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Stress Response of Juvenile Sockeye Salmon (*Oncorhynchus nerka*) to the Butoxyethanol Ester of 2,4-Dichlorophenoxyacetic Acid

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The presence of eurasian milfoil, Myriophyllum spicatum, in the Okanagan basin lakes has been identified as a nuisance by the lakeshore residents and visiting tourists. These lakes situated in sourthern central British Columbia, form part of the Columbia River basin and contain many valuable fish stocks including kokanee (Oncorhynchus nerka), lake trout (Salvelinus namaycush) and rainbow trout (Salmo gairdneri). Concern that the weed might spread was justified when in 1977, milfoil was found in Cultus Lake. The latter lake, located in the western region of southern British Columbia, forms part of the Fraser River system and serves as an important homing, spawning and rearing ground for sockeye salmon (Oncorhynchus nerka) (FOERSTER 1968). Milfoil is not common in Canadian waters, but is well established in many parts of the United States where it has been linked to losses in commercial fishing, smothering of shellfish beds and clogging of water ways.

Methods for eradication and/or control of the weed can be grouped into 3 general categories: i) Mechanical harvesting - partially successful, but mowing must be continued throughout the growing season. Equipment and manpower requirements make this a costly process. ii) Biological control - success has been reported in controlling milfoil following the introduction of grass carp, Ctenopharyngodon idella Val. This species may, however, create serious ecological imbalances and also, compete directly for feed etc., with indigenous fish populations (BEACH et al. 1976). iii) Chemical control - success has been credited to herbicides, but the possibility of inducing secondary toxic effects has led to strict laws regulating their application (ANON 1979).

As part of a general study to assess the effect(s) of the butoxyethanol ester of 2, 4-dichlorophenoxyacetic acid (BEE 2, 4-D) application on juvenile Pacific salmon, this report documents the sublethal histopathological and stress responses of sockeye salmon (Oncorhynchus nerka) fry and smolts to the herbicide. The acute toxic responses LC_{50} (96 h) for these fish have been reported earlier (MARTENS et al. 1980).

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MATERIALS AND METHODS

BEE 2, 4-D

The butoxyethyl ester (BEE) formulation of 2, 4-D, commercial preparation (Aqua-Kleen®, Amchem Products, Incorporated) was used in these tests. The herbicide solutions were renewed daily. However, degradation of the ester to the less toxic acid equivalent (a.e.) is know to occur (MARTENS et al. 1980). In this report, for the sake of simplicity, all dosages of the herbicide are recorded as the nominal concentration.

Histopathology

Only a brief outline of the details relating to the fish and the toxicity bioassays is given as these topics have been described in detail elsewhere (MARTENS et al. 1980).

Bioassay

The sockeye fingerlings and smolts from which the tissues were taken for histological examination were from the same groups of fish used in the LC_{50} (96 h) acute toxic bioassays. However, for the sublethal portion of the study the exposure period to the herbicide was varied from 24 to 96-h, a time period more in line with field application of the compound. Moreover, to simulate the effect of migration to seawater after herbicide exposure, one group of these fish was exposed to BEE 2, 4-D, then transferred to fresh water followed by sea water.

Tissue Preparation

Processing of the tissues for histological examination was as previously noted (McBRIDE & VAN OVERBEEKE 1971, VAN OVERBEEKE & McBRIDE 1971). In the case of the fry, serial sections of the whole fish were prepared with every second slide being stained.

Stress, Plasma Cortisol

This section of the study was conducted separately from the histopathology. To ensure adequate plasma volumes 20 to 40 g sockeye post-smolts reared in fresh water at the Cultus Lake Laboratory, International Pacific Salmon Fisheries Commission, were used for the corticosteroid determinations. The fish were acclimated for 3 days (2 fish per tank) in identical light tight test tanks as described by DONALDSON & DYE (1975). A temperature of 12.0 - 12.5°C was maintained by keeping the tanks partially immersed in running well water. A 1 mm diameter polyethylene tube was inserted through the black plexiglas cover of each tank and 50 mL of BEE 2, 4-D solution was added, followed by 50 mL of distilled water using two 50 mL syringes connected with a 3-way valve. This procedure allowed addition of the herbicide solution without disturbing the fish.

After 1 h of exposure to the BEE 2, 4-D, 2 fish were removed simultaneously from each tank, stunned by a blow on the head and blood samples taken (McBRIDE et al. 1979). Cortisol determinations were carried out on duplicate µl samples using the Clinical Assay Gamma Coat ^{125}I cortisol radioimmunoassay kit.

RESULTS

Histopathology

Sockeye fry:

Histological examination of the integument, lateral muscle, heart, kidney, spleen, alimentary tract and gonad revealed no apparent pathological changes in any of the test groups. Structural alterations indicative of pathology were recorded in the gill, liver and interrenal of those fry exposed to 0.7 and 1.0 ppm concentrations of the herbicide.

In contrast to the distinct interlamellar spaces noted in the gills of the unaffected fish, the injured gills of some treated fish showed hypertrophy and hyperplasia of the epithelial cells, although rarely to the extent of complete fusion of the lamellae. The effect was most pronounced in the fish exposed to the highest concentration of BEE 2, 4-D for 48 h.

The histopathological changes in the liver were focal and generally confined to those hepatocytes in close association with the numerous blood vessels common to this organ. The principle lesion was the vacuolar degeneration of the cytoplasm. Pyknotic nuclei were not commonly noted, rather the nuclei were displaced to the periphery of the cell. In a few extreme cases, noted only in the fish exposed to 1.0 ppm BEE 2, 4-D, focal areas displayed an absence of cell integrity and cord-like organization.

In the controls the interrenal cells showed a round nucleus containing a fine reticular chromatin, a single inconspicuous nucleolus and a fine evenly dispersed granular cytoplasm. Mitotic activity was rarely noted. This cytology was maintained in those fish exposed to the lowest concentration (0.1 ppm) of BEE 2, 4-D for all time periods tested. Exposure of the fry for 48 h to 0.7 and 1.0 ppm BEE 2, 4-D induced a significant hypertrophy of the interrenal nuclei (Table 1). Moreover, a visible increase in mitotic activity clearly indicated a developing hyperplasia. In 2 of the 4 fish exposed to 1.0 ppm of the herbicide, hemorrhages were noted in the cortical tissue. Following 96 h exposure a significant interrenal response was evident in the fry exposed to 0.3 ppm of BEE 2, 4-D.

TABLE 1

Effect of BEE 2, 4-D exposure on the interrenal nuclear diameter (mean ± SD) of sockeye fry (O. nerka). All treatments carried out in fresh water with each evaluation based on results of 4 fish. Significant differences from control fish denoted by *, $P < 0.05$.

Nominal concentration 2, 4-D (mg/L)	Exposure (h)	Interrenal nuclear diameter (μm)
0	48	6.12 ± 0.29
0.1	48	6.09 ± 0.33
0.3	48	6.15 ± 0.33
0.7	48	6.82 ± 0.42*
1.0	48	7.18 ± 0.58*
0	96	6.04 ± 0.34
0.1	96	6.19 ± 0.37
0.3	96	6.64 ± 0.43*
0.7	96	All fish dead
1.0	96	All fish dead

Sockeye smolts:

In the case of sockeye smolts the only tissue to show a response to BEE 2, 4-D exposure was the interrenal (Table 2). A significant hypertrophy of the interrenal nuclei was noted at the end of 48 h exposure to 1 mg/L of the herbicide. By the end of 96 h the smolts in the 1.0 mg/L group were all dead and those exposed to the 0.7 mg/L concentration of the herbicide displayed a signficant hypertrophy of the interrenal nuclei. In the case of fish exposed first to BEE 2, 4-D for a period of 24 h and then transferred to uncontaminated fresh water or fresh water followed by sea water, the interrenal nuclear hypertrophy induced at the 1 mg/L concentration was reversed (Table 3).

Stress, Plasma Cortisol

The investigation of the effect of BEE 2, 4-D was conducted in two phases. In the initial trial conducted in June (Table 4) there was no significant difference in plasma cortisol concentrations of 0.5, 1.58 and 5.0 mg/L BEE 2, 4-D. The second trial conducted on larger fish during August and September revealed lower and less variable cortisol concentrations in control fish compared to control fish in June. In this experiment significant stress responses were observed in juvenile sockeye at nominal BEE 2, 4-D concentrations of 5.0, 15.8 and 50.0 mg/L.

TABLE 2

Effect of BEE 2, 4-D exposure on the interrenal nuclear diameter (mean ± SD) of sockeye smolts (*O. nerka*). All treatments carried out in fresh water with each evaluation based on results of 4 fish. Significant differences from control fish denoted by *, $P < 0.05$.

Nominal concentration 2, 4-D (mg/L)	Exposure (h)	Interrenal nuclear diameter (μm)
0	48	6.17 ± 0.21
0.1	48	6.27 ± 0.31
0.3	48	6.22 ± 0.35
0.7	48	6.19 ± 0.32
1.0	48	6.97 ± 0.32*
0	96	6.27 ± 0.32
0.1	96	6.22 ± 0.29
0.3	96	6.41 ± 0.32
0.7	96	6.98 ± 0.29*
1.0	96	All fish dead

TABLE 3

Interrenal nuclear diameter (mean ± SD) of sockeye smolts (*O. nerka*) exposed to BEE 2, 4-D in fresh water (FW) for 24 h followed by transfer to uncontaminated fresh water or fresh water then sea water (SW). Significant differences from corresponding control fish denotd by *, $P < 0.05$.

No. of fish	Treatment	Interrenal nuclear diameter (μm)
3	Control: FW for 24 h	5.98 ± 0.41
4	0.7 mg/L of 2, 4-D for 24 h	6.25 ± 0.36
4	1.0 mg/L of 2, 4-D for 24 h	6.86 ± 0.42*
3	Control: FW for 144 h	6.16 ± 0.27
3	0.7 mg/L of 2, 4-D for 24 h, transfer to FW for 120 h	6.04 ± 0.16
3	1.0 mg/L of 2, 4-D for 24 h, transfer to FW for 120 h	5.97 ± 0.34
4	Control: FW for 48 h, transfer to SW for 96 h	5.99 ± 0.35
4	0.7 mg/L of 2, 4-D for 24 h transfer to FW for 24 h, transfer to SW for 96 h	6.17 ± 0.37
4	1.0 mg/L of 2, 4-D for 24 h, transfer to FW for 24 h, transfer to SW for 96 h	5.96 ± 0.49

TABLE 4

Effect of 1 h exposure to BEE 2, 4-D on plasma cortisol concentration on sockeye salmon post-smolts during June and Aug.-Sept.

JUNE			
Nominal concentration BEE 2, 4-D	N	Mean weight	Cortisol concentration µg/100 mL
0	8	23.9 ± 4.6	8.22 ± 6.99
0.5	8	20.8 ± 5.6	6.93 ± 5.61
1.58	8	22.6 ± 6.9	7.40 ± 6.22
5.0	8	23.8 ± 4.5	10.47 ± 4.71
15.8	-	-	-
50.0	-	-	-

AUG.-SEPT.			
Nominal concentration BEE 2, 4-D	N	Mean weight	Cortisol concentration µg/100 mL
0	22	38.1 ± 10.3	1.95 ± 1.79
0.5	-	-	-
1.58	9	38.6 ± 9.2	1.33 ± 1.59
5.0	12	39.2 ± 7.4	5.16 ± 5.06*
15.8	10	35.7 ± 9.9	5.34 ± 1.94**
50.0	10	36.4 ± 5.0	44.59 ± 11.14**

* $0.02 > P > 0.01$

** $P < 0.001$

DISCUSSION

The finding that the sockeye fry were more adversely affected by the presence of the herbicide, as measured by interrenal nuclear hypertrophy, than the larger smolts, is consistent with the LC_{50} mortality data of POST & SCHROEDER (1971), MEHAN et al. (1974) and MARTENS et al. (1980). In the present study, a clear stress response, as shown by interrenal hypertrophy, was indicated in the fry exposed to concentrations of BEE 2, 4-D of 0.7 mg/L for 48 h and 0.3 mg/L for 96 h. While all of the fry were alive, albeit in a state of stress, at the end of 48 h exposure to 0.7 mg/L of the herbicide, continuance of exposure for an additional 24 h period resulted in complete mortality.

Evidence that the stressful effect(s) resulting from exposure to this hebicide may be quickly reversed are indicated in the interrenal responses of smolts. While a significant interrenal hypertrophy was noted in smolts exposed to 1.0 mg/L of BEE 2, 4-D for 24 h, the subsequent transfer of these fish to fresh water or fresh water followed by sea water for a period of 120 h, resulted in a reversal of the stress state as indicated

by a normalization of the interrenal structure.

Plasma cortisol concentrations, unlike the tissue structure assessments, offer the advantage of evaluating the response of the animal to very short exposure periods of stress.

Using a 1 h exposure period, the first attempt in June to identify the minimum concentration of the herbicide required to induce a significant change in cortisol levels of post-smolts failed. A second attempt, using a wider range of BEE 2, 4-D concentrations, however, was successful. In the latter study a significant increase in plasma cortisol occurred at a herbicide concentration of 5 mg/L. Interestingly, this concentration of 5 mg/L did not evoke a clear response in the first study. No obvious explanation for the discrepancy between the two studies is evident. In the first study, the cortisol values for the control as well as the groups exposed to the lower concentrations of BEE 2, 4-D were somewhat elevated and this may have masked the significance of the response to the higher concentration of the herbicide. While tissue residue analyses for BEE 2, 4-D were not carried out in this study, RODGERS & STALLING (1972) noted not only that the uptake and elimination of BEE 2, 4-D varied with species, but also that peak tissue accumulation of the herbicide occurred between 2nd to 6th h of exposure.

The results of this study indicate that i) the minimal concentration of BEE 2, 4-D required to induce a stress response is only marginally below the lethal level and ii) sockeye smolts stressed as a result of exposure to BEE 2, 4-D can, on transfer to an uncontamined environment, revert relatively quickly to an unstressed state. The recommended dose for 2, 4-D application (nominal concentration) to control aquatic weeds is 1 to 5 mg/L (CAST 1975). Thus, the degree of safety for sockeye exposed to BEE 2, 4-D in terms of either concentration of herbicide or duration of exposure appears to be marginal.

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