

Molecular Investigation on Clinopathological, Genetic and Biochemical Changes in *Channa punctata* Infected with Internal Parasites and Subjected to Metal Pollution in Chittagong, Bangladesh

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Abstract

This study was carried out to investigate the effects of parasites and metals in the fishes of fresh water sources of three different stations of Hathazari Upazilla, Chittagong, Bangladesh, by analyzing blood biochemical parameters and body composition of *Channa punctata*. The results indicated that, the highest rate of parasitic infection change the blood parameters spontaneously. Findings of blood biochemical factors (blood sugar, hemoglobin and creatinine) revealed significant variation between infected and uninfected fishes. Considerable works mainly on systematics, nature of infestation and pathology of different groups of fish parasites - Cystode, Trematodes and Nematodes have been done. Different species of parasites as endoparasitic Cystode and monogenetic Trematodes were recorded mainly from fish species of different water sources. One helminth parasite Nematode also reported from those fishes. Much attention has been given on observing intestine, liver and kidney. Molecular observation including DNA extraction has been done from both infected and healthy fish blood samples. Purification of DNA has been confirmed by Nano drop. Recommendation has been made for the future works on parasitology for sustainable production of healthy fishes by heavy metal detection, molecular observation as well as mutation detection.

Keywords: Fish parasites; Parasitic infection; Blood parameter; Body composition.

Introduction

Fish is an essential component of daily diet of many people in Bangladesh. Our country is the land of rivers and floodplains, which has a high potential of aquatic resources where fish plays a very important role. Traditionally, people of Bangladesh like to eat fresh fishes where chilled and dried fishes are also marketed in large quantities around 70 % fresh fish, 25% dried and the other forms of locally processed fishes include fermented and frozen products [1]. Like human and other animals, fish suffers from diseases and infected with parasites. Fish defenses against diseases are specific and non-specific, where non-specific defenses include skin and scales, as well as the mucus layer secreted by the epidermis that traps microorganisms and inhibits their growth. If pathogens breach these defenses, fish can develop inflammatory responses that increase the flow of blood to infected areas and deliver white blood cells that attempt to destroy the pathogens. According to previous records, blood parameters serve as reliable indicators of fish health as many parasites can live in a fish, where fishes died due to their toxicity [2]. Therefore, the changes associated with hematological parameters due to various parasites can serve important information which could be used for disease diagnosis and guidelines for the implementation of the treatment in the future. This knowledge would be very much helpful in fish farming and fish industry [3]. Blood constituent analysis is considered as a physiological indicator of the fish body which has importance in diagnosis of the structural and functional status of fishes caused by pollutants [4]. There are many reports indicate that, evaluation of disturbance caused by parasitism in fishes, depend on the determination of their blood parameters. Therefore, objective of this study is to investigate the impact of internal parasites on some physiological parameters related to both liver and kidney functions of *Channa punctata* as well as to determine the relation between metal pollution and the infection with internal parasites.

In the environment, usually fish can accumulate elevated metal

concentrations. In laboratory and nature there is a big relationship between metal concentrations in water and fishes [5-9]. Due to water pollution and fish species the rate of metal accumulation differ even they live in the same water source [10]. Environmental pollution and parasitic infection might be the main causes of most of the fish diseases [11]. Knowledge of fish parasites is of particular interest in relation not only to understand fish health but also ecological problems [12]. Negative effects of the health of the respective organisms as well as fishes are caused due to aquatic pollution which is still a problem in many freshwater and marine environments [13]. Few researches have done on the investigation of the effects of pollutants and concurrently occurring in parasites [14]. However, the effect of environmental pollutants on fish parasites varies depending on the particular parasite and specific pollutant that interact [15]. Pollutants may affect the immune system of the fish either directly or by changing water quality; that in turn may reduce the fish immunity to parasites [16]. Water pollution may accelerate the life cycle of the internal parasites and promote their spread [17]. So, another objective of this study is to detect the relation between metal pollution and the infection with internal parasites and further attempt can be taken to detect the molecular and genetic changes due to the infection.

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Purpose of this Research

Aquaculture plays an important role in nutrition, income, employment and foreign exchange earnings in Bangladesh. In our country people intake 80% of the animal protein from fish sources. Approximately, 1.4 million people directly involve themselves in fisheries, 11 million in part-time fishing and another 3 million in aquaculture in this country. An estimated 73% of rural households are engaged in some sort of fishing activity and fisheries comprise around 7% of the total employment (around 28 million) in Bangladesh. By exporting fish and fisheries products, we earn about US \$ 230 million foreign currency per annum. The fisheries of Bangladesh are very diversified and are involved of inland open water capture fisheries, inland and coastal aquaculture and marine fisheries. These contribute 46.5%, 28.5% and 25.0%, respectively to the total fish production of 1.04 million tons. Freshwater aquaculture consists of poly culture of Carps mainly, monoculture of Tilapia and various Catfish in ponds. Integrated fish farming with paddy-poultry or duck-fish is also practiced in our country. In Bangladesh, major limitations to both fresh and salty water aquaculture development is identified due to lack of infrastructure in terms of storage, transport, communication, electricity, marketing and inadequate feed and seed supplies. Environmental factors and man-made involvements in riverine morphology, the disruption of ecology, severe agricultural practices and modern developments have had a negative impact on aquaculture development, is a major problem of Chittagong city. It is affecting the normal condition of the living system of the rivers and another water sources as well. Thus, fishes are being infected and becoming toxic. Level of metal is increasing day by day. Genetic changes of fishes have been reported in various recent research works. In addition, natural disasters such as flood, drought and cyclones also have a negative impact. In our country, legislation and regulations for fish conservation are in existence but enforcement is difficult and these regulatory mechanisms are inadequate for protecting aquaculture. As a fish keeper, familiarization with various common fish parasites is very important. Early stage of parasite infestations observation is easier to cure comparatively severely infested fishes. By learning about fish parasites and the techniques to recognize them bring the possibility to decrease the risk of bringing home infected fishes from the fish shops. This study will show parasitic infection, the level of water contamination, and biochemical changes among the fishes of some specific village areas in Chittagong. Identification of particular biochemical changes due to parasitic infections will bring benefits for the drug designer as well as manufacturer to evaluate drugs against their resistant one. It will also help the fishermen and fish industry to take precautions against parasitic infections. This study will evaluate the metal absorption and relation with parasitic infections which could indicate the effect of metals in Bangladeshi rivers and its impact on fishes. The information from this research will help to continue further research to identify specific genes responsible for those changes.

Materials and Methods

Fishes were collected from some fresh water sources (some local rivers and ponds) of Najumiahat, Madunaghat, Hathazari Upazilla, Chittagong during different time (September, November and January) to observe the common parasites, to determine the level of metal absorption and to assess whether any biochemical change occurs or not due to the parasitic infections. Observations of correlation among metal absorption, parasitic infection and various biochemical parameters have also been done. Extraction of fish blood DNA was done for further process to quantify the extracted blood DNA and

finally visualized it on agarose gel electrophoresis.

A. Fish Samples

About 200 live fishes (*Channa punctata*) were collected from different fresh water sources (some local rivers and ponds) of Najumiahat, Madunaghat, Hathazari Upazilla, Chittagong by the aid of fisherman and transported them alive to the laboratory where they were examined immediately

B. Parasitological Examination

Detection of parasite: Fishes were dissected in order to observe the internal parasites.

Procedure

The fishes were placed on the dissecting tray; then dissections were started from anal opening by using fine scissors and at the time of dissection saline solution was used to avoid bacterial infection. By using scissor and forceps, the gastrointestinal tracts of the fishes were taken in the petriplate containing 0.7% saline solution. In the petriplate, the intestines, livers and stomachs were separated from gastrointestinal tracts and were placed them on separate petriplates. Then, those were cleaned by removing the fat body. After that, the stomachs and intestines were dissected properly in order to detect parasites. Then the intestines, livers and stomachs were observed under the microscope for detecting parasites. The parasites (Cestodes, Trematodes and Nematodes) were found and detected them carefully according to the following procedure. After detecting parasites, two drops of water were taken in the watch glasses where the parasites were also taken by using a clean brush or dropper. The watch glasses containing parasites were kept for 4-5 minutes. Then, alcohol formal acetic solution (for Platyhelminthes) was taken into a test-tube and boiled on a burner. After that, the boiled solution was poured in the watch glasses containing Platyhelminthes instead of directly pouring on the parasite. Glycerin acetic solution (for Nematode) was taken into a test-tube and it was boiled on a burner and same procedure was followed as was done with the Platyhelminthes. Finally, after 2-4 minutes the parasites were preserved in separate vials containing Lactophenol.

C. Detection of Ectoparasites

Monogenea: Monogenea were taken in a small petri-dish and washed several times with water to remove debris and mucous. After that, worms were kept at 4°C followed by 5% formalin treatment for permanent preparation. Then the worms were washed several times to remove formalin and stained with Semichon's acetocarmine for 5-10 minutes. By this time, the specimens were passed through an ethanol treatment (30, 50, 70, 90% and absolute gradually) for dehydration. Finally, prior to observe under Binocular Dissecting Microscope, they were cleared in clove oil, xylene and then mounted in Canada Balsam while the unstained were mounted in glycerin jelly [18,19].

Protozoa: Some of the positive slides were stained according to Klein's 'Dry silver impregnation method'. In this method, the slides were air-dried and covered with 2% AgNO₃ solution, followed by rinsing with water and sunlight exposure for 1-2 hours. The slides were dried and mounted with Neutral Canada Balsam [20].

Crustacea: The detected crustacean parasites were collected by a needle and brush. Then taken into a petri-dish and cleaned with lactophenol, which was followed by mounting with polyvinyl alcohol [21].

D. Blood Samples

Biochemical and Hematological Measurements: Fresh blood samples were collected in EDTA tube. The needle was kept in the middle line just behind the anal fin in a dorso-cranial direction till striking the vertebrate. Two blood samples were collected by caudal venipuncture. One sample was collected with a syringe containing anticoagulant 10% EDTA and the other without it. These procedures were carried out very swiftly to minimize stress. The collected blood then centrifuged at 3000 rpm for 10 minutes to separate serum and was used later for various biochemical analyses. Two aliquots of EDTA containing blood samples were prepared. One was used to determine the RBC count, haemoglobin and creatinine concentration and the other for measurement of plasma glucose by glucose oxidase. An automatic blood cell counter was used to perform RBC determination.

E. Extraction of DNA from Fish Blood Sample

Procedure: 200µl of blood sample was taken from fish into a 1.5µl sterile centrifuge tube. 20µl Proteinase K and 200µl of FABG buffer was added to the sample mixture. Then it was mixed thoroughly by vortexing followed by incubation at 60°C for 15 min to lyase the sample. It was vortexed at every 3-5 minute during incubation. 200µl of 97% ethanol was added to the sample, which was followed by mixing, again by vortexing. An FABG Mini column was placed into a collection tube. The sample was transferred into the column, centrifuged and the flow through was discarded. After that, the column was placed in a new collection tube. The FABG column was washed with 500µl W1 buffer by centrifugation and the flow through was discarded. The FABG column was washed with 750µl Wash Buffer by centrifuging for 1 minute and the flow through was discarded. FABG Column was placed to Elution tube. 50-200µl elution buffer or ddH₂O was added to the membrane center of the column. The column was kept for 3 minutes prior to further centrifugation. Then the column was centrifuged for 2 minutes to elute total DNA. Total DNA was stored at 4°C or -20°C. DNA was quantified using a Nanodrop (Thermo Scientific) which was then visualized by gel electrophoresis.

F. Procedure of Agarose Gel Electrophoresis: 200 ml 1XTAE (prepared from the 50Xstock) and 1% agarose gel (0.3gm agarose to 30ml 1XTAE) were prepared separately. Agarose was then dissolved in the microwave. 6.0µl of ethidium bromide was added to the dissolved agarose and mixed. Melted agarose was poured onto the gel plate in the electrophoresis box. Extracted DNA was put with bromophenol blue in every well. Marker was used to compare with sample DNA. After 20 minutes the gel was put into the gel documentation and visualized it.

G. Steps for Quantifying DNA in Nano Photometer

Nano drop: UV-VIS spectrophotometry is easy for micro-volume samples by using Nanodrop 2000/2000c spectrophotometers, where it is ideally suited for measuring. The Nano Photometer analyzes ultra-low sample volumes of 0.3µl while maintaining high accuracy, reproducibility and speed. The tests were designed to evaluate the performance of low sample volume on DNA and proteins together with the overall speed of analysis and sample stability.

Procedure: All tests were performed with freshly prepared samples. The tests were performed under described procedures. All tests were repeated several times to ensure reproducibility. The sampling arm was raised and pipetted the sample onto the lower measurement pedestal. After that, the sample arm was dropped and initiated a spectral

measurement using the software on the computer. The sample column was automatically drawn between the upper and lower pedestals and the measurement was made. When the measurements was completed, raised the sampling arm and wipe the sample from both the upper and lower pedestals using a dry, lint-free laboratory wipe. Simple wiping prevents sample carryover in subsequent measurements for samples varying by more than 1000 fold in concentration.

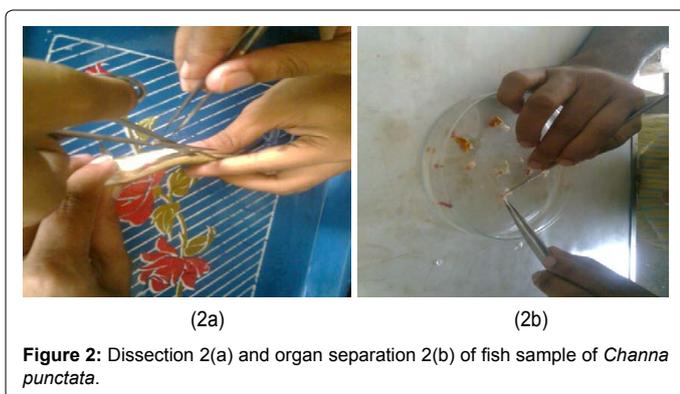
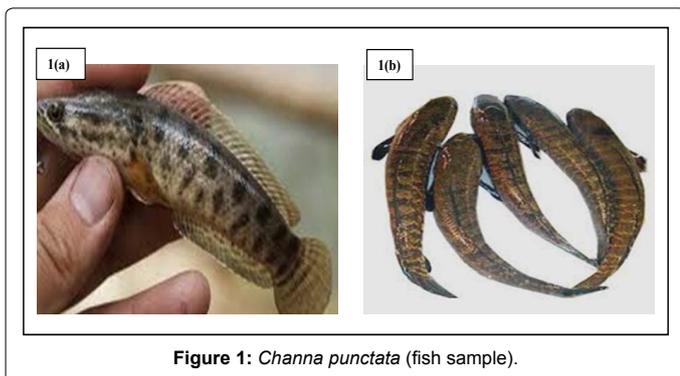
DNA Quantification: A small 218ng/µl DNA (Sigma) test sample was measured several times on both systems to compare accuracy and to ensure reproducibility. A sample volume of 0.3µl, 1.0µl, and 2.0µl was used for the Nano Photometer. The Nano Photometer can give comparable results with 0.3µl, 1.0µl and 2.0µl sample volumes.

Results and Discussion

A. Detection of Parasites

Water pollution is a major problem of Chittagong city in the urban areas as well as in the village areas. It is not only affecting the usual condition of the ecosystem of rivers but also many other fresh water sources of fishes like ponds, lagoons in our country. Many studies have been done on the fish ecosystems and impact of environmental pollutions on their life cycle especially on renowned rivers in Chittagong city like Halda, Karnaphuli, etc. However, there is no significance report in our country on the fresh water ecosystems of specific region named Najumiahat, Madunaghat, Hathazari Upazilla in Chittagong, Bangladesh during specific time. Therefore, remarkable research is the prerequisite for those countryside areas where villagers are very much dependent on the fishes as they usually collect from their nearby fresh water sources. During our observations, we have studied to find out the effects of metal absorption, parasitic infection, and biochemical changes on blood parameters due to any kind of environmental and genetic changes of the fresh water fishes *Channa punctata* from those selected areas. *Channa punctata* (Figure 1a,1b), is a common fish in those areas prefers its habitat in dead shallow ponds with mud substrate and vegetation. They can also be found in slow muddy streams [22,23] and in canals, reservoirs, lakes, and rivers.

According to the 'National Control and Management Plan for the Northern Snakehead (*Channa* sp)' by the 'Northern Snakehead Working Group (page 1-41)' during cold temperature, *Channa* has a reduced metabolism and oxygen demand. That's why they can survive under ice [23,24]. The fishes were gone through a replicate treatment. They were held at concentrations of 0, 3, and 10 ppt salinity. A fourth treatment increased salinity by one ppt per day until it is lethal. Among ectotherms, aquatic organisms represent a large number of species that are directly and acutely exposed to environmental changes. It is amazing that in the same time (January, 2013) those fishes being infected by internal parasites as well (Figure 3, Figure 4). From our results high amount of parasite in the intestine of fishes found in-January 2013 compared to another two months (September and November, 2012) results that the environment becomes toxic due to dry season and application of insecticides (Figure not shown). There are different kinds of clinical symptoms of parasitic diseases in fish, which are actually unclear. The signs in fish could be swimming alone, slowly, swimming on its side, moving gills more rapidly or losing weight. White colored spots could be seen on the skin or gills of sick fishes. Some fishes may have the appearance of swollen abdomen. Most parasitic organisms are opportunistic, but when fish is in stressed condition, then disease can be appeared. A number of factors such as, number of fish present in



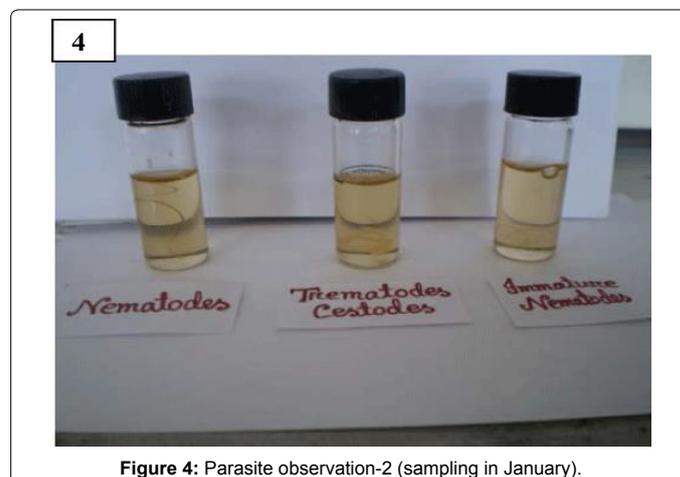
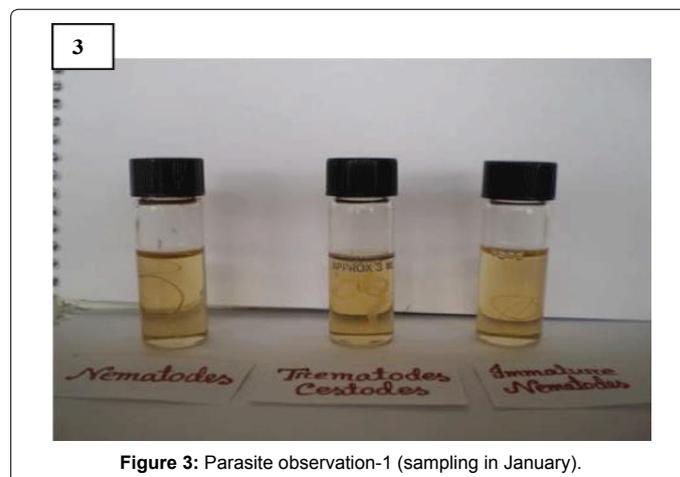
a tank, temperature of water, pH, lighting, filtration system etc. have role in the health of fish. The prevalence of parasite in this study was coincided with [25], who reported that during autumn the infection rate was lowest. This may be because of difference in localities, water quality in these areas as well as the effect of the seasonal variation in the present study. This result is contrary with [26,27], where their result was in the highest rate of infection during autumn. However, the rate of infection was highest in winter season when considering the seasonal dynamics of internal parasites from our study. This result is agreed with [28] detected the crustacean food in winter season which was contrast with [29], who declared that the rate of protozoan infection was high in spring. Differences in the rates and the seasonal dynamics of infection between the different areas may be regulated by several factors such as environmental conditions, fish species, number of examined samples, and the degree of water pollution.

B. Concentrations of Metals from Fresh Water Sources

Water samples were collected during September-2012, November-2012 and January-2013. Concentration of four inorganic elements (Sodium), Li (Lithium), K (Potassium) and Ba (Barium) was analyzed in the water sources by using Flame photometer BWB XP 2011. During three months (September-2012, November-2012 and January-2013) we have collected the data, where we found in January, the level of Na, K and Li is significantly highest in the water of those water sources compare with control (distilled water) and other sampling time water (September and November). This may be due to the water level become low during January because of high rate of water evaporation during winter season (Table 1, Figure 5, and Supplementary file 1). The concentration of Ba is also comparatively higher at the same time but not that much significant. Among all known species, 41.24% of fishes are found in fresh water. Freshwater fishes can spend their lives in fresh

water with a salinity of <0.05%. These environments are different from marine conditions in many ways, and level of salinity is a major fact. To survive in the fresh water, the fishes are to adapt themselves. Na, K, Ba and Li in small quantities are essential for growth and metabolic reactions in animals and plants. Supply of small amount of these metals is as nutrients in most fresh waters lives, causing significant growth and having a large effect on the aquatic ecosystem. Nevertheless, extreme amount of these inorganic elements from animal wastes, sewage, detergent, fertilizer, disturbed land, and road salts usually in the winter is harmful for fresh aquatic ecosystem.

Most studies on the investigation of metal concentrations in the liver, kidney or muscle have been done previously. If there is a high extent of metal pollutants in the river, its exposure induces to immune cells high which affect their function. Such imbalance of immune response can be a reason of infectious diseases in fishes. Metals have a great influence to the function of immune competent cells. The immune response can be suppressed or enhanced by a particular metal, its speciation, period of exposure, concentration, and bioavailability. The major insecticides, used in agricultural sections are organophosphate, organochlorines, carbamate and pyrethroids. Intensive agriculture and surface runoff of water is considered as a major reason of water contamination. Usually most of insecticides are highly toxic to non-targeted organisms. This does not only affect those organisms; also inhibit natural environments close to agricultural fields. Several studies reported that surface waters



and surrounding environments were contaminated with a number of insecticides.

For human, fishes are a good supply of proteins and lipids. Therefore, health of fishes is important for human beings. During the life cycle of fish, it can be subjected to wide range of metals that come from insecticides or from other different kinds of pollutants. Skin, gills or alimentary ducts can act as the entry point of different insecticides. Fishes are sensitive to the environmental condition of water. So, different pollutants can damage the physiological and biochemical process, when these pollutants enter into the organs of fishes. Therefore, the effects of different kinds of pollutants on the fishes are of great importance. Our report is similar with some reports that the mechanism of metals in higher concentration come from insecticides and other pollutants can cause the damage in fishes which finally effects on the whole aquatic ecosystem.

C. Biochemical Tests of Fish Blood

Another comparative study showed (Table 2, Figure 6 and Supplementary file 2) that the infected fishes contain more creatinine level compared to healthy fishes. The blood sugar and haemoglobin level decreased significantly in the case of infected one. It may be caused by parasite feed blood and sugar from host. One reason behind increasing creatinine level in infected fish is kidney failure because of parasitic infection. Another can be for high blood pressure or kidney stone due to parasitic infection. The reason is that kidneys cannot function normally and not able to filter the creatinine. As a result, creatinine levels become high. The stones in kidneys are very painful and may cause injuries. When the stone moves around the kidneys, it can rupture the kidney membrane and can resulting blood in the urine. Kidneys and stones do not go well together. Our results showed that the river and fresh water sources are highly polluted in those selected areas in January, so the infected fishes which collected from those areas contain high creatinine level, lower blood glucose and decreased Hb level.

D. Blood DNA Samples Visualization

The DNA extraction was made by the Blood DNA extraction kit. Table 3 shows the result of actual quantity of DNA measured by Nanodrop (Supplementary file. 3) Isolation of fish DNA has been done perfectly because the ratio of DNA range was 1.88-2.18 (two samples). Extracted DNA was visualized by gel electrophoresis to compare with a ladder where DNA band of three samples shown the presence of DNA (Figure 7). Our results originated all the requirements to determine

Measured Elements (in ppm)	Control (Distilled water)	Sample A (September, 2012)	Sample B (November, 2012)	Sample C (January, 2013)
Na	3.07	3.3	5.5	136.70
K	2.5	2.9	15.1	70.4
Li	0.8	1.39	2.3	6.4
Ba	5.2	16.8	12.7	19.6

Table 1: Concentration of metals from three fresh water samples of different time (September, November, 2012 and January, 2013) from selected areas.

Test no.	Test Name	Control (1)	Control (2)	Control (average)	Infected (sample1)	Infected (sample2)	Infected (sample3)	Infected (average)
1	Blood Glucose (mg/dL)	118.00 mg /dL	117.65 mg /dL	117.83 mg /dL	60.40 mg/dL	69.77 mg/dL	50.77 mg/dL	60.08 mg/dL
2	Hb (mg/dL)	8300 mg/dL	8050 mg/dL	8175 mg/dL	3002 mg/dL	3000 mg/dL	2800 mg/dL	2934 mg/dL
3	Creatinine (mg/dL)	0.35 mg /dL	0.36 mg /dL	0.355 mg /dL	0.71 mg/dL	0.66 mg/dL	0.69 mg/dL	0.687 mg/dL

Table 2: Data of blood biochemical test of *Channa punctata*.

the further steps of mutational changes occurred in the infected fishes compare with healthy fishes. The blood DNA from fishes were isolated by DNA extraction kit and visualized by gel running. Three samples of DNA bands were visualized and compared with marker by agarose gel electrophoresis.

Finally, our study indicated that the high level of water contamination, elevated parasitic infection and significant biochemical changes occurred among the fishes of those areas in Chittagong during January. Genetic changes of fishes have been reported in various research works. Further study will be helpful to identify the molecular characters, and specific genes responsible for these changes.

Conclusion

From this study, it can be assumed that Trematodes, Nematodes and Cystodes are the common parasites in *Channa punctata* from those specific areas in Najumiahat, Madunaghat, Hathazari Upazilla, Chittagong. As it is related to creatinine, kidney dysfunction can be caused by any abnormalities in urea cycle. Haemoglobin and blood sugar level decreased due to those taken by parasite from host. Finally, our research shows a comparative study which will help for further research for getting a better treatment of fish to make human healthy. Not only the liver and kidney dysfunction is related to biochemical test, but also related with metal exposure. Because metal can change the blood parameters of body and it is related with environmental pollution. The pure water fishes do not expose with metal and their

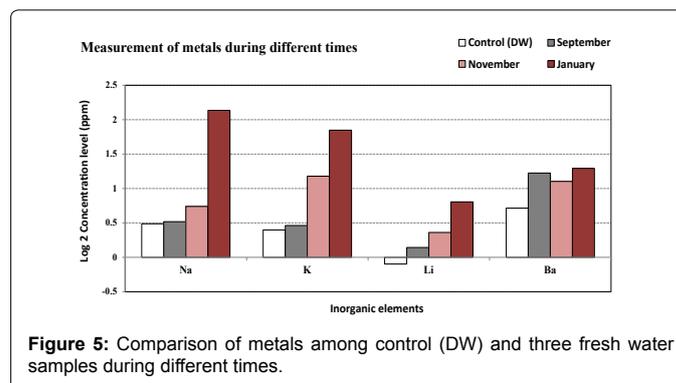


Figure 5: Comparison of metals among control (DW) and three fresh water samples during different times.

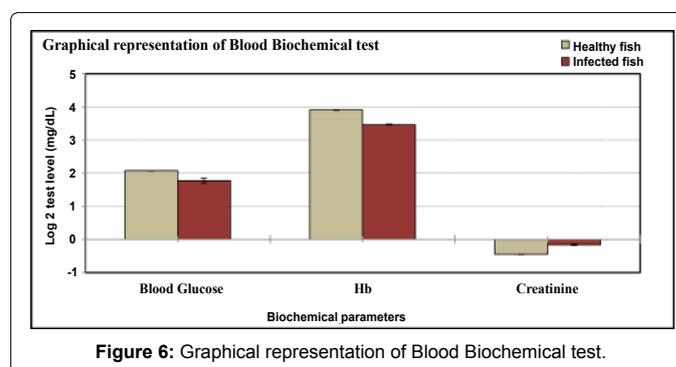
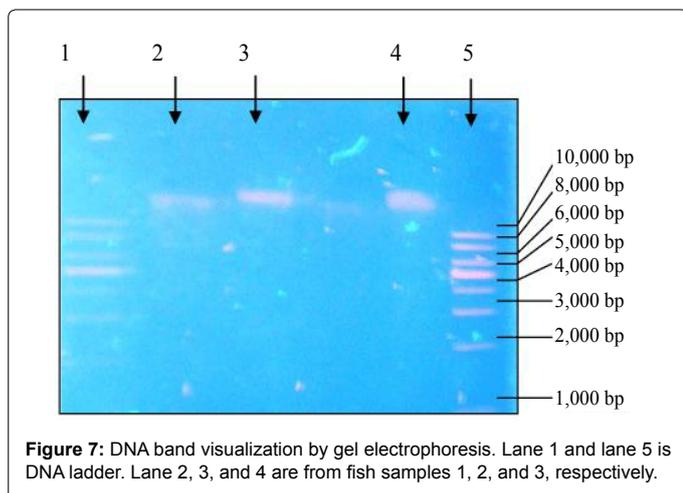


Figure 6: Graphical representation of Blood Biochemical test.

Serial No	Quantification	Ratio (260/280)
Sample 1	294.70	1.88
Sample 2	20.9	2.18
Sample 3	55.1	1.28

Table 3: Quantifying Fish Blood DNA.



haematological changes are minor. According to data analysis, we found environmental pollution is responsible for physiological changes of fishes. These parameters also showed toxic stress in the treated fishes especially on blood and blood forming organs. Blood biochemical parameter is an important diagnostic tool can be used for the detection of abnormalities in the liver and other tissues. The concentrations of metals are considered very important, as some are essential at low concentration but toxic at higher levels. With increasing public concern regarding environmental contamination, there is a growing need to monitor, manage and remedy of ecological damage. Rivers, lakes, ponds and lagoons are exposed to atmospheric deposition of anthropogenically derived trace elements. These elements can create harmful effects on environment as well as on human health due to their toxicity and bioaccumulation. The concentration of sodium increased in winter season when the water level in those water sources was low and decreased in summer season when water flow is high. The higher concentration of sodium limits the biological diversity due to osmotic stress. High Na contents in the form of chloride and sulfates make the water salty. High Na content in irrigation water brings about puddling of soil. K plays the same role in water as sodium. It occurs in small amounts and is regarded as an important macronutrient in the metabolism of freshwater environments. But excess amount of K is harmful for fish survival.

Our further approach is to analyse the mutation in extracted DNA of infected fish. This result may give a clarification whether there is any genetic cause and relationship for these physiological changes. Further research can determine the genomic changes and evaluation of the specific mutation in the gene due to the parasitic infection and metal exposure.

Additional Material

Supplementary File 1: Log 10 data of concentrations from metals of three fresh water samples at different time (September-2012, November-2012 and January, 2013) from selected areas

Supplementary File 2: Data of blood biochemical tests of

Channa punctata [Data showed in Log 10]

Supplementary File 3: Fish Blood DNA Quantification by using Nano drop (Three)

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