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Effect of Pulp and Paper Effluent on a Marine Fish, *Pseudopleuronectes americanus*

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Several studies have reported that aquatic organisms are affected by the effluent discharged from pulp and paper mills, especially benthic species living downstream (Lindström-Seppa and Oikari 1989). Some of these effects include impairment of growth, skin disruption, liver disfunction, kidney damage, vertebral deformation, histopathological and physiological changes (Anderson et al. 1988; Couillard et. al. 1988; Hardig et al. 1988; Santos et al. 1990; Whittle and Flood 1977). Components in pulp and paper mill effluent include resin and fatty acids, chlorophenols, dioxins and furans that are produced when chlorination is used in the bleaching process (Waldichuk 1990). Some of these toxins can persist in the environment and there is evidence of bioaccumulation which ultimately might cause reproductive failure and malignancies.

The purpose of the present study was to ascertain the effect of untreated effluent from two pulp and paper mills on a benthic marine fish, Pseudopleuronectes americanus, living downstream from the outfall. A reference site was chosen in an area where there is no evidence of discharge from pulp and paper mills. Included in this study is a comparison of the parasitofauna of flounder taken from the three areas in 1990 and data that were obtained from the same fish species at one of the sites in 1978 before the pulp and paper mill commenced its operation.

MATERIALS AND METHODS

Winter flounder were collected by SCUBA divers in late June 1990 from three areas in western Newfoundland, Port Harmon (48° 32'N,58°33'W) and Birchy Cove (48°57'N, 58°00 W)] located about 1 and 4 km, respectively, from two pulp and paper mills and an unpolluted (reference) site, Norris Point (49°32'N,

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57°52 W). Both plants use chlorine for bleeching and discharge untreated effluents into inlets of the ocean. The plant near to Port Harmon once produced lineboard products but later (1982) changed to pulp and paper production. Flounder were collected during daylight (1000-1500 hr) at depths of 6 to 15 m. Samples of fish were also obtained by hook and line in June 1978 and by SCUBA divers in early May 1990 at Port Harmon. Water temperature at the three areas in June 1990 varied from 4 to 8°C as it did in June 1978 and about 2°C in May 1990. The bottom sediment at Port Harmon and Birchy Cove was composed of a dark, muddy sand with conspicuous quantities of bark fragments and wood fiber as well as patches of microbial films whereas at Norris Point it was sandy with clear visibility.

All of the fish collected in June 1990 were bled shortly after capture for determination of hemoglobin levels. The total length, body and organ [liver, spleen and digestive tract (gut)] weights of each fish were recorded. Tissue samples, which included spleen, were fixed in 10% buffered formalin, processed by conventional histological methods and sections, 6 μm in thickness, stained with hematoxylin and eosin. Sections of spleen were also stained with Perl's Prussian blue for hemosiderin. The gut of each fish was frozen and subsequently examined for food contents and endoparasites. In the 1978 fish sample from Port Harmon, the length and weight of each fish were recorded but only blood and parasites were taken for examination. Samples of flounder, collected in early May, 1990 were frozen shortly after capture and examined later for parasites.

The data was statistically compared between groups using the one or two-way ANOVA for differences related to treatment. Mean and standard errors were determined for condition (k) factor (W/L^3), organ somatic indices (organ weight/total body weight), hematological variables and parasitism. Differences were considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

Winter flounder collected at Port Harmon in June 1990 were emaciated, had pale pink gills and about 90% suffered from acute fin and tail necrosis (Fig. 1). The majority were infected with numerous metacercariae of the digenetic trematode, most likely <u>Cryptocotyle lingua</u>. Both fin erosion and metacercariae were considerably lower in the sample from Birchy Cove whereas no fin damage but a low prevalence of metacercariae was observed in the sample from Norris Point (Fig. 2). Comparison of k-factor, organ somatic

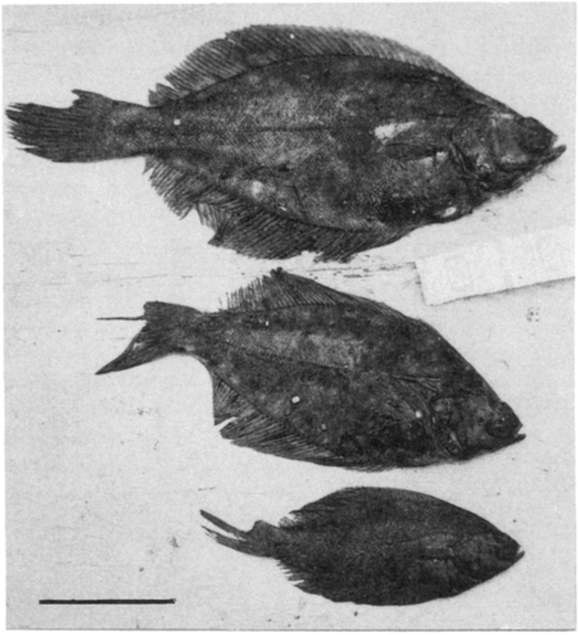

Figure 1. Fin necrosis in winter flounder from Port Harmon (June 1990). (Scale bar = 5 cm).

indices and hemoglobin in winter flounder taken from the three areas indicated all were significantly lower in fish from Port Harmon than from Norris Point (Table 1). Although several of the values in the sample from Birchy Cove were lower than those at Norris Point, only length, weight and hemoglobin levels were significantly lower. However, the number of melanomacrophage centers containing hemosiderin were substantially greater in flounder taken from the two contaminated sites than from the reference area.

Differences were also observed in the stomach contents of winter flounder examined from the three areas. Eighty five percent (22) of 26 fish from Norris Point had fed on polychetes and bivalve molluscs. At Birchy Cove 59% (16) of 27 flounder had been feeding mostly on polychetes, amphipods (Gammarus spp.) and crabs (Hyas spp). In contrast, 87% (40) of 46 flounder, collected at Port Harmon, had empty stomachs and the remaining were feeding mainly on algae and bivalves which, in the mid-intestinal region, were undigested when compared to the samples examined at Norris Point. The prevalence of parasitism varied in flounder collected from the three areas. The anisakid nematode was observed in fish from all areas but was the least prevalent in samples taken at Norris Point (Fig. 2).

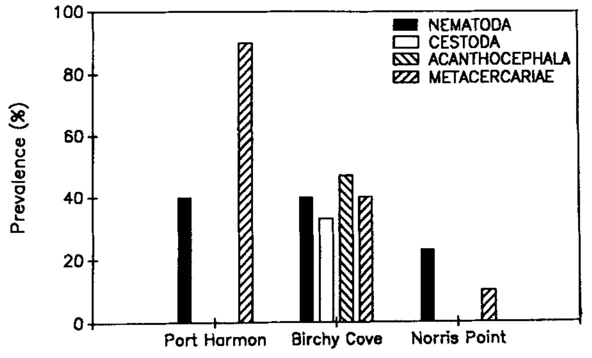

Figure 2. Prevalence (%) of anisakid nematodes, unidentified cestodes, Acanthocephala (Echinorhynchus sp.) and metacereariae of Cryptocotyle lingua in winter flounder collected at Port Harmon, Birchy Cove and Norris Point, western Newfoundland.

Table 1. Comparison of variables in winter flounder, Pseudopleuronectes americanus, taken from two pulp and paper effluent discharge sites (P.H. and C.B.) and an unpolluted area (N.P.).

Variable	Port Harmon (n=46)	Birchy Cove (n=27)	Norris Point (n=26)
Length (cm)	22.0 ± 0.7*	27.5 ± 0.1*	30.5 ± 1.2
Weight (g)	108 ± 15	218 ± 17*	326 ± 38
Condition Factor (W/L^3)	0.79 ± 0.04*	1.00 ± 0.02	1.07 ± 0.03
Liver s.i.	1.8 ± 0.3	3.8 ± 0.5$^+$	4.3 ± 0.7
Spleen s.i.	0.10 ± 0.01*	0.25 ± 0.05$^+$	0.36 ± 0.10
Gut s.i.	9.3 ± 1.4*	20.3 ± 2.2$^+$	18.7 ± 2.9
Hemoglobin (g%)	2.4 + 0.3*	2.8 + 0.4*	6.3 + 0.3

* significantly < (0.05) Norris Point sample
\+ significantly > (0.05) Port Harmon

No differences in intensity were observed in flounder from the three areas. Acanthocephala (Echinorhynchus sp. (intensity, 2.5 ± 0.3) and unidentified cestodes (intensity, 0.4 ± 0.1) were observed only in fish taken at Birchy Cove.

Comparisons of variables in flounder collected at Port Harmon in 1978 and 1990 suggests major differences (Table 2). No signs of fin erosion were observed in the fish sampled in 1978. The total length, body

Table 2. Comparison of variables in winter flounder, P. americanus, collected at Port Harmon, Newfoundland in 1978 and 1990.

Variable	Year 1978 (n = 17)	Year 1990 (n = 46)
Length (cm)	34 ± 0.9	22 ± 0.07*
Weight (g)	500 ± 39	108 ± 15*
Condition factor (W/L^3)	1.27 ± 0.08	0.79 ± 0.02*
Hemoglobin (g%)	5.9 ± 0.4	2.4 ± 0.3*
Glugea stephani (# infected)	9	0
Metacercariae (# infected)	6	41
Echinorhynchus sp. (# infected)	7	0

* significantly <(0.05) 1978 sample

weight and k-factor were significantly greater ($P \leq 0.05$) in fish taken in 1978 than in 1990. Moreover, the occurrence of cysts of Glugea stephani (Microspora) on the wall of the gut was observed only in fish collected in 1978. In contrast, the prevalence of metacercariae was substantially greater in flounder examined in 1990 (90%) than in 1978 (35%). Additionally, seven fish were infected with Echinorhynchus sp. (intensity 1.3 ± 0.2) in 1978 whereas none was observed in 46 fish examined in 1990.

Tumors were noted in the kidney of six of 15 winter flounder sampled in May 1990 (Fig. 3). These included four females and two males. Twelve fish displayed evidence of fin necrosis and 13 were infected with numerous metacercariae of the digenean trematode, C. lingua. No food was present in the stomach of the fish as they do not feed during winter and early spring. No tumors were observed in flounder taken from the same site about 6 wk later (June 1990).

This is another report of neoplasms occurring in adult flounder associated with pulp and paper mill effluents. Two additional studies attributed high prevalences of neoplastic lesions in the liver and skin tumors to pulp and paper effluents (Malins et al. 1983; Kimura et al. 1984). The reason for the occurrence of tumors only in the sample taken at Port Harmon in early May and not in late June 1990 is unknown. Origin of the tumors is speculative pending chemical analysis of the outfall. Dioxins and furans are produced as biproducts during the bleaching process and have been reported to be carcinogenic especially following long-term exposure

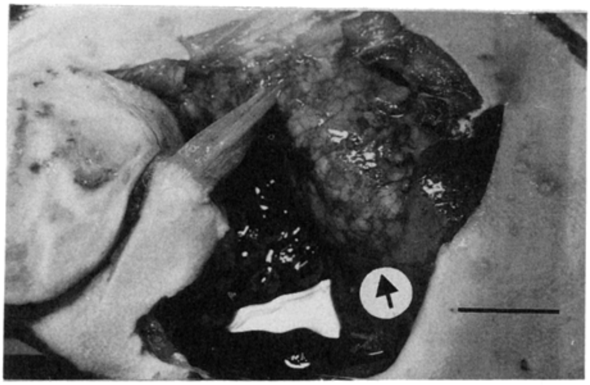

Figure 3. Kidney tumor (arrow) in female winter flounder collected at Port Harmon in May 1990. (Scale bar = 0.25 cm).

(Waldichuk 1990). Possibly, the flounder, which tend to migrate into estuarine areas over winter/spring to spawn/spermiate and where nursery grounds are located, might have been exposed to the effluent for some time. Flounders, moreover, are more likely to be affected by effluents than other species of fish since they tend to remain in local areas for extended periods of time, feed mainly on benthic organisms and bury themselves in sediment when not foraging for food. Further studies are required to clarify this situation.

Additional adverse effects associated with the discharge in flounder were reflected in low k-factor, organ somatic indices and hemoglobin and levels; all of these are suggestive of impairment of growth and hemopoiesis and/or increased blood loss reported previously in other studies (Whittle and Flood 1977; Hardig et al. 1988). The increase of melanomacrophage centers in fish from the contaminated sites in the present and another study (Santos et al. 1990) is a reflection of excessive erythrocytic destruction. Moreover, the presence of fin erosion in association with low lymphocyte levels suggests that defence mechanisms were compromised (Hardig et al. 1988). Fin necrosis has been observed also in fish exposed to pulp mill effluent (Couillard et al. 1988; Lindesjöö and Thulin 1990). It is known that exposure of fish to bleached kraft mill effluent induces stress which is linked to suppression of the immune system and mediated through the release of catecholamines and ACTH (McLeay and Brown 1979; Andersson et al. 1988). Alteration of susceptibility in flounder probably permitted opportunistic bacteria and/or viruses to establish an infection which caused tail necrosis after disruption of the mucus barrier (Couillard et al. 1988). It is, therefore, not surprising that the parasite fauna of the fish in the present and other studies were also altered. The effluent appeared to have had a negative affect on the spores of G. stephani which are transmitted directly from fish to fish. However, it appears that molluscs (Littorina saxatilis and L. littorea), which serve as intermediate hosts of C. lingua, were not affected as the parasite was substantially greater in the 1990 than in the 1978 sample. Three additional studies have reported an increase in prevalence and intensity of ectoparasites (trichodinid ciliates and monogenetic trematodes) in fish exposed to pulp and paper effluents (Lehtinen et al. 1984; Thulin et al. 1988; Valtonen and Koskivarra 1989). However, intestinal trematodes and ectoparasitic copepods in some fish were absent or decreased in prevalence when in contact with the effluent (Valtonen and Koskivaara 1989; Thulin et al. 1988).

Major aspects of long-term pollution caused by effluents from pulp and paper mills include the negative effects on fish populations and communities. In Scandinavia, salmonid species disappeared from polluted waters and were replaced by species such as pike, perch and roach (Lindström-Seppa and Oikari 1989). Moreover, mortality rates were higher in perch populations in a Baltic pulp mill effluent area than from a reference site (Sandstrom and Thoresson 1988). Another aspect involves bioaccumulation of some biproducts in the effluent, i.e., dioxins and furans, which are known to be carcinogenic and can be transmitted through the food chain (i.e., fish and shellfish) to fish-consuming birds and mammals, including man (Waldichuk 1990). In view of these reports, a study on the ecological and health consequences of the pulp and paper mill effluent, especially at Port Harmon, is needed to assess its long-term impact.

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