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Two Species of Commercial Flatfish, Winter Flounder, *Pleuronectes americanus*, and American Plaice, *Hippoglossoides platessoides*, as Sentinels of Environmental Pollution

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Abstract Two species of marketable-size flatfish, winter flounder (*Pleuronectes americanus*) and American plaice (*Hippoglossoides platessoides*) from a fjord, were examined as sentinels for the effects of effluent discharged by a pulp and paper mill in Newfoundland, Canada. Reference samples for comparison were obtained from a pristine fjord. Condition factor, lymphocyte levels and the abundance of two intestinal parasites were significantly greater in flounder and plaice sampled at the reference site while macroscopic and microscopic lesions, and hepatic somatic indices were greater in flatfish taken from the contaminated site. These results suggest that both species of commercial flatfish are useful as sentinels for studying environmental pollution.

Keywords Flatfish · Sentinels · Paper mill · Pollution

Some persistent organic pollutants (POPs) in the aquatic environment are known to disrupt homeostasis in fish following chronic exposure and eventually affect reproduction especially effluent discharged by pulp and paper mills (Goksoyr et al. 1996; Hewitt et al. 2008; McMaster et al. 2006; Munkittrick et al. 2002 and references therein). Resin acids and phytosterols have been identified as the main components in the outfall that cause these changes in both fresh water and marine fish. Abnormalities include an enlarged liver, elevated levels of detoxifying enzymes, blood anomalies, disruption of reproductive hormones and fecundity (McMaster et al. 2006). Species of flatfish such as winter flounder (*Pleuronectes americanus*) and American plaice (*Hippoglossoides platessoides*) are known to inhabit sediment where many POPs tend to accumulate and exhibit stress-related abnormalities (Khan 2000; Khan and Billiard 2007). Both flounder and plaice are exported from Newfoundland for human consumption but little is known of the quality of the product. Moreover, biotransformation of POPs can release metabolites that are genotoxic, carcinogenic (Singh and Singh 2008) and accumulate in adipose tissue in the fillets. Consequently, the aim of the present study was to assess the health of a commercial sample of flounder and plaice from a fjord receiving effluent from a pulp and paper mill as sentinels based on the premise that the product was destined for human consumption. A number of bioindicators including body and hepatic indices, blood values, external and tissue lesions and selected parasites infecting the digestive tract was used to compare these groups with samples that originated from another fjord that was considered a pristine site. The rationale to use only certain parasites was based on observations that many parasitic infections in nature are too low grade to be useful as bioindicators between groups of impacted and reference fish (Khan and Thulin 1991; Marcogliese et al. 1998).

Materials and Methods

Winter flounder and American place were purchased from a commercial fisherman after capture offshore at Benoit's Cove (49°03′N, 58°13′W) in the Humber Arm shortly after arrival at a port. This site is located about 5 km down-current in the line of flow from the pulp and paper mill. Wet debarking of logs, mainly black spruce (*Picea mariana*) and balsam fir (*Abies balsamea*), has led to an

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accumulation of fibre and bark at the bottom of the fjord (Khan and Billiard 2007 and references therein). The mill produced newsprint by thermomechanical and sulphite-bleaching processes up to 1992 but the latter was discontinued subsequently. The effluent containing suspended solids, resin acids and tannins was discharged without treatment until 1997. Only fish that displayed some form of movement were selected for sampling. A note was made of any external macroscopic lesions and then each fish was bled from the caudal vein with a heparinized syringe and needle for determination of hemoglobin, using a hemoglobinometer, and lymphocyte levels from smears on glass slides after staining with Giemsa. Total body length, eviscerated body and liver weights were recorded before samples of gill, liver and spleen were removed for histological assessment following conventional methods of fixation and staining with hematoxylin and eosin. Additional sections of spleen were stained with Perl's Prussian blue for hemosiderin, its concentration estimated by digital image analysis and expressed as a percentage of the area scanned (Khan and Nag 1993). The digestive tract was removed, the food contents recorded and metazoan parasites removed, enumerated, fixed and stained by conventional methods for identification. Two commonly occurring metazoan parasites, a digenean, *Steringophorus furciger*, and an acanthocephalan, *Echinorhynchus gadi*, in the digestive tract were selected for comparison. Reference samples of flounder and plaice of comparable size were obtained from a commercial catch using a line trawl in a pristine fjord, Bonne Bay, at Rocky Harbour (49°59′N, 57°97′W). These fish were examined and autopsied similarly to others reported previously.

Condition (k) factor (eviscerated body weight/length^3 and hepatosomatic index (liver weight/eviscerated body weight) were compared for significance by the ANOVA. Means and standard errors for the variables were calculated for each fish group. The non-parametric test of Kruskal–Wallis compared parasitic abundance between groups while the Chi-square test was used to compare prevalence (%). Mean abundance and prevalence follow the terminology suggested by (Bush et al. 1997).

Results and Discussion

Some differences were detected between the groups of winter flounder and American place sampled from the Humber Arm and the reference site. External lesions, primarily fin necrosis and epidermal ulcers were observed in all fish from the Humber Arm but not in any from the reference site (Table 1). K-factor and hepatosomatic index were significantly different between the groups of flounder and plaice sampled at the two sites. Additionally, both groups of flounder and plaice from the Humber Arm had significantly lower hemoglobin and lymphocyte levels than those from the reference site.

Lesions were more prevalent in both the winter flounder and American plaice captured from the Humber Arm than from the reference site. Severe hyperplasia of the secondary branchial lamellae and interlamellar troughs resulted in partial fusion of adjacent filaments. Lesions in the liver included clear cell foci, characterized by aggregates or linear arranged vacuolated cells. Microscopic pericholangial fibrosis with a monoclonal inflammatory infiltrate and multifocal macrophage aggregates, were also observed only in samples from the Humber Arm (Table 1). A greater concentration of hemosiderin pigment was noted in the spleen of flounder and plaice taken down-current from the paper mill.

The type of food ingested and parasites in the digestive tract also differed in fish sampled from the two sites. Both flounder and plaice in the Humber Arm fed mainly on algae, unidentified gastropods and fish offal while a greater variety of prey was observed in the groups from the reference site. Flounder at the latter location fed on polychetes (*Nereis* sp), bivalves (*Mytilus edulis*), amphipods (*Gammarus* spp.) and fish eggs while remains of shrimp (*Pandalus borealis*), polychetes, mussels and fish eggs were present in the stomach of plaice. Two dominant metazoan parasites, viz., a digenean *S. furciger*, and an acanthocephalan, *E. gadi*, were significantly greater in abundance and prevalence in both species of reference fish than in samples from the Humber Arm (Table 1).

Results from the present study suggest that the two species of flatfish of marketable-size, sampled in a fjord receiving untreated effluent from a pulp and paper mill, exhibited evidence of stress-related abnormalities and were useful as sentinels for assessing chronic exposure to toxic waste. This report supports previous observations that fish inhabiting the inlet are affected by the discharge (Billiard and Khan 2003; Khan and Billiard 2007). These changes included a higher prevalence of external lesions probably associated with the toxic effluent, a lower condition factor caused either by inadequate food or metabolic disturbance but a greater liver somatic index, a reflection of elevated detoxifying enzyme activity than in fish sampled at a reference site (Khan and Payne 2002). Similar results have been reported in other species of fish, especially flatfish, living in environments degraded by anthropogenic discharges (Khan 2000). Additionally, discharges by pulp and paper mills have affected several species of fish inhabiting fresh water (Munkittrick et al. 2002; McMaster et al. 2006; Hewitt et al. 2008 and references therein).

Microscopic anomalies including hemoglobin and lower levels of circulating lymphocytes and a high prevalence of toxicopathic lesions in tissues, impairing some

Table 1 Comparison of biological variables (0 ± SE) in marketable-size female winter flounder and American plaice sampled down-current from a pulp and paper mill (PPE) at Benoit's Cove and at a pristine reference (Ref) site at Rocky Harbour, Newfoundland in 1998

Variable	Winter flounder		American plaice	
	Ref	PPE	Ref	PPE
n	21	17	19	14
Length (cm)	30 ± 2.0	33 ± 2.1	40 ± 3.2	43 ± 2.4
Weight (g)	298 ± 10	317 ± 14	446 ± 16	369 ± 23
K-factor ($\times 10^{-3}$) 0.05	1.04 ± 0.07*	0.86 ± 0.08	0.76 ± 0.05*	0.62 ± 0.05
Liver s.i. ($\times 10^{-2}$)	2.8 ± 0.3	7.0 ± 0.9*	2.9 ± 0.5	9.8 ± 1.2*
Hemoglobin (g%)	5.6 ± 0.4	3.1 ± 0.2	5.8 ± 0.5	3.4 ± 0.3
Lymphocytes/1,000 RBC	72 ± 6*	48 ± 4	62 ± 5*	21 ± 7
Lesions				
Skin				
Fin necrosis (%)	0	100*	0	100*
Epidermal ulcers (%)	0	100*	0	100*
Gills				
Hyperplasia				
Lamellar (%)	0	100	0	100
Interlamellar (%)	0	100	0	100
Liver				
Clear cell foci (%)	0	100	0	100
Pericholangial fibrosis (%)	0	100	0	100
Macrophage aggregates (%)	0	100	0	100
Spleen				
Hemosiderin (%/mm^2)	0	8.2 ± 1.2*	0	7.1 ± 0.9*
Parasites				
Steringophorus furciger [0 (%)]	6.2 ± 0.7 (100)*	0.9 ± 0.1 (47)	9.6 ± 1.1 (100)*	1.1 ± 0.4 (43)
Echinorhynchus gadi [0 (%)]	11.4 ± 1.1 (100)*	1.8 ± 0.5 (53)	7.2 ± 0.6 (100)*	0.9 ± 0.2 (50)

* Significantly different from other group

physiological functions, of flounder and plaice inhabiting the Humber Arm were different from samples taken at the reference site. Hyperplasia of the secondary branchial lamellae probably affected oxygen exchange and foraging activity. Lesions in the liver including widespread clear cell foci, pericholangial fibrosis and multifocal macrophage aggregates were observed only in samples from the Humber Arm. Some of these abnormalities have been reported previously in fish inhabiting degraded habitats (Moore and Stegeman 1994). Additionally, excessive deposition of hemosiderin, resulting from erythrocytic destruction, in the spleen, has been observed before in fish exposed to environmental contaminants (Khan and Billiard 2007 and references therein). Low lymphocyte levels was probably associated with a decline of the immune response and an increase of susceptibility to disease, i.e., fin necrosis, while toxicity of the discharge was likely responsible for the tissue lesions.

The prevalence and abundance of two commonly occurring metazoan parasites in the digestive tract of flounder and plaice supports previous observations that some parasites can be useful as bioindicators of environmental change (Khan and Thulin 1991). Both the digenean, *S. furciger*, and the acanthocephalan, *E. gadi*, were more abundant in fish taken at the reference site than in the Humber Arm. These differences were probably associated with an interruption of transmission to the fish host or voiding of the parasites after infection. Laboratory studies support the view that some parasites are voided caused by changes in gut physiology but toxicity of the latter to sensitive larval stages of some appear also to occur in the field (Khan and Thulin 1991; MacKenzie et al. 1995; Marcogliese et al. 1998).

Flatfish are bottom feeders where POPs tend to accumulate and in fish these can potentially bioconcentrate and biomagnify via the food chain following chronic exposure (Singh and Singh 2008, and references therein). Biotransformation of POPs is known to result in metabolites that are genotoxic and carcinogenic following detoxification and can accumulate in adipose tissue (Singh and Singh 2008). Since winter flounder from the Humber exhibit evidence of hepatic detoxifying enzymes, it is possible that metabolites of PPE such as resin acids and wood sterols in sediment might accumulate in the fillets (Lappänen and Oikari 1999; Khan and Payne 2002; Meriläinen et al. 2006). Local residents report an off-flavour in all fish species from the Humber Arm (Khan, unpubl. data). In view of these reports, the anomalies noted in the present study, and fish tainting downstream from discharges by pulp and paper mills, an investigation in the occurrence and quantity of these metabolites in the fillets of winter flounder and American plaice would be relevant to ascertain their potential impact on human health (Heil et al. 1989; Kenefick et al. 1995; Redenbach 1997).

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