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Physiological Changes in Prickly Sculpin (*Cottus asper*) Inhabiting a Lake Used by Jet-Propelled Watercraft

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The use of jet-propelled watercraft in lakes and inland waterways has dramatically increased in the last two decades, raising concerns about the effects of the hydrocarbon discharge on the demersal fishes in those waterways. These craft, known by their brand names such as Jet Skis, Waterbikes, and Sea-Doos, are powered by a water jet drive that allows them to operate close to shore. Officially designated personal watercraft (PWC), there are an estimated 1.3 million of these recreational vehicles in use in the United States with an additional 200,000 sold each year (Martin 2003). This amounts to nearly one-third of new boat sales annually. Older models can discharge up to one-third of their fuel into the water. In a single hour of operation, a PWC can release between 14 and 17 L of fuel, mostly uncombusted oil, into the environment (California Environmental Protection Agency 1999). Two-stroke engines spill an estimated 15 times more oil annually in United States waters than the *Exxon Valdez* (Mele 1993). Some lakes, especially in national parks, prohibit or limit the use of these vehicles.

Among contaminants that can enter the watershed from PWC activity, polycyclic aromatic hydrocarbons (PAH) are the most toxic to aquatic life and the most persistent. Released into the environment through either combustion or weathering of oil products, some PAH are known carcinogens and mutagens. Several studies have linked increases in watershed PAH with summertime motorboat activity. In a drinking water reservoir, surface PAH levels as high as 19 μg/L were measured in June at the height of the boating use, but no PAH were detected in October when boating activity was low (Mastran et al. 1994). Similar correlations in peak boating activity and water-soluble hydrocarbon concentrations have been reported for Lakes Metigoshe in North Dakota (Brammer and Puyear 1982) and Tahoe in Nevada (Oris et al. 1998).

Auke Lake, inhabited by 8 species of fish, is a popular site for personal watercraft use near Juneau, Alaska. Motorized recreation has increased in the last few years, with a current estimate of 1000 boat hr of use per yr. Both boat use and PAH concentration in the top meter of the water column increase as the summer progresses and decline with the coming of fall (Rice et al. in preparation). This use is correlated with a seasonal increase in PAH in the areas of heaviest personal watercraft activity each year as measured by passive sampling devices (Rice et al.

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in preparation). Changes in water quality and habitat are suspected of curtailing the previously robust Pacific salmon runs into Auke Lake. If chronic exposure to low levels of toxic hydrocarbons were affecting any of the biota of the lake, it would be more evident in the prickly sculpin (*Cottus asper*) than in the anadromous fish that spend less of their life span in the lake. The prickly sculpin is a year-round resident of the lakes of southeast Alaska, living in the shallow nearshore benthic habitat. Several bioindicators of stress including length/mass relationships, hematology, histology, and parasitism were compared among sculpin sampled from an area heavily used by PWC, a nearby lake without PWC use, and a reference area in a remote lake with no motorized recreation. These bioindicators have proven to be useful in assessing the effects of chronic exposure to pollutants (Khan 1999).

MATERIALS AND METHODS

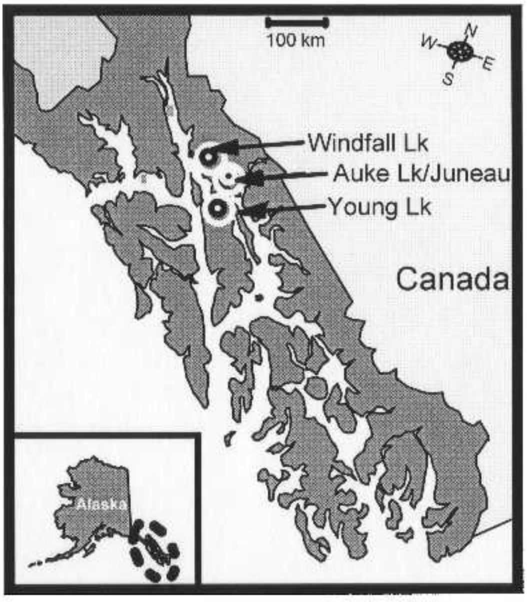

Figure 1. Location of sculpin sampling sites at Windfall Lake, Auke Lake, and Young Lake.

The heavy use lake, Auke Lake, is a 70-ha lake in Southeast Alaska with a maximum depth of 31 m, located in the city of Juneau, Alaska (Figure 1). There are two permanent inflows that drain a watershed of 1000 ha. A 500 m stream, Auke Creek, connects the lake to the ocean. It is the principal recreational lake for a town of 30,000 people. The lake has endemic populations of prickly sculpin, 5 species of anadromous salmon, and 3 species of resident trout. An average of 150,000 salmonids migrate from the lake annually.

Windfall Lake, a popular sport fishing destination, is located 24 km north of Auke Lake and 7 km from the ocean with access via an 11 km hiking trail. Although motorboat and aircraft activity occur during the summer, there is no PWC access because of the lake's remote location. The 46-ha lake has a maximum depth of 4.5 m, and two inlet streams drain a watershed of 2908 ha. It supports populations of prickly sculpin, 3 species of anadromous salmon, and 3 species of char or trout. The only estimate of fish abundance is the return of about 3,500 sockeye salmon to the lake each year. Our reference lake was Young Lake in the Admiralty Island National Monument Wilderness, a lake with little or no motorized activity. A 9 km stream, Admiralty Creek, connects the 78-ha lake to the ocean. The lake drains a wilderness watershed of 1900 ha and is located 19 km SSW of Juneau. With a depth of 60-100 m, the lake supports unknown numbers of prickly sculpin, 1 species of anadromous salmon, and 2 species of trout. Very little else is known about the biology of the two remote lakes.

Low-density polyethylene passive sampling devices were deployed near the outlet of Auke Lake and Young Lake for 3 weeks (June 15-July 6) to confirm the presence of PAH in Auke Lake and their corresponding absence from the reference lake. To avoid contamination, passive samplers were frozen except during deployment; however this proved impractical for the remote Windfall Lake. Total PAH sequestered in the matrix of the polyethylene was calculated as the sum of 39 analytes ranging from the 2 ring napthalene to the 5 ring benzo(ghi)perylene. See Carls et al. (2004) for details on the passive samplers including a list of aromatic hydrocarbons analyzed, analytical methods, chemical kinetics, detection limits, sample recoveries, and reliability of the devices.

In June, July, and August of 2000, at the height of the PWC season, prickly sculpin were captured with minnow traps near the outlets of all 3 lakes. The outlet area of Auke Lake forms a lagoon that is the only launching site for personal watercraft into Auke Lake. Operators of jet-propelled watercraft power up their 2-stroke engines in this isolated area and run them for several minutes before leaving the area for the open lake. The outlet areas of the other lakes were less accessible to boats of any sort. Fifteen fish were harvested from each trap monthly. Three fish from each sample were transported live to the laboratory, then killed and immediately dissected to remove liver, spleen, and gills for histology. Organs were processed and analyzed for abnormalities using the methods of Marty et al. (1999). No histology was done on fish from Windfall Lake due to logistical and budgetary constraints. The remaining 12 fish were measured, sexed, and weighed, and tissues were removed. Hepatosomatic (HSI) and gastrointestinal (GI) indices were calculated as a percent of total body wet weight. All tissues were examined for gross pathology and parasites. A blood smear was stained with Giemsa for estimation of the number of lymphocytes per 1000 erythrocytes.

The length/mass and lymphocyte data were analyzed using 2-way ANOVA to test for significant differences due to month of sampling or lake x month interactions ($P = 0.87$-0.97). Data from all months were then combined for a total of 36 fish per lake. Differences in means for the length/mass and lymphocyte counts from sculpin from the 3 lakes were tested using 1-way ANOVA. Dunnett's test was used to test between-lake means and the means for the reference lake with significance set at $P < 0.05$. The proportion of fish infected with individual species of parasites was compared between the test lakes and Young Lake using 2 x 2 contingency tables analysis. Differences in the prevalence of tissue lesions between lakes were not assessed statistically owing to the small number of fish sampled (9 from only two lakes).

RESULTS AND DISCUSSION

There is strong evidence that prickly sculpin in Auke Lake are undergoing physiological changes that are not observed in sculpin from lakes with far less motorized activity. The chronic input of PAH every summer in the same locale as the sculpin sampling certainly suggests that PAH were the probable source of these alterations. This summer input of PAH is the result of increased use by motorboats, particularly jet-propelled watercraft (Rice et al. in preparation).

Auke Lake experienced a chronic input of PAH consistent with PWC exhaust. The mean concentration of PAH in passive samplers deployed at the lagoon site in the summer of 2000 was 1468 ±11.8 ng/g total PAH. The mean relative composition of the PAH samples was 40% naphthalenes, 18% fluorenes, 2% dibenzothiophenes, 22% phenanthrenes, and 6% each fluoranthene and pyrene. This combination of burned and unburned fuel is what would be expected from the operation of a two-stroke engine (Mastran et al. 1994). During the period 1999-2003, PAH concentrations sequestered in passive samplers over 2-3 week intervals in Auke Lake at the peak of the boating season ranged from 1184 ng/g to 6834 ng/g (Rice et al. in preparation).

Passive samplers evolved as surrogates for biological tissue uptake to detect intermittent pulses of contaminants in water. To put the concentrations detected with our passive samplers in context, a concentration in excess of 1100 ng/g PAH was detected in mussel tissue at only 15% of the 263 sites sampled during Mussel Watch and was sufficiently high to cause biological damage (O'Connor 2002).

Sculpin examined in 2002 from the lake heavily used by jet-propelled watercraft differed in several respects from fish sampled from the other two lakes in the study. Sculpin sampled from the popular recreational Auke Lake had lower condition factor, gastrointestinal indices, and lymphocytes than did fish collected from the lesser used Windfall Lake and the reference site of Young Lake (Table 1). Sculpin from all three lakes ranged from 12 to 14 cm in total length and 19 to 31 grams in weight, but the mean lengths and weights at all three sites were

Table 1. Mean (±SE) length, weight, condition factor, liver (HIS) and gastrointestinal (GI) indices, and blood values of *C. asper* from 3 lakes. Asterisk (*) indicates statistical difference from fish from reference site ($P < 0.05$).

Variable	Auke L.	Windfall L.	Young L.
Length (cm)	12.54 ± 0.23	13.0 ± 0.11	12.79 ± 0.19
Weight (g)	22.54 ± 1.56	28.6 ± 1.23	26.32 ± 1.18
Condition Factor	1.09 ± 0.02*	1.30 ± 0.02	1.24 ± 0.02
Sex Ratio (F;M:I)	53:38:9	45:30:25	48:36:16
HSI	0.85 ± 0.04	0.83 ± 0.08	0.87 ± 0.07
GI	4.79 ± 0.13*	6.93 ± 0.51	6.38 ± 0.43
Lymphocytes	10 ± 1.6*	19.7 ± 3.3	17.5 ± 2.9

similar and did not differ statistically. Despite this similarity in size, the proportion of immature sculpin was lowest in Auke Lake, at only 9% versus 16-25% at the other lakes. The gastrointestinal index was 33% and 45% greater at the lightly used Windfall Lake and the reference Young Lake respectively than at the heavily used Auke Lake. Less mesenteric fat was observed on the visceral surfaces of sculpins from Auke Lake. The presence of less mesenteric fat may explain the lower condition factor among Auke Lake fish. The sculpin from this recreational lake also had only 10 lymphocytes per 1000 erythrocytes, in contrast to nearly double that number in sculpin from the other two lakes.

The prevalence of certain parasites differed between Auke Lake and Young Lake (Figure 2). The protozoan gill parasite *Epistylis* sp. and the protozoan gall bladder parasite *Myxobolus* sp. were present in a higher proportion of sculpin from Auke Lake than from Young Lake. In contrast, the digenetic trematodes *Crepidostomum isotumum* and *Tetracotyle* sp., the cestode *Proteocephalus* sp., and the glochidia *Anodonta* sp. were absent from Auke Lake sculpins but common (prevalence of 19, 39, 33, and 28% respectively) in Young Lake fish. The nematodes *Eustrongylides* sp. and *Pseudocapillaria salvelini* were present in similar proportions in both lakes. *Neoechinorhynchus rutili*, an acanthocephalan species, was absent from Auke Lake but only noted in three fish from Young Lake, an insignificant difference. Parasites from Windfall Lake were similar to those in fish from Young Lake, and those data are not presented here.

Parasites are sensitive indicators of environmental health. In a healthy ecosystem, the parasitic fauna is often as diverse and abundant as the many hosts and habitats present in the system. Landsberg et al. (1998) speculate that parasites with few intermediate hosts may flourish in fish undergoing chronic stress. Exposure to petroleum hydrocarbons can reduce immune response, providing a habitat for gill parasites to reproduce (Moles and Wade 2001). Conversely, toxic conditions can disrupt the life cycle of parasites that require several intermediate hosts. *Epistylis* sp. has a direct lifecycle, unlike the trematodes and cestode in the present study, which require mollusk and crustacean intermediate hosts to complete their life cycles. The lack of glochidia (larval stage of the freshwater mussel *Anodonta* sp.) in Auke Lake fish is not surprising given that the once abundant adult mussels are now rare in Auke Lake.

Microscopic lesions in the liver are indicative of chronic toxicity. Four types of lesions were more prevalent in livers from Auke Lake sculpin than from reference lake sculpins (Figure 2). Hepatic macrophage aggregates were noted in the 78% of the Auke Lake sculpin livers but in only 44% of the Young Lake fish livers. This higher prevalence could indicate that the Auke Lake fish were older, despite the similarity in size, or that the fish had been exposed to toxicants. The lower proportion of immature fish in the high use lake suggests that the Auke Lake fish grew less over time. Lipid vacuoles were twice as prevalent (44%) in the livers from Auke Lake fish as in the livers from reference lake fish (22%). Apoptosis, or single cell necrosis, of the hepatic cells was noted in 22% of the fish from Auke Lake but was not observed in the livers of fish from the reference lake. There was also slightly more hepatocellular megalocytosis in sculpin from the recreational Auke Lake (78%) than in fish from the reference lake (56%). All of these liver lesions are known responses to toxicants. The combination of all four lesions coupled with the differences in lymphocytes counts and parasites suggests reduced health among the Auke Lake fish. The spleen and gill sections did not have any differences that could be attributed to sampling site.

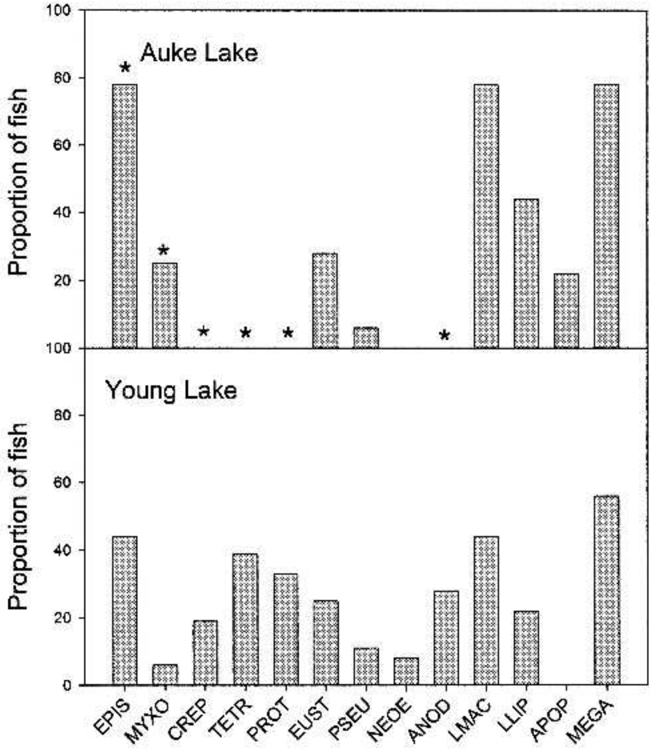

Figure 2. Proportion of sculpin infected with parasites (n=36) or exhibiting liver lesions (n=9) in Auke Lake and Young Lake, Alaska. EPIS=*Epistylis* sp., MYXO =*Myxobolus* sp., CREP=*Crepidostomum isotomum*, TETR=*Tetracotyle* sp., PROT=*Proteocephalus* sp., EUST=*Eustrongylides* sp., PSEU= *Pseudocapillaria salvelini*, NEOE=*Neoechinorhynchus rutili*, ANOD=*Anodonta* sp., LMAC= macrophage aggregates, LLIP=liver lipidosis, APOP=apoptosis, MEGA= megalocytosis. Asterisks denote significant difference from reference lake.

The burned and unburned fuel that constitutes the effluent of PWCs can have a deleterious effect on aquatic organisms. In laboratory tests, water from an area of Lake Tahoe that had high levels of motorcraft use reduced survival and reproduction of lake zooplankton and reduced growth of the larval fish species *Pimephales promelas*s (Oris et al. 1998). In other studies, perch (*Perca fluviatilis*) exposed to two-stroke exhaust components had elevated DNA adducts in blood, liver, and kidneys (Tjarnlund et al. 1995). Similarly, rainbow trout injected with exhaust extract exhibited DNA damage and altered carbohydrate metabolism (Tjranlund et al. 1996).

Low concentrations of PAH can have a deleterious effect on early life stages of fishes. These hydrocarbons, because they are larger and heavier than moncyclic aromatics, are released from oil films and droplets at progressively slower rates with increasing molecular weight and can remain bound to sediments and tissues for several years. Salmon embryos that accumulated PAH from aqueous concentrations as low as 1 ng/g had reduced survival and growth, probably as the result of damage during early embryonic development (Heintz et al. 2000). Animals that are capable of bioaccumulating these compounds, such as the bivalve intermediate hosts for digenetic trematodes, could sequester sufficient PAH in their tissues over time to induce deleterious effects.

The quality of fish rearing habitat within the Auke Lake watershed may be declining due to human activities such as hydrocarbons from jet-propelled watercraft. In the last decade, salmon returning to the lake have declined from 32,000 fish to 16,000 fish. Sticklebacks (*Gasterosteus* sp.) and freshwater mussels (*Anodonta* sp.), once very commonly found in the littoral zone were not found during a recent unpublished survey. Although changing natural factors, such as long-term climactic trends or differences between sculpin populations due to food abundance, fish movement or maturity rates may be contributing to these changes, the alterations in sculpin GI index, parasites, and liver histology indicate toxic agents are affecting these benthic fish. Limiting PWC use to the more fuel-efficient models could produce improvement in the water quality of lakes.

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