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# Comparison of Tissue Lesions in Four Species of Benthic Fish Sampled in 1972-1973 and 1997-1998 on the Grand Banks off Newfoundland

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Lesions in fish under normal conditions tend to be associated with old age groups. However, when environmental degradation occurs following disposal of anthropogenic compounds, chronic exposure could affect homeostasis and culminate in tissue changes in additional age groups. A number of methods have been devised to detect these changes but in recent years, histopathology has gained prominence as a useful approach in biomonitoring programs (Adams 1990; Hinton and Lauren 1990). Several studies have focussed on the liver because of its detoxicating function and susceptibility to neoplastic and nonneoplastic conditions (Myers et al. 1994; Bucke and Feist 1993; Vethaak 1996). Moreover, there is increasing evidence that hepatic lesions are linked to xenobiotics in fish based on both laboratory and field studies (Heansly et al. 1982; Gardner and Yevich 1988; Johnson et al. 1993; Vethaak 1996).

During a survey of blood protozoans infecting fish on the Grand Banks off Newfoundland (North Atlantic Fisheries Organisation (NAFO) subarea 3N in 1972-3, tissues were taken from four benthic species which included Laval's (Lycodes lavalei) and Vahl's eelpout (L. vahli), striped wolffish (Anarhichas lupus) and American plaice (Hinnoplossoides platessoides) for examination for additional proliferative stages (Khan et al. 1980). Lesions were rare in the liver, spleen and kidney. Prior to and since this time, an intensive fishery transpired in this area. Overexploitation, recruitment failure and lower than normal water temperature are some factors associated with depletion of fish stocks. Since fish health was not considered a factor in demise of the fishery, a histological study was conducted to compare samples taken in 1972-3 with 1997-8 of the same species. The results, reported herein, reveal a significant change in the prevalence and occurrence of histopathological lesions in all four piscine species.

## MATERIALS AND METHODS

The four species of fish were taken by otter trawl by survey ships of the Department of Fisheries and Oceans viz., A.T. Cameron in spring of 1972-3 and the W. Templeman in 1997-8 in NAFO subarea 3N (area encompassing 43°40' - 44°30'N, 49°35'-50°30'W). Total body length (cm) of the fish which included both genders in 1972-3 and 1997-8 respectively were: Laval's eelpout 50±5 and 41±4, Vahl's eelpout 40±4 and 27±3, striped wolffish 54±6 and 47±5 and American plaice 42±4 and 27±2. All samples taken in 1972-3 were sexually mature whereas in 1997-8, 46% of 13 Laval's eelpout, 45% of 20 Vahl's eelpout, 50% of 12 striped Wolffish and 52% of 21 American plaice were immature. Tissues which included the liver (-4 mm in thickness from the distal portion of the long axis), spleen and hind kidney, were fixed in 10% buffered formalin, processed by conventional histological methods and sections, 5$\mu$m in thickness, stained with hematoxylin and eosin. Additional sections of spleen were stained with Perl's Prussian blue for hemosiderin which was estimated by digital image analysis (Khan and Nag 1993). Data of the latter were compared for each species by year by the ANOVA and Tukey's studentized range test (HSD) using the SAS system. Means and standard errors were calculated for all fish groups.

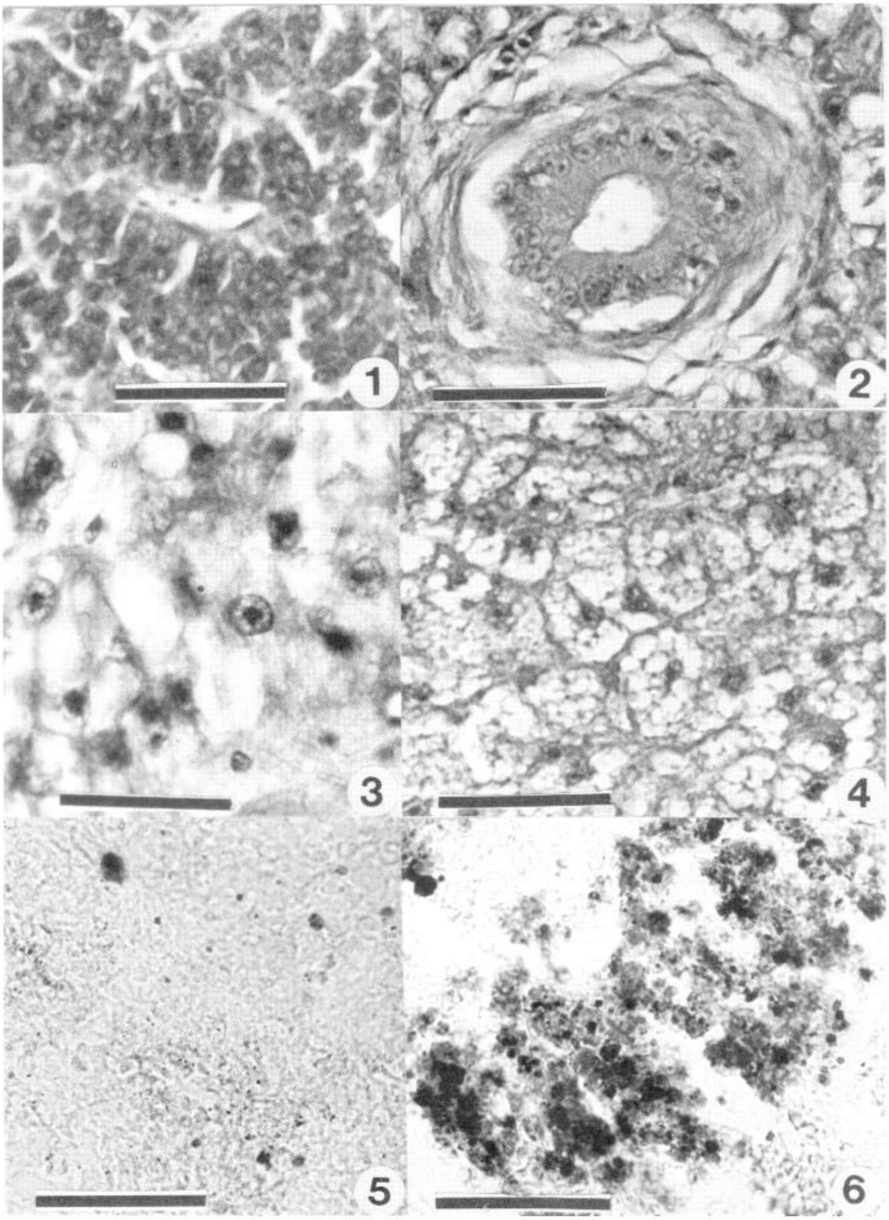
## RESULTS AND DISCUSSION

Examination of tissues from the four species of fish revealed significant changes between samples taken in 1972-73 and 1997-8. No differences were apparent between sexually mature and immature fish taken in 1997-8. Main histopathological

**Table 1.** Comparative occurrence (%) of bile ductule hyperplasia (Bdh). hypervacuolation with microvesicles in hepatocytes (V) and hypertrophied, vesicular nuclei (H) in the liver and concentration of hemosiderin (%/mm$^2$) in the spleen between Laval's and Vahl's eelpouts, striped wolffish and American plaice sampled in 1972-3 and 1997-8 from the Grand Banks.

| Species | Year | n | Liver (%) | | | Spleen (%) |
|---|---|---|---|---|---|---|
| | | | Bdh | V | H | $\bar{x}$ ±s.e. |
| Laval's eelpout | 1972-3 | 12 | 17 | 0 | 0 | 1.56 ± 0.16 |
| | 1998 | 13 | 77 | 0 | 0 | 5.56 ± 0.57* |
| Vahl's eelpout | 1972-3 | 12 | 17 | 0 | 0 | 1.17 ± 0.11 |
| | 1997 | 20 | 15 | 85 | 85 | 7.51 ± 0.98* |
| Striped wolffish | 1972-3 | 10 | 30 | 0 | 0 | 0.69 ± 0.04 |
| | 1998 | 12 | 100 | 75 | 25 | 4.48 ± 0.56* |
| American plaice | 1972-3 | 10 | 0 | 0 | 0 | 0.81 ± 0.10 |
| | 1998 | 21 | 86 | 71 | 67 | 5.06 ± 0.51* |

*significantly different ((P<0.05 ) from corresponding group.




Comparative tissue sections of fish originating from the Grand Banks in 1972-3 and 1997-8. Scale bar is 100 $\mu$m in Fig. 2 and 50 $\mu$m in other figures. Fig. 1. Reference liver (American plaice - 1973). Fig. 2. Liver exhibiting bile ductule hyperplasia (striped wolffish - 1998). Fig. 3. Liver showing nuclear hypertrophy (Laval's eelpout - 1998). Fig. 4. Megalocytes with microvesicles in the liver (Vahl's eelpout - 1997). Figs. 5 and 6. Hemosiderin in the spleen of American plaice taken in 1973 and 1998 respectively.

alterations in the liver included bile ductule hyperplasia, hypertrophied vesicular nuclei and hypervacuoled hepatocytes with microvesicular formation (megalocytic hepatosis) (Figs. l-4). Anomalies were minimal in the 1972-3 samples (Table 1). However, bile ductule hyperplasia increased significantly in Laval's eelpout (17 to 77%) striped wolffish (30 to 100%) and American plaice (0 to 73%) but remained unchanged in Vahl's eelpout. Additionally hypertrophied vesicular nuclei of hepatocytes occurred in Vahl's eelpout (85%), striped wolffish (25%) and American plaice (67%). The occurrence of hypervacuolated hepatocytes with microvesicles, not observed in the 1972-3 samples, was noted in Vahl's eelpout (85%), striped wolffish (75%) and American plaice (71%). There was increase of hemosiderin in the spleen of the 1997-8 samples (Figs. 5 and 6, Table 1). Macrophage aggregates, not recorded in the kidney of any of the 1972-3 fish samples, were observed in Laval's (46%) Vahl's eelpouts (60%), striped wolffish (25%) and American plaice (100%).

Results from the present study, although based on a limited number of samples, reveal temporal differences in the prevalence of lesions in three tissues of four species ofbenthic fish sampled between 1972-3 and 1997-8. Some of these lesions have been reported in fish from areas such as the North and Baltic Seas and off the northeast and northwest coasts ofNorth America where chemical contaminants have been identified (Wolke et al. 1985; Peters et al. 1987; Kohler et al. 1992; Bucke and Feist 1993; Vethaak and Wester 1996; Myers et al. 1998). Data from these studies suggest a strong link between xenobiotics and histopathological lesions in the liver. Haensly et al. (1982) reported a high prevalence of histological lesions in the organs of plaice (Pleuronectes platessa) that were exposed to crude oil spilled by the Amoco Cadiz on the French coast (Brittany) in 1978 and attributed these to the xenobiotic. While bile ductule hyperplasia has been reported in fish exposed to several xenobiotics, hepatocytic hypertrophy with microvesicular formation was the most prominent lesion observed in Atlantic cod (Gadus morhua) exposed to water-accommodated oil fractions containing polycyclic aromatic hydrocarbons (PAHs) and pearl date (Mareariscus margarita) sampled at a diesel-fuel contaminated stillwater pond (Khan and Kiceniuk 1984; Khan 1999). This hypervacuolation (steatosis) is indicative of a high lipid content (Peters et al. 1987; Kohler et al. 1992; Bucke and Feist 1993). Similarly, nuclear pleomorphism, including cellular hypertrophy, observed in three species of fish in the present study, has been reported previously as symptoms of toxic injury (Kohler et al. 1992). These degenerative lesions are considered early indicators of hepatotoxin-carcinogen exposure (Myers et al. 1994). Possibly, further studies might reveal additional lesions in fish as noted at other contaminated sites (Peters et al. 1987; Kohler et al. 1992; Bucke and Feist 1993; Vethaak and Wesler 1996).

The present study has also shown that the concentration of hemosiderin in the spleen and macrophage aggregates in the kidney were significantly greater in the four species of fish sampled in 1997-8 than in 1972-3. Macrophage aggregates which appear as pigmented cells containing melanin, lipopigments and hemosiderin following erythrocytic breakdown, tend to increase after excessive blood loss (Khan and Nag 1993). Laboratory studies have shown that hemosiderin (ferric iron) concentration increased temporally in fish exposed to petroleum-contaminated sediment and also sediment originating from the vicinity of a pulp and paper mill

(Khan and Nag 1993; Khan et al. 1994). Moreover, there was a decreasing concentration gradient from the vicinity of a pulp and paper mill to sites further away (Khan 1998). Some of these studies also revealed a link between hepatic lesions and the concentration of hemosiderin (Khan et al. 1994; Khan 1998,1999). These results support the view proposed by other studies that hemosiderin concentration and/or macrophage aggregates can represent a useful tool for monitoring fish health (Bucke et al. 1984, 1992; Wolke et al. 1985; Peters et al. 1987).

The high prevalence of the spectrum of lesions in both immature and mature fish noted in the present study is suggestive of chronic exposure to xenobiotics. The source of the presumed stressors is unknown but might be associated with the indiscriminate disposal of petroleum-contaminated bilge water by fishing boats over the last five decades. The Grand Banks has been and still is an area where more than a thousand vessels fish annually (Fisheries and Oceans, unpubl. data). Oil slicks have been observed in the area (NAFO subareas 3N-3O) since the 1970s (Canadian Coast Guard, unpubl. data) and consequently, it is likely that bioconcentration and bioaccumulation of PAHs occurred in fish over the years. A recent study, which detected metabolites of polycyclic aromatic hydrocarbons (PAH) at depths of 1500-1800m in bile of five species of fish in the NW Mediterranean Sea, is supportive of the view of PAH contamination in the deep-sea environment (Escartin and Porte 1999). The Grand Banks are also partially under the influence of the Gulf Stream which could represent another source of contamination originating from the south. Moreover, reports of synergy between some pollutants and parasites in fish might have contributed to morbidity (Khan 1987). Future studies, using additional biomarkers including synergism between parasites and pollutants, should focus on length-age relationship in these and other species of fish along a transect from coastal Newfoundland to the Grand Banks (NAFO subareas 3L, 3N and 3O).

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