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Effects of Exposure to Contaminated Sediments on the Parasite Fauna of American Plaice (*Hippoglossoides platessoides*)

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Toxic contaminants in the environment can affect parasite species in different ways (Poulin, 1992). Parasitism in a host can be promoted due to an impaired immune response resulting from pollution-induced stress, or because of enhanced survival and reproduction of intermediate hosts involved in the parasite's life cycle (Overstreet 1988; Khan and Thulin 1991; MacKenzie et al. 1995). Alternatively, parasitism in a host may be hindered due to toxicity to free-living stages of the parasite or its intermediate hosts, or because of changes in host physiology in polluted conditions (Overstreet 1988; Khan and Thulin 1991; MacKenzie et al. 1995). Generally, the effects of pollution on parasites depends on the life cycle of the parasite, and the concentration and type of pollutant (Overstreet and Howse 1977).

There is much indirect evidence suggesting that fish possess an increased susceptibility to trichodinid and monogenean ectoparasites, which possess direct life cycles, after long-term exposure to toxicants (Khan and Thulin 1991; MacKenzie et al. 1995). Endoparasites also can be affected directly within a host either by exposure to contaminants or by biochemical and physiological changes to the host (Khan and Kiceniuk 1983). Gastrointestinal helminths undergo decreases in prevalence and intensity in marine fishes exposed to crude oil in sediments and water (Khan and Kiceniuk 1983; Khan 1991). Ingestion of contaminants may cause death or expulsion of intestinal parasites (Kahn and Kiceniuk 1983). Exposure to the same type of contaminants leads to increases in ectoparasitism by trichodinids and monogeneans on marine fish (Khan and Kiceniuk 1983; Khan 1990, 1991; Khan et al. 1994).

In this study, American plaice *(Hippaglossoides platessoides)* were exposed to contaminated sediments characterized by high levels of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Ecto-and endoparasites from the fish were identified and enumerated after exposure to sediments from a heavily-contaminated site and from a reference site to determine the response of the parasite populations in and on fish to organic contaminants. Flatfish are appropriate for exposure to contaminated sediments because they live

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in close association with the sediments and may absorb through the skin more contaminants than other fishes (Khan 1991).

MATERIALS AND METHODS

This study was performed in conjunction with another to evaluate the effects of contaminated sediments on reproductive success of male American plaice (see Nagler and Cyr 1997). Fish were collected by otter trawl from the St. Lawrence estuary near Matane, Quebec, approximately 7 months prior to experimental exposure. Fish were maintained in a fiberglass holding tank (3.6 x 1.2 x 0.45 m) supplied with aerated filtered flowing seawater, at ambient temperature and natural photoperiod for 4 months prior to the experiment. Fish were fed previously-frozen capelin twice weekly and fed capelin-based food pellets supplemented with vitamins at every 6th feeding (see Nagler and Cyr 1997 for references).

Baie des Anglais on the St. Lawrence estuary, near the town of Baie Comeau, Quebec, Canada is recognized as an area of environmental concern regarding sediment contamination. The bay receives effluent from an aluminum smelter, a pulp and paper mill and has a port. Two sites, designated "polluted" and "reference" in and near Baie des Anglais were chosen based on previous sediment chemical analysis (Nagler and Cyr 1997). The reference site was used as the control in the companion study by Nagler and Cyr (1997). The polluted site is located at 49° 15.05' N, 68° 7.38' W, and the reference site at 49° 14.95' N, 68° 4.07' W. The difference in contaminant levels between sites is approximately 100-fold. Sediments contain mainly organic compounds, characterized by high levels of PAH (39.8 µg/g dry sediment), PCB 77 (7 000 pg/g dry sediment), PCB 105 (43 000 pg/g dry sediment), PCB 118 (31 000 pg/g dry sediment), PCB 126 (230 pg/g dry sediment) and PCB 169 (3.2 pg/g dry sediment) at the polluted site. Metal levels are low and similar to background levels throughout the St. Lawrence River and estuary (Nagler and Cyr 1997).

Surface sediments (0- 15 cm) were collected over a 2-d period with a 32-L Van Veen grab. Multiple grabs were taken at each site and pooled in l-m^3 polyethylene containers on board ship. Approximately 600-650 L were collected at each site. The sediments were homogeneously mixed with a shovel as they were collected. Containers were sealed and stored at l-5° C.

Sediments were shoveled into dry fiberglass exposure tanks (1.2 x 0.9 x 0.6 m) to a depth of 15 cm. Two replicate exposure tanks were set up per sediment sampling site. Seawater was continuously passed through a convoluted arrangement of Tygon tubing at the bottom of each tank beneath the sediments to maintain a temperature similar to the overlying water. Once the sediments were added, ambient filtered seawater was supplied to each tank at a flow rate of 1 L/min. In January, 6 immature females and 6 immature males were added to each

tank and kept under conditions of ambient temperature and natural photoperiod. During winter months, a heat exchanger was used to keep the temperature from dropping below 4-5° C. Five months later (June) fish were anaesthetized and treated (including removing a small piece of liver) according to protocols for the study by Nagler and Cyr (1997), after which they were frozen for subsequent parasitological analysis. An additional 15 fish were removed from the holding tank, frozen, and necropsied as a second set of controls for the fish put in the experimental basins.

Ten and 2 fish died during confinement in the two replicates containing polluted sediments, and were not available for the analysis. Fish were thawed, measured for total length and weighed. The external surface was rinsed, and the rinse allowed to settle. After decanting, the sediment was examined for ectoparasites with a stereomicroscope. Gills were removed and rinsed, and the arches examined. The eyes and fins were removed and examined separately. The internal organs were inspected for parasites free or encapsulated on the viscera. The stomach and intestine were separated and slit open longitudinally, and their contents rinsed into beakers and allowed to settle. Walls of the stomach and intestine, and the spleen, kidney, heart and remaining liver were compressed between glass plates and examined for parasites. The body cavity was rinsed, and the rinse then examined for parasites after settling. Squash preparations made from the liver, kidney, brain, and gonads, and scrapings of the urinary bladder and gall bladder were examined for microparasites with a compound microscope at 400X. Each tissue preparation was examined for 5 min. The entire body musculature was removed, separated from the skin, thin sliced, and examined for parasites with a stereomicroscope. All parasites were sorted into taxa, counted and fixed in 10% glycerol in 70 % ethanol (Nematoda) or alcohol-formalin-acetic acid (Platyhelminthes, Acanthocephala) for later identification.

Parasite data are presented as prevalence (percent of fish infected), abundance (number of parasites in an individual host), and mean abundance (mean number of parasites per host in a sample) as recommended by Bush et al. (1997).

All samples were examined for heterogeneity of variances using an F_{max}-test prior to analysis. If the F_{max}-test was significant, values were $\log_{10}(n + 1)$ transformed to minimize the heterogeneity of variances. Means between replicate treatments were compared using t-tests. If not significant, the replicates were pooled. Regression analysis was performed to test for relationships between fish length and parasite abundance within each treatment for each parasite taxa. Treatments (polluted, reference, holding tank) were compared using an analysis of variance (SAS general linear models procedure) followed by a Student-Newman-Keuls multiple comparison among means. Analyses were performed on fish length, and on each parasite taxon except those which only occurred sporadically. In addition to analysis by species, gastrointestinal digeneans were combined and compared across treatments. Myxozoan and protozoan data (presence/absence) were

converted into binary form and subsequently analyzed. Significance is defined as *P < 0.05).*

RESULTS AND DISCUSSION

American plaice were infected with 18 taxa of helminths, 4 myxozoans and 1 protozoan. Digeneans included *Derogenes varicus, Steringophorus furciger, Stenakron vetustum, Steringotrema ovacutum, Brachyenteron pycnorganum, Bacciger* sp. (gastrointestinal tract); *Aporocotyle simplex* (gills); and *Stephanostomum baccatum* metacercariae (musculature at base of tins). Nematodes included *Pseudoterranova decipiens* (musculature), *Anisakis simplex* (viscera), *Hysterothylaium aduncum* (larvae on viscera, adults in intestine), and Contracaecinea (viscera). Cestodes included larval forms (*Scolex pleuronectis* in intestine, *Grillotia* sp. on viscera). One species of acanthocephalan, *Echinorhynchus laurentianus,* occurred in the intestine. A monogenean, *Gyrodactylus* sp., was found on the body surface and fins. This is probably a previously-undescribed species belonging to an inshore marine clade of *Gyrodactylus* found on marine fishes *(D. Cone, St. Mary's University, pers. comm.). The* myxozoans *Ortholinea* sp., *Myxidium* sp., *Ceratomyxa* sp., and *Myxoproteus* sp. were found primarily in the urinary bladder and the gall bladder. The protozoan *Trichodina* sp. was found on the external surface. Infection statistics are found in Table 1.

Mean lengths of American plaice did not differ statistically among treatments. None of the regression analyses of parasite abundance, or in the case of myxozoans and protozoans, presence/absence, and fish length were significant, with the exception of *S. vetustum* ($F = 6.799$, $P = 0.0120$). For most parasite taxa, there was no difference in mean abundance, or in the case of myxozoans and protozoans, presence/absence, among treatments, with the following exceptions. For Contracaecinea, mean abundance in replicate 2, reference treatment, was significantly higher than those in reference replicate 1 and the control holding tank, but not from that in the polluted treatment (ANOVA, $F = 3.93$, $P = 0.0140$). Mean abundance of *P. decipiens* differed between the control holding tank and the reference treatment (ANOVA, $F = 3.69$, $P = 0.0323$). Mean abundance of *Anisakis simplex* was higher in reference replicate 2 than in the other replicate and treatments (ANOVA, $F = 5.86$, $P = 0.0017$). Mean abundance of *Gyrodactylus* sp. was significantly higher in the polluted treatment than in the reference and holding tanks (ANOVA, $F = 9.82$, $P = 0.0003$). *Ceratomyxa* sp. occurred on significantly fewer fish in reference replicate 1 than in other the replicate and treatments (ANOVA, $F = 4.01$, $P = 0.0127$). *Trichodina* sp. was found on significantly more fish in the control holding tank than in the other treatments (ANOVA, $F = 24.72$, $P < 0.0001$).

There are three results of biological significance obtained from this study, those for *Gyrodactylus* sp., *Trichodina* sp., and the gastrointestinal digeneans. The higher abundance of *Gyrodactylus* sp. on fish exposed to heavily contaminated

Table 1. Prevalence (P) and mean abundance (A ± SE.) of parasites infecting American plaice (*Hippoglossoides platessoides*) maintained in experimental tanks containing sediments from a contaminated site and a reference site, and in a control holding tank.

	Contaminated sediments		Reference sediments		Holding tank	
No. of fish	12		24		15	
Mean length (cm)	321.9 ± 5.9		326.9 ± 7.5		329.9 ± 3.5	
Parasite	P (%)	A ± S.E.	P (%)	A ± S.E.	P (%)	A ± S.E.
Protozoa						
Trichodina sp.	8	---	0	---	67	---
Myxosporea						
Ceratomyxa sp.	83	---	75	---	87	---
Myxidium sp.	50	---	63	---	73	---
Myxoproteus sp.	0	---	4	---	27	---
Ortholinia sp.	17	---	17	---	47	---
Monogenea						
Gyrodactylus sp.	100	217.5 ± 50.4	100	36.4 ± 12.7	93	107.2 ± 32.3
Digenea						
Aporocotyle simplex	100	7.4 ± 3.0	83	9.3 ± 2.2	93	6.8 ± 1.4
Bacciger sp.	0	---	0	---	17	0.2 ± 0.1
Brachyenteron pycnorganum	0	---	0	---	7	0.1 ± 0.1
Derogenes varicus	17	0.2 ± 0.1	38	0.5 ± 0.2	53	0.7 ± 0.2
Stephanostomum baccatum	58	6.4 ± 4.2	79	32.7 ± 15.5	67	19.8 ± 11.0
Stenakron vetustum	25	0.9 ± 0.5	30	4.0 ± 1.7	13	0.8 ± 0.6
Steringophorus furciger	42	1.6 ±0.7	63	4.9 ± 1.9	53	8.4 ± 6.0
Steringotrema ovacutum	25	0.3 ± 0.1	13	0.1 ± 0.1	0	---
Cestoda						
Grillotia sp.	0	---	9	0.1 ± 0.1	20	0.5 ± 0.3
Scolex pleuronectis	17	0.2 ± 0.1	25	0.5 ± 0.2	13	1.2 ± 1.1
Nematoda						
Anisakis simplex	58	1.3 ± 0.5	54	1.8 ± 0.4	40	0.7 ± 0.3
Contracaecinea	83	17.6 ± 5.4	92	19.4 ± 4.7	67	7.7 ± 4.7
Hysterothylacium aduncum	0	---	12	0.1 ± 0.1	7	0.1 ± 0.1
Pseudoterranova decipiens	50	0.8 ± 0.3	71	1.1 ± 0.2	27	0.3 ± 0.2
Acanthocephala						
Echinorhynchus laurentianus	0	---	21	0.3 ± 0.1	13	0.1 ± 0.1

sediments might be attributed to the proliferation of this parasite on stressed, immunocompromised fish. Other experimental and field studies demonstrate that monogenean abundance increases in polluted systems (Skinner 1982; Khan and Kiceniuk 1988), though different responses can be observed depending on the parasite species involved (Siddall et al. 1997). Numbers of *Gyrodactylus* spp. increased on Atlantic cod (*Gadus morhua*) exposed for 4 months to crude oil extracts containing high and low concentrations of hydrocarbons (Khan and

Kiceniuk 1988). While it is conceivable that the polluted conditions in those and in our experiments directly created conditions favorable for survival and reproduction of *Gyrodactylus* spp., it is more likely that the experimental fishes were stressed and immunologically compromised. This supposition is reinforced by the results of immunological studies, using head kidney macrophages from American plaice exposed to Baie des Anglais sediments, which indicate that these sediments strongly inhibit phagocytic activity of these cells (Lacroix et al., unpublished results). In polluted conditions, immunosuppression can result from suppression of the humoral antibody response, damage to the lymphoid organs, changes in leucocyte numbers, reduction in phagocyctosis, and damage to the protective mucous layer (Poulin, 1992; MacKenzie et al. 1995). The similar results between our studies and those of Khan and Kiceniuk (1988) cannot solely be a result of gill damage as suggested by the latter authors, because the monogeneans in our experiments occurred on the fins and body surface.

The observed decrease in *Trichodina* sp. on fish in the experimental tanks containing sediments from the polluted or the reference site, compared to on those from the holding tank, is opposite to what usually may be expected for ectoparasites which have direct life cycles and can undergo reproduction and transmission within experimental enclosures (Khan and Thulin 1991; MacKenzie et al. 1995). Trichodinids infected 67% of the fish from the control holding tank, but only 1 of 12 exposed to highly contaminated sediments, and none of those in the reference site tanks. This particular species of *Trichodina* may be highly susceptible to organic pollutants, even at lower concentrations. However, most studies examining levels of *Trichodina* spp. in polluted conditions show an increase in levels of infection (Lehtinen et al. 1984; Axelsson and Norrgren 1991; Khan 1991; Khan et al. 1994), although *Trichodina* spp. were more common on perch from an oligotrophic reference lake than on perch from 3 eutrophic polluted lakes (Halmetoja et al. 1992). The species of *Trichodina* infecting American plaice in the St. Lawrence estuary should be identified and studied further, as it may prove to be a sensitive early warning indicator of contamination by organic pollutants in coastal habitats.

An intriguing result obtained herein is the lack of change observed among gastrointestinal digeneans in plaice after exposure to contaminated sediments. This is especially true in light of the results obtained elsewhere on marine flatfish (Khan and Kiceniuk 1983; Khan 1991). Khan and Kiceniuk (1983) found a decrease in the prevalence and abundance of the trematode *Steringophorus furciger* in winter flounder (*Pleuronectes americanus)* and of the acanthocephalan *Echinorhynchus gadi* in Atlantic cod after exposure to sediments or water contaminated with crude oil extracts. They proposed the decline in gastrointestinal helminths to be the result of direct toxicity of the contaminants to the parasites or from modification of intestinal physiology, rendering the gut less suitable for parasite habitat. Digeneans also were absent from longhorn sculpin (*Myxocephalus octodecempinosus*) exposed for 6 months to crude oil contaminants. Siddall et al. (1994) observed inconsistent patterns in the

distribution of gastrointestinal digeneans, including *S. furciger, S. ovacutum,* and *D. varicus,* in *H. platessoides* at contaminated dump sites compared to reference sites. Given that the major contaminants in our study and those of Khan and colleagues are PAHs, the contrasting results may be explained by the availability of the contaminants to the fish. Unfortunately, the contaminant levels were measured differently in the two studies. The level of PAHs at our contaminated site was 39.8 µg/g, whereas in the studies of Khan and Kiceniuk (1983), levels in the water column measured 2600 µg/L. Conceivably, the contaminants in our experiments were bound to the sediments and not as accessible to the fish as would be waterborne contaminants. Indeed, water soluble crude oil extracts seem to have a more pronounced effect on the parasite fauna than do oil-contaminated sediments (Khan and Kiceniuk 1983). In addition, the sediments used by Khan and Kiceniuk (1983) contained 2300-4500 µg/g of Venezuelan crude oil (Fletcher et al. 1981). PAHs and PCBs *can* make up 15-20% of total oil (*K Lee, Dept. of Fisheries and Oceans, pers. comm.*), providing sediment concentrations of 350-900 µg/g of these contaminants, much higher values than occurred in our studies. Thus, concentrations of contaminants in Baie des Anglais sediments may not have been high enough to directly affect gastrointestinal helminths.

For most endoparasites, especially those located outside the gastrointestinal tract, changes in abundance resulting from polluted conditions are more often attributable to changes in the populations of other hosts in the life cycle or to direct effects of the contaminant on short-lived, sensitive, free-living stages of the parasites (MacKenzie et al. 1995). No. significant changes in abundance were observed for most parasites infecting American plaice in our experiments. Similarly, no changes were detected in the abundance of a variety of parasites infecting longhorn sculpins exposed to contaminated sediments, including *Anisakis* sp. *Contracaecum* sp., *Echinorhynchus* sp., *Gyrodactylus* spp., and *Myxidium* sp. (Khan 1991).

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REFERENCES

Axelsson B, Norrgren L (1991) Parasite frequency and liver anomalies in three-spined stickleback, *Gasterosteus aculeatus* (L.), after long-term exposure to pulp mill effluents in marine mesocosms. Arch Environ Contam Toxicol 21:505-513

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575-583

Fletcher GL, Kiceniuk JW, Williams UP (198 1) Effects of oiled sediments on mortality, feeding and growth of winter flounder *Pseudopleuronectes americanus.* Mar Ecol Prog Ser 4:91-96

Halmetoja A, Valtonen ET, Taskinen J (1992) Trichodinids (Protozoa) on fish from four central Finnish lakes of differeing water quality. Aqua Fenn 22: 59-70

Khan RA (1990) Parasitism in marine fish after chronic exposure to petroleum hydrocarbons in the laboratory and to the Exxon Valdez oil spill. Bull Environ Contam Toxicol 44:759-763

Khan RA (1991) Effect of oil-contaminated sediment on the longhorn sculpin (*Myoxocephalus octodecemspinosus*) following chronic exposure. Bull Environ Contam Toxicol 47:63-69

Khan RA, Kiceniuk J (1983) Effects of crude oils on the gastrointestinal parasites of two species of marine fish. J Wild1 Dis 19:253-258

Khan RA, Kiceniuk J (1988) Effects of petroleum aromatic hydrocarbons on monogeneids parasitizing Atlantic cod, *Gadus morhua* L. Bull Environ Contam Toxicol 41:94-100

Khan RA, Thulin J (199 1) Influence of pollution on parasites of aquatic animals. Adv Parasitol 30:201-238

Khan RA, Barker DE, Williams-Ryan K, Hooper RG (1994) Influence of crude oil and pulp and paper mill effluent on mixed infections of *Trichodina cottidarium* and *T. saintjohnsi* (Ciliophora) parasitzing *Myoxocephalus octodecemspinosus* and *M. scorpius.* Can J Zoo1 72:247-251

Lehtinen KJ, Notini M, Landner L (1984) Tissue damage and parasite frequency in flounders, *Platichtys flesus* (L.) chronically exposed to bleached kraft pulp mill effluents. Ann Zool Fennici 21:23-28

MacKenzie K, Williams HH, Williams B, McVicar AH, Siddall R (1995) Parasites as indicators of water quality and the potential use of helminth transmission in marine pollution studies. Adv Parasitol 35:85-144

Nagler JJ, Cyr DG (1997) Exposure of male American plaice (*Hippoglossoides plutessoides*) to contaminated marine sediments decreases the hatching success of their progeny. Environ Toxicol Chem 16: 1733-1738

Overstreet RM (1988) Aquatic pollution problems, southeastern U.S. coasts: histopathological indicators. Aquat Toxicol 11:213-239

Overstreet RM, Howse HD (1977) Some parasites and diseases of estuarine fishes in polluted habitats of Mississippi. Ann NY Acad Sci 298:427-462

Poulin R (1992) Toxic pollution and parasitism in freshwater fish. Parasitol Today 8:58-61

Siddall R, Koskivaara M, Valtonen ET (1997) *Dactylogyrus* (Monogenea) infections on the gills of roach (*Rutilus rutilus* L.) experimentally exposed to pulp and paper mill effluent. Parasitology 114:439-446

Siddall R, Pike AW, McVicar AH (1994) Parasites of flatfish in relation to sewage sludge dumping. J Fish Biol 45:193-209

Skinner RH (1982) The interrelation of water quality, gill parasites, and gill pathology of some fishes from south Biscayne Bay, Florida. Fish Bull 80:269-280