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Establishing cause effect of multistressor environments on *Oreochromis niloticus* and its parasite communities

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This study investigated the composition and structure of the parasitic communities in *Oreochromis niloticus* with respect to levels of water quality in three different ecosystems. A total of 366 *O. niloticus* were examined from three water courses: CLAR ponds, (unpolluted), Abbassa privet ponds (slightly polluted) and Manzalla ponds (polluted). Trematodes, monogeneans, cestodes and acanthocephalans were found in all the sample sites. Trematodes dominate the parasite population. *Clinostomum tilapiae*, *Euclinostomum heterostomum, Diplostomum spathaceum*, *Posthodiplostomum minimum*, *Mesostephanus appendiculatus, Haplorchoides cahirinus*, *Cichlidogyrus aegypticus*, *Acanthosentis tilapiae* and larval stages of *Polyonchobothrium clariass* were found in all the sampled sites, but the distribution of these parasitic species varied between the three ponds. Meanwhile, *P. clarias*, *E. heterostomum*; and *A. tilapiae* were completely absent in the severely polluted site. The observed composition and structural changes among the sites were studied; the variability of the calculated infection indices (prevalence, mean abundance and mean intensity); and the degree of interactivity among parasites, as well as parameters of species richness and diversity suggest that the structure of parasite communities are affected by the water contamination levels.

Key word: *Oreochromis niloticus, Polyonchobothrium clarias, Euclinostomum heterostomum, Acanthosentis tilapiae,* multistressor environments.

INTRODUCTION

Parasites occur in nearly every population, they often interact in complex ways with other stressors. In some cases, the interaction may lead to a disproportionately negative effect on the host population. In other cases, the stressor may ameliorate the effects of parasitism (Lafferty and Kuris, 1999). In an aquatic environment, there is a profound and inverse relationship between environment quality and disease status of fish and parasites are one of

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the most serious limiting factors in aquaculture (Gupta et al., 2012). Most of reliable technologies for detection of pollutants and policies regulatory framework for managing the aquatic environment are expensive and time consuming. The use of parasites of fish and macro-invertebrates are considered one of the cheapest and reliable ways of tracking environmental perturbation in the aquatic system (Orzell and Platt, 2008). Aquatic pollution is still a problem in many fresh and marine environments since it has negative effects on the health of the respective organisms (Fent, 2007). However, the effect of environmental pollutants on fish parasite varies depending on the particular parasite and pollutant that interact (Lafferty and Kuris, 1999). Pollutants may affect the immune system of the fish either directly or by changing the water quality; that in-turn may reduce the fish immunity to parasitism (Poulin, 1992). Fish parasites are used as bio-indicators for monitoring environmental perturbations in aquatic ecosystems. Specific species of parasites or even the parasitic community as whole are utilized as indicators for pollution, as any change in the prevalence and intensity of infection in certain parasite taxa and the composition of parasitic communities are used to assess pollution impact on the aquatic environment (Nachey and Sures, 2009). Man-made pollutants and/or intensification of fish culture resulted in an increase of environmental changes, which may be stressful to fish (Lio-Po and Lim, 2002). Reviews have clearly demonstrated that many chemical contaminants modulate immune functions and affect disease resistance. (Galloway and Depledge, 2001). Anthropogenic stressors are a pervasive problem in freshwaters because these ecosystems can act as conduits or sinks that accumulate and concentrate contaminants (Relyea, 2009). Understanding how aquatic systems respond to stressors, which is fundamental to their management, is not straight forward because often multiple stressors are in operation (Culp and Baird, 2006). Stressors can have both lethal and non-lethal effects that impact on individuals and populations, and these may be additive antagonistic or synergistic factors (Hu et al., 2009). Hosts may become more susceptible to the morbidity effects of disease without any change in per capita infection because contaminants can induce cortisol production and suppress blood leukocytes (Forson and Storfer, 2006). Moreover, the diversity of fish parasites in contaminated water probably reflects two points: (1) disturbance of the entire parasite community and/or (2) a reduction in the immunological response of fish that might facilitate infection by parasite species with a relatively low epidemiological potential. Parasites are indicative of many biological aspects of their hosts, including diet, migration, recruitment and phylogeny (Williams et al., 1992). They may also be useful direct indicators of environmental quality status (Marcogliese and Cone, 1997). Parasites can thus be considered complementary to chemical analysis or traditional biological surveys as indicators of dysfunction at the ecosystem level. These findings suggest that parasites are characterized as fingerprint at community level according to the quality level of the water body in which they complete their life cycles.

Oreochromis niloticus was chosen to study the impact of pollution in the three locations due to its wide range of food selection, and its tolerance to chemicals and physical pollutant factors. The present study aimed to highlight the relationship between composition and structure of parasite communities hosted by *O. niloticus* in the three different located sites with varying levels of pollution and water quality. The results of this study may contribute to the understanding of changes in communities of metazoan parasites of chub due to pollution in freshwater ecosystems. And it demonstrate the eco-toxicological relevance of the biodiversity of metazoan communities fractionated according to deterrent criteria and demonstrate the suitability of a logical set of biodiversity measures which integrates standard diversity indices and a novel approach to the quantitative analysis of cumulative species.

MATERIALS AND METHODS

O. niloticus were sampled in three different ecosystems that differed according to their water quality.

Location of sampling ponds

The first site is CLAR ponds (central laboratory for Aqua-culture research in Abbassa), which is supplied with water from the Ismalia canal by way of the El Wadi El Quadim supply canal that originates from the Nile River. Water enters the CLAR ponds through sedimentation pond. The second site is Abbassa privet ponds for fish aquaculture, that is supplied with water from the Ismalia canal. The third water body is Manzalla ponds which is a brackish lake, sometimes called a lagoon, in northeastern Egypt, and served as a significant source of inexpensive fish for human consumption, but pollution and lake drainage have reduced the lake's productivity.

Water quality

The water quality, assessed by means of Woodwiss's Extended Biotic Index according to Ghetti (1986), and some microbiological (total coliforms and fecal coliform) and chemical pollution (lead, nitrate and phosphrous) indicators were estimated. A total of 366 fish were caught from April 2012 to August 2013.

Fish examination

The number of fish analyzed per sampling site is given as follows: 145 from CLAR ponds; 130 from Abbassa privet ponds; 91 from Manzalla ponds. Eyes, gills and gut of the fish were examined for external parasites and internal parasites in the laboratory and each fish was measured and weighed to estimate the coefficient of condition K (derived from the formula: $K = W\ 105 / L3$, where W and L, respectively, represent the individual weight in grams and the standard length in centimetres (Lévêque et al., 1988).

Table 1. Water quality data evaluated in the three sites.

Water quality parameter	CLAR ponds	Abbassa privet ponds	Manzalla ponds
	Non polluted	Signs of pollution detected	Polluted
Total coliforms (/100 ml)	2300	6100	31 000
Faecal coliforms (/100 ml)	240	1500	18 000
Conductibility (μs/cm)	241	276	1.9430
Nitrite (mg/L)	<1	<1	>1
Nitrate (mg/L)	0.03	0.05	0.07
Total phosphorous (mg/L)	<0.03	<0.03	0.09
Lead (μg/L)	<1	1	7

Table 2. Host condition as a constraint for parasites in the three ponds.

Parameter	Measurements	CLAR ponds	Abbassa privet ponds	Manzalla ponds
Fish length (cm)	Min-max	15.5-19.5	17-23	20.5-33
	Mean	17.5	20	26.5
	Standard deviation	3.21	3.9	3.53
Fish weight (g)	Min-max	50-330	120-250	155-340
	Mean	241	185	247.5
	Standard deviation	65.6	117	71.8
Coefficient of condition (*k*)	Min-max	1.2-3.6	0.7-2.1	0.9-2.9
	Mean	2.0	1.8	2.1
	Standard deviation	0.3	0.21	0.42

Parasitological studies

The level of parasite infection was quantified by using: prevalence (P), intensity (I), mean intensity and mean abundance (A) according to Bush et al. (1997). Species richness, dominance and diversity indices were used to compare parasite community structures. In addition to the application of standard indices of richness, that is, Margale f index, diversity, Shannon-Wiener, and dominance Simpson and Berger-Parker (Magurran, 1983), differences in parasite community structures among the sampled sites were also evaluated.

Statistical analysis

One way ANOVA was applied to test the significant differences between the mean values of intensity, abundance and prevalence in the three ponds.

RESULTS

The water quality, assessed by means of Woodwiss's Extended Biotic Index according to Ghetti (1986), and some microbiological and chemical pollution indicators are shown in Table 1. The total coliform, lead, nitrate and phosphrous estimated in a high values in Manzalla ponds (polluted) while the same Parameters were less in Abbassa privet ponds (slightly polluted).

Nine parasite species were recorded from the examined 366 *O. niloticus*, 300 *O. niloticus* were infected with one or more species of parasites. Trematodes were the predominant taxa comprising six species, *Clinostomum tilapiae*, *Euclinostomum heterostomum, Diplostomum spathaceum*, *Posthodiplostomum minimum*, *Mesostephanus appendiculatus, Haplorchoides cahirinus* (metacercaria); monogeneans were represented by one species *Cichlidogyrus aegypticus*, while cestodes were represented by larval stages of *Polyonchobothrium clarias*, and acanthocephalans were represented by one species, *Acanthosentis tilapiae.*

Fish size, weight and the coefficient of condition are shown in Table 2, the mean fish total length ranged between 17.5 and 26.5 cm, while the mean fish total weight ranged from 185 to 247.5 g. The condition factor did not differ significantly in the three localities (as estimated by *k*) and seem to be slightly higher at Manzalla ponds, and allow the exclusion of any consistent effect of the fish health status on the variability of parasite community.

The values of prevalence, intensity, mean abundance for each parasite are given in Table 3. *C. tilapiae*, *E. heterostomum, C. aegypticus*, *D. spathaceum*, *P. minimum*, *M. appendiculatus, H. cahirinus*, *A. tilapiae,* and larval stages of *P. clarias* were found in the three localities (unpolluted, slightly polluted and heavily polluted).

Table 3. Metazoan parasite species with their main eco-parasitological characteristics.

Parasite	CLAR ponds			Abbassa privet ponds			Manzalla ponds		
	I	A	P (%)	I	A	P (%)	I	A	P (%)
Clinostomum tilapiae	2.3 (1-6)	1.0	41.0	7.1 (1-28)	3.6	51.0	4.8 (1-18)	2.8	59.0
Euclinostomum heterostomum	14.7 (1-58)	13.4	94.0	8.7 (1-24)	0.7	8.0	0.0	0.0	0.0
Cichlidogyrus aegypticus	5.4 (1-24)	2.6	48.5	1.6 (1-3)	0.4	23.0	17.3 (1-80)	11.0	63.4
Diplostomum spathaceum	1.7 (1-3)	0.2	11.4	1.5 (1-2)	0.1	10.2	1.6 (1-5)	0.5	34.1
Posthodiplostomum minimum	8.4 (1-47)	4.3	51.4	30.0 (2-93)	28.7	94.8	5.5 (1-18)	4.1	75.6
Mesostephanus appendiculatus	12.2 (3-31)	1.7	11.4	25.6 (1-98)	13.8	53.8	18.0 (1-83)	6.6	36.6
Haplorchoides cahirinus	2.4 (1-5)	0.3	14.0	10.0 (1-67)	5.6	56.0	1.7 (1-7)	0.4	24.0
Polyonchobothrium clarias	0.0	0.0	0.0	1.00 (1)	0.03	3.0	0.0	0.0	0.0
Acanthosentis tilapiae	14.7 (1-58)	13.4	94.0	8.7 (1-24)	0.7	8.0	0.0	0.0	0.0

I: Intensity, A: abundance, and P: prevalence.

Table 4. Comparison between the total mean values of intensity, abundance and prevalence in the three ponds using one way ANOVA.

Parameter	Different locations			P value	$F_{2,26}$
	CLAR ponds	Abbassa privet ponds	Manzalla ponds		
Intensity (I)	6.87	10.47	5.43*	P<0.01	8.55
Abundance (A)	4.1	5.96	2.8*	P<0.01	5.25
Prevalence (P)	40.63%	34.2%	9.7%*	P<0.01	7.99

*Significant differences between locations using one way ANOVA, P<0.01.

Table 5. Values of diversity, richness of species, equitability and dominance.

Site	Diversity	Richness of species	Equitability	Dominance	Dominance
CLAR ponds	1.64 (± 0.11)	2.1 (± 0.18)	0.68 (± 0.04)	0.29 (± 0.04)	0.547 (± 0.44)
Abbassa privet ponds	1.55 (± 0.10)	1.81 (± 0.15)	0.62 (± 0.01)	0.27 (± 0.03)	0.446 (± 0.35)
Manzalla ponds	1.52 (± 0.06)	1.6 (± 0.10)	0.66 (± 0.03)	0.28 (± 0.02)	0.500 (± 0.40)

In the heavily polluted site (Manzalla ponds), the prevalence was the highest in *P. minimum*, *C. aegypticus*, and *C. tilapiae* (75.6, 63.4 and 59%, respectively) and it was less in *H. cahirinus*, *D. spathaceum* and *M. appendiculatus* (24, 34.1 and 36.6%, respectively), while prevalence was 0% *in* both *E. heterostomum*, *A. tilapiae* and *P. clarias*.

In the slightly polluted site, Abbassa privet ponds, the prevalence was high in both *P. minimum*, *H. cahirinus*, *M. appendiculatus* and *C. tilapiae* (94.8, 56, 53.8 and 51%, respectively), it was decreased (23%) in *C. aegypticus* where it was minimum in the other species.

In the unpolluted pond, the prevalence was the high in *A. tilapiae*, *E. heterostomum*, *P. minimum*, *C. aegypticus* and *C. tilapiae* while it was 0% in the case of *P. clarias*.

Table 4 shows the significant differences between the data obtained from the Manzalla ponds and the other studied locations concerning intensity, abundance and prevalence using one way ANOVA. The total intensity value of the parasitic infection in Manzalla ponds was significantly low (5.43) as compared to 6.87 and 10.47 in CLAR ponds and Abbassa privet ponds respectively. The same trend of significance was recorded in the total abundance and prevalence values of parasitic infection between the three selected ponds in this study

Values of Margalef, Shannon-Wiener, dominance, Simpson's and Berger-Parker indices are shown in Table 5 which show that, the mean values of parasite species richness and diversity, according to Margalef and to Shannon-Wiener indices, showed significant increase from the polluted Manzalla ponds (1.52) to the unpolluted site CLAR ponds (1.64) which present the highest dominance value Simpson dominance (0.547), for the Berger-Parker index, the values are not structured along the pollution gradient.

DISCUSSION

Aquatic pollution is still a problem in many fresh and marine environments, it has a negative effects on the health of the respective organisms (Fent, 2007). According to their effects, pollutants can either be lethal or sub-lethal. The effects may manifest immediately (acute toxicity) or after prolonged exposure to the pollutant (chronic toxicity) (Sures, 2008). The number of studies investigating effects of pollutants and occurring parasites is still relatively low (Sures, 2006, 2007). Environmental stress has been suggested to increase host susceptibility to infections and reduce host ability to resist parasite growth and reproduction, thus benefiting parasites. This prediction stems from expected costs of immune defense; hosts in poor condition should have less resource to be allocated to immune function.

As a contribution to the ongoing debate on the role of environmental parameters on parasite community structure, this study provides evidence on the effect of water quality both on the level of parasite species richness as well as on the degree of interactivity of the component communities (Lio-Po and Lim, 2002).. Each parasite can interact differently with each stresses (Lafferty, 1997). In the present study, the monogenean *C. aegypticus* shows a good resistance to pollution stresses affecting other more sensitive gill parasites, while the digeneans *E. heterostomum* and *P. clarias*, are absent in the severely polluted site, but show highest levels of infection indices in the moderately polluted site. These values could be attributed to the use of a common intermediate host, the gasteropod pulmonate *Lymnaea stagnalis*, that is sensitive to chemical contaminants but tolerating a high organic pollution (Girod et al., 1980). The pollution, in this case, influenced the prevalence and the abundance of these parasites through the abundance of their intermediate host (Kiely et al., 2004). Even the digenean *P. minimum*; *M. appendiculatus* and *C. tilapiae* appeared to prefer habitats with moderate water pollution (94.8, 56.0 and 51.0%, respectively of *O. niloticus* were infected by these species), so that both unpolluted and polluted sites were less suitable for these species. Moderate organic pollution (Abbassa privet ponds) could increase the number of macrobenthic species, probable intermediate parasite hosts, and therefore the numbers of parasites themselves. Clearly, due to the stressing effects of pollution, fish defense responses against pathogenic agents, including parasites, could be reduced, making digenean easier.

Sindermann (1979) summarized some of the recent supporting evidence that toxins have a deleterious effect on the immune response of fishes. This study of Biscayne Bay fishes suggests that, in the presence of sub-lethal quantities of pollutants in a natural marine environment, fish suffered from gill damage which produced stress, physiological and physical compensation, leading to weakening, reduced immunity, and heavy parasitic infestation. It is very important to mention that the statistical analysis using one way analysis of variance (ANOVA) of the total values of intensity, abundance and prevalence between the tree water bodies indicated that the significantly lower values of these parameters in Manzalla ponds reflects the strong stressful impact of pollution on the parasitic communities on *O. niloticus* as compared to the two other sites. According to Hoffman (1976), eutrophication and pollution probably affect helminthes parasites as well as the hosts. The acanthocephalan *A. tilapiae* was collected only in the unpolluted sites. The absence of *A. tilapiae* in polluted ponds can be attributed to the sensitivity of the intermediate host, the *Cyclop* and *Mesocyclop strenus* which is known to be highly affected by pollution.

According to Mojetta (1998), cyclops show a high density in the CLAR ponds and Abbassa privet ponds and are completely absent in the Manzalla ponds sampled sites. Species richness and diversity indices show that parasite communities are affected by the different status of environmental conditions. For instance, when comparing the values of the Shannon Index with total coliforms the Pearson correlation value is 0.98 when comparing the three sites, while this value decreased to 0.78. By excluding site 3, the value rises to 0.99. This finding indicates that the differences between sites 1, 2 and 3 are not as striking.

The present data suggest that, parasite sensitivity to water pollution can be considered as indicators of water quality. Parasites represent an ubiquitous component of animal communities and are more abundant than their hosts.

In addition, a large number of parasite species require, to complete their life cycle, many types of verte-brate and invertebrate organisms acting as intermediate or definitive hosts; therefore, changes in the structure of a parasitic community reflect differences in the composition of the aquatic species (phyto and zooplankton, and benthos) commonly used as indicators of water quality. Parasites move through the food web and are situated at its top, integrating the adverse effects of various contaminants. The pollution ecology of many parasites remains unknown and not all host fishes are good models for environmental research. On the other hand, parasite species that are directly or indirectly sensitive to pollution have been found to disappear as the pollution levels increase, and may be considered good indicators for early detection of adverse environmental effects. Despite these contrasting pieces of evidence, the analysis of freshwater fish parasites could offer a useful and reliable indication or monitor of environmental quality in our case.

Conflict of Interests

The authors have not declared any conflict of interests.

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