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Pollutant-induced effects on immunological and physiological interactions in aquatic host–trematode systems: implications for parasite transmission

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Abstract

Under conditions of pollution both host and parasite are susceptible to the pathogenic effects of toxicants, which in turn may result in detrimental changes to their immunological and physiological processes. Digenetic trematodes, which encompass species of both medical and economic importance, possess complex life cycles and are common parasites of both vertebrates and molluscs. The combined stress induced by pollution and parasitism influences the physiology of the host which can have implications not only on host survival but also on the functional biology of resident parasite populations. The present paper reviews the effects of pollutants on the immunology and physiology in both vertebrate and molluscan host–trematode systems and the implications for parasite transmission.

Introduction

The interaction between anthropogenic pollution and disease, which induces stress, is an increasing area of concern within the fields of environmental, veterinarian and medical sciences. Under the combined conditions of exposure to pollutant and parasitism, the 'fitness' of an organism, as measured by immunological and physiological processes, plays an important role in ensuring survival and reproductive potential at both the individual and population levels. 'Fitness' can readily be compromised by exposure to toxic pollutants and/or parasitism, which either directly affects or increases the vulnerability of an organism to any subsequent abiotic or biotic stressor. Conversely, a parasite may also be susceptible to a pollutant which can influence both its transmission and reproductive success, and in some cases the parasite can even be more sensitive to pollution than its host. The effect of parasitism on the host is a product of the localization of the parasite, the intensity of the infection, and the life-stage of the parasite (Stadnichenko, 1984). Superimposed on this is the realization that host–parasite interactions can be influenced by a range of biotic factors which can influence the transmission success of subsequent free-living parasite stages (Morley & Lewis, 2004). This has been eloquently shown in the relationships between trematodes and their vertebrate and invertebrate hosts. Digenetic trematodes, which comprise species of both medical and economic importance, possess complex life-cycles dependent on a molluscan first intermediate host and one or more vertebrate hosts with transmission occurring via a number of free-living stages. The majority of studies on toxicity using digeneans has focused on the pathology of medically and veterinary important species such as *Schistosoma mansoni* and *Fasciola hepatica* and the impact of toxicants which are commonly encountered by humans or livestock. The present review focuses on the effects which pollutants have on the physiological and immunological parameters in a range of host–trematode interactions, in both vertebrates and molluscs, and assesses the implications of pollution on parasite transmission and establishment.

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Molluscan–trematode interactions

Physiological interactions

Impaired survival of trematode-infected molluscs on exposure to pollutants has been extensively studied (Morley *et al.*, 2003a). In general, snails carrying a primary infection (sporocysts and rediae) have an increased and more rapid mortality than uninfected ones. However, this increase in susceptibility is influenced by a range of parameters. For example, laboratory infected snails are more susceptible than naturally infected ones (Guth *et al.*, 1977) and may demonstrate a species-dependent susceptibility to toxicant exposure (Morley *et al.*, 2004b), whilst overwintered naturally-infected *Lymnaea peregra* showed reduced survival under cadmium exposure compared to seasonally infected snails (Morley *et al.*, 2003b). Interestingly, Heinonen *et al.* (2001) found an increased survival and lethal body burden (concentration of xenobiotic inside an organism at the time of death) of infected compared to uninfected bivalves exposed to pentachlorophenol (PCP). It was suggested that these differences may be associated with the higher lipid content found in parasites which could change the internal distribution of PCP. The system is further complicated in that snails collected from polluted sites can be less susceptible to copper than snails collected from unpolluted sites (Misechko & Stadnichenko, 1988) which implies that this intermediate host might acclimate to the pollutant.

The impaired survival of infected molluscs in polluted conditions may be due to an alteration in toxicant accumulation in infected molluscs compared to uninfected ones. An increase in the accumulation of heavy metals and organic halogen compounds has been reported for infected freshwater molluscs compared to uninfected controls (e.g. Kraak & Davids, 1991; Pokora *et al.*, 1993; Pellinen *et al.*, 1994; Kirichuk *et al.*, 2002; Kirichuk & Stadnichenko, 2004). On the contrary, a decreased accumulation of heavy metals was reported for infected marine molluscs (Evans *et al.*, 2001). A variable temperature-dependent and trematode species-dependent accumulation occurs in freshwater molluscs exposed to hydrophobic organic chemicals (Heinonen *et al.*, 1999, 2000), whilst Cross *et al.* (2003), in contrast, found that the metal levels of *Littorina littorea* collected from a polluted site over time varied with snail sex and parasite infection. In general, infected snails showed reduced copper and iron concentrations and an increased zinc concentration compared to uninfected snails. However, snails infected with the more pathogenic *Cryptocotyle lingua* trematode compared with those infected with *Renicola roscovita* had lower copper concentrations. Recent investigations have speculated on a link between infection and the presence of metal-binding proteins in the molluscan host (Baudrimont *et al.*, 2003). The levels of these low molecular weight metal-binding proteins, such as metallothionein, play an important role in detoxification of toxic metals and the homeostasis of essential metals but may vary according to season and reproductive status (Leung *et al.*, 2000). Baudrimont *et al.* (2003, 2006) found that a trematode infection significantly interfered with the metallothionein synthesis of cockles (*Cerastoderma edule*) by affecting their reproductive cycle. These authors cited unpublished laboratory studies indicating that infected cockles accumulated higher quantities of cadmium than controls, and noted that this accumulation correlated with a lower production of metallothionein (Baudrimont *et al.*, 2003).

Changes in the accumulation rate of toxicants in combination with the stress of trematode infection in snails will result in a range of physiological effects on the host and a number of studies have documented changes in molluscan physiology under various biotic and abiotic stressors. For example, cardiac activity is significantly increased under the influence of trematode infections, which is correlated with changes in feeding behaviour and locomotor activity (Thompson, 1997). In contrast, heavy metals can decrease cardiac activity in molluscs (Cheng & Sullivan, 1973). Under the combined effect of pollutants (metals and synthetic detergents) and trematode infections, a reduction in the cardiac rate occurred in both bivalve and pulmonate molluscs compared with controls (Stadnichenko *et al.*, 1994; Vyskushenko, 2001). This decrease in cardiac activity is probably associated with a change in the metabolism of infected snails under polluted conditions which in turn may affect such physiological parameters as behaviour, feeding and respiration (e.g. Stadnichenko *et al.*, 1999). Stadnichenko & Kotsyuk (1990) found that exposure to detergents caused a decrease in the amount of food consumption with an increase in digestion time in *Lymnaea stagnalis* infected with *Echinostoma revolutum*. However, in uninfected snails there was an increase in food consumption accompanied by a large increase in the time of digestion. These authors suggested that the parasite may be inhibiting the ability of snails to adapt to the unfavourable conditions. Respiration can also be seriously compromised in toxicant exposed infected snails. Stadnichenko *et al.* (1992) found that lead nitrate intoxication caused a depression in the respiration of *Planorbarius corneus* with shortening of air intakes and an increase in intervals between them which was achieved more rapidly and prominently in infected than in uninfected snails. Similar results occurred in infected *L. stagnalis* exposed to high concentrations ($50–85\,mg\,l^{-1}$) of lead nitrate, but at a lower concentration ($15\,mg\,l^{-1}$) the snails demonstrated an 'defensive-adaptive reaction' with an increase in the length and rate of inspirations (Stadnichenko *et al.*, 1996a). Nevertheless, at this lead concentration oxygen diffusion across respiratory surfaces (lung and skin) is sharply reduced with a greater proportion of gaseous exchange occurring across the skin (Stadnichenko *et al.*, 1996b). In addition, Stadnichenko *et al.* (1995) found that the cilia locomotor function of the branchial respiratory system of infected *Anodonta cygnaea* was impaired by exposure to potassium nitrate, but only in females carrying a branchial pregnancy. The duration and activity of the movement of cilia were significantly reduced with increasing temperature or toxicant concentration.

The molluscan haemolymph is particularly susceptible to the effects of pollution, especially in snails harbouring a trematode infection. Toxicant exposure of infected compared to uninfected molluscs caused significant changes in haemolymph density, viscosity, pH, residual nitrogen content, and protein content (e.g. Stadnichenko *et al.*, 1987, 1993; Stadnichenko & Golovacheva, 1989,

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1990; Stadnichenko & Kirichuk, 2000, 2002). A large proportion of the protein content of molluscan haemolymph comprises respiratory pigments, either haemoglobin or haemocyanin. Respiratory pigments are known to readily bind to heavy metals under pollution conditions, increasing their oxygen affinity and thereby reducing the degree of cooperative oxygen binding (Martin & Rainbow, 1998; Leung *et al.*, 2000). Under the influence of trematode infections, concentrations of haemoglobin are reduced in snail haemolymph (Thompson, 1997). Stadnichenko *et al.* (1987, 1994, 2003) also noted a decrease in haemoglobin content in the haemolymph of pollutant-exposed infected snails compared with uninfected ones. Pollutants and infections appear to affect the respiratory capacity of the molluscan host, as shown by Stadnichenko & Golovacheva (1990) who found that infected molluscs, after exposure to surfactants, were unable to switch from aerobic to anerobic respiration as efficiently as uninfected molluscs and this subsequently led to an increase in molluscan mortality.

Infections and pollutants can also cause other effects on molluscan metabolism. Changes in the levels of aldolase, an enzyme involved in glycolytic (anaerobic) carbohydrate metabolism, have been observed in the digestive gland of infected snails (Marshall *et al.*, 1974; Narayanan & Venkateswara Rao, 1981), whilst toxicant exposure can lead to an increase in aldolase activity in some uninfected molluscan species (Moorthy *et al.*, 1985). Under the combined stress of toxicant and trematode parasite, Stadnichenko *et al.* (1988) found that exposure of *Planorbarius banaticus* to nitroammophoska (a common fertilizer used in the former USSR) caused an increase in aldolase activity, which was significantly greater in snails infected with *Cotylurus cornutus*. Such a high rate of carbohydrate expenditure resulted in large mortalities, especially in infected snails. In contrast, studies on the aerobic metabolism of PCP-exposed *Pisidium amnicum*, as measured using animal heat output, found increased metabolism but a decreased duration in the active periods with no apparent difference between responses in PCP-exposed infected and uninfected molluscs. However, the trematode infections did induce a general reduced level of metabolism (Heinonen *et al.*, 2003).

Ascorbic acid (vitamin C) is an essential nutrient for the maintenance of normal physiological functions in animal cells (Mai, 1998). However, for the majority of molluscs the dietary requirements of this vitamin, or indeed, whether molluscs can synthesize vitamin C is unknown (Mai, 1998). Infection with trematodes affects the ascorbic acid balance of molluscs with very high infections causing increased concentrations within the haemolymph along with a decrease in their reserves within the body tissues (Stadnichenko, 1984). This reaction appears to be affected by pollution as exposure to copper induces a decrease in the concentration of ascorbic acid in the haemolymph of both infected and uninfected *L. stagnalis* and *P. corneus* with a significantly greater decrease in concentration occurring in the snail tissue (Stadnichenko *et al.*, 1986). Phenol toxicity also leads to a decrease in ascorbic acid in the haemolymph which occurs more rapidly in infected compared with uninfected snails (Stadnichenko *et al.*, 1985a).

Carotenoid pigments have been widely studied in molluscs (Vershinin, 1996) although their exact function remains unknown. It has, however, been speculated that these pigments most probably function by stabilizing cell membrane fluidity (Vershinin, 1996). Molluscs with a high carotenoid concentration in their haemolymph are known to be strongly resistant to pollution (Karnaukhov *et al.*, 1977; Tewari *et al.*, 2001). However, in the presence of primary trematode infections the concentration of carotenoid pigments can decrease due to their consumption by the parasite and the necrotic decompostion of the parasite-damaged hepatopancreas (Stadnichenko, 1984). When uninfected and trematode-infected snails are exposed to phenol, a reduction in carotenoid concentrations occurs in both groups of snails, but this is more significant in infected snails, especially at lower concentrations (Stadnichenko *et al.*, 1985b). In contrast, exposure to copper sulphate showed that uninfected snails demonstrated a greater carotenoid reduction at low concentrations ($0.2\,mg\,l^{-1}$) than infected snails. This situation was reversed at high concentrations ($2\,mg\,l^{-1}$) with the highest carotenoid reduction occurring in infected snails (Misechko & Stadnichenko, 1988).

Immunological interactions

The combined effects of pollutants and trematode infections on the immune system of molluscs has been poorly studied, although it has been suggested that immunocomprised hosts lead to an increase in parasite prevalence in toxicant exposed snails (e.g. Morley *et al.*, 2002). Russo & Lagadic (2000) compared the immune response of *Lymnaea palustris* individually exposed to either pesticide, atrazine or hexachlorobenzene, or *Metaleptocephalus* sp., an immunologically compatible parasite. Infection with the trematode or exposure to atrazine generated similar responses from circulating haemocytes of *L. palustris*, whereas hexachlorobenzene-exposed snails showed a different pattern of immune response. However the trematode, in comparison to pesticide exposure, did not elicit a strong response from the circulating haemocytes, although some mobilization of immunocompetent haemocytes (hyalinocytes and granulocytes) had occurred.

Exposure of snails to silica or urethane, an anaesthetic, affected the number of circulating haemocytes (Granath & Yoshino, 1985; Martins-Souza *et al.*, 2003), but only silica exposure increased snail susceptibility to trematode infection. Interestingly, Martins-Souza *et al.* (2003) found a different response in two strains of *Biomphalaria tenagophila* infected with *Schistosoma mansoni* infection following silica exposure. The 'Cabo Frio' strain demonstrated a high susceptibility to infection, accompanied with shortening of the parasite's intramolluscan phase and an increase in the output of sporocysts and cercariae. On the other hand, the 'Taim' strain, naturally resistant to *S. mansoni*, demonstrated no increase in susceptibility. Salice & Roesijadi (2002) showed that parasite-resistant strains of snails were more susceptible to cadmium exposure. The authors speculated that an increase in cadmium sensitivity was the cost of being able to resist parasitic infection and that in polluted environments there was a greater chance of survival of potential hosts

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which in turn were more likely to disseminate disease. However, interactions between pollutants and parasites are more complex than this, involving a variety of factors which influence immune responses of snails to trematodes. For example, different trematode species can induce different immune responses in the same snail host species, e.g. *S. mansoni* and *Echinostoma paraensei* in *Biomphalaria glabrata* (Loker & Adema 1995), suggesting that under pollution conditions the prevalence of individual trematodes may increase or decrease depending on the specific impairment of host immune systems by individual toxicants.

Vertebrate–trematode interactions

Physiological interactions

In vertebrates, exposure to parasites and pollutants have been noted to induce extensive morphological changes and alterations in a range of physiological parameters. For example, Polyakova-Krusteva *et al.* (1988a) found that 90% mortality occurred in rats exposed to *F. hepatica* and diethylnitrosamine, a nitrosamine solvent used in tanneries and textile industries and found in certain pesticides. Death was preceded by an imbalance in the trace element content of the liver (Tsocheva & Gabrashanska, 1992) and an increase in the weight of the liver, spleen and body of the host (Polyakova-Krusteva *et al.*, 1988b; Tsocheva *et al.*, 1990a, 1991a). Tsocheva *et al.* (1990a) assumed that this was associated with a disruption of the rats' endocrine regulative system. In addition, the number of cancerous tumour nodes in rats was dependent on the level and nature (chronic or acute) of the parasite infection. Low numbers of tumours occurred during the chronic phase of fascioliasis with tumours increasing during the acute phase of reinfection with *F. hepatica* (Tsocheva *et al.*, 1990a). El-Khafif *et al.* (2001) also found that combined exposure of lead and infections with *S. mansoni* caused extensive pathological changes to the liver and kidneys of mice. These effects are not only limited to the interactions between parasites and mammalian hosts. Gross morphological deformities of limbs have been associated with trematode infections in wood frogs *Rana sylvatica* collected from pesticide-contaminated field sites (Kiesecker, 2002). This may be associated with impaired immune function in polluted environments which leads to an increase in parasite prevalence in developing tadpoles. More deformities were found in adult frogs implying that pesticide exposure and trematode infection may contribute to the global decline of amphibian populations. Giesy & Smith (1985) found that exposure of livebearing female mosquitofish *Gambusia affinis* infected with *Diplostomulum scheuringi* to cadmium completely inhibited host reproduction as all pregnant fish were found to be uninfected. These authors considered that exposure to both parasite and toxicant may have induced fish to abort their pregnancies.

The physiology of the host is not only affected by direct exposure to pollution, but also when the host is infected with pollutant-exposed cercariae. For example, Okwuosa & Osuala (1993) found that anaemia, as measured by haemoglobin concentration and packed cell volume, in mice infected with detergent-exposed *S. mansoni* cercariae was significantly reduced compared with infected controls.

In animals hepatic cytochrome P450 s (CYP) enzymes and other mixed function oxidase systems play an important role in the metabolism of xenobiotics and drugs, with each CYP isoenzyme able to respond differently to exogenous chemicals in terms of induction and inhibition (Sheweita *et al.*, 2002). Trematodes, especially those species which utilize the liver as the main site of infection, have been found to impair hepatic xenobiotic-metabolizing activity (e.g. Tekwani *et al.*, 1988). Tsocheva *et al.* (1992a,b) found that under the combined exposure of diethylnitrosamine and *F. hepatica* infection, the liver content of CYP and the activity of ethylmorphine-N-demethylase and aniline hydroxylase were decreased to a greater extent than with exposure to only one stressor. In contrast, the activity of dimethylnitrosamine demethylase I, dimethylnitrosamine demethylase II, hepatic aminopyrine demethylase and aniline hydroxylase in mice infected with *S. mansoni* significantly increased after exposure to lindane compared to uninfected controls (Mostafa *et al.*, 1984; El-Bassiouni *et al.*, 1984). A small increase in hepatic and serum glutathione and malondialdehyde levels occurred in hamsters infected with either *S. mansoni* or *S. haematobium* and exposed to lead compared with hamsters exposed only to lead (El-Gohary *et al.*, 2003). However, when lead-exposed infected hamsters were treated with the antioxidant 'Antox' a significant reduction in the oxidative stress parameters was noted. A consequence of an increase in activity of these oxidative stress parameters is a greater chance of DNA damage occurring. When DNA is oxidized, several types of damage are produced and one product of such damage is an increase in the levels of hepatic 8-oxodeoxyguanosine phosphate (8-Ox-Dg). El-Gohary *et al.* (2003) noted that levels of 8-Ox-Dg were greater in combined lead-exposed and parasite-infected hamsters compared with unexposed and uninfected controls, whilst the administration of 'Antox' resulted in a significant reduction in 8-Ox-Dg to levels comparable to control hamsters. In rats exposed to both diethylnitrosamine and *F. hepatica*, Stefanova *et al.* (1990) found a decrease in the levels of DNA and an increase in the levels of RNA compared to unexposed and uninfected controls. However, in rats reinfected with *F. hepatica*, the quantities of nucleic acids were significantly reduced. In studies on the oxidative stress of the yellow perch *Perca flavescens* infected with *Apophallus brevis*, Marcogliese *et al.* (2005), observed that there was no change in the reduced glutathione levels between infected fish from polluted and control sites. The authors considered that this may reflect a low sensitivity to glutathione as measurements of lipid peroxidation showed higher levels in infected perch from the polluted site compared with controls. A change in the ability to metabolize pollutants under trematode exposure may be the cause of many physiological responses of the host subjected to pollution.

Within the liver of rats exposed to diethylnitrosamine and infected with *F. hepatica*, a number of functional changes in enzyme activity have been recorded. Tsocheva *et al.* (1988) reported that the

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activity of glucose-6-phosphatase and succinate dehydrogenase were reduced but that of acid phosphatase was increased compared with controls. In contrast, Mizinska-Boevska *et al.* (1991) found that the activity of succinate dehydrogenase was increased whilst that of glucose-6-phosphatase, acid phosphatase and adenosine triphosphatase was reduced in diethylnitrosamine-exposed and *F. hepatica*-infected rats. When diethylnitrosamine-treated rats were given two separate infections of *F. hepatica*, all enzyme activity was low except for adenosine triphosphatase which was raised in some parts of the liver, suggesting severe impairment of hepatocyte organelles and a raised membrane permeability (Mizinska-Boevska *et al.*, 1991). Tsocheva *et al.* (1990b), using histoautoradiography on the proliferative activity of rat hepatocytes with the same trematode–toxicant system, found increased stimulation of hepatocytes over 27 weeks, especially during the acute phase of a repeated *F. hepatica* infection following the application of diethylnitrosamine. Additional studies by Tsocheva *et al.* (1991c) showed that the proportion of different hepatocyte lysosomes changed under the combined trematode–toxicant exposure, accompanied by an increase in Golgi complex vesicles, multivesicular bodies, and primary lysosomes in the hepatocytes which in turn was associated with an increase in APase activity. These changes suggest an increase in the functional activity of lysosomes and the likely activation of hepatocyte protein synthesis not found on exposure of rats to a single stressor. The increased APase activity, however, may be associated with the direct effects of either disintegrated cell products or diethylnitrosamine causing liberalization of the lysosome membrane or inhibition of *F. hepatica* cortisol secretion due to diethylnitrosamine-induced structural damage.

One of the most prominent consequences of toxicant–trematode interactions in vertebrates is an increase in the occurrence of cancerous tumours. Schistosomiasis is one of the primary risk factors for the development of bladder cancer in mammals (WHO, 1986), whilst other trematode infections, such as *Opisthorchis viverrini* and *Clonorchis sinensis*, have been associated with the development of cholangiocarcinomas, (Mostafa *et al.*, 1999). A number of studies have shown that interactions between mutagenic chemicals and trematode infections can increase the risk of tumour development. These have included: carcinogens such as benzo(a)pyrene, a polycyclic aromatic hydrocarbon, and *S. mansoni* (Awney *et al.*, 2001); nitrosamines and *O. viverrini* (Thamavit *et al.*, 1978; Flavell & Lucas, 1983), *S. mansoni* (Mostafa *et al.*, 1994), *S. haematobium* (Hicks *et al.*, 1980), *Fasciola hepatica* (Tsocheva *et al.*, 1990a, 1991a), or *C. sinensis* (Lee *et al.*, 1993); and aromatic amines and *S. haematobium* (Hashem & Boutros, 1961; Al-Aaser *et al.*, 1978), *S. japonicum* (Miyasato, 1984), *S. mansoni* (Kakizoe, 1985) or *C. sinensis* (Iida, 1985). Although the exact nature of the interactions between carcinogens and trematodes in the occurrence of cancerous tumours has not been elucidated, the development of bladder cancer during exposure to schistosomes and mutagenic chemicals may be linked with chronic inflammation of the bladder lining and an associated increase in cell proliferation following infection with *S. haematobium*. Such cells are more sensitive to the induction of DNA damage and are more likely to propagate mutagenic events into daughter cells rather than dormant cells which will favour the initiation of carcinogenesis from exposure to environmental mutagens (Gentile, 1985). Indeed, it has been shown that individuals who have inherited certain polymorphic metabolizing genes that can increase the body burden of reactive metabolites from chemicals, have an increased risk of developing bladder cancer. Any of these individuals who become infected with schistosomes may therefore be more susceptibile to the induction of cancer by environmental mutagens (Au, 2001).

Similar trematode–toxicant interactions may also be associated with the induction of cancers in lower vertebrates. For example, the development of fibropapillomatosis tumours in turtles has been associated with spirorchid blood fluke infections (Dailey & Morris, 1995) and also with areas of high pollution (Foley *et al.*, 2005). It is possible that these trematodes may be able to act synergistically with pollutants to induce tumours in areas of high pollution in a similar way to mammalian–trematode systems.

Immunological interactions

Studies on cellular immunology in higher vertebrates under combined toxicant–trematode exposure have been limited. Pistl *et al.* (1995) found heavy metal exposed lambs subsequently infected with *F. hepatica* showed no significant changes in the leukocyte migratory index or the index of phagocytic metabolic response. In contrast, Benkova *et al.* (1993) reported significant decrease in the phagocytic activity and index of phagocytic activity in polymorphonuclear leukocytes in lambs exposed to industrial heavy metal emissions and *F. hepatica*. The activity of complement, as determined by CH_{50} haemolytic assay, also decreased whereas an alternative haemolytic complement assay (AH_{50}) showed no significant changes. Tsocheva *et al.* (1991a) studied pathohistological changes in the spleen of *F. hepatica*-infected rats exposed to diethylnitrosamine. In single exposures to either *F. hepatica* or toxicant, the T-dependent zone of the Malpighian bodies was either stimulated by the parasite or atrophied after exposure to diethylnitrosamine. This combination which induced proliferative changes to the T-dependent zone of the Malpighian bodies, suggested that cellular immunity by the host was strongly activated. This may be associated with cell-mediated cytotoxicity induced by fascioliasis, which is known to have an anticarcinogenic effect. When rats were infected twice with *F. hepatica* and treated with diethylnitrosamine during the acute phase of reinfection, diffuse distrophic changes in the spleen occurred. Tsocheva *et al.* (1991a) considered that this was probably the result of reinfection activating the humoral immune response, stimulating the production of IgG which blocks the anti-tumour effects of the T-lymphocytes and IgE. Using a similar toxicant–trematode exposure system, Dimov *et al.* (1985) observed that stimulation of both phagocytic and microbicidal activities in peritoneal exudative macrophages, and normally associated with a *F. hepatica* infection, was inhibited under the combined exposure

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to parasite and diethylnitrosamine. In contrast, Mizinska-Boevska *et al.* (1990a,b) found that ultrastructural changes and the phagocytic activity of peritoneal macrophages were reduced under the combined exposure to *F. hepatica* and diethylnitrosamine compared with single-stressor exposures. This may be due to a reduction in parasite metabolism caused by toxicant damage or the parasite's metabolites may be slightly neutralizing the toxicity of diethylnitrosamine (Mizinska-Boevska *et al.*, 1990b). Tesana *et al.* (2000) studied the effect of immunization on the development of carcinogenesis in *O. viverrini*-infected hamsters exposed to dimethylnitrosamine. Pre-immunizing with a crude somatic antigen of the parasite accelerated the development of cancer in hamsters subsequently infected with *O. viverrini* and exposed to dimethylnitrosamine compared with non-immunized animals. This may have been the result of immunization which increases proliferation of the macrophage population leading to an increase in the production of nitric oxide. The latter reacts with amine and nitrosating agents producing cancer-causing nitroso-compounds.

In lower vertebrates cellular immune responses under the combined stress of parasite and pollutant have demonstrated some interesting results. Kiesecker (2002) exposed tadpoles of the wood frog *Rana sylvatica* to three types of pesticides (atrazine, malathion, and esfenvalerate) and found in the blood a significant decrease in the number of circulating eosinophils, which play an important role in macroparasite infections. This was correlated with a subsequent increase in the prevalence of encysted metacercariae of *Ribeiroia* sp. and *Telorchis* sp. within the frog tissues. Linzey *et al.* (2003) studied splenic lymphocyte blastogenesis of marine toads *Bufo marinus* collected from a cadmium-polluted site where infections with *Mesocoelium monas* were between 90–95%. Lymphocytes demonstrated a reduced proliferation when stimulated with the mitogen lipopolysaccharide when compared to lymphocyte proliferation of toads from a control site. Although there was no direct relationship between trematode prevalence and inhibited immune responses, the incidence of *M. monas* in the toad population at the polluted site had increased from 2% in 1992 to $>$ 90% between 1996 and 1999, suggesting that parasitic infection was an additional source of stress for the amphibians.

Jacobson *et al.*, (2003) studied the cumulative effects of polychlorinated biphenyl (PCB) exposure on Chinook salmon infected with *Nanophyetus salmincola.* In combination, the trematode and PCB exposure caused a lower primary haemolytic plaque-forming cell response in the anterior kidney leucocytes than with either stressor alone, although no comparable significant difference was found with splenic leucocytes. By comparing these results with models of multiple stress effects the authors were able to conclude that the two stressors were acting independently as separate mechanisms of immune suppression and could act interchangeably in the same step of a multistep process of stress inducement (Jacobson *et al.*, 2003).

Schuwerack *et al.* (2001, 2003) found that exposure of carp infected with *Sanguinicola inermis* to either ammonia or cadmium resulted in organ- and time-specific immunological and structural changes in the lymphoid organs not found in unexposed infected fish. A differential response between the number of lecucocytes from the thymus and pronephros occurred which was associated with the relative positions of the two organs in the body. It was speculated that the close proximity of the thymus to the branchial chamber of the fish may result in increased exposure to pollutants compared with the pronephros. *In vitro* proliferation of carp pronephric lymphocytes from fish infected with *S. inermis* was increased with mitogen stimulation in infected carp that had been exposed to ammonia and cadmium for 48 h. In cadmium-exposed infected fish, lymphocyte blastogenesis after stimulation with cercarial extract was significantly increased compared to that in unexposed infected fish. In a reversal of these experiments, Hoole *et al.* (2003) exposed carp to either ammonia or cadmium followed by infection with *S. inermis* and found that the pollutant type and the order in which the fish experience the parasite and toxicant significantly influenced the ultrastructural appearance and cellular content of the pronephros and thymus. This was reflected in the intensity of infection where the pollutant appeared to have less of an effect on worm establishment.

Studies on interactions between trematodes and pollutants and their effects on the humoral immune system of vertebrates are limited. In mammals infected with *F. hepatica* there appears to be no significant reduction in the levels of circulating antibodies after exposure to either diethylnitrosamine (Tsocheva *et al.*, 1991b) or heavy metals (Boroskova *et al.*, 1994).

Although there have been no investigations on the humoral immune response of lower vertebrates in relation to interactions between trematodes and pollutants, both infection and pollution are known to individually affect the humoral immune system (Hoole, 1997; Roberts *et al.*, 2005). Studies on other metazoan parasites have, however, suggested that pollutants and parasites can interact to affect the humoral components of the immune system of fish. Studies on eels exposed to PCB 126 and infected with the acanthocephalan *Anguillicola crassus* have revealed that the production of antibodies is suppressed in PCB 126-exposed hosts. In contrast, exposure of infected eels to cadmium results in a negligible effect on antibody production (Sures & Knopf, 2004).

Parasite transmission

The transmission of trematodes can be affected by the 'fitness' of both source and target hosts in pollution conditions. Successful transmission is considered to be more likely in healthy source hosts (Krist *et al.*, 2004). Studies have revealed that target hosts exposed to toxicants often have shown an increase in parasite prevalence and intensity (e.g. Krupicer *et al.*, 1996; Morley *et al.*, 2002; Tantawy, 2002). Successful trematode establishment is dependent on the balance between parasite infectivity and host susceptibility. Infectivity has previously been shown to be affected by a wide range of pollutants (Morley *et al.*, 2003a) and the present study has shown how host physiological and immunological

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functions can be detrimentally affected by toxicants. Although a reduction in host 'fitness' may at first appear to be beneficial to the parasite it may, under certain conditions have detrimental consequences. For example, the formation of granulomas around eggs of *S. mansoni* is stimulated by the host immune response but induces pathological changes within infected tissues. Although granulomas are themselves pathogenic, they also serve as an essential host-protective function by encapsulating toxic excretory/secretory egg products which may otherwise induce host mortality and in turn the parasite (El-Ansary, 2003). The immune processes also facilitate in the excretion of parasite eggs and enable completion of the life-cycle (Doenhoff *et al.*, 1985). In severe combined immunodeficient infected mice, granulomas fail to develop and liver failure can occur (El-Ansary, 2003). Under polluted conditions chronically immunocompromised hosts may therefore initially possess high trematode prevalences but in the long term the poor 'fitness' of the host could interfere with successful parasite dissemination and lead to an eventual decline in the parasite population.

Trematodes residing in both molluscan and vertebrate hosts can absorb a range of toxicants from exposed hosts which may subsequently affect their transmission efficiency (e.g. Booth & Schulbert, 1968; Delak & Radakovic, 1978; Giesy & Smith, 1985; Mutafova *et al.*, 1986; Ibrahim *et al.*, 1992; Sures *et al.*, 1998; Ruus *et al.*, 2001). A number of studies have noted that exposure of a host to a toxicant may affect the physiology of trematodes residing in their tissues. Gorchilova *et al.* (1986) found that the tegument and intestinal wall of *F. hepatica* residing in diethylnitrosamine-exposed rats suffered extensive degenerative and necrotic changes. These morphological and functional changes of the parasite may have been caused either indirectly via toxicant altering the structure and metabolism of the host liver tissue or by the pollutant directly affecting *F. hepatica*. Additional studies by Mutafova *et al.* (1986) and Gabrashanska & Tsocheva (1992) showed that diethylnitrosamine host-exposure disturbed chromosome function in the interphase nucleus, caused malformation of spermatozoids, and altered the content of trace elements in *F. hepatica.* El-Khafif *et al.* (2001) demonstrated that lead exposure of mice infected with *S. mansoni* resulted in degenerative changes to the parasite. Giesy & Smith (1985) found that cadmium exposed mosquito fish *G. affinis* infected with *D. scheuringi* led to a reduced parasite size compared to controls. These authors suggested that such an effect on size could have resulted from cadmium directly affecting parasite growth rate or the parasites' maximum limit on attainable size or indirectly by decreasing available nutrients, reducing vitamin B_{12} concentrations, or altering the enzyme profile in liver tissues of the fish. Studies on infected intermediate hosts in trematode life cycles have revealed that pollutants may influence parasite transmission in a variety of ways. In infected molluscs exposed to toxicant, a delay in sporocyst development with a reduced cercarial production has been noted (Hira & Webbe, 1972; Yescott & Hansen, 1976). Emergence of cercariae may also be completely inhibited by certain pesticides (Ibrahim *et al.*, 1992; Morley *et al.*, 2003c), but this inhibition may only be temporary as emergence recommences once the pollutant has been withdrawn (Ibrahim *et al.*, 1992). It is possible that the pollutant targets the maturing cercariae, leaving the sporocyst unharmed (Ibrahim *et al.*, 1992). This supposition is supported by the work of Yescott & Hansen (1976) who found that sporocysts of *S. mansoni* in *B. glabrata* exposed to manganese contained cercariae which appeared sluggish or immobile and the majority of snails contained no emerging cercariae within their haemolymph. Morley *et al.* (2004a) noted that in snails infected with *Echinoparyphium recurvatum*, and exposed to cadmium, developing cercariae absorbed the metal whilst residing within the redia and subsequently encysted without emerging from the snail host. Such a change in transmission strategy may ensure that at least a proportion of the cercarial population will form metacercariae whatever the status of the aquatic environment, although an increase in first intermediate host encystment may reduce the chances of successful transmission as only a small proportion of snails contain primary infections (Morley *et al.*, 2004a). Furthermore, Morley *et al.* (2004b) established that toxicant-induced mortality within an *E. recurvatum* metacercarial population residing in second intermediate snail hosts was dependent on which host species was being exposed.

Many pathological effects on trematodes may result from their apparent limited detoxification mechanisms (Barrett, 1997). For example, trematodes lack cytochrome P450 mono-oxygenases (Barrett, 1998) which hinders their ability to detoxify xenobiotic compounds and thus there is a reliance primarily on reductive or hydrolytic reactions for metabolism, although sequestration of toxicants by lipid-binding proteins may be used as an alternative (Barrett, 1997). Parasites would therefore appear to be heavily dependent on the detoxification mechanisms of their hosts for protection against toxicants, even though a frequent consequence of a parasitic infection is a decrease in host detoxification enzymes (Barrett, 1997, 1998). This somewhat paradoxical situation means that trematodes may be susceptible to pollutant-induced damage which may affect their ability to absorb essential nutrients thereby reducing their reproductive potential, or causing mutagenic damage to developing embryos, all of which may lead to a reduced transmission. El-Khalif *et al.* (2001) and El-Gohary *et al.* (2003) found that in mammals infected with *S. mansoni* and *S. haematobium* and exposed to lead, a significantly reduced worm burden, tissue egg load and ova excretion occurred compared to controls. Morley *et al.* (2005) showed that the survival characteristics of *Diplostomum spathaceum* cercariae emerged from cadmium exposed snails were altered compared with controls. These changes may be associated with the accumulation of cadmium by developing cercariae within the exposed host which subsequently inhibited the glycogen utilization. Cross *et al.* (2001) observed a reduction in the horizontal swimming rate and survival of cercariae emerged from snails collected at a metal-polluted site compared with controls. Cross *et al.* (2005) later established that the changed swimming rate was due to slower swimming rather than disorientation and longer swimming pathways and they suggested that pollutants accumulated by developing cercariae in the snail host

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affected the neuromusculature associated with swimming. These authors also transplanted infected snails from a 'clean' to a polluted site and found that cercarial survival and swimming rates were affected. It was considered that transplanted snails may have exhibited a stress response, possibly resulting in reduced feeding, which in turn affected cercarial development.

It is apparent from laboratory-based studies that complex interactions occur between host and parasite in polluted environments. At present, however, it is not clear how these interactions are manifested in the field, nevertheless it is likely the 'fitness' of hosts may be compromised in a range of different and interacting ways. However, a degree of caution must be taken into account when extrapolating simplified laboratory experiments into diverse and complex ecosystems which encounter a variety of pollutant stresses. In such environments, a range of factors may influence trematode transmission. For example, the majority of trematode species utilize hosts which may reside in different kinds of habitats, only some of which may be subjected to pollution. This scenario is exemplified by *S. mansoni*, which is transmitted through an aquatic phase encompassing a freshwater snail host via free-living miracidia and cercariae. The definitive host is usually a terrestrial mammal whose contact with the aquatic environment is intermittent and transitory. Toxic effects of pollutants may therefore influence only one of the habitats and hosts, as part of the life cycle of the trematode. Cross *et al.* (2005) speculated that high trematode prevalences in snails from polluted sites were due to their continued introduction by large infected-bird populations attracted to feed at these habitats from other areas. How trematodes may react to such oscillating host conditions from 'clean' to polluted over successive parasite generations remains to be determined.

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