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Study of Pearl Dace (*Margariscus margarita*) Inhabiting a Stillwater Pond Contaminated with Diesel Fuel

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Petroleum-derived hydrocarbons represent one of the major pollutants that occur in the aquatic environment. It has been shown that the polycyclic aromatic components (PAHs) such as benzenes, xylenes and toluence, are water soluble, highly volatile and acutely toxic to aquatic life. In fish, an enzyme system, P-450-dependent mixed function oxygenase (MFO) which occurs mainly in the liver is responsible for detoxication (Neff 1985). Sexual differences in activity levels are known especially in fish during reproduction as MFO activity in males is greater than in females and in some instances it might be inhibited altogether (Neff 1985; Jimenez and Stegeman). Several field and laboratory studies have reported that chronic exposure to petroleum hydrocarbons can affect feeding, growth, reproduction and cause irreversible tissue damage (Haensley et al. 1982; Khan and Kiceniuk, 1984; Kiceniuk and Khan, 1987; Solangi and Overstreet 1982). Structural damage to the gills impair gas exchange, induce hypoxic stress and result in the release of catecholamines (Alkindi et al. 1996). Lesions can also be induced in the liver causing degenerative changes that impair MFO activity (Kohler and Pluta 1995). A number of reports also suggest that parasitic levels, coincident with pathological lesions, were also affected in fish following prolonged exposure to PAHs (Khan and Thulin 1991; Moles and Norcross 1998).

A study on diesel fuel contaminating a series of ponds recently reported that pearl dace, *Margariscus (= Semotilus) margarita* Cope, captured at one of the sites contaminated were contaminated with PAHs and exhibited severe pathological changes such as epithelial hyperplasia in the gills, excessive lipid accumulation in the liver andnumerous macrophage aggregates in the spleen (Beatty et al. 1994). It concluded that the petroleum hydrocarbons and flocculent material had a negative impact on fish habitat and other aquatic life and it would be unlikely that a suitable environment for aquatic organisms could be restored. Since no information was available on growth, reproduction, sex ratios, length, class distribution and body condition of pearl dace, a study was initiated in 1995 to investigate these variables in a stillwater pond that was contaminated with petroleum hydrocarbons and a reference site. Biomarkers such as K-factor, histopathology, MFO activity and parasitic abundance were also used to assess the effect of chronic exposure to the pollutant (Goede and Barton 1990; Hinton and Lauren 1990; Jimenez and Stegeman 1990). The results of this study are reported herein.

MATERIALS AND METHODS

Pearl dace were captured August 29 to September 1, 1995 using minnow traps (48 x 22 x 11 cm) baited with beef liver at a reference location and a site contaminated with diesel fuel and where the water flow was extremely slow (a stillwater pond, Fig. 1). The latter was approximately 600 x 15-95 meters with a depth of 0.9-1.2 meters. Dissolved oxygen at the surface was 6.2-11.9 mg. The area was continuously contaminated by a discharge of fuel

from an underground source that was estimated at 2.3-3.9 million litres (Beatty et al. 1994). There was a strong odor of fuel (benzene 0.37 mg/l, xylenes 3.07 mg/l in surface water samples) in the air (temperature, 18-22°C) and an oily sheen was observed on the pond's surface which was littered with dead branches, brush and debris originating from a landfill situated nearby. The reference pond was situated about 5 km west but upstream (Churchill River Road). The fish, after capture, were measured, sexed, weighed and tissues (liver, gill, spleen, kidney and gonad) removed subsequently for histological processing. Tissues were routinely stained with hematoxylin and eosin (H & E) but the spleen was also stained with Pearl's Prussian blue for hemosiderin and gills with periodic acid Schiff (PAS) for mucopolysaccharides (Durry et al. 1967). A blood smear was also prepared and stained subsequently with Giemsa for estimating the number of lymphocytes/1000 erythrocytes. A sample of liver from 10 female fish was taken at each site (pooled subsequently) and frozen in dry ice for determination of mixed function oxygenases (MFO) levels following the fluorometrical procedure of Porter et al., (1989). The gastrointestinal tract was examined for parasites.

Tabulations were made of maturity and sex ratio, length (L) class distribution, eviscerated weight(W) and K-factor (W/L^3) values of pearl dace captured at the stillwater and reference site. An ANOVA was used to compare K-factor and other variables in the two fish groups using SPSS™ software package for differences.

RESULTS AND DISCUSSION

Comparison of discrete biological variables of pearl dace captured at Goose Bay revealed distinct differences between the oil-contaminated and reference sites. Differences were observed in length-class distribution, length-weight relationships and sex ratio between samples captured at the stillwater and reference sites. Fish in the smaller length classes, i.e., 2.1-4.0 and 4.1-6.0 cm, were absent in the stillwater pond but abundant (71%) at the reference site (Fig. 2). Moreover, dace in the length classes 10.1-12.0 and > 12.1 cm were rare in the sample from the reference site in contrast to substantially greater percentages at the impacted site. Comparison of eviscerated body weight in male fish from the two sites revealed no differences but females at the impacted site were significantly greater (13.9±0.2 g) than at the reference location (7.8±0.1 g). Moreover, the K-factor value at the stillwater site ($96\pm1x10^{-4}$) was significantly greater than that at the reference site ($90\pm1x10^{-4}$). There was also a preponderance of prespawning female dace at the stillwater site in contrast to an equal sex ratio with females in a post-spawned condition at the reference site. Female fish constituted 70% of the total sample captured at the stillwater site whereas only 16% (males 18% and the remaining were juveniles of undetermined sex) occurred at the reference site. Additionally, eggs of dace examined from the stillwater site were bright yellow in color in contrast to a smaller size and opaque colour in reference fish.

Examination of sections of the tissues of samples from the stillwater pond revealed histopathological including reproductive, MFO and lymphocytic changes. Structural anomaly was evident in the interlamellar region of the branchial tissue between adjacent secondary lamellae in which there was a substantial hyperplasia in fish from the stillwater site culminating in fusion compared to the reference location (Figs. 3-4). Additionally, there was evidence of more mucus-secreting cells in the gills of fish taken at the impacted site than at the reference location (Figs. 5-6). In contrast to basophilic-staining hepatocytes in the liver of reference fish (Fig. 7), widespread vacuolation (lipidosis) was the most consistent and prevalent anomaly varying from moderate to severe in fish from the affected site (Fig. 8). Similarly, unusually large macrophage aggregates occurred only in the kidney and spleen of samples from impacted site and both lipofuchsin and hemosiderin were observed (Figs. 9-12). Examination of gonadal tissues revealed differences primarily in female dace. Spermatogonia and spermatocytes were the predominant stages in the testes

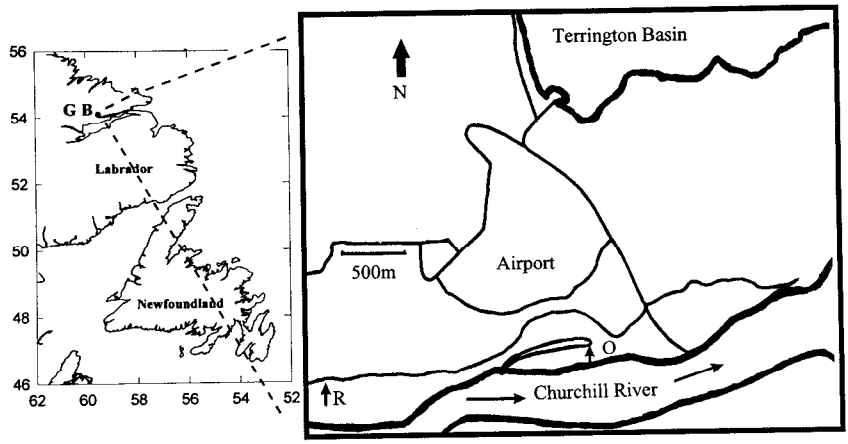


Figure 1. Reference (R) and diesel fuel oil-contaminated (O) sites where pearl dace were sampled at Goose Bay (GB), Labrador. Scale bar is two (2) km.

of males from both sites. In females, the previtellogenetic phase and post-ovulatory follicles were predominant at the reference site while advanced vitellogenesis was apparent at the fuel-contaminated location (Figs. 13-14). MFO levels determined by ethoxyresorufin o-deethylase (EROD) activity of pooled livers suggested minimal activity that was not significantly different between reference and impacted sites. However, comparison of lymphocyte counts per 1,000 erythrocytes in stained blood smears revealed that significantly higher levels were present in pearl dace captured at the reference site ($\overline{x}$89µ6.2) than at the stillwater location ($\overline{x}$34±1.4).

Gastrointestinal parasites were observed only in pearl dace sampled at the reference site. All samples (n=25; length 8.1-10.0 cm) originating from the reference site were infected with digenetic trematodes with a mean abundance of 12.4 ± 1.2/fish. These were tentatively identified as species of *Bunodera, Brachyphallus* and *Crepidostomum.* None was found in pearl dace (n=57; length classes 8.1-10.0 and 10.1-12.0 cm) taken from the oil-impacted site. These differences were probably associated with food preferences or availability of prey as stomach contents of samples from the reference site contained exoskeletal fragments of arthropods whereas filamentous algae and organic debris were observed in fish taken from the oiled site. Additionally, a visible invertebrate fauna, comprised of amphipods, copepods, larval insects, lymnaeid gastropods, etc. were noted at the reference pond whereas none was seen at the oiled site.

The results of the present study indicated that there was a wide length-class range of pearl dace at the reference site in contrast to the stillwater site; female dace at the reference site probably spawned earlier in the season in July as suggested by Scott and Crossman (1973) than fish from stillwater area and there appeared to be selection for females, probably as a result of environmental influences, at the stillwater site. Additionally, fish at the stillwater pond possessed a significantly greater K-factor, a reflection of body condition, than the sample from the reference site. The latter finding suggests that there was little competition for food resources at the stillwater pond. Moreover, the absence of young fish and the presence of late-maturing females at the impacted site towards the end of summer is suggestive of reproductive asynchrony and delayed spawning, culminating in little or no survival of the offspring, an observation made also in the previous year (Khan, unpubl. data). Only mature (n=17) three-spined sticklebacks (*Gasterosteus aculeatus*) were captured also at the oil-contaminated pond in contrast to thousands of various lengths that inhabited the reference site. Additionally, the presence of numerous young (5-12 cm in length)

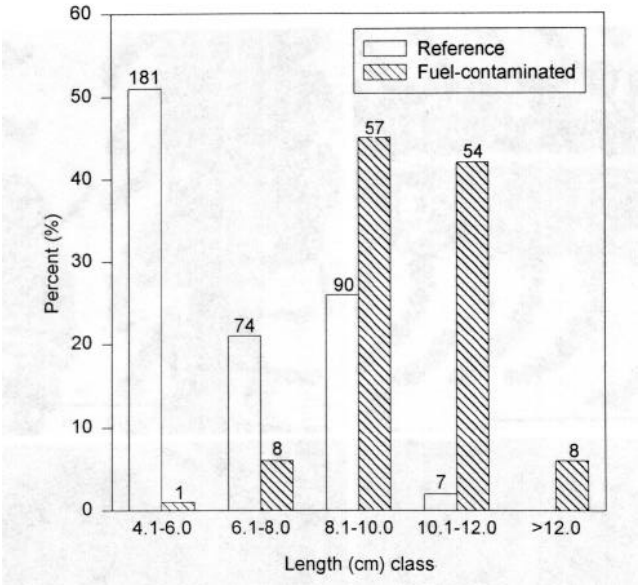
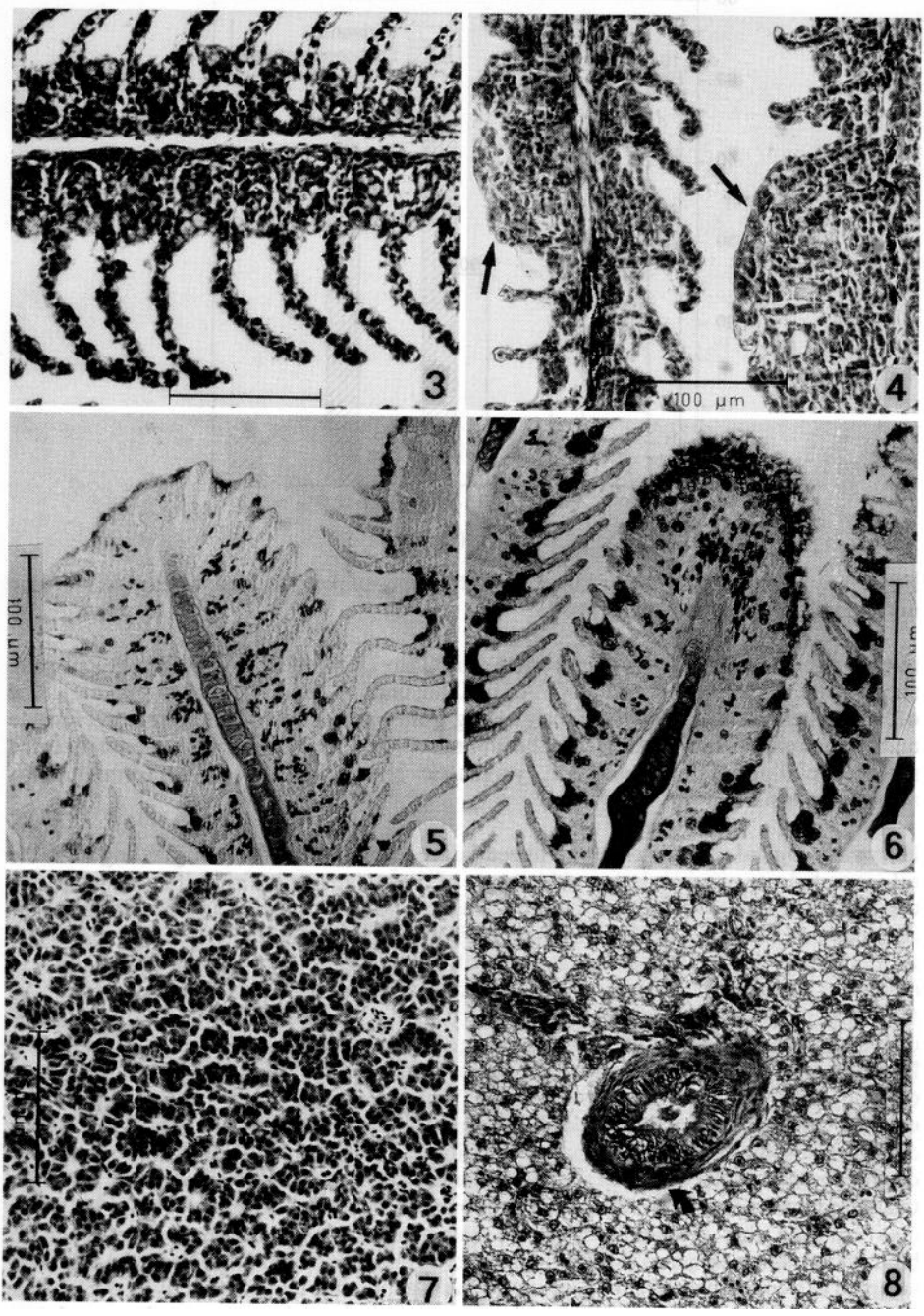


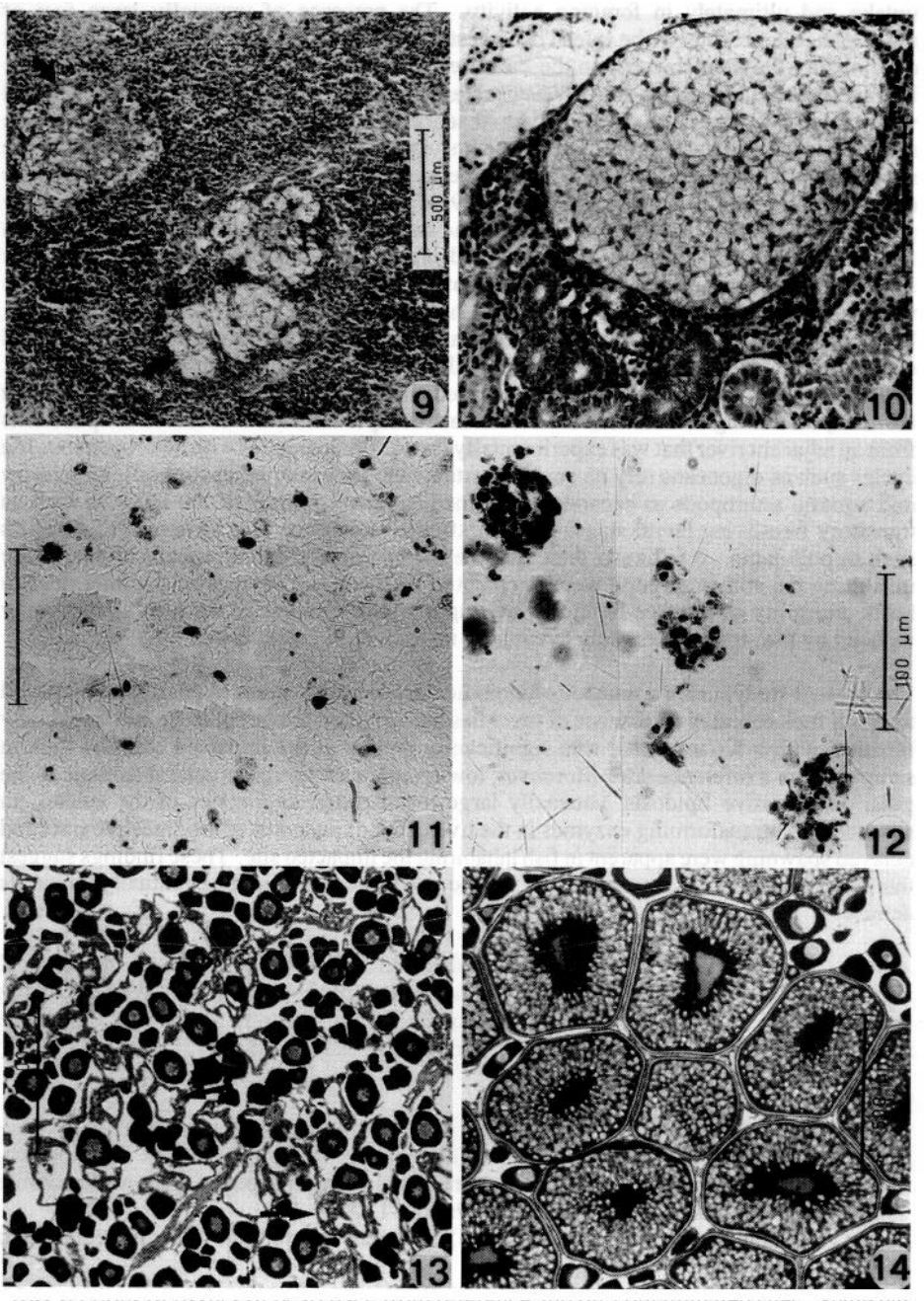
Figure 2. Comparison of two (2.0) cm length classes of pearl dace captured at a reference and diesel fuel-contaminated sites.

rainbow trout (*Salvelinus fontinalis*) and Arctic Charr (*S. alpinus*) only at the reference site supports the view of the severity of PAHs (Khan, unpubl. data). Recent studies on larvae of Pacific herring (*Clupea pallasi*) originating from egg masses collected from a reference and two petroleum-contaminated beaches after the Exxon Valdez oil spill in Alaska revealed more morphological deformities and cytogenetic abnormalities in samples from the oiled sites (Hose et al. 1996).

Histopathological changes observed in pearl dace in the present study originating from the oil-impacted site was consistent with reports of tissue damage in fish following long term exposure to petroleum hydrocarbons (Haensly et al., 1982; Khan and Kiceniuk, 1984; Solangi and Overstreet 1982). These findings were also similar to those reported in 1994 for the same fish species captured at the identical stillwater site one year before (Beatty et al. 1994). The most prominent histological change in pearl dace from the affected site was fatty degeneration (lipidosis) in the liver. The latter organ is the main site for food conversion, storage (lipid and glycogen), production of a variety of proteins including lysosomes, biotransformation and detoxication of anthropogenic compounds. Accumulation of lipid in hepatic cells probably caused injury to the cellular components, especially enzyme systems, impaired the catabolism of organic pollutants and their metabolities causing lysosomal injury and ultimately pathological disorders. Ourresults, however, based on the absence of MFO (EROD) activity confirm that dace at the oil-affected site were unable to metabolise hydrocarbons that were bioaccumulated. Kohler and Pluta (1995) have also reported that lysosomal injury is accompanied by a decrease or absence of biotransformation enzymes (MFOs) in the liver of flounder (*Platichthys flesus L.*) that were sampled at a contaminated site. Moreover, it is also possible that MFO activity declined in the prespawning pearl dace as reproductive steroids have been reported to modulate detoxication enzymes during the spawning season in several species of fish (Jimenez and Stegeman 1990). Pathological changes in other tissues such as the gill and spleen indicated damage to these systems. Excessive mucus secretion, a protective device to prevent injury to the gills and an increase of the interlamellar spaces could culminate in impaired oxygen



Comparative cross sections of tissues from reference and diesel-affected pearl dace. Scale bar is 100 µm. Figure 3. Reference gill (H & E). Figure 4. Gill from affected dace showing fusion (arrows) of secondary lamellae (H & E). Figure 5. Reference gill exhibiting (PAS). Figure 6. PAS positive-staining mucuous cells (dark colour) in affected dace are more abundant than in Figure 5. Figure 7. Reference liver. Note basophilic-staining cytoplasm. (H & E). Figure 8. Liver from affected fish exhibiting lipidosis (vacuoloated cells) and pericholangitis (bile ductule fibrosis - arrow, H & E).



Cross sections of tissues of pearl dace from reference and oil-contaminated sites. Scale bar is 500 µm except in Figures 11 and 12 (100 µm) Figures 9 and 10. Kidney and spleen of diesel fuel-affected dace showing macrophage aggregates (arrows) with pale-staining lipofuchsin (H & E). Figures 11 and 12. Macrophage aggregates in spleen ofreference and affected fish stained with Perl's Prussian blue. Note larger aggregates in Figure 12. Figure 13. Ovary of reference dace in post-spawning condition. Note post-ovulatory follicles (arrows) and developing oocytes in primary growth phase (H & E). Figure 14. Ovary from oil-affected dace showing advanced vitellogenic oocytes (H & E).

uptake and ultimately in foraging activity. The presence of unusually large foci of macrophage aggregates in the spleen is suggestive of excessive erythrocytic destruction and catabolism of hemoglobin within these centres. None of the preneoplastic and neoplastic lesions reported in English sole (*Pleuronectes vetulus*) exposed to PAHs in Puget Sound were observed (Myers et al. 1994). An absence of digenetic trematodes in pearl dace sampled at the stillwater pond in the present study and their presence at the reference site supports the view that some parasites can be useful indicators of environmental change (Khan and Thulin 1991; Khan and Payne 1997). Some of the trematodes observed in the present study have been reported in threespine stickleback (*Gasterosteus aculeatus*) from Labrador (Hanek and Threlfall l970). Limited data on abundance of similar enteric digenes in threespine sticklebacks (n=15) at the uncontaminated location ($\bar{x}$2.6 ± 0.3) in contrast to their absence at the oiled site is further evidence of ecosystem stress (Khan, unpubl. data). Previous studies also indicate a decline of gastrointestinal parasites following prolonged exposure to PAHs and a number of other pollutants (Khan and Thulin 1991; Moles et al. 1993). Marcogliese and Cone (1997) observed an absence of digeneans in eels (*Anguilla rostrata*) inhabiting an acidified river whereas multiple infections occurred in specimens from an adjacent river that was experimentally limed. Fish parasites with heterogeneous life cycles such as digeneans rely on some invertebrates, for example, gastropods, as primary and aquatic arthropods as secondary intermediate hosts. Some of the latter as well as transitory free-living larval stages can be extremely sensitive to environmental stressors such as pollutants. It is likely, then, that the life cycles of some digeneans of pearl dace inhabiting the stillwater pond were interrupted by the disappearance of the intermediate hosts, mortality of the free-living larvae stages and/or changes in the host's physiology induced by the stressor, resulting eventually in voiding of the parasites.

The present study, using a number of bioindicators to assess stress in pearl dace exposed to diesel fuel, revealed an absence ofjuveniles but a greater number of large fish, especially females, with a K-factor that was significantly greater at the impacted location than in samples from a reference site. Moreover, low lymphocyte levels, structural damage to the gills, degenerative lipidosis, unusually large macrophage aggregates in the spleen, an absence of biotransforming enzymes in the liver, lack of parasites in the digestive tract and delayed spawning were apparent in fish inhabiting the impacted site. These findings suggest that PAHs have had a severe impact on the population and could eventually cause its demise.

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