellet carp feed was used (proximate composition was: 27.26% protein, 7.20% lipid, 11.54% ash, 13.70% moisture, 5.90% fiber and 34.40% carbohydrate). To prepare the 15 kg feed with hormone, first 150 mg (at the rate of 10 mg/kg feed) Methyl testosterone (marketed by 'Argenta' in Bangladesh) were diluted in 15 ml of lab grade ethanol. Then it was made 250 ml by adding tap water. Then the 250 ml solution was sprayed over the 15 kg feed and mixed up well so that the feed soaked up all the liquid containing 150 mg methyl testosterone. Then the moist feed was air dried in the shed. Despite being air dried the feed contained extra moisture, therefore, to avoid the fungal growth, the feed was preserved in the refrigerator, in a plastic bag.

2.2. Feeding of the Fish

The prepared feed was fed to the fish in the *hapa* at the rate of 3% of fish body weight once on daily basis for 30 days in the month of June. Feeding was maintained at the rate of 3% of body weight per day to ensure that all the feed is consumed by fish. The stock was maintained for another month with normal diet (without testosterone).

2.3. Collection of Blood Samples

Blood samples were collected from 2 to 3 male and female on every 5 days following the process mentioned above to monitor the serum testosterone level. After collection of the blood sample, the fishes were released in the *hapa* again. Because there were limited number of fish, in this case fish were not dissected after the collection of blood sample, rather sex was identified by observing their genital opening. Fish from different *hapa*s were collected on rotational basis for sampling to allow the fish recover from the physical injury that incurred in the process of blood collection. Thus, over the period of one month the fish were fed with methyl testosterone with feed and blood samples were collected to monitor the change in serum testosterone level in*O. niloticus*. Blood samples were kept collecting till another month after feeding of testosterone was stopped.

2.4. Determination of Testosterone Level

Serum testosterone level were determined by 'Das Plate Reader'- manufactured in Italy (http://www. dasitaly. com/ en/prodotti/Plate% 20 Reader), marketed by Bio-Trade International in Bangladesh, using 'Accu Bind ELISA Microwells' for Testosterone from 'Monobind Inc.', USA, following the prescribed systems/procedure of the ELISA kit using the reagents supplied with the kit (Monobind Inc., 2018) in the laboratory of Royal Hospital (pvt.) Ltd located in Laxmipur area of Rajshahi city. Since the ELISA kit is customized for human use it can determine the maximum concentration of 12 ng/ml of testosterone. After testing the blood serum, for those samples where values were over 12 ng/ml, were diluted with the diluent and tested again for determining the actual testosterone concentration.

2.5. Statistical Analysis

Comparison of means test (t test for independent samples) was used to determine whether there is any difference between the testosterone level of regular tilapia and sex inversed tilapia of marketable size.

3. Results and Discussion

Average testosterone level in male adult *O. niloticus* in normal population found to be 7.3 ng/ml of blood serum. While in female the value was 1.95 ng/ml. But the distinct feature of the testosterone level of both male and female is high internal variability. The highest value of testosterone in normal male population was 26.4 ng/ml, while the lowest was 0.6 ng/ml. For female in normal population the range was 0.01 to 10.3 ng/ml (Table 1).

Table 1. Testosterone level (ng/ml) of adult male and female in normal and sex inversed population of *O. niloticus*

Population Category	Sex	Number of Individuals	Testosterone Level (ng/ml)	Range of Value	Score for Appearance of Sex Organ
Normal Population	Male	7 (13.2% of total)	16.7±7.3	8.2-26.4	6
	Male	14 (26.4% of total)	3.9± 3.7	0.9-10.1	5
	Male	3 (5.66% of total)	0.8±0.4	0.6-1.2	3
	Combined	**24 (45.3% of total)**	**7.3±7.8**	**0.6-26.4**	
	Female	22 (41.5% of total	2.5± 2.9	0.04-10.3	5
	Female	7 (13% of total)	0.16±0.36	0.01-1.04	3
	Combined	**29 (54.7% of total)**	**1.95±2.8**	**0.01-10.3**	
Sex Inversed Population	Male	6 (12% of total)	19.4±11.6	4.35-36.8	6
	Male	14 (28% of total)	2.4±1.16	0.66-4.13	5
	Male	4 (8% of total)	2.85±1.04	2.22-4.42	4
	Male	10 (20% of total)	0.37±0.22	0.09-0.75	2
	Combined	**34 (68% of total)**	**4.87±8.29**	**0.09-36.8**	
	Female	5 (10% of total)	2.34±1.18	1.42-3.87	5
	Female	6 (12% of total)	1.38±0.83	0.25-2.69	3
	Female	5 (10% of total)	0.26±0.39	0.01-0.95	1
	Combined	**16 (32% of total)**	**1.33±1.17**	**0.01-3.87**	

Note: 6 means very well developed, 5 means well developed, 4 means one testis/ovary well developed, 3 means less developed, 2 means poorly developed, 1 means very poorly developed and functionally not ready to breed.

Average testosterone level (4.87 ng/ml) of male individuals of sex inversed population was lower (though non-significant, P=0.2648 at t test) than the normal population with high internal variability like the normal population (Table 1). A total of 68% male individuals in sex inversed population (compared to only 45% in normal population) was inclusive of many of the sex inversed phenotypic male (but genetically female) have resulted in with higher number of males with poorly or less developed testis and lower level of testosterone production. The range of testosterone level varied from 0.09 to 36.8 ng/ml. Average testosterone level (1.33 ng/ml) of female individuals of sex inversed population was also lower (also non-significant, P=0.4041 at t test) than that of normal population with high internal variability. The testosterone value form female individuals in sex inversed population ranged from 0.01 to 3.87 ng/ml. Wahbi and Shalaby (2010) reported significantly decreased plasma testosterone level in six month's old sex inversed males of *O. niloticus* compared to the control. While Khalil *et al.* (2011) found significantly different level of serum testosterone in methyltestosterone treated male and untreated control group of *O. niloticus*, testosterone levels were higher in treated male in most months during observation but for couple of months testosterone level was several times higher in untreated control group.

In this study the sampling was done over the course of four months form May to the August, and the range of timing may have an impact on the testosterone level and could be the reason of high level of variability of testosterone level in group. Month to month variation of plasma testosterone level in *O. mossambicus* has also been reported by Cornish (1998). Varying testosterone level in *O. niloticus* in different months has also been reported by Khalil *et al.* (2011). The high variability of serum testosterone level in this study may also be attributed to the size of fish. Cornish (1993) found the inverse relationship in-between size and plasma testosterone level in *O. mossambicus*, means larger specimen has relatively lower plasma testosterone level.

Ideally sex inversed population is expected to have all male individual, however, since the samples of this study were collected from market, there was no control of the researcher on the sex inversion process. Poor male percentage (68%) indicates that when sex inversion was done commercially to produce sex inversed population, it wasn't done properly. Contrary to the normal population, the sex inversed population consisted of 68% male and 32% female, though 10% fish (categorized within female) had very poorly developed ovary that was not ready or suitable for breeding, and functionally were serving as sterile individuals. Also, only 10% fish (female) of sex inversed population had well developed ovary. Unlike the normal population a total of 30% fish (combined male and female) in sex inversed population had poorly developed testis and ovary, it could be result of interaction between poor masculinization and expression of inherent genetic characters. Macintosh *et al.* (1988) reported testicular degeneration in tilapia when fed with 17-alpha methyltestosterone at the rate of 60 mg/kg feed at early developmental stage for sex reversal. Similar phenomenon was evident in decreased Gonado Somatic Index (GSI) for both male and female treated with high (5 to 40 mg/kg feed) methyl testosterone doses (Ahmed *et al.*, 2002).

The positive correlation between serum testosterone level and appearance of sex organ in normalmale (r value 0.72) was stronger than male (r value 0.55) of sex inversed population. However, the correlation between serum testosterone level and appearance of sex organ in female of normal population was weaker (r value 0.36) than the female (r value 0.64) of in sex inversed population of *O. niloticus*.

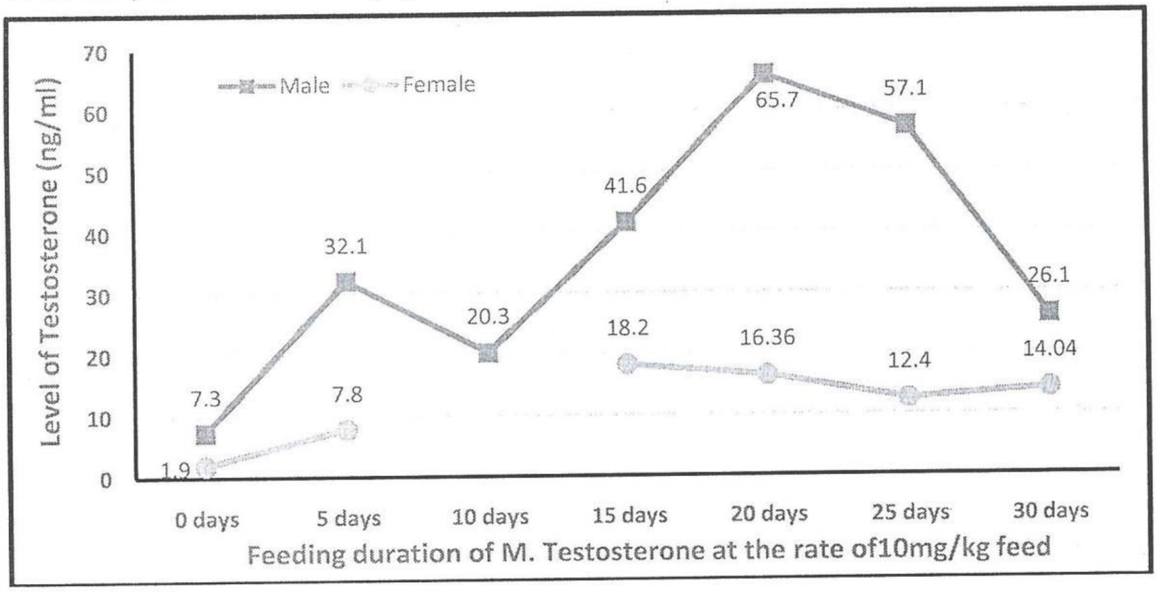

Figure 1. Testosterone level (ng/ml) in Adult *O. niloticus* serum when fed with methyltestosterone at the rate of10 mg/kg feed as growth promoter.

Serum testosterone level of both male and female tilapia increased with the feeding of methyl testosterone as growth promoter (Figure 1) at lower dose (10 mg/kg) compared to what is used (60 mg/kg) in sex reversal process. Since at each sampling in different days different individuals were collected from the treatment pool, the average level value of subsequent samplings is of different individuals. Therefore, it was not possible to determine the change pattern in testosterone in one individual fish. However, this graph gives a general idea of increase of testosterone level in the treated population. During the feeding stage highest level of blood serum testosterone in male tilapia was 70.8 ng/ml on the 20th days of feeding, compared to the highest value of 26.4 ng/ml found in male of normal population.

Since the fishes were not dissected for confirmation of sex after collection of blood sample, (sex was confirmed by observing the difference in genital opening), it is possible to have error in identification of sexes, which practically means male and female may have been labeled as opposite and may have impacted the value presented in the graphs, based on its grouping as male or female. Therefore, the overall trend of higher testosterone level is more important rather than specific value (testosterone level) at any certain point of Figures 1 and 2.

While it is expected to increase the level of serum testosterone when methyltestoster one is fed to the treated group, some unexpected behavior was observed. Generally, it is hard to get free ranging male and female tilapia to breed naturally when put in captivity or confinement. However, in this experiment though the adult male and female tilapia (previously free ranging) were put in confinement (in *hapa*) due to the low dose (10 mg/kg) feeding of testosterone, fish bred frequently. As a result, during each sampling many of the hatchlings had to be taken out of the treatment pool and mouths of the female. This phenomenon continued even after the end of feeding of hormone. This unusual and increased mating activity might have positively influenced the serum testosterone level of the treated male and female. Significant increase of testosterone level in normal male *O. niloticus* has been attributed to the male paring with female during mating season by Khalil *et al.* (2011).On the other hand, Smith and Haley (1988) recorded high plasma testosterone in female mouth brooders (*O. mossambicus*) in the latter half of brooding period while testosterone level dropped at the end of brooding.

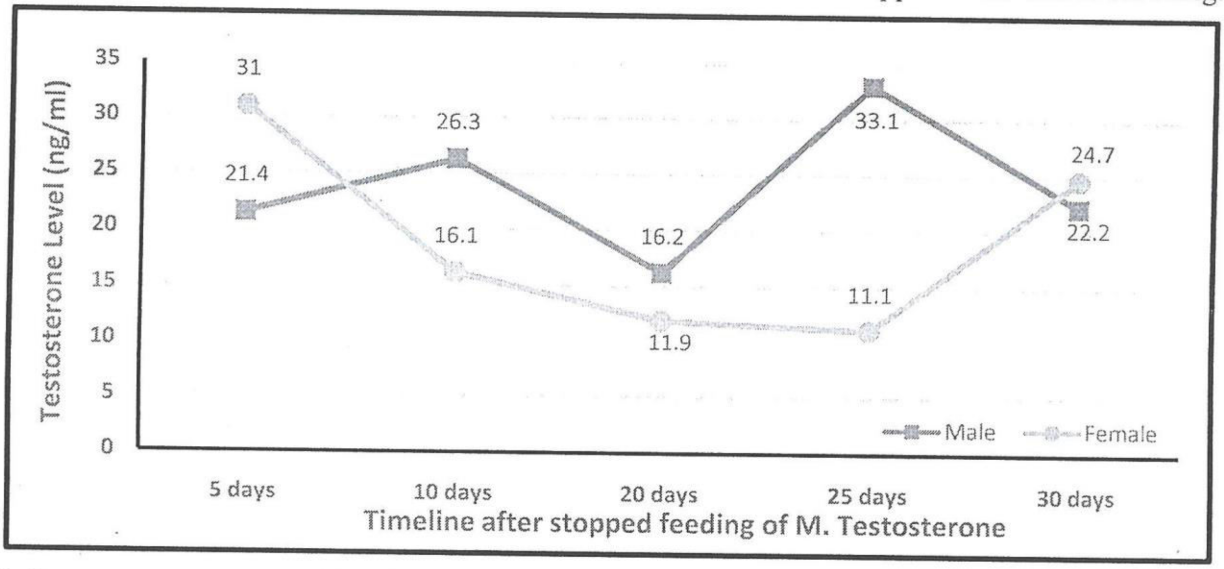

Figure 2. Serum testosterone level in adult *O. niloticus* after stopped feeding of methyltestosterone.

Serum testosterone level in adult *O. niloticus* found to be higher till the end of observation period after one month of stopped feeding of methyltestosterone (Figure 2). There was no indication of reducing the serum testosterone level even at the end of observation period (one month after stopping of methyl testosterone feeding). The highest value of serum testosterone level in female was found to be 40.3 ng/ml at the 5 days point after stopped feeding of methyltestosterone; compared to the highest testosterone value of 10.3 ng/ml in female of normal population. Since it takes several months to get to plasma testosterone level to normal in sex inversed male tilapia (Wahbi and Shalaby, 2010; Rizkalla *et al.*, 2004; and Khalil *et al.*, 2011), it seems in adult population where methyltestosterone is used as growth promoter, resulted in high serum testosterone will also take several months to get the level of serum testosterone normal. However, this increase in testosterone level can not only be attributed to the feeding of methyl testosterone as growth promoter, because external factors like seasonality (Khalil *et al.*, 2011) and breeding cycle (Smith and Haley, 1988) has its effect on tilapia's testosterone level

4. Conclusion

About 6 to 8-month-old sex inversed tilapia found to have serum testosterone level similar to the normal population, therefore it should be equally safe for human consumption like the normal tilapia in regards to serum testosterone level. On the other hand, when methyltestosterone was fed as growth promoter in growing stage of tilapia, serum testosterone level was found to be higher even after one month of withdrawal period. Despite the rise of testosterone level, based on the available data it cannot be said that using methyltestosterone as growth promoter makes tilapia unsafe for human consumption even in short term.

Acknowledgement

Md. Mahbubur Rahaman, Assistant Professor, Biochemistry Department, Rajshahi Medical College, Rajshahi; for his assistance and cooperation regarding the assessment of serum testosterone level in the laboratory of Royal Hospital (pvt) Ltd. in Laxmipur, Rajshahi city.

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