Bull. Environ. Contam. Toxicol. (1997) 58:442-447

Environmental Contamination and Toxicology

Stress-Related Changes in Cunner, *Tautogolabrus adspersus* Living Near a Paper Mill

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Received: 20 May 1996/Accepted: 1 November 1996

Several species of fish have been reported to be affected by the effluent discharged after bleaching of wood for the production of pulp and paper and linerboard products (Owens 1991). The bleaching process uses either chlorine or sulphur derivatives, and the discharge includes dioxins, furans, resin acids and many other chemicals, some of which are believed to be toxic to aquatic life (Munkittrick *et al.* 1991; Råbergh *et al.* 1992). Physiological irregularities reported in fish also include lesions (Munkittrick *et al.* 1992a), tainting (Munkittrick *et al.* 1992b), changes in growth (Munkittrick *et al.* 1992a), elevated levels of detoxifying enzymes (McMaster *et al.* 1991; Munkttrick *et al.* 1991; Lindström-Seppä *et al.* 1992), skeletal deformities (Bengtsson *et al.* 1988), impaired reproduction (Munkittrick *et al.* 1992a) and possibly mortality (Lindström-Seppä and Oikari, 1990; Munkittrick *et al.* 1991; Neuman and Karås 1988). These reports initially implicated dioxins and furans, byproducts of chlorine bleaching, as the main causes of the anomalies. But recent studies suggest that these manifestations also apply to fish living in areas where sulphite-bleaching is used (Munkittrick *et al.* 1994). Khan *et al.* (1992, 1994); in a sulphite-bleaching study on winter flounder, *Pleuronectes (Pseudopleuronectes) americanus,* collected in the vicinity of a pulp and paper mill in Newfoundland reported skin lesions, tumours, impairment of growth, low hemoglobin and organ somatic indices, histopathology and changes in the parasite fauna. These abnormalities were not observed in samples collected at two reference sites. A study on longhorn Myoxocephalus octodecemspinosus and shorthorn *(M. scorpius)* sculpins sampled near the same pulp mill also reported some effects but these were less pronounced than in winter flounder (Barker et al. 1994b). In view of these reports, the current study was conducted to ascertain whether or not changes occur in the tissues of cunner, (Tautogolabrus adspersus) an inshore, non-migratory species collected near the same pulp and paper mill in contrast to samples collected at the same up-current reference site.

Since the cunner is a year-round, inshore resident living on or near the bottom in shallow waters, it has the potential of being an indicator of both short- and longterm changes in environmental conditions (Green, 1975).

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MATERIALS AND METHODS

Adult cunners were captured by SCUBA divers at depths of 5 metres and by hook and line in August, 1992 from the study site at Port Harmon (48°31' N, 058°33' W) situated about 1 km from a pulp and paper mill and from the reference site, St. George's, located about 12 km across the inlet, up-current in St. George's Bay (see Barker *et al*, 1994a).

A total of 49 cunners were captured at the two study sites, killed by spinal dislocation and the mean length, eviscerated body and gonadal weights recorded. Twenty gills, 19 liver and spleen and 18 hind kidneys were removed and fixed in 10% neutral buffered formalin for histopathology from cunners at each site (Port Harmon and St. George's). The tissues were dehydrated, embedded in wax and sections of tissue from each fish cut at 7mm in the thickness were stained with hematoxylin and eosin for histological assessment, whereas, kidney and spleen tissues were also stained with Perl's Prussian blue method for hemosiderin. Some gill sections were also stained with periodic acid-Schiff, a diagnostic stain for carbohydrates in mucous cells. Quantitative analysis of hemosiderin in splenic tissue of cunner was determined by digital image analysis (Khan and Nag 1993). Estimates of hemosiderin concentrations were expressed as percentages of the standardized examined area- the ratio of hemosiderin present to the total area examined. Metacercariae of Cryptocotyle lingua was by enumerated as the number on 10 primary gill lamellae. In addition, hyperplasia of the epithelial cells in the gills of cunner from both study sites was arbitrarily divided into three categories: slight, moderate and severe.

An ANOVA was used comparing variables in fish taken from the two sites (Port Harmon) and the reference site (St. George's). Kruskal-Wallis ANOVA tests were used to determine the significance between the abundance and intensity of the metacercariae of the digenetic trematode, *C. lingua* infections on the gills of cunner (see Sekhar and Threlfall 1970) from the two study sites as well as to evaluate the significance of splenic and of renal hemosiderin deposits from both study sites. A chi-square test was used to evaluate the significance between the numbers of cunners in St. George's, Port Harmon with hepatic vacuolation. Differences were considered significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

A comparison of k-factor and organ somatic indices between cunner sampled at the reference site and the paper mill revealed no significant differences (Table 1). Similarly, no differences were seen in two species of sculpins (Myoxocephalus spp.) captured at the same impacted and reference sites but in winter flounder the differences were significant (Barker et al. 1994a, 1994b). Generally, flounder captured near the paper mill had lower k-factor, but hepatic and gonadal somatic indices were significantly greater than in samples taken at the reference sites (Barker et al, 1994a).

Examination of gill sections from cunner taken from Port Harmon revealed a greater occurrence of lesions than in samples taken from the reference site

(Table 1). The majority of fish (55% of 20) taken from St. George's showed normal gill architecture or slight hyperplasia. Nine fish (45%) from those sampled in St. George's showed evidence of slight hyperplasia. While slight hyperplasia occurred in six fish (30%) sampled from near the pulp mill, 14 (70%) displayed moderate to severe hyperplasia. Previous studies on winter flounder and sculpins revealed that hyperplasia in the gill was also more pronounced near the paper mill than at a reference site (Khan et al. 1994; Barker et al. 1994b). Similar findings have also been reported in fish living under the influence of pulp mill discharge (Lehtinen *et al.* 1984; Lindström-Seppå and Oikari 1990). At least two studies have shown an increase in ciliated protozoa parasitizing the gill lamellae of fish living near the pulp mill (Lehtinen *et al.* 1984; Barker *et al.* 1994b). However, the prevalence and abundance of the metacercariae of the digenetic trematode *C. lingua* in cunners from St. George's and Port Harmon were not significantly different although these variables of the parasite on the fins and gills were greater on flounder captured near the mill than at the reference site (Barker et al. 1994a).

Hepatic abnormalities occurred in fish taken from both sites. The liver in the cunner, and some other teleosts, is a site for energy storage (lipid and glycogen) in addition to other functions. Lipid is recognized by the vacuolated hepatic cells whereas, cells devoid of lipid, tend to stain basophilic (Bucher *et al.* 1992). Eighty-four percent of cunner sampled at the reference site showed evidence of vacuolation in the liver contrast to 10 specimens (53%) from the vicinity of the pulp and paper mill. Lipid depletion was also recognised in sculpins captured near to the same pulp mill (Barker et al. 1994b). Such depletion might be associated with reduced feed intake as noted recently in the redbreast sunfish (Lepomis auritas) (Adams et al. 1992).

Hemosiderosis was the major lesion observed in the spleen and kidney, in samples taken at the reference site and near the pulp and paper mill. The size of melanomacrophages (MMC's /mm^2) in the spleen of fish from Port Harmon was larger and occupied a greater percentage of total area than in the fish from the reference site. Similarly, cunner from Port Harmon had a higher mean number of hemosiderin deposits than did samples collected at St. George's. This abnormality has been reported previously for other species of fish exposed to pulp mill effluent (Khan et al. 1994; Barker et al. 1994b)

Gastrointestinal parasites were observed only in cunners captured at St. George's (Table 1). Forty- three percent of the fish taken at St. George's were infected with the acanthocephalan Echinorhynchus gadi, and its abundance was 2.6 worms/fish. In contrast, none was infected at Port Harmon.

The results of the present study provide evidence that histopathological changes were more pronounced in cunners sampled near the pulp and paper mill than at the

reference site in spite of the observations that no significant differences occurred in body length, eviscerated body weight, condition factor or gonadal somatic indices. The presence of lesions in the gills, liver, spleen and kidney of cunner taken at Port Harmon confirm the usefulness of histopathology in assessing health of the cunner exposed to discharge from a pulp and paper mill (Hilton and Lauren 1990). These stress-related lesions, however, were not as pronounced in the cunner as in winter flounder inhabiting the same site but appear similar to those observed in the two species of sculpins. This observation appears to be likely as flounder tend to submerge themselves in sediment whereas sculpins and cunner lie above the sediment. Consequently, exposure to xenobiotics which can gain entry through various sites in submerged fishes have been shown to induce major physiological irregularities. Sulphite-bleaching mills release in the effluent a variety of byproducts which are believed to be toxic to fish. It is likely then, that since these histopathological changes were fewer in samples from the reference site, the underlying abnormalities noted can probably be attributed to effluent discharge from the paper mill. Analysis of sediment and water conducted in June, 1992 revealed the presence of a variety of resin acids especially dehydroabietic acid (see Khan et al. 1994) which has been reported to impair detoxification enzymes namely, mixed function oxygenases (Lindström-Seppå and Oikari 1990). It is likely that the benthic mat at Port Harmon, which has a high odour of hydrogen sulphide might also be contributing to the anomalies observed in the cunner (see Barker *et al.* 1994a). Further studies should investigate the impact of the effluent on reproduction and/or detoxifying enzymes as two additional criteria used to assess fish health at the pulp and paper mill site.

Table I. Comparison of variables ($\bar{x}$±s.d.) in cunner, captured near a paper mill at Port Harmon and a reference site at St. George's, Newfoundland,

Variable	Port Harmon	St. George's
n♂	9	16
n♀	11	13
Length (cm, pooled)	15 ± 2.4	16 ± 2.3
Weight (g, pooled)	51 ± 25.3	65 ± 30.6
K-Factor ($x10^{-2}$, pooled)	1.38 ± 0.10	1.34 ± 0.12
Gonad s.i.♂ ($x10^{-2}$)	1.45 ± 0.55	1.16 ± 0.61
Gonad s.i.♀ ($x10^{-2}$)	2.13 ± 0.07	2.07 ± 1.16
Gill Hyperplasia		
Moderate-Severe (no.)	14	0
Liver-lipid storage (%)	53	84
%Hemosiderin-spleen(mm^2)	4.1 ± 4	2.5 ± 0.2
%Hemosiderin-kidney (cm^2)	11.5 ± 1.5	7.9 ± 1.3
Echinorhynchus gadi		
Prevalence (%)	0	43
Abundance (no./fish)	0	2.6 ± 0.2

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