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Factors Influencing EROD Activity in Feral Winter Flounder (*Pleuronectes americanus*) Exposed to Effluent from a Pulp and Paper Mill in Newfoundland

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Aquatic organisms can biotransform organic chemicals by producing a series of enzymes which include the mixed function oxygenase (MFO) system (Payne *et al.* 1987; Jimenez and Stegeman 1990; Collier *et al.* 1998). The liver is the major site that metabolizes these xenobiotics in fish. Although several bioindicators of stress are used to assess exposure to contaminants, the MFO system is used more often because of its short induction period (Jimenez and Stegeman 1990). However, a number of variables such as species of fish, sex, age, temperature, nutritional status, heavy metals, pathological changes in the liver, etc., can have a profound influence on the rate and extent of biotransformation (Eggens *et al.* 1995; Andersson and Forlin 1992; Kohler and Pluta 1995; Karels and Oikari 2000; Mathieu *et al.* 1991; Lindstrom-Seppa and Stegeman 1995; Viarengo *et al.*, 1997; Williams *et al.*, 1998). Enzyme activity is usually reduced in nutritionally-deficient fish, after exposure to low temperatures and also shortly before spawning in females (Jimenez and Stegeman 1990). Effluent discharged by pulp and paper mills have been reported to induce MFO activity in a number of fish species (Andersson and Forlin 1992). Previous studies on winter flounder (*Pleuronectes americanus*), a sediment-inhabiting, non-migratory species inhabiting a fjord receiving effluent from a sulphite-bleaching pulp and paper mill, revealed that it was sensitive to pollutants and exhibited MFO activity (Payne *et al.* 1987; Khan and Payne 1997). The fish in the north temperate region lives in shallow water (<15m), remains in the substrate when not foraging, has a limited home range and consequently is useful as a sentinel monitoring species. Spawning occurs in late spring. In view of these observations, a field study was conducted to ascertain the influence of gender, sexual maturity, water temperature and proximity to the pulp and paper mill on MFO activity in flounder living in the inlet. Additionally, it also compared condition (K) factor and hepatosomatic index (HSI) as indicators of nutritional status and gonadosomatic index (GSI) as a measure of gonadal maturity in pre-spawning, post-spawned and mature, non-spawning fish.

MATERIALS AND METHODS

Winter flounder were captured at depths of 5 to 10 m by SCUBA divers at three sites, Birchy Cove, located two km down current and at two and five km across the

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embayment at Summerside and Meadows respectively from the untreated outfall of a pulp and paper mill located at Corner Brook (49°12'N, 58°21'W), Newfoundland. There is no evidence that fish migrate between the collecting sites since a deep channel separates Birchy Cove from the two sites on the opposite shore. The mill has been operating since the 1920s and, for about eight decades, logs of black spruce (*Picea mariana*) and balsam fir (*Abies balsamea*) were transported via a river into the fjord where wet debarking occurred. Consequently, a considerable quantity of bark residue and fiber occur throughout the inlet increasing in quantity towards the mill. The bottom substrate of the fjord consists of soft, muddy sediment mixed with bark and fibre and has an odor of hydrogen sulphide. A superficial brackish-water layer overlies high salinity (~25 ‰) oceanic water. Untreated effluent discharged by the mill, which used thermomechanical and sulphite-pulping processes for newsprint production, was composed of suspended solids (79-93 mg/L), tannin and resin acids (27 mg/L), especially dehydroabietic (600-800 μg/L) and abietic (600-1200 μg/L) acids (Environ. Canada, unpubl. data). The effluent was visible at the surface up to two km from the outfall.

Flounder were sampled when the bottom water temperature was 5° (May), 7° (June) and 14°C (August) in 1995. Between 41 and 69 fish were captured at each site during each month and 10 samples for comparison selected on the basis of gender and maturity. Mean length (L) in cm, eviscerated weight (W) in g and age (A) in years were determined for mature-females (L, 29±0.7; W, 310±22; A, 5-8), mature males (L, 23 ± 0.9; W, 150 ± 24s, A, 5-7), subadult females (L, 21 ± 0.3; W, 146 ± 18; A, 3-4) and subadult males (L, 15 ± 0.2; W 114 ± 12; A, 3-4). Ages were estimated from cleaned otoliths by counting the number of annuli.

Following capture, liver and ovarian weight were also recorded and samples of liver were removed at autopsy subsequently and frozen in dry ice (-60°C) for determination of EROD activity within 2-4 wk after collection. Liver was homogenized in ice-cold 50 mM Tris-HCL (pH 7.5). Mixture for the reaction (final volume, 1.25 ml) contained 53 nmol Tris-sucrose buffer (50 mM, pH 7.5), 50-100 μl of homogenate, 2.25 nmol 7-ER (150 μM) and 0.16 mg NADPH (1.25 mg/ml). The reaction was terminated after 15 min incubation at 25°C by addition of 2.5 ml of ice-cold spectro-analysed methanol. EROD activity was then assayed fluorimetrically (Porter *et al.* 1989). Influence of gender, sexual maturity and temperature on EROD activity were also compared in groups of flounder, especially in post-spawned females since enzymic activity is disrupted just prior to spawning (Porter *et al.* 1987). Condition (K) factor (W/L^3), hepatosomatic (HSI) and gonadosomatic indices (GSI) and EROD activity values from groups of flounder (n=10) were compared by the one-way ANOVA using a SAS software package and Tukey's studentised range test for differences which were considered significant when $P \leq 0.05$.

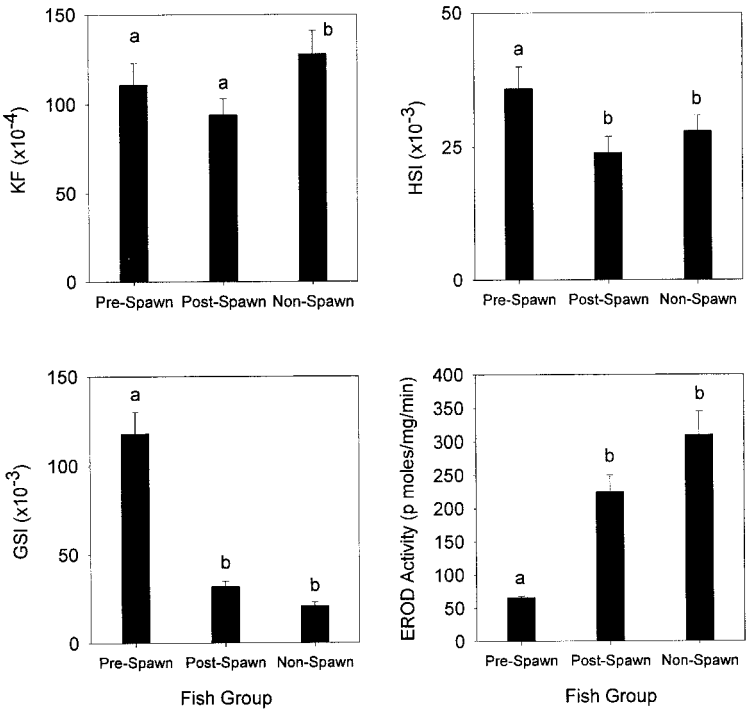

Figure 1. Comparison of K-factor, HSI, GSI and EROD activity in three groups of mature, female winter flounder sampled two km downcurrent from a pulp and paper mill in pre-spawning (Pre-sp) post-spawned (Post-sp) and non-spawning (Non-sp) condition, in which no ovarian growth occurred, at 7°C. Letters above bars indicate significant differences between groups.

RESULTS AND DISCUSSION

EROD activity varied depending on water temperature, gender, sexual maturity and site of sampling in feral winter flounder inhabiting the fiord receiving effluent from the pulp and paper mill. There was an inverse relationship between GSI and EROD activity in pre-spawning females sampled two km down current from the outfall (Fig. 1). HSI and GSI values were significantly greater in pre-spawning than post-spawning (spent) female flounder but EROD activity was significantly elevated in the latter group. In adult female flounder, in which the ovaries did not develop, K-factor and EROD activity were significantly greater than in pre-spawning or spent females.

Comparison of EROD activity in relation to water temperature, sexual maturity and gender in flounder sampled at Birchy Cove revealed that significantly higher values

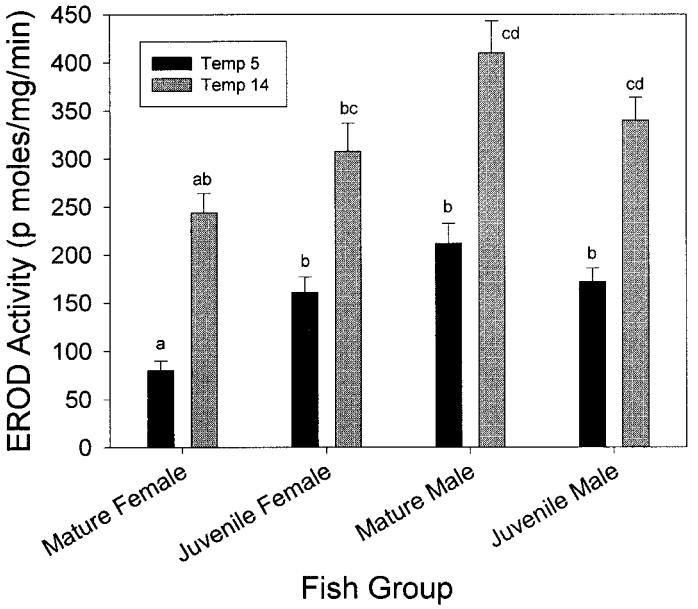

Figure 2. EROD activity in spent, female winter flounder sampled two km downcurrent at Birchy Cove (BC) and two and five km at Summerside (SS) and Meadows (M) respectively on the opposite side of a fjord receiving effluent from a pulp and paper mill in spring (5°C) and summer (14°C). Letters above bars indicate significant differences between groups.

occurred in spring (5°C) and summer (14°C) in mature and immature males and female fish than in pre-spawning (spring only) or spent female (summer only) fish (Fig. 2). Moreover, EROD activity values were significantly greater in mature male flounder at both 5° and 14°C than in immature males and females. Comparison of EROD activity in relation to site of sampling and water temperature suggested that spent flounder collected 2 km down current (Birchy Cove) from the pulp and paper mill showed significantly greater values than their counterparts collected two (Summerside) or five km (Meadows) across the inlet in spring (5°C) (Fig. 3). However, EROD activity values during the summer (14°C) were not significantly different in samples from Birchy Cove and Summerside but were significantly greater than in fish originating from Meadows. K-factor, HSI and GSI values were not significantly different among the groups of fish sampled at 5°and 14°C.

It is evident from the present study that effluent discharged by the pulp and paper mill induced EROD activity in feral winter flounder inhabiting the Humber Arm, especially in samples originating from Birchy Cove and Summerside. Our findings

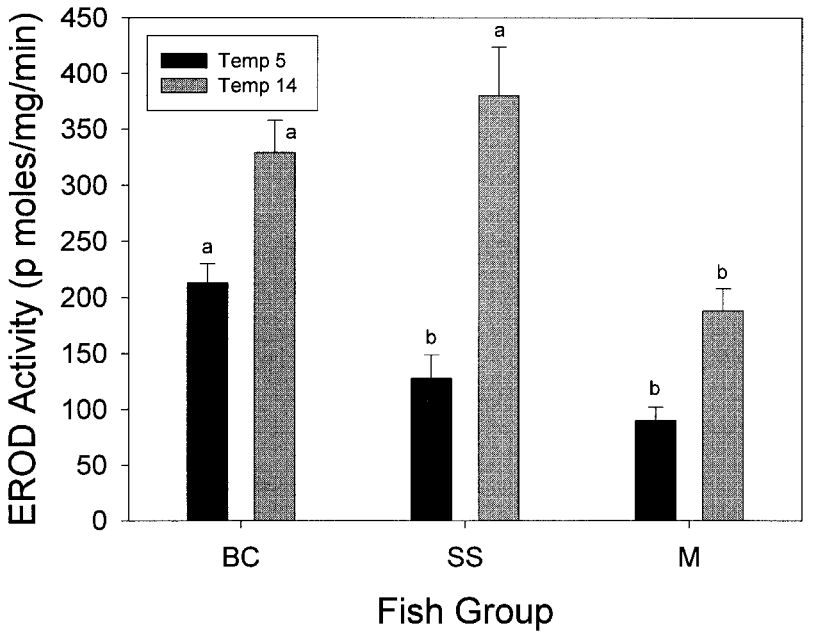

Figure 3. Comparison of EROD activity in adult female (pre-spawning in spring), sub adult female, adult male and juvenile male winter flounder sampled two km down-current at Birchy Cove (BC) and at two and five km at Summerside (SS) and Meadows (M) respectively on the opposite side of a fjord receiving effluent from a pulp and paper mill in spring (5°C) and summer (14°C). Letters above bars indicate significant differences between groups.

also suggest that although EROD activity was significantly lower in prespawning than in post-spawned or mature, non-spawning fish and nutritional state, based on K-factor and HSI, was not the underlying cause. The inverse relationship between GSI and EROD in the pre-spawning flounder is more likely related to sex steroids. Previous studies have reported significantly lower EROD activity in pre-spawning than in immature, post-spawned (spent) females (Elskus *et al.* 1992; Goksøyr *et al.* 1996). Current evidence indicates that elevated levels of sex steroids disrupt EROD activity, especially in females with mature gonads (Jiminez and Stegeman 1990; Munkittrick et al. 1994). GSI values normally decrease to their lowest level during summer when plasma sex steroids are at their minimum and EROD activity is at its peak (Goksøyr *et al.* 1996). Although steroid hormones were not measured in the present study, it is clear that the elevated water temperature during summer resulted in increased levels of EROD activity in winter flounder. Several reports have indicated that water temperature has a profound influence on EROD activity (Andersson and Förlin 1992; Goksøyr and Förlin 1992).

In the present study, winter flounder sampled in the fiord receiving effluent from the pulp and paper mill showed a gradient of EROD activity depending on proximity to the outfall. In spring (water temperature at 5°C), a significantly greater level of EROD activity was observed in fish sampled two km down current from the outfall (Birchy Cove) than across the embayment also about two km (Summerside) or at the most distant site (Meadows) located about five km away. Moreover, the occurrence of EROD activity in fish at the latter site is suggestive of widespread dispersal of the effluent throughout the fiord (Khan and Payne 1997). Other studies on the impact of pulp and paper mill effluent have also reported a gradient of responses in fish decreasing in effects with increasing distance from the outfall (Munkittrick *et al.* 1994). The high values of EROD activity recorded in fish collected at Summerside during summer might be attributed to exposure to an excessive quantity of effluent carried by the prevailing wind which was southerly (gail force) rather than easterly one week prior to sampling. This was evident as a considerable quantity of bark from an uncovered pile, located immediately west of the mill, was swept by the wind into the ocean and carried across the inlet, appearing as a brownish layer above the sediment (Khan, unpubl. data).

In conclusion, EROD activity in feral winter flounder inhabiting a fiord receiving effluent from a pulp and paper mill was influenced by temperature, reproductive status, maturity, gender and distance from the outfall. Activity was greater in post-spawned and non-spawning than in pre-spawning females in spring, greatest in males in both spring and summer than spent females or both immature males and females. Samples taken at the most distant sites had lower levels of EROD activity. These results suggest that sampling for EROD activity is better in summer than at other times of the year and adult male flounder exhibit higher values than female adults or juvenile fish of both sexes.

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