Fish hepatic glutathione-S-transferase activity is affected by the cestode parasites *Schistocephalus solidus* and *Ligula intestinalis*: evidence from field and laboratory studies

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SUMMARY

The activity of hepatic glutathione-S-transferase (GST) was analysed in 3 different fish species with respect to fish sex and infection with parasites. In both sexes of laboratory bred three-spined sticklebacks (*Gasterosteus aculeatus*) experimentally infected with *Schistocephalus solidus* (Cestoda), a significantly lower GST-activity was found for infected fish compared to control. After field sampling of roach (*Rutilus rutilus*) from Lake Müggelsee (MS) and the Reservoir Listertalsperre (LTS), the GST-activity showed significantly lower values for males infected with *Ligula intestinalis* from MS (25%) and for infected females from LTS (55%). *L. intestinalis*-infected female chub (*Leuciscus cephalus*) from LTS also appeared to have a lower GST-activity. Thus, it could be shown that the presence of parasites significantly affects GST-activity in different fish species resulting in a decreased GST-activity due to infection. Our results therefore emphasize the need for more integrative approaches in environmental pollution research to clearly identify the possible effects of parasites in an effort to develop biomarkers for evaluating environmental health.

Key words: *Ligula intestinalis*, *Schistocephalus solidus*, environmental pollution, stickleback, roach, chub.

INTRODUCTION

The variety of pollutants in the aquatic environment continues to increase, necessitating means to detect and assess the impact of pollution. Particularly low concentrations of chemicals, as well as complex pollutant mixtures, are difficult to detect by applying only chemical analyses. This has generated the idea of using molecular biotic indicators (biomarkers) to unravel exposure to, and effects of, contaminants on organisms (Livingstone *et al*. 1994). However, the evaluation of biomarkers for environmental contaminants is usually done in laboratory experiments which inherently lack complex environmental interactions. Considering that biomarkers are also applied under field conditions, the artificial nature of laboratory analyses can be compensated for, as it should be with natural stressors which may influence a biomarker response.

Total hepatic glutathione-S-transferase (GST) activity has been a commonly applied biomarker for assessing different groups of pollutants for several years. The GST enzyme is an important intracellular enzyme belonging to the second stage of xenobiotic metabolism by virtue of catalysing the conjugation of the tripeptide glutathione and electrophilic substances of exogenous origin (Eaton and Bamler, 1999). In contrast to a wealth of reports on the modulation of GST-activity in organisms by chemicals, only a few studies have been published dealing with the impact of parasites on GST-activity in fish – showing an increased anti-oxidant response in carp parasitized by the cestode *Ptychobothrium* sp. (Dautremepuits *et al*. 2002, 2003). Another parasite known for its significant impact on common biomarkers used in aquatic ecotoxicology is the cestode *Ligula intestinalis*. Due to its most striking effect, the inhibition of fish host's sexual maturation, previous work focused on biomarkers for endocrine disruption such as vitellogen in bream (*Abramis brama*), chub (*Squalius cephalus*) and roach (*Rutilus rutilus*) (Hecker and Karbe, 2005; Schabuss *et al*. 2005; Hecker *et al*. 2007; Geraudie *et al*. 2010; Trubiroha *et al*. 2009, 2010, 2011). With regard to using a laboratory model to investigate host-parasite interactions under controlled conditions, three-spined stickleback (*Gasterosteus aculeatus*) infected with

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S. solidus is recommended (Barber and Scharsack, 2010). An additive effect of Cd exposure and parasite infection was already demonstrated by decreased survival rates compared with uninfected fish (Pascoe and Cram, 1977; Pascoe and Woodworth, 1980). Both parasites are characterized by a 3-host life cycle involving copepods as first intermediate hosts, fish as second intermediate hosts and birds as final hosts (Dubinina, 1980). The parasitic stage in the fish intermediate host, the so-called plerocercoid is located in the body cavity, usually of cyprinids for *L. intestinalis* and the three-spined stickleback for *S. solidus*. Roach (Van der Oost *et al*. 1994; Machala *et al*. 2000), chub (Vigano *et al*. 1998; Krca *et al*. 2007; Havelkova *et al*. 2008) and three-spined sticklebacks (Sanchez *et al*. 2007, 2008; Björkblom *et al*. 2009) are recently used as sentinels for freshwater monitoring in biomarker studies. However, the knowledge of parasites as modulators of GST-activity in fish hosts is rare despite parasites being a factor commonly found in every fish population. Therefore we investigated the influence of infections with the diphyllobothridean cestodes *S. solidus* and *L. intestinalis* on GST-activity of their intermediate fish hosts *G. aculeatus, R. rutilus* and *S. cephalus*.

MATERIALS AND METHODS

Sampling design

Three different host-parasite systems were investigated (Table 1). In order to evaluate effects of parasites under controlled conditions, three-spined sticklebacks experimentally infected with *S. solidus* were chosen as a laboratory host-parasite system (LAB). To transfer our results into the wild, cyprinids (i.e. roach and chub) naturally infected with *L. intestinalis* were chosen. They were collected from 2 sites in Germany where fish are known to be infected with the respective parasites. One sampling site was located at Lake Mueggelsee (MS) (5°26′N, 13°39′E), a polymictic and eutrophic shallow lake (surface area of 7·3 km^2, mean depth of 5 m) in the area southeast of Berlin which is flushed by the river Spree (Driescher *et al*. 1993). The second sampling site was at the Reservoir Listertalsperre (LTS) (51°5′38″N, 7°50′15″E), a mesotrophic dam built in 1912 (surface area of 0·79 km^2, max depth of 39 m) south of Meinerzhagen. At both sampling sites *L. intestinalis*-infected roach were collected; additionally, chub infected with this parasite were also caught at LTS. Accordingly, this sampling design allowed us not only to compare the same host-parasite system from different independent sites but also to compare the effects of the same parasite on 2 different hosts.

Fish collection and tissue sampling

Three-spined sticklebacks (*Gasterosteus aculeatus*) originating from the lake Großer Plöner See were bred in the laboratory and experimentally infected with *Schistocephalus solidus* as described by Scharsack *et al*. (2007), based on Smyth (1946). After 2 weeks of acclimatization in an 80 L aquarium, 44 fish were held under constant conditions for 10 weeks in aerated tap water at a water temperature of 17 °C and a photo-period of 12 h light:12 h dark. Uninfected and infected sticklebacks were maintained together in the same aquaria and fed 3 times a week with tubificids; the water was changed twice weekly.

Roach (*Rutilus rutilus*) were caught by electrofishing from MS during autumn 2007 and dissected within the capture day. Roach and chub (*Squalius syn. Leuciscus cephalus*) from the water reservoir Listertalsperre were caught by electrofishing in June 2008 and transferred to the institute laboratory in aerated tanks. Roach were held in a 200 L aquarium with aerated tap water at approximately the same water temperature as in the reservoir (22 °C) until they were dissected within the next 3 days. In order to reduce variations due to catching stress, chub were held for 7 weeks in the laboratory at a photo-period of 12 h light:12 h dark before being killed and dissected. During maintenance the water was changed once a week and fish were fed 5 times a week with tubificids.

For sampling, the dissecting procedure was the same for all fish species. After anaesthetization with ethyl 3-aminobenzoate methansulfonate (MS222, Sigma) the fish were decapitated. The fish were measured (to the nearest mm) and weighed (to the nearest mg); organs and parasites were removed, weighed (to the nearest mg) and immediately frozen in liquid nitrogen. All samples were stored at −80 °C until further analysis was performed.

GST analyses

Liver samples (60 mg) were homogenized with a sonicator (Sonoplus HD 2070, Bandelin, Germany) in adequate volumes of phosphate buffer (50 mM KH_2PO_4, 1 mM EDTA, pH 7·0) and centrifuged at 10 000 ***g*** for 10 min at 4 °C. The glutathione-S-transferase-activity was evaluated using the GST Assay Kit from Sigma-Aldrich (Germany) utilizing 1-chloro-2,4 dinitrobenzene (CDNB) as substrate – as previously described by Habig *et al*. (1974). The measurements were performed with the supernatant fraction kept at 25 °C within 6 min using a microplate reader (Infinite M200, Tecan, Austria). The total protein concentration was determined in each supernatant fraction using the Pierce BCA Protein Assay Kit (Pierce Biotechnology, Rockford, Illinois, USA).

Data analysis and statistics

The following morphological and parasitological indices were calculated: the gonadosomatic index (GSI) as fish gonad mass/fish somatic mass × 100, the condition factor (CF) as fish somatic mass × 100/fish total length^3, and the parasitization index (PI) as

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Table 1. Morphological parameters for host fish

(Abbreviations: HSI, hepatosomatic index; GSI, glutathione-S-transferase-activity; CF, condition factor; PI, parasitization index; TL, total length; GSI, gonadosomatic index. Data are given as mean (± S.D.). Values not sharing a common letter for the same parameter from the same site are statistically different from each other (Student's *t*-test, $P<0{\cdot}05$), with *n*, number and na, not analysed. Within each sex group the uninfected fish (uninf) were compared to the infected ones (inf). Comparing the different sexes, only the individuals with the same infection status were analysed.)

Sites	Sex (uninf/inf)	*n*	TL (cm)	CF	HSI	GSI	PI
LAB (stickleback)	M (uninf)	5	4·15 (0·32)^{a}	0·72 (0·03)^{a}	3·26 (0·50)^{a}	na	
	M (inf)	4	4·44 (0·53)^{ab}	0·65 (0·02)^{b}	2·62 (0·33)^{b}	na	37·51 (5·02)^{a}
	F (uninf)	6	4·88 (0·32)^{b}	0·74 (0·03)^{cab}	4·21 (0·50)^{cb}	na	
	F (inf)	14	4·51 (0·45)^{ab}	0·68 (0·07)^{dab}	2·62 (0·43)^{dab}	na	37·28 (6·53)^{a}
MS (roach)	M (uninf)	10	14·79 (2·50)^{ab}	0·82 (0·07)^{a}	na	3·11 (1·28)^{a}	
	M (inf)	11	13·75 (0·93)^{a}	0·76 (0·05)^{a}	na	0·41 (0·13)^{b}	10·68 (4·09)^{a}
	F (uninf)	11	14·85 (0·9)^{ab}	0·88 (0·12)^{a}	na	5·58 (1·96)^{c}	
	F (inf)	13	15·14 (1·74)^{b}	0·77 (0·08)^{a}	na	1·08 (0·36)^{d}	9·50 (1·90)^{a}
LTS (roach)	M (uninf)	5	8·58 (0·78)^{a}	0·95 (0·09)^{ab}	1·38 (0·13)^{a}	0·47 (0·21)^{a}	
	M (inf)	5	9·48 (0·82)^{ab}	0·88 (0·04)^{a}	1·21 (0·30)^{a}	0·36 (0·20)^{a}	9·97 (4·82)^{a}
	F (uninf)	5	9·93 (0·22)^{b}	0·90 (0·04)^{ab}	1·32 (0·13)^{a}	0·61 (0·24)^{a}	
	F (inf)	5	10·01 (1·06)^{ab}	1·04 (0·13)^{b}	1·27 (0·32)^{a}	0·55 (0·14)^{a}	9·51 (4·01)^{a}
LTS (chub)	M (uninf)	9	9·18 (0·70)^{a}	0·77 (0·03)^{a}	1·01 (0·35)^{a}	0·25 (0·09)^{a}	
	M (inf)	6	8·35 (0·65)^{b}	0·73 (0·07)^{ab}	1·64 (0·62)^{b}	0·43 (0·41)^{a}	15·75 (5·92)^{a}
	F (uninf)	5	8·66 (0·68)^{ab}	0·72 (0·02)^{b}	1·20 (0·29)^{ab}	0·32 (0·17)^{a}	
	F (inf)	9	8·82 (1·01)^{ab}	0·75 (0·10)^{ab}	1·24 (0·71)^{ab}	0·29 (0·10)^{a}	19·85 (9·22)^{a}

parasite mass/fish somatic mass × 100. For GSI and CF, fish somatic mass was determined without parasite mass. As all data were normally distributed, pair-wise analysis was done by Student's *t*-test. Significance was accepted when $P \leqslant 0{\cdot}05$.

After confirming normality and homogeneity of variance with Kolmogorov-Smirnow test and Levene test, respectively, the differences among GST-activities were assessed using a univariate analysis of variance (ANOVA) with GST-activity as dependent factor and host sex and infection status as independent factors. For the analysis of roach data from the field the sampling site was used as an additional independent factor. When necessary, the ANOVA was followed by Student's *t*-test to compare the uninfected fish with the infected ones within the same sex and site.

Spearman's rank correlation coefficient was used to investigate the associations between morphological parameters/indices and GST-activity, if a sufficient number of data points was given ($n>6$). Accordingly, a lack of significant correlations might simply be the result of a low number of data pairs.

All statistical analyses were performed using Statistica 6.0; except the analysis of variance including the Kolmogorow-Smirnow test and the Levene test that were performed with SPSS, PASW 18.

RESULTS

Meristic parameters

Total length, CF, HSI, GSI and PI of all fish are summarized in Table 1. Fish of each group consistently showed a similar size range; although significant differences occurred between groups. In the groups LAB and LTS, the uninfected females were significantly larger than the uninfected males; for MS this was also the case for infected fish. For LTS-chub a difference was only found between uninfected and infected males; with infected fish being significantly smaller. Roach from MS (Min/Max = 10·8/19·6 cm) were significantly larger than roach from LTS (Min/Max = 7·3/11·8 cm).

For sticklebacks, CF and HSI showed reduced values due to infection in both genders. Only infected male chub from LTS had a higher HSI than the uninfected males. In roach from MS a significant reduction of GSI by parasite infection in both genders occurred. Furthermore the GSI for MS females was higher than for males.

For sticklebacks, significant correlations were only found in infected females, for which CF showed a negative relationship with parasite burden ($r=-0{\cdot}56$). For MS, negative correlations were found between CF and PI for both genders ($r=-0{\cdot}67$ (female); $-0{\cdot}64$ (male)), albeit the CF difference between the uninfected and infected fish of each sex was not significant. Roach from LTS showed a negative correlation between CF and HSI for infected females ($r=-0{\cdot}93$). For uninfected male chub the CF showed significant correlations with the total length ($r=0{\cdot}72$), whereas the CF of infected females was significantly correlated with the GSI ($r=-0{\cdot}67$).

Sticklebacks showed the highest PI (37%), without differences between sexes. The PI was lowest for

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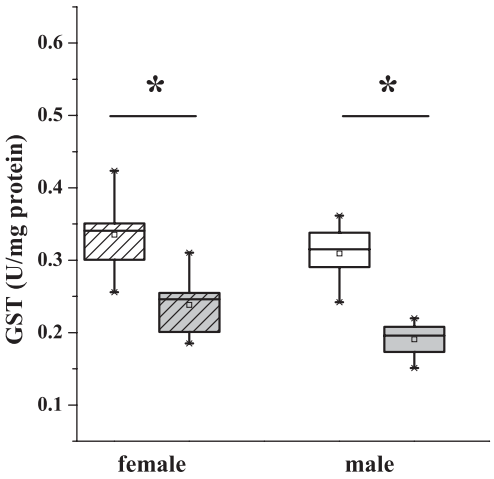

Fig. 1. GST-activity (U/mg protein) of three-spined sticklebacks differentiated by fish sex and infection status (female, striped boxes; male, empty boxes; uninfected, white boxes and *S. solidus*-infected, grey boxes). Stars indicate significant differences for infection status (*ANOVA; F = 39·284; $P < 0·001$). Each box represents the interquartile range (box range: 25–75% percentile; whisker range: 1–99% of values) with the empty dot showing the mean and the horizontal line showing the median. The asterisks show the minimum and maximum values of the dataset. Numbers are listed in Table 1.

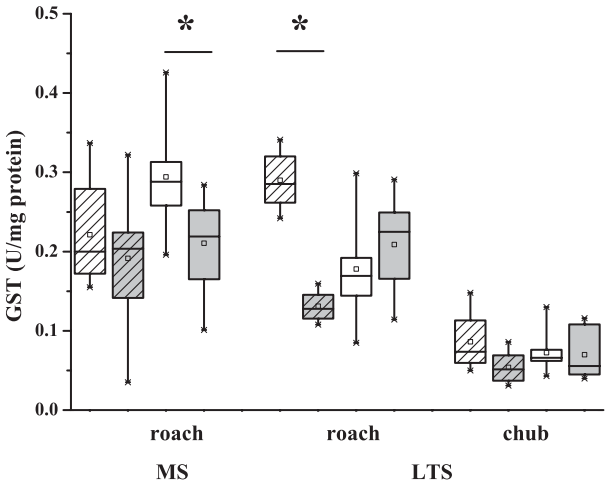

Fig. 2. GST-activity (U/mg protein) of roach from MS and LTS and of chub from LTS; each time differentiated by sex (female, striped boxes; male, empty boxes) and infection (uninfected, white boxes; *L. intestinalis*-infected, grey boxes). Stars indicate significant differences between groups ($P < 0·05$, *t*-test). Each box represents the interquartile range (box range: 25–75% percentile; whisker range: 1–99% of values) with the empty dot showing the mean and the horizontal line showing the median. The asterisks show the minimum and maximum values of the dataset. Numbers are listed in Table 1.

roach with approximately 10% for both sites and sexes. Chub infected with the same parasite as roach showed a PI around 17%.

Total hepatic GST-activity and correlation with morphological parameters

Hepatic GST-activities in sticklebacks are presented in Fig. 1. Analysis of variance indicated a slight significant effect of host sex (F = 4·591; $P = 0·042$) on stickleback's GST-activity with females showing 8–18% higher values than males. However, the effect of infection with *S. solidus* plerocercoids was found to be highly significant (F = 39·284; $P < 0·001$). The extent of reduction on GST-activity was the same in both host sexes (39%). The only significant correlation found for sticklebacks was a positive relationship between GST-activity and HSI in infected females (r = 0·65).

Hepatic GST-activities in roach are presented in Fig. 2. Analysis of variance indicated no effect of sampling site (F = 3·253; $P = 0·078$) or host sex (F = 1·803; $P = 0·186$). Infection with *L. intestinalis* was the only factor causing a significant difference in GST-activity of roach (F = 12·295; $P \leqslant 0·001$). The small but significant interaction between host sex and infection status (F = 4·764; $P \leqslant 0·05$) indicated 32% higher GST-activities for infected male roach in comparison to infected females. The differences between GST-activities within the same sex were different between sites (F = 6·095; $P \leqslant 0·05$), whereas the level of difference between infected females or males, respectively, was the same for both sites (F = 0·477; $P = 0·493$). Because of the significant interaction between host sex, infection status and site (F = 16·434; $P \leqslant 0·001$) a Student's *t*-test was performed between each pair of uninfected and infected roach of each sex per site, indicating that the significant reduction of GST-activity occurred at site MS only for males (25%) and at site LTS only for females (55%).

For infected females from MS, the CF was positively correlated with the GST-activity (r = 0·62). For roach from LTS the only correlation between GST-activity and fish size was found for infected females (r = −0·92). At site LTS, roach were not the only fish analysed. Similar to LTS roach, the LTS chub showed a 35% reduction in GST-activity only for infected chub females. However, this difference was not significant (F = 1·549; $P = 0·225$) (Fig. 2). The GST-activity of infected chub females was significantly correlated with the GSI (r = 0·75). For male chub the GST-activity showed significant correlations with the total length (r = 0·73 (uninfected); 0·82 (infected)).

In summary, while the results for *S. solidus* revealed a significant reduction of hepatic GST-activity in both sexes of infected sticklebacks, the impact of *L. intestinalis* was not the same for the analysed cyprinids. For female roach and chub at LTS, reduced GST-activities were found. On the contrary, for MS roach a reduced GST-activity was detected only for infected males.

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DISCUSSION

Our results confirm the previously held assumption of parasites being a natural stressor affecting biomarker response in fish. In the present study, hepatic glutathione-S-transferase (GST)-activity was reduced due to parasite infection in 3 different host-parasite systems. The experiment with laboratory bred and laboratory infected fish showed a significant reduction of GST-activity in sticklebacks maintained under controlled conditions. In addition, the comparison of similar host-parasite systems from 2 different field sites, as well as the comparison of 2 different host-parasite systems from the same site, indicates the interference of cestode plerocercoids with host GST-activity as a general effect. As fish were caught at different seasons of the year fish maturation and thus the stage of gonad development may explain part of the differences between roach caught at different sites.

Even if infected fish can be disregarded in biomarker studies due to the obvious presence of large plerocercoids, there is a wealth of parasites living in nearly every organ of the host. Parasitological studies show that under natural conditions almost every fish is infected with at least 1 parasite species (Lamková *et al*. 2007; Nachev and Sures, 2009). This will certainly also affect the host's physiological homeostasis and thus might change biomarker levels (Sures, 2008). With respect to cestodes, most adult species are dwelling in the intestines of fish. This organ is usually not checked for the presence of parasites when the expression of biomarkers (e.g. in the liver) is analysed. However, Dautremepuits *et al*. (2003) observed an increased GST-activity in carp parasitized by the intestinal cestode *Ptychobothrium* sp. when compared with uninfected fish. Because of the lack of contact between the parasite and the analysed organ, it was suggested that the observed increase in different anti-oxidant defences (liver catalase-, activities of GST and glutathione reductase) in infected carp is due to the modulation of metabolic activity by the parasite. A further possibility involves the parasite's release of molecules in order to suppress its host's oxidative response or other actions of the immune system, respectively. A broad range of enzymes and other molecules secreted by helminths is known to subvert the host immune defence in order to maintain a long-term persistence within the host (Dzik, 2006). Examples are given for helminths secreting GST enzymes or presenting them on their body surface to ensure parasite survival by neutralizing the toxins acting against them. For cestodes, GSTs are suggested as biochemical systems that protect the parasites against the host's immune attack (Brophy and Pritchard, 1992). Barber and Scharsack (2010) mentioned that *S. solidus* is apparently capable of manipulating (evading) immune traits that are specifically directed against parasite antigens. To what extent the possible secretion/presentation of parasite GST is linked with the host's GST response is not clear. As we only analysed the GST-activity, it is impossible to distinguish between a reduced enzyme synthesis and a reduced enzyme activity due to blocked binding sites. However, reduced hepatic GST-activity has also been reported for 2 other host-parasite systems. Infection with either *Dicrocoelium dendriticum* or *Fasciola hepatica* is known to reduce hepatic GST-activity in their respective final hosts (Galtier *et al*. 1983; Skálová *et al*. 2007). Both parasites are known to live for many years in the bile ducts of their host's liver. It was recently shown that a sigma class glutathione transferase (rFhGST-si), a recombinant form of an *F. hepatica* secreted molecule, modulates host immunity by suppressing responses associated with chronic inflammation (Dowling *et al*. 2010). This confirms the notion that there are additional factors influencing the observed reduction of GST-activity other than only incidental side-effects (for example liver injury or nutritional drains).

The driving force for biomarker research is their possible use to study pollution effects in animals from the field. However, all populations of aquatic organisms are usually infected with parasites (Kuris *et al*. 2008). Van der Oost *et al*. (2003) reviewed more than 40 papers dealing with the effects of various pollutants on GST-activity in different fish species under laboratory conditions and in the wild. By and large, the authors reported conflicting results concerning the correlation between pollution and GST-activities in fish. According to our results on GST-activity in sticklebacks, we showed that significant differences between sticklebacks were not observable before we considered infection with *S. solidus*. This underlines the extent of a parasite's impact on the use of biomarkers. Thus, for environmental studies, it is not only difficult to find pristine reference conditions in order to describe the expression level of a biomarker at physiological homeostasis, but it is also important to evaluate the effects of parasites on the level of biomarkers in fish and to describe biomarker levels of infected, unexposed fish. In summary, it is conceivable that even more parasites are interacting with different physiological parameters commonly used as biomarkers for pollution. Therefore it is difficult to understand why exposure studies are usually done without considering parasites, despite repeated demonstration of their effects on the physiological homeostasis of their hosts (e.g. Sures *et al*. 2006; Sures, 2008). The results of the present study accentuate the need for more integrative approaches to environmental pollution research, similarly raising the question as to how the two parasites manipulate their host's physiology.

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REFERENCES

Barber, I. and Scharsack, J.P. (2010). The tree-spined stickleback-*Schistocephalus solidus* system: an experimental model for investigating host-parasite interactions in fish. *Parasitology* **137**, 411–424.

Björkblom, C., Högfors, E., Salste, L., Bergelin, E., Olsson, P.E., Katsiadaki, I. and Wiklund, T. (2009). Estrogenic and androgenic effects of municipal wastewater effluent on reproductive endpoint biomarkers in three-spined stickleback (*Gasterosteus aculeatus*). *Environmental Toxicology and Chemistry* **28**, 1063–1071.

Brophy, P.M. and Pritchard, D.I. (1992). Immunity to helminths: Ready to tip the biochemical balance? *Parasitology Today* **8**, 419–422.

Dautremepuits, C., Betoulle, S. and Vernet, G. (2002). Antioxidant response modulated by copper in healthy or parasitized carp (*Cyprinus carpio* L.) by *Ptychobothrium* sp. (Cestoda). *Biochimica et Biophysica Acta* **1573**, 4–8.

Dautremepuits, C., Betoulle, S. and Vernet, G. (2003). Stimulation of antioxidant enzymes levels in carp (*Cyprinus carpio* L.) infected by *Ptychobothrium* sp. (Cestoda). *Fish and Shellfish Immunology* **15**, 467–471.

Dowling, D.J., Hamilton, C.M., Donnelly, S., La Course, J., Brophy, P.M., Dalton, J. and O'Neill, S.M. (2010). Major secretory antigens of the helminth *Fasciola hepatica* activate a suppressive dendritic cell phenotype that attenuates Th17 cells but fails to activate Th2 immune responses. *Infection Immunity* **78**, 793–801.

Driescher, E.H., Behrendt, H., Schellenberger, G. and Stellmacher, R. (1993). Lake Müggelsee and its environment – natural conditions and anthropogenic impacts. *International Revue der Gesamten Hydrobiologie und Hydrogeographie* **78**, 327–343.

Dubinina, M.N. (1980). *Tapeworms (Cestoda, Ligulidae) of the Fauna of the U.S.S.R.* Amerind Publishing Co., New Dehli, India.

Dzik, J.M. (2006). Molecules released by helminth parasites involved in host colonization. *Acta Biochimica Polonia* **53**, 33–64.

Eaton, D.L. and Bammler, T.K. (1999). Concise review of the glutathione S-transferases and their significance to toxicology. *Toxicological Sciences* **49**, 156–164.

Galtier, P., Battaglia, A., Moré, J. and Franc, M. (1983). Impairment of drug metabolism by the liver in experimental fascioliasis in the rat. *The Journal of Pharmacology and Pharmacology* **35**, 729–733.

Geraudie, P., Boulange-Leconte, C., Gerbon, M., Hinfrav, N., Brion, F. and Minier, C. (2010). Endocrine effects of the tapeworm *Ligula intestinalis* in its teleost host, the roach (*Rutilus rutilus*). *Parasitology* **137**, 697–704.

Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry* **249**, 7130–7139.

Havelkova, M., Blahova, J., Kroupova, H., Randak, T., Slatinska, I., Leontovycova, D., Grabic, R., Pospisil, R. and Svobodova, Z. (2008). Biomarkers of contaminant exposure in chub (*Leuciscus cephalus* L.) – Biomonitoring of major rivers in the Czech Republic. *Sensors* **8**, 2589–2603.

Hecker, M. and Karbe, L. (2005). Parasitism in fish – an endocrine modulator of ecological relevance? *Aquatic Toxicology* **72**, 195–2007.

Hecker, M., Sanderson, J.T. and Karbe, L. (2007). Suppression of aromatase activity in populations of bream (*Abramis brama*) from the river Elbe, Germany. *Chemosphere* **66**, 542–552.

Krca, S., Zaja, R., Calic, V., Terzic, S., Grubesic, M.S., Ahel, M. and Smital, T. (2007). Hepatic biomarker responses to organic contaminants in feral chub (*Leuciscus cephalus*) – laboratory characterization and field study in the Sava River, Croatia. *Environmental Toxicololgy and Chemistry* **26**, 2620–2633.

Kuris, A.M., Hechinger, R.F., Shaw, J.C., Whitney, K.L., Aguirre-Macedo, L., Boch, C.A., Dobson, A.P., Dunham, E.J., Fredensborg, B.L., Huspeni, T.C., Lorda, J., Mababa, L., Mancini, F.T., Mora, A.B., Pickering, M., Talhouk, N.L., Torchin, M.E. and Lafferty, K.D. (2008). Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature, London* **454**, 515–518.

Lamková, K., Simková, A., Paliková, M., Juradjida, P. and Lojek, A. (2007). Seasonal changes of immunocompetence and parasitism in chub (*Leuciscus cephalus*), a freshwater cyprinid fish. *Parasitology Research* **101**, 775–789.

Livingstone, D.R., Förlin, L. and George, S.G. (1994). Molecular biomarkers and toxic consequences of impact by organic pollution in aquatic organisms. In *Water Quality and Stress Indicators in Marine and Freshwater Systems: Linking Levels of Organization* (ed. Suthcliffe, D.W.), pp. 154–171. Freshwater Biological Association, Ambleside, Cumberland, UK.

Machala, M., Ulrich, R., Neca, J., Vykusova, B., Kolarova, J., Machova, J. and Svobodova, Z. (2000). Biochemical monitoring of aquatic pollution: Indicators of dioxin-like toxicity and oxidative stress in the roach (*Rutilus rutilus*) and chub (*Leuciscus cephalus*) in the Skalice river. *Veterinary Medicine* **45**, 55–60.

Nachev, M. and Sures, B. (2009). The endohelminth fauna of barbel (*Barbus barbus*) correlates with water quality of the Danube River in Bulgaria. *Parasitology* **136**, 545–552.

Pascoe, D. and Cram, P. (1977). The effect of parasitism on the toxicity of cadmium to the three-spined stickleback, *Gasterosteus aculeatus*. *Journal of Fish Biology* **10**, 467–472.

Pascoe, D. and Woodworth, J. (1980). The effect of joint stress on sticklebacks. *Zeitschrift für Parasitenkunde* **62**, 159–163.

Sanchez, W., Ait-Aissa, S., Palluel, O., Ditche, J.M. and Porcher, J.M. (2007). Preliminary investigation of multi-biomarker responses in three-spined stickleback (*Gasterosteus aculeatus* L.) sampled in contaminated streams. *Ecotoxicology* **16**, 279–287.

Sanchez, W., Katsiadaki, I., Piccini, B., Ditche, J.M. and Porcher, J.M. (2008). Biomarker responses in wild three-spined stickleback (*Gasterasteus aculeatus* L.) as a useful tool for freshwater biomonitoring: A multiparametric approach. *Environment International* **34**, 490–498.

Schabuss, M., Gemeiner, M., Gleiß, A., Lewis, J.W., Miller, I., Möstl, E., Schober, U., Tschulenk, W., Walter, I. and Grillitsch, B. (2005). *Ligula intestinalis* infection as a potential source of bias in the bioindication of endocrine disruption in the European chub *Leuciscus cephalus*. *Journal of Helminthology* **79**, 91–94.

Scharsack, J.P., Koch, K. and Hammerschmidt, K. (2007). Who is in control of the stickleback immune system: interactions between *Schistocephalus solidus* and its specific vertebrate host. *Proceedings of the Royal Society of London, B* **274**, 3151–3158.

Skálová, L., Krizová, V., Cvilink, V., Szotáková, B., Storkánová, L., Velík, J. and Lamka, J. (2007). Mouflon (*Ovis musimon*) dicrocoeliosis: effects of parasitosis on the activities of biotransformation enzymes and albendazole metabolism in liver. *Veterinary Parasitology* **146**, 254–262.

Smyth, J.D. (1946). Studies on tapeworm physiology 1. The cultivation of *Schistocephalus solidus* in vitro. *Journal of Experimental Biology* **23**, 47–70.

Sures, B. (2008). Host-parasite interactions in polluted environments. *Journal of Fish Biology* **73**, 2133–2142.

Sures, B., Lutz, I. and Kloas, W. (2006). Effects of infection with *Anguillicola crassus* and simultaneous exposure with Cd and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) on the levels of cortisol and glucose in European eel (*Anguilla anguilla*). *Parasitology* **132**, 281–288.

Trubiroha, A., Kroupova, H., Frank, S.N., Sures, B. and Kloas, W. (2011). Inhibition of gametogenesis by the cestodes *Ligula intestinalis* in roach (*Rutilus rutilus*) is attenuated under laboratory conditions. *Parasitology* **138**, 648–659.

Trubiroha, A., Kroupova, H., Wuertz, S., Frank, S.N., Sures, B. and Kloas, W. (2010). Naturally-induced endocrine disruption by the parasite *Ligula intestinalis* (Cestoda) in roach (*Rutilus rutilus*). *General and Comparative Endocrinology* **166**, 234–240.

Trubiroha, A., Wuertz, S., Frank, S.N., Sures, B. and Kloas, W. (2009). Expression of gonadotropin subunits in roach (*Rutilus rutilus*, Cyprinidae) infected with plerocercoids of the tapeworm *Ligula intestinalis* (Cestoda). *International Journal for Parasitology* **39**, 1465–1473.

Van der Oost, R., Beyer, J. and Vermeulen, N.P.E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology* **13**, 57–149.

Van der Oost, R., Van Gastel, L., Worst, D., Hanraads, M., Satumalay, K., Van Schoten, F.-J., Heida, H. and Vermeulen, N.P.E. (1994). Biochemical markers in feral roach (*Rutilus rutilus*) in relation to the bioaccumulation of organic trace pollutants. *Chemosphere* **29**, 801–817.

Vigano, L., Arillo, A., Melodia, F., Arlati, P. and Monti, C. (1998). Biomarker responses in cyprinids of the middle stretch of the river Po, Italy. *Environmental Toxicology and Chemistry* **17**, 404–411.