JAEHR Journal of Advances in Environmental Health Research
Environmental Health Research Center

Assessment of Aquatic Environmental Quallty Using *Gyrodactylus* sp. as a Living Probe: Parasitic Biomonitoring of Ecosystem Health

Jayanta Kumar Biswas[1*], Sasanka Pramanik[2]

1 International Centre for Ecological Engineering and Department of Ecological Studies, University of Kalyani, Kalyani- 741235, West Bengal, India
2 Department of Zoology, Sreegopal Banerjee College, Bagati, Mogra, Hooghly- 712148, West Bengal, India

Original Article

Abstract

Biological indicators are species that can be employed to monitor environmental quality and ecosystem health. Different groups of organisms such as plants, animals, bacteria and parasites regularly produce certain molecular signal in response to changes in their environmental milieu. Parasites are important tools for providing wealth of information on physicochemical quality, environmental stressors, trophic interactions, population structure, biodiversity, etc. Given that environmental degradation impacts occurrence frequency and intensity of fish parasites, they may serve as sensitive living probes to monitor environmental factors and ecological status of the water body. Population dynamics of parasites of fresh water fish have been studied involving several host species infected by monogenetic parasite, *Gyrodactylus* sp. The parasitological parameters such as prevalence, mean intensity, mean abundance of parasite were used for such aquatic biomonitoring purpose. There appeared to be distinct variation on parasitization and relationship between host and prevalence of infection. The present study indicates significant interaction between water quality variables and parasitism. Temperature seems to be the most important abiotic parameter that affected parasitic prevalence and load of infection. Under the pH range and dissolved oxygen level as encountered in the polluted sites, fish became stressed and vulnerable to be affected with parasitic infection. The present study presents a comprehensible view on how *Gyrodactylus* sp. can be championed as a sensitive and meaningful model for aquatic environmental study and an effective management tool for aquatic biomonitoring.

KEYWORDS: Biomonitoring, monogenean parasite, *Gyrodactylus* sp., fish, health, parasitisation, environmental degradation, water quality

Date of submission: 14 Dec 2016, ***Date of acceptance:*** 06 Mar 2017

Citation: Biswas JK, Pramanik S. **Assessment of Aquatic Environmental Quallty Using *Gyrodactylus* sp. as a Living Probe: Parasitic Biomonitoring of Ecosystem Health.** J Adv Environ Health Res 2016; 4(4): 219-226

Introduction

The increasing pressure of industrialization and anthropogenic activities has led increase in the levels of contamination in aquatic ecosystems. To maintain ecosystem health and to ensure ecosystem goods and services, monitoring of the presence and effects of pollutants are necessary. Pollutants affect the quality of aquatic water inclusive of dissolved oxygen, dissolved carbon dioxide, free carbon dioxide, pH, alkalinity and salinity. Health status of the aquatic ecosystem directly or indirectly influences fish health, thereby affecting immunity of fish and leading to disease susceptibility. Fishes in polluted water bodies are more susceptible to diseases. In aquatic ecosystems, the water quality parameters and some environmental parasitological parameters such as prevalence, mean intensity, mean abundance of parasite indicate a distinct relationship between water quality and parasitic infection or fish susceptibility to parasitic infection.[1] Several factors such as changes in physicochemical

Corresponding Author:
Jayanta Kumar Biswas
Email: biswajoy2000@yahoo.com

realm, stress, presence of pathogens can increase the host susceptibility to parasites and provoke an imbalance in the host-parasite-environment system.[2] The aquatic environment can be studied either directly by a regular monitoring of water quality parameters or indirectly by using bioindicators, like fish parasites.[3,4] These organisms interact and react with environmental conditions or their alteration. It provides some cues which could be used for a wide spectrum of applications such as biomonitoring of water quality,[5,6] environmental stress,[7] pollution,[8,9] distinguished accumulation and effect of bioindicators; organisms efficiently take up substances in the former while the latter was used to detect environmental impact. Studies have demonstrated that parasites can be used as gauges by measuring concentrations of toxicants in them relative to various host tissues. Both experimental studies and natural parasitic infections demonstrate the usefulness of parasites as indicators of the fate of specific contaminants in the biota.[10]

In recent years, fish parasites have gained increasing interest from an environmental perspective.[11-13] Many studies show the close relationship between parasitism and ecological conditions in a given milieu and portray how parasites can be used to extend knowledge on ecosystem function and integrity.[14] There is an intimate interaction between environmental factors and parasitism.[15,16] Fish generally harbors a wide range of ecto - and endo-parasites. Pollutants might induce increased parasitism in case of aquatic animals, especially fish by impairing the host's immune response or favouring the survival and reproduction of the intermediate host.[8,17] As common ectoparasites of fish gills and skin, some monogenean parasites remain in permanent contact with the ambient environment. Abiotic factors of the water environment play a significant role on the presence, intensity and diversity of the parasite.[18,19] Some studies have demonstrated their close relation to eutrophication[15] and other types of pollution arising from industrial effluents.[20] The role of fish parasites with special reference to monogenean parasites for monitoring of water quality and ecosystem health has not been properly assessed. There remains a paucity of information on this aspect on a global scale in general, and the Gangetic region in particular. The present study attempts to investigate if *Gyrodactylus* sp. could be used as biological indicators of ecosystem health as well as impact indicators to describe the relationship between pollution and the parasitic abundance and load. It has also given due consideration to proper selection of the parasites and their specific life cycle stages which are prerequisites for such potential applications. The aim of this study was to examine the bioindicator capacity of monogenean parasites harbouring the fish and to assess the relationship between water quality and fish health in both polluted source of water in Ganges river and the less polluted water of Murti river in Jalpaiguri. Hence, the objective of this paper is to expound the value of parasites as tools for assessing their role as biomonitor of aquatic habitat health.

Materials and Methods

Selection of ectoparasite

Gyrodactylus sp. was selected as a representative monogenean flatworm of fresh water fishes. It possesses a high degree of host specificity with a few of these parasites infecting Salmonids worldwide.[21]

Systematic position

Kingdom – Animalia
Phylum – Platyhelminthes
Class – Monogenea
Order – Monopisthocotylea
Family – Gyrodactylidae
Genus – *Gyrodactylus*

Morphological description

Gyrodactylus sp. is an ectoparasitic flatworm with a length less than 1mm and body width of about 0.1 mm. Nevertheless, soft body parts of *Gyrodactylus* sp. are affected by compression during slide preparation and their dimension was not used for diagnosis. In contrast, hard parts are of taxonomic importance. The ventrally directed opisthohaptor is equipped with two large hamuli and 16 marginal hooklets (Figure 1). Shapes and dimensions of these sclerotized parts are used for diagnosis.
The dimensions of the hard structure are negatively correlated to temperature. As a result, if parasites are propagated at comparatively low temperature, the anchors increased in size alongside ducts producing secretions. These structures and secretions aid attachment, for instance, during migration of the worm from one host to the other or to

objects in the aquatic environment. Using light microscope, the internal caeca, pharynx and cirrus are clearly visible. The most prominent character is the uterus where embryo develops. The parasite is hyperviviparous i.e. it gives birth to a living young about the same size as the mother which may already have its own embryo. This spectacular organization with three generations in one parasite specimen has inspired the parasitologists to call it "Russian Doll" arrangement.[22]

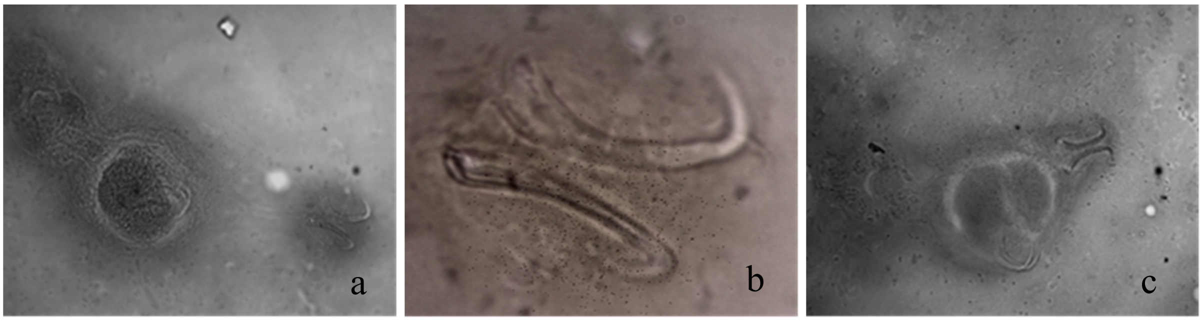

Figure 2. Morphological structure of *Gyrodactylus* sp. a. Adult stage, b. Adult with embryo, c. Hamuli.

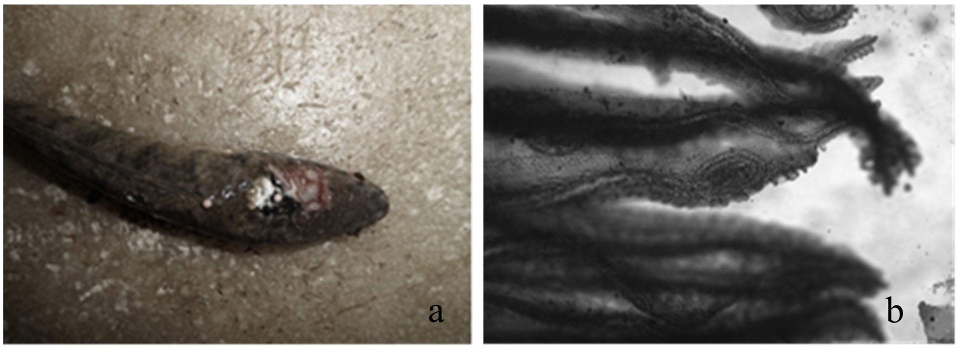

Figure 2. Infected fish (*Channa* sp.) (a) and immature stage of *Gyrodactylus* sp. attached with its gills (b).

Location on the host

Gyrodactylus sp. infects the fins of fishes, but may also be found on the body and head (including nostril, mouth cavity and cornea). The gills are only rarely infected. Nevertheless, the location on the host is influenced by the host strain, parasitic load, and duration of infection. During its initial colonization, *Gyrodactylus* sp. selects preferentially fins, especially the pectoral fins. During the course of eight weeks infection , the relative occurrence in pectoral fins decrease whereas a large part of the parasite population can be found on the caudal fin. Heavily parasitized fish can harbour parasites all over the body including skin, fins and corneal surfaces. Moderately infected fish harbour the major part of the parasites population on the dorsal, anal and pectoral fins.

Clinical signs and effects

Fishes infected with monogeneans were found lethargic, swimming near the surface. They had clamped fins, took the sides of the pond and had diminished appetite. They may be seen rubbing the bottom or sides (flashing) of the tank. Scale loss occurred, where the monogeneans were attached and the skin varied in colour when laden with parasites. Heavy gill infestation (Figure 2) resulted in respiratory disease. Gills became swollen and pale. Respiration rate increased as the fish were subjected to low ambient oxygen level that was manifested by fish in severe respiratory distress gulping air at the water surface. Large numbers of monogeneans on either the skin or gills may result in significant damage and mortality. Secondary infection with bacteria and water moulds were also encountered on tissue damaged by monogeneans.

Collection of fish samples

Water samples and fishes were collected from polluted water bodies (local ponds and water reservoir near the west side of the river Ganges). The fish samples collected from Murt River, Jalpaiguri were considered as control for the study. The present study was carried out for about one year, from August 2013 to July 2014. A total of 80 individual host fish belonging to five genera were collected from different water bodies, out of which 16 host were found to be infected with different parasites.

Preparation of parasitological slides

At first, the collected fish specimen were examined externally. The main examined areas are gills, operculum, fins, scales and the mucous covering of the body. Then fishes were opened dorsoventrally and internal organs like liver, kidney and different part of digestive tract were thoroughly examined. Infected portion of fish was put in a watch glass containing normal saline water. A clean grease free slide was coated with Mayer's albumin and a thin smear was prepared with the infected portions of the fish. The slide was air dried for 1 min. The slide was immediately placed into 90% alcohol for 15 min for fixation of parasites. It was passed gradually through down-graded alcohols (70, 50, 30%) followed by distilled water. Staining was done with haematoxylin. Sequential dehydration through up-graded alcohols (50, 70 and 90%) was followed by staining with Eosin. After washing with 90% alcohol and cleaning in xylene, the slide was finally mounted with DPX. The prepared slide was observed under light microscope[19].

Study of parasitological parameters

Fish samples belonging to five genus were examined to determine prevalence, mean abundance and mean intensity of parasites.

Prevalence

Prevalence is the number of individuals of the hosts infected with particular parasite species or with total parasites divided by the number of hosts examined. Prevalence is expressed in terms of percentage (%).

$$\text{Prevalence (\%)} = \frac{\text{Number of infected fishes} \times 100}{\text{Total Number of Fish Examined}}$$

Mean abundance

Mean abundance is the total number of individuals of a particular parasite species in a sample of particular host species divided by the total number of hosts of that species examined (including both infected and uninfected hosts).

$$\text{Mean abundance} = \frac{\text{Total number of individuals of a parasite species}}{\text{Total number of host fishes examined}}$$

Mean intensity

Mean intensity is the average intensity of total number of individuals of a particular parasite species in a sample of host species or total number of individuals of all parasites found divided by the total number of host species.

$$\text{Mean intensity} = \frac{\text{Number of collected individuals of a particular parasite species}}{\text{Number of infected fishes}}$$

The analysis of parasites is not necessarily simple because not all hosts serve as good models and because the number of species, presence of specific species, intensity of infections, life histories of species, location of species in hosts, and host response for each parasitic species have to be addressed individually to assure usefulness of the tool.

Water quality analyses

The water samples were collected regularly from polluted water bodies during the period of investigation for monitoring physicochemical parameters of water. Water temperature, pH, and dissolved oxygen concentration were measured using specific probes of a Multiline system (F/SET-3, Best-Nr. 400327, WTW Wissenschaftlich-technische Werkstatten 82362 Weilheim, Germany). Dissolved carbon dioxide level, alkalinity and salinity were determined by standard titrimetric methods.[22]

Statistical analyses

All data obtained in the study were subjected to appropriate statistical analyses. Difference of mean values of different parameters were compared following one way analysis of variance (ANOVA) and LSD test by using the statistical package EASE and M-STAT, respectively. The degree of correlations among different physicochemical factors of water were determined by regression analysis using the statistical package Origin. The level of statistical significance was accepted at $p < 0.05$.

Results and Discussion

The fishes *Barilius bendelisis*, *Badis* sp.,*Mastecembelus armatus*, which were collected from the Murti river (less polluted water) were found to be free from any parasitic infection, whereas the fishes (*Channa* sp., *Anabas* sp. and *Clarias* sp.) collected from the water bodies contaminated with pollutants from mainly domestic sewage were found to be infected with an ectoparasite *Gyrodactylus* sp. (Figure 3).

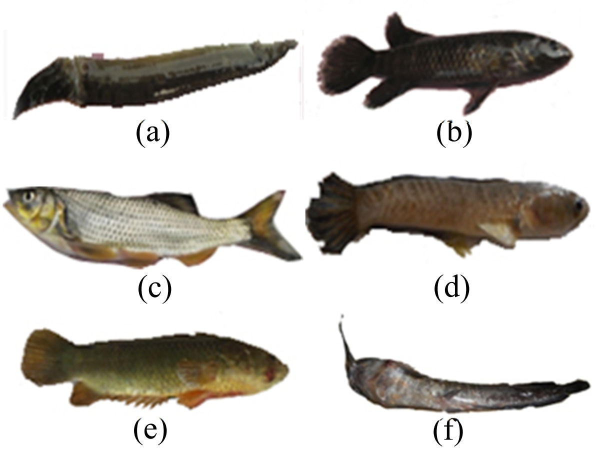

Figure 3. Fishes collected from Murti River (the comparatively less polluted reference site) free from parasitic infection: (a) *Mastecembelus armatus*, (b) *Badis* sp., (c) *Barilius bendelisis*; fishes (from polluted water bodies) infected with parasites: (d) *Channa* sp., (e) *Anabas* sp. and (f) *Clarias* sp.

Water quality

The amount of dissolved oxygen in water varied between 4.6 to 5.8 mg/l in the water during the period of study where infection was found. In the water where no infection was found, the range of DO levels was 7.5-10.4 mg/l (Table 1). The concentration of dissolved CO_2 in water samples collected from sources showing fish infection and without any infection were 2.7 - 4.2 mg/l and 1.2-1.8 mg/l, respectively.

Temperature is one of the most important factors in our work because the life cycle of parasite was found to be dependent on temperature. The occurrence and prevalence of monogeneans correlated with seasonal changes in water temperature. The water temperature of the sites showing *Gyrodactylus* sp. infections ranged between 20 and 28°C, while that in the reference site ranged from 16 to 22 °C (Table 1). It can be corroborated with the observation of Blažek et al.[23] who revealed that the relationships of the water temperature and parasitisation intensities of different *Gyrodactylus* spp.

Table 1. Major water quality parameters of aquatic habitats from which fishes were sampled during August, 2013 to November, 2013. Note: Uninfected fishes were collected from Murti river (Jalpaiguri district in West Bengal, India) which served as a non-polluted reference site.

Sites		Temperature (°C)	pH	Dissolved Oxygen (mg/l)	Dissolved Carbon dioxide (mg/l)	Alkalinity (mg/l)	Salinity (mg/l)
Polluted sites	Range	20 -28	6.1-7.2	4.6 -5.8	2.7-4.2	180 -218	550- 611
	Mean (±SE)	25± 1.2	NA	5.2±0.6	3.7±0.8	206.5±8.5	575±31
Reference site (non-polluted)	Range	16-22	8.2-8.5	7.5-10.4	1.2-1.8	196.6- 235	690-696
	Mean (±SE)	20.5±1.5	NA	8.8 ±0.7	1.5±0.2	218±12	693±3

(*Gyrodactylus gobiensis*, *G. gobii* and *G. gasterostei)* were negatively correlated and the intensity of *D. cryptomeres* peaked when the water temperature increased above 14°C. Several other studies also presented similar conclusion on water temperature dependent population dynamics of the monogeneans where reproduction and survival of parasites were found to be directly affected [24,25] and altered immune response of the host was elicited.

pH of the sample water where infection was found was 6.1-7.2 and pH of the sample water where infection was not found was 8.2 to 8.5. Fish have an average blood pH of 7.4, the pH range between 7.0 to 8.5 is optimum for primary productivity and conducive to fish life. According to Santhosh and Singh,[26] the suitable pH for fish culture ranges between 6.7 and 9.5 and pH between 7.5 to 8.5 favours optimum growth. Under the pH range as encountered in polluted sites, fishes became stressed and vulnerable to be affected with parasitic infection. This is also in line with the observation of Ekubo and Abowei[27] who stated that a pH less than 6.5 or greater than 9 poses stress to fish while pH less than 4 or higher than 11 can even have lethal effect leading to death of the fish.

The mean alkalinity of water samples (Table 1) were 196.6 and 210.47 mg/l in the polluted aquatic systems and the non-polluted reference water body, respectively. It showed that there is a little difference in alkalinity of water samples among the sites which implies that alkalinity did not have any significant impact on parasitic prevalence and degree of infection. The ranges of salinity in polluted and non-polluted water bodies were 550 to 611 mg/l and 690 to 696 mg/l, respectively.

There appeared a significant variation in the levels of salinity (LSD test; $p<0.05$) between the two types of aquatic systems. The aquatic habitats where parasitic infestation was found exhibited lower level of salinity, in comparison to the non-polluted reference water body.

Parasitic prevalence, abundance and intensity

The parasite demonstrated significant differences in prevalence among the fish species examined (LSD test; $p < 0.05$). The order of parasitic prevalence was *Heteropneustes* sp. > *Clarias* sp. > *Channa* sp. > *Anabas* sp. > *Labeo* sp. ((Fig. 4). However, the mean intensity was maximally encountered in *Clarias* sp. and minimum in *Labeo* sp. The fish species examined showed the following order of variation in parasitic intensity: *Clarias* sp. > *Anabas* sp. > *Channa* sp. > *Heteropneustes* sp. > *Labeo* sp. The parasite was found to be the most abundant among *Clarias* sp. (0.75), followed by *Heteropneustes* sp. (0.6), *Channa* sp. (0.54), *Anabas sp.* (0.5) and *Labeo* sp. (0.15) (Figure 4).

It is obvious from the present study that fish have become very susceptible to parasitic infection under deteriorated water quality conditions in polluted water bodies while no infection was observed in case of fish living in non-polluted Murt River, Jalpaiguri. The seasonal fluctuation in certain factors like water temperature and dissolved oxygen had a remarkable influence in monogenean life cycle while amplifying their proliferation at higher temperature and lowered dissolved oxygen level subject to organic loading and oxygen sag. This can be corroborated with the finding that organic loading followed by eutrophication increased parasitic infection where the monogenean species responded positively.[28-30] In general, infections with ectoparasites tend to increase, whereas infections with endoparasitic helminths tend to decrease with increasing levels of pollution.[11] Marcogliese et al.[31] stated that monogeneans tend to increase in number when subjected to low and medium pollutant concentrations, but decrease or disappear at high concentration.

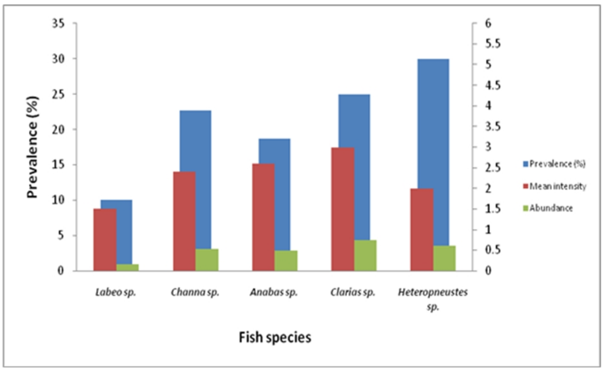

Figure 4. Variation of abundance, prevalence and mean intensity of monogenean parasite (*Gyrodactylus* sp.) harbouring different fish host investigated.

The poor water quality may be responsible for poor health of fish with lower immunity and resistance against parasites. On the contrary, parasites with direct life cycle (monoxenous) are less affected under eutrophic state of the aquatic system. It can be explained by the fact that these monoxenous monogeneans are ectoparasites and are in direct contact with the ambient water. They are more capable of adapting to changes in environmental conditions in comparison to the heteroxenous parasites.[15,6] Thus with a direct life cycle and better adaptability, the monogeneans are considered as good bioindicator candidate in comparison to those having multiple life cycles. The presence of optimum concentration of dissolved oxygen, alkalinity, salinity but low dissolved carbon dioxide may be conducive for viability and sound health of fish. Therefore, fish in non-polluted habitat endowed with high host resistance that normally keeps the monogenian parasite in check were capable of resisting parasites. When parasites harbour and multiply in their host, they may obtain more food in the form of mucus and sloughed off epithelium. Under pollution stress, the intensity of parasitic infection as well as the parasitic load per fish are increased. Contamination with domestic waste water and storm water run-off (both surface and agricultural run-off) can contribute organic loading enriched with surface and agricultural run-off) can contribute organic loading enriched with nutrients and bacteria that may serve as a nutritional source for the parasite. The parasites are characterized by an activity of constantly probing and trying to occasion a breach of the immune system of the fish with the objective of gaining a strong foothold. Studies indicate that for them to gain a strong foothold as desired, there has to be an underlying predisposing factor, such as poor environmental conditions, poor nutrition, overcrowding, poor water quality, or a combination of these factors. The prevalence of these circumstances gives rise to a situation of stress, which has the effect of depressing the immune system of the fish and subsequently encourages increased numbers of opportunistic infections and pathogens.[32]

It can be concluded that fish health is related to ambient water quality, and fish parasites like *Gyrodactylus* sp. can be used as an important tool for monitoring the health of aquatic ecosystem. Usually overlooked as biological models for expressing the effects of contamination and the status of environmental health, a fish parasite (*Gyrodactylus* sp.) can provide a useful, powerful, reliable and economical tool to telescope the status, either an acute or chronic view of the biota and ecosystem health.

Acknowledgements

The authors gratefully acknowledge the infrastructural support extended by the University of Kalyani and Sreegopal Banerjee College, Hooghly.

References

1. Lafferty KD, Allesina S, Arim M, Briggs CJ, De Leo G, Dobson AP, et al. Parasites in food webs: the ultimate missing links. Ecol. Let.2008; 11: 533-546.
2. Coutant CC. What is normative for fish pathogens? A perspective on the controversy over interactions between wild and cultured fish. J Aquat Anim. Health Bethesda 1998; 10: 101-106.
3. Galli P, Crosa G, Mariniello L, Ortis M, D'Amelio S. Water quality as a determinant of the composition of fish parasite communities. Hydrobiologia 2001; 452:173–179.
4. Palm HW, Kleinertz S, Ruckert S. Parasite diversity as an indicator of environmental change? An example from tropical grouper (Epinephelus fuscoguttatus) mariculture in Indonesia. Parasitology 2011; 138(13): 1793-1803.
5. MacKenzie KH, Williams H, Williams B., McVicar A H, Siddall R. Parasites as indicators of water quality and the potential use of helminth transmission in marine pollution studies. Adv Parasitol 1995; 35:85-144.
6. MacKenzie K. Parasites as pollution indicators in marine ecosystems: a proposed early warning system. Mar Poll Bull 1995; 38: 955-959.
7. Nachev LM. Bioindication capacity of fish parasites for the assessment of water quality in the Danube River. Ph.D. Dissertation, Universität Karlsruhe (Karlsruhe Institute of Technology) ; 2010.
8. Khan RA, Thulin J. Influence of pollution on parasites of aquatic animals. Adv Parasitol 1991; 30:201–239.
9. Vidal-Mart´ınez VM, Pech D, Sures B, Purucker ST, Poulin R. Can parasites really reveal environmental impact? Trends Parasitol 2010; 26:44–51.
10. Sures B. Accumulation of heavy metals by intestinal helminths in fish: an overview and perspective. Parasitology 2003; 126 (Suppl.): S53-S60.
11. Sures B. Effects of pollution on parasites, and use of parasites in pollution monitoring. In: Rohe K, editor. Marine Parasitology. CSIRO Publishing, Collingwood, 2005; 421-425pp.
12. Sures B. How parasitism and pollution affect the physiological homeostasis of aquatic hosts. J Helminthol 2006; 80:151-157.
13. Sures B. Environmental Parasitology. Interactions between parasites and pollutants in the aquatic environment. Parasite 2008; 15:434-438.
14. Hudson PJ, Dobson AP, Lafferty KD. Is a healthy ecosystem one that is rich in parasites? Trends Ecol Evol 2006; 21:381–385.
15. Valtonen ET, Holmes JC, Koskivaara M. Eutrophication, pollution, and fragmentation: effects on parasite communities in roach and perch in four lakes in central Finland. Parasitologia 1997; 39(3): 233-236.
16. Marcogliese, D.J. Parasites: small players with crucial roles in the ecological theatre. EcoHealth 2004; 1, 151–164.
17. Poulin R. Toxic pollution and parasitism in freshwater fish. Parasitol. Today 1992; 8: 58-61.
18. Buchmann K, Uldal A. Gyrodactylus derjavini infection in four salmonids: comparative host susceptibility and site selection of parasites. Dis Aquat Organ 1997; 28: 201–209.
19. Buchmann K, and Bresciani J. Microenvironment of Gyrodactylus derjavini on rainbow trout Oncorhynchus mykiss:

association between mucous cell density in skin and site selection. Parasitol Res 1998; 84(1): 17–24.

20. Boyoumy EM, Osman HAM, Laya F, Bana EI, Hassanain MA. Monogenean parasites as bioindicators for heavy metals status in some Egyption Red Sea fishes. Glob Vet 2008; 2 (3): 117 – 122.
21. Bakke TA, Cable J, Harris PD. The biology of gyrodactylid monogeneans: the “Russian Doll-killers”. Adv Parasitol 2007; 64: 161-376.
22. APHA (American Public Health Association), American Water Works Association, Water Pollution Control Federation. Standard Methods for the Examination of Water and Wastewater, 22nd ed. American Public Health Association, New York, NY ; 2012.
23. Blazek R, Jarkovsky J, Koubkova B, Gelnar M. Seasonal variation in parasite occurrence and microhabitat distribution of monogenean parasites of gudgeon *Gobio gobio*. Helminthologia 2008; 45: 185-191.
24. Jansen P A, Bakke TA. Temperature-dependent reproduction and survival of *Gyrodactylus salaris* Malmberg, 1957 (Platyhelminthes: Monogenea) on Atlantic salmon (*Salmo salar* L.). Parasitology 1991; 102: 105 – 112.
25. Andersen PS, Buchmann K. Temperature-dependent population growth of *Gyrodactylus derjavini* on rainbow trout, *Oncorhynchus mykiss*. J Helminthol 1998; 72: 9-14.
26. Santhosh B, Singh NP. Guidelines for water quality management for fish culture in Tripura, ICAR Research Complex for NEH Region, Tripura Centre, Publication no. 29; 2007.
27. Ekubo AT, Abowei, JFN. Aspects of aquatic pollution in Nigeria. Res J Environ Earth Sci 2011; 3(6): 673-693.
28. Lafferty KD. Environmental Parasitology: What can parasites tell us about human impacts on the environment? Parasitol. Today 1997; 13(7): 251-255.
29. Lafferty KD. Ecosystem consequences of fishparasites. J Fish Biol 2008; 73:2083–2093
30. Lafferty KD, Allesina S, Arim M, Briggs CJ, DeLeo G, Dobson AP, et al. Parasites in food webs: the ultimate missing links. Ecol Lett 2008; 11: 533-546.
31. Marcogliese DJ. Parasites of the superorganism: Are they indicators of ecosystem health? Internat J Parasitol 2005; 35: 705–716.
32. Klinger RE, Francis-Floyd R. Introduction to Freshwater Fish Parasites, CIR, 716: Institute of Food and Agricultural Sciences, University of Florida ; 2002.