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In Situ Experimental Assessment of Lake Whitefish Development Following a Freshwater Oil Spill

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Wabamun Lake (Alberta, Canada) has been subject to ongoing contamination with polycyclic aromatic hydrocarbons (PAHs) from multiple sources for decades and in August 2005 was exposed to ca. 149 500 L of bunker C oil following a train derailment. We compared the pattern, frequency, and severity of deformity in larvae of lake whitefish (*Coregonus clupeaformis*) incubated in situ in areas of Wabamun Lake exposed only to "background" PAH contamination and in areas additionally exposed to PAHs from the oil. All sites in the lake (including reference areas) showed incidences of deformity higher than are typically observed in laboratory studies. A small number of oil-exposed sites showed higher incidences of some teratogenic deformities and a tendency to exhibit deformities of higher severity than sites not exposed to oil. The frequency of moderate to severe deformities in 8 of 16 classes was correlated with PAH exposure. Nonmetric multivariate ordination of deformity data revealed a general pattern of increasing incidence and severity of several skeletal (lordosis, scoliosis) and craniofacial (ocular, jaw) deformities at sites with relatively high exposure to oil-derived PAHs. A simultaneous consideration of incidence, severity, and pattern of deformity enabled us to detect a consistent (overall ∼5% above background) response to the oil despite high variability and high background deformity rates in this historically contaminated environment.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a complex class of organic compounds containing two or more fused aromatic rings. PAHs are released into the environment in association with seeps, spills, and volatilization of fossil fuels (petrogenic sources) or during incomplete combustion of organic matter (pyrogenic sources). Hundreds of PAH congeners have been identified in environmental samples, typically including a complex mixture of parent and substituted forms. The toxicity of mixtures of PAHs to early life stages of fish has been attributed to a combination of multiple modes of toxic action, including narcosis, AhR agonism, and "alkylphenanthrene toxicity" (*1*−*3*).

Sublethal effects of chronic PAH exposure in developing fish embryos have been shown in numerous laboratory studies (e.g., refs *3*−*6*), but field-based assessments are rare. The *Exxon Valdez* oil spill in Prince William Sound, Alaska, represents one of the few instances in which developmental effects have been assessed on wild fish larvae in areas exposed to an oil spill (*7*−*9*). The objective of our study was to bridge the gap between laboratory studies and field observations with an in situ experimental incubation, combining the ecological relevance of a field study (i.e., approximating exposure conditions of wild embryos) with the logistical advantages of a laboratory study (i.e., control over oil exposure, control for parental factors, complete recovery of larvae).

Wabamun Lake (Alberta, Canada) has been subject to ongoing contamination with PAHs from multiple sources for decades (*10*) and in August 2005 was exposed to 149 500 L of bunker C oil following a train derailment. As part of a larger program to assess possible ecological effects of the oil release (*11*), we conducted an in situ experiment to determine whether oil exposure resulted in a change in the pattern, incidence, or severity of larval deformity in lake whitefish (*Coregonus clupeaformis*). A simultaneous consideration of incidence, severity, and pattern of deformity enabled us to detect a consistent response to the oil despite high variability and high "background" deformity rates in this historically contaminated environment.

Experimental Section

Study Location. Wabamun Lake (53°32′ N, 114°34′ W) is large (8180 ha) and shallow (z_{mean} = 6.3 m) and currently has no appreciable outflow due to the construction of a weir in 1927 (residence time >100 y; *12*). The lake is situated in the North Saskatchewan River basin, adjacent to the villages of Wabamun and Fallis, the Paul Band First Nation Reserve, and several cottage developments. Coal mines are located north and south of the lake, two coal-fired power plants discharge cooling water to the northeast (directly) and southeast (via cooling ponds) of the lake, and two additional coal-fired power plants operate within a 35 km radius (*10*). As well, the lake has several marinas and extensive recreational boat use. PAHs have been detected more frequently and at higher concentrations in Wabamun Lake than in other regional lakes; possible sources include water and air emissions from power plants, leaching from coal naturally present in and near the lake, creosote-treated structures, and combustion and spillage of boat fuel (*10*, *13*).

About one-third of Wabamun Lake is <5 m depth, with a predominance of gravel and cobble substrates along the eastern and southern nearshore areas (*13*), including a large shoal at Blueberry Point (Figure 1). Shallow gravel and cobble substrates provide a spawning habitat for lake whitefish, and this species has supported a year-round sport fishery, a winter commercial fishery (currently closed), and year-round subsistence fishing by members of the Paul Band First Nation (*12*, *14*).

On Aug 3, 2005, a train derailment released 712 000 L of bunker C oil and 88 000 L of Imperial pole treating oil to the ground north of Wabamun Lake (Figure 1). An estimated 149 500 L of bunker C reached the north shore of the lake, where it was transported by prevailing northwesterly winds to portions of the south and east shores. The western basin of the lake, including several areas of potential whitefish

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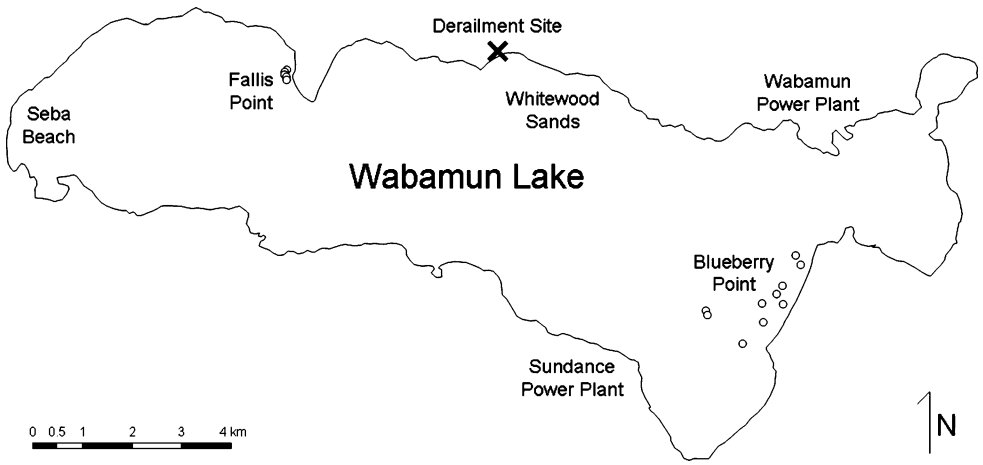

FIGURE 1. Location of study areas at Fallis Point (reference, $n = 10$; individual stations cannot be distinguished due to the small size of the spawning shoal) and Blueberry Point (oil-exposed, $n = 10$), Wabamun Lake.

spawning habitat, was not exposed to oil at the time of the spill.

In November 2005, we established four study areas in Wabamun Lake. Reference areas were established in the western basin on the north shore at Fallis Point (cobble substrate) and on the west shore at Seba Beach (sand substrate). Oil-exposed study areas were established in the eastern basin on the east shore at Blueberry Point (cobble substrate) and on the north shore near the spill site at Whitewood Sands (sand substrate). Complete egg mortality occurred at all 10 stations at Seba Beach and more than half of the stations at Whitewood Sands, concomitant with observations of fungal infection, predation by planarian worms and other invertebrates, and sedimentation with silt, organic detritus, and small coal particles. The following analysis therefore focuses on a comparison between the two cobble-substrate areas, where egg survival was relatively high. Stations at Fallis Point were distributed over a patch of cobble substrate approximately 200 m in length, 100 m from shore, and at 1.5−2.7 m depth. Stations at the Blueberry Point shoal were distributed along 2.1 km of shoreline, ranging from 100 to 1400 m from shore and at 1.8−3.5 m depth (Figure 1).

Exposure Assessment. Exposure of whitefish eggs to PAHs was assessed using triolein-filled semipermeable membrane devices (SPMDs; Environmental Sampling Technologies (EST), St. Joseph, MO). SPMDs were received in stainless steel cans, handled with clean stainless steel forceps, and placed in perforated stainless steel canisters directly on the substrate. Thirty-two SPMDs were deployed from February to April 2006. At Blueberry Point, SPMDs were deployed within 2−5 m of incubation trays at eight sites and midway between the remaining two sites (<50 m from each); at Fallis Point, three SPMDs were deployed, each estimating exposure for a group of incubation trays within 2−40 m. Upon retrieval, SPMDs were removed from their canisters with clean stainless steel forceps, placed in 250 mL heat-treated glass jars with Teflon-lined lids, and returned on ice to EST for dialysis. The extract from the SPMDs was analyzed for parent and alkylated PAHs by USEPA method 3510/8270-GC/MS following gel permeation chromatography cleanup (ALS, Edmonton, AB, Canada). Detection limits for individual PAHs were 0.05−0.2 μg/SPMD.

Egg Incubation. Fish sampling for broodstock was carried out Nov 2−4, 2005, at the east end of Wabamun Lake. Fish were sampled by gill netting and boat electrofishing. Gill nets (3.8−6.4 cm stretched mesh, 74.3 m^2 total area) were deployed for 30 min intervals to minimize mortality. Electrofishing was conducted using a Smith-Root (Vancouver, WA) type VIa electrofisher unit and two fixed-boom anode arrays (60 Hz, 672 V dc, 3−4 ms pulse width, 5−6 A). Ripe fish were placed in a holding cage until a sufficient number of fish were obtained. Eggs and milt were stripped from 11 ripe males and 3 ripe females into a stainless steel bowl and gently mixed with a feather to promote fertilization. After 10 min, the eggs were rinsed with clean lake water, transferred to an aerated container, and allowed to water-harden in clean lake water for a minimum of 2 h. The eggs were then transferred over a period of 2 days into aerated incubation trays using disposable plastic pipets. The incubation trays were constructed of 15 × 25 cm sheets of 0.9 cm thick Lexan with 0.95 cm diameter holes drilled completely through, sandwiched between sheets of 1.5 × 2 mm nylon mesh held in place by thinner sheets of Lexan (Figure 1 in ref *15*). One egg was placed in each of 100 separate compartments per tray.

The egg trays were deployed Nov 4−7, 2005. Two trays were placed on the spawning substrate at each of 10 locations within each of the 4 study areas. The water temperature was recorded throughout the incubation period by temperature data loggers (VEMCO, Halifax, NS, Canada) deployed in each study area. One tray from each location was removed Feb 20−22, 2006, to assess egg survival (results in the Supporting Information), and the other was removed April 26−29, 2006 (after ice melt). One pair of trays at Fallis Point and one pair at Blueberry Point could not be located under the ice, so both trays from these two sites were removed in April (denoted 19a/19b and 36a/36b).

Assessment of Larvae. The larvae in each tray at the end of the incubation period were classified as missing, dead, or hatched, then transferred into Stockard's solution, and stored at 4 °C. Hatching success was expressed as the percentage of the 100 eggs in each tray that produced live larvae. The larval length was measured from the snout to the tip of the notochord. Deformity incidence was expressed as the fraction of successfully hatched larvae exhibiting a particular deformity. Larval deformities were assessed on the basis of refs *7*, *16*, and *17*. Four categories of externally visible morphological deformities were scored: skeletal (lordosis, kyphosis, scoliosis, stunting), craniofacial (eye, head, jaw), finfold (fin size/shape, fin presence), and edema (head, yolk sac, pericardial). Each deformity type within these four categories was scored using a graduated severity index (GSI; Table S1, Supporting Information). In addition, each larva was given a GSI score for the overall magnitude of combined deformity types in each of the four categories. A total of 712 larvae from Fallis Point and Blueberry Point were assessed. All larvae from a randomly selected subset of seven trays ($n = 150$ larvae) were subjected to blind reassessment. In 11 of the 16 deformity categories, the absolute incidence of deformities among the 150 larvae differed by no more than 2 between

assessments. Jaw deformity had the highest rate of difference, with five larvae receiving a different evaluation on reassessment.

Statistical Analysis. Mann−Whitney *U* tests were used to test for a difference between sites in the mean incidences of deformities in each of the 16 types. To incorporate information about the severity of deformity as well as incidence, separate tests were conducted for different levels of severity (slight, GSI = 1; moderate to severe, GSI = 2−3; any severity, GSI = 1−3) within each deformity type. Spearman rank correlation was used to test for relationships between total detectable PAHs in SPMDs (as an index of oil exposure) and incidences of different levels of severity of deformity in each class (as above). Incidences of different classes of deformity and different levels of severity within a deformity class are expected to be correlated, so inflation of type 1 error in multiple tests was expected to be slight and type 1 error correction was not applied. Nonmetric multidimensional scaling (NMDS) was used to explore gradients in the overall pattern of deformity within the lake. Incidences of slight and moderate to severe deformity in each class were included in NMDS analysis separately to incorporate information about both incidence and severity in this assessment of pattern. The dimensions identified by NMDS were interpreted by conducting Spearman rank correlations of dimension scores with each individual deformity variable. All statistical analyses were conducted using SYSTAT version 11 (Systat Software Inc., Richmond, CA).

Results

Exposure. Two PAHs (*C*3-naphthalene and phenanthrene) were above detection in only 1 of 13 SPMDs deployed in reference areas. At oil-exposed sites, 1 or more PAHs were detected in 13 of 19 SPMDs, including parent naphthalene, methyl-, *C*2-, *C*3-, and *C*4-naphthalene, parent phenanthrene/anthracene and methyl- and *C*2-phenanthrene/anthracene, parent fluorene and *C*2-fluorene, *C*4-dibenzothiophene, and *C*2-biphenyl. The dominant PAHs detected in samples of bunker C obtained at the derailment site were alkylnaphthalenes (64% of detected PAHs) and alkylphenanthrenes (14%), consistent with the PAHs detected in SPMDs in the potentially oil-exposed areas of the lake (*18*; details in the Supporting Information). Total detectable PAH concentrations in these SPMDs were 0−0.30 μg/SPMD at reference sites (Seba Beach, ND in all cases, $n = 9$; Fallis Point, 0.075 ± 0.15, $n = 4$) and 0−7.8 μg/SPMD at oil-exposed sites (Whitewood Sands, 1.2 ± 2.5, $n = 10$; Blueberry Point, 1.3 ± 1.7, $n = 9$). Mean water temperatures over the incubation period were similar at all locations (mean [range]: Fallis Point, 2.7 °C [1.1−10.5 °C]; Blueberry Point, 2.9 °C [0.0−9.9 °C]; Seba Beach, 2.7 °C [0.4−17.5 °C]; Whitewood Sands, 3.2 °C [0.8−13.0 °C]).

Effects. Neither hatching success nor the mean larval length differed significantly between Fallis Point (reference) and Blueberry Point (oil-exposed). Hatching success was 38 ± 16% (mean ± SD, range 13−64%) at Fallis Point and 29 ± 16% (13−63%) at Blueberry Point (Mann−Whitney $U = 36$, $p = 0.18$). the mean larval length was 1.17 ± 0.05 mm (1.06−1.22 mm) at Fallis Point and 1.19 ± 0.03 mm (1.14−1.23 mm) at Blueberry Point ($U = 65.5$, $p = 0.46$). At a coarse level, i.e., considering all deformities to be equal, there was no significant difference between exposed and reference areas in deformity incidence. Incidence of any deformity in successfully hatched larvae was 86.0 ± 13.6% (53−100%) at Fallis Point and 88.3 ± 8.9% (73−100%) at Blueberry Point ($U = 55$, $p = 1.0$). Percent normal survival (i.e., the number of 100 eggs that produced larvae with no deformities) was 6.6 ± 8.7% (0−30%) at Fallis Point and 3.6 ± 3.5% (0−11%) at Blueberry Point ($U = 46.5$, $p = 0.54$).

The distribution of GSI scores for the larvae recovered from each tray in April varied greatly among trays (Figure 2). Both areas exhibited a highly variable proportion of edemas: 8−100% of larvae at Fallis Point and 4−86% of larvae at Blueberry Point exhibited some level of edema. Every tray contained at least one larva with some type of deformity.

Larvae incubated at Blueberry Point had significantly higher mean incidence (at any level of severity) of kyphosis and overall skeletal and fin presence deformities (Table 1). These significant differences were primarily driven by higher incidence of relatively severe deformities: no deformity type showed a significant difference between sites in incidence of slight deformity (GSI = 1), but comparing sites with respect to the incidence of moderate to severe deformity only (GSI = 2−3) revealed significant differences for lordosis, kyphosis, and fin presence deformity and marginally significant differences for scoliosis and jaw and overall skeletal deformity. Overall skeletal deformity was the category with the highest mean incidence (at any level of severity) at both sites, increasing from 58% at Fallis Point to 73% at Blueberry Point, whereas the mean incidence of moderate to severe overall skeletal deformity increased from 23% at Fallis Point to 36% at Blueberry Point. Moderate to severe lordosis, kyphosis, and fin presence and jaw deformities were rare at Fallis Point (0−2% of larvae) but occurred in 5−10% of larvae at Blueberry Point. There was no significant difference between sites for any class of edema.

Multivariate ordination by NMDS identified two dimensions that accounted for 91% of the variance (stress 0.13) in the original deformity data (Figure 3). Correlating these dimensions with the original variables revealed that the dominant pattern in the data (dimension 1) was a general trend of covariance in the incidence of several skeletal (lordosis, scoliosis, overall), craniofacial (ocular, overall), and edema (yolk sac, overall) deformity types (Table S2, Supporting Information). All of these types showed covariance in the incidence of moderate to severe deformity, although there were also significant positive correlations of dimension 1 with slight lordosis, ocular deformity, and yolk sac and overall edema and a weak ($R < 0.5$) negative correlation with slight kyphosis. Dimension 2 represented a gradient among sites in incidence of slight kyphosis and moderate to severe jaw, overall craniofacial, and overall skeletal deformity and an opposing gradient of (predominantly slight) yolk sac and overall edema.

Exposure vs Effects. No class of deformity showed a significant correlation between incidence of slight deformity and PAH exposure (Table 2); however, incidence of moderate to severe deformity was correlated with PAH exposure for all of the skeletal deformity types (except stunting, for which only slight deformities were observed) and fin presence, jaw, ocular, and overall craniofacial deformity (Figure S1, Supporting Information). In all of these deformity types, correlations for moderate to severe deformities alone were stronger (and more often significant) than correlations with any severity of deformity. Stations at Blueberry Point with relatively high PAH exposure (depicted in Figure 3 by the size of the symbols) also tended to have higher scores for dimension 1 and lower scores for dimension 2, reflecting a general pattern of higher incidence of moderate to severe deformities in several classes.

Discussion

Several stations in the oil-exposed portion of the lake exhibited higher incidence and higher severity of some deformity types relative to the reference area. These stations also had the highest levels of oil-associated PAHs, indicating that "hot spots" of oil exposure are a likely explanation for observed increases in deformity end points. Overall, however, the oil-exposed areas did not exhibit large absolute changes

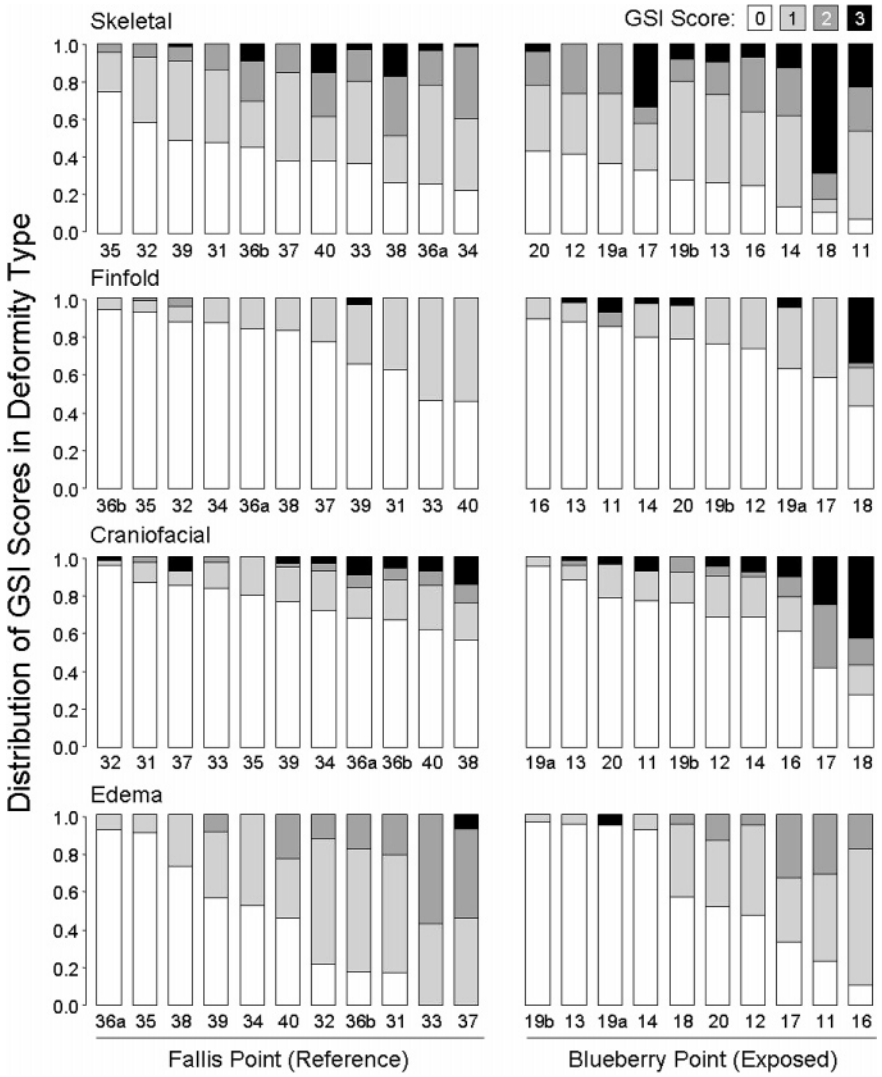

FIGURE 2. Distribution of GSI scores among larvae within each individual egg tray incubated in Wabamun Lake. GSI scores are for overall assessment of the severity of all deformity types within the four major deformity classes. Tray numbers are shown below each bar for comparison with other figures. Tray 15 (Blueberry Point) had no surviving larvae.

relative to the baseline level of deformities in this lake. Most of the significant increases in mean deformity incidence were for moderate to severe deformities in classes that were very rare or absent in the reference area, but that occurred in 5−10% of larvae at Blueberry Point (i.e., lordosis, kyphosis, and fin presence and jaw deformity). The incidence of kyphosis (and consequently overall skeletal deformity) was an exception to this, exhibiting a significant ∼15% increase in overall incidence (any level of severity) at Blueberry Point relative to Fallis Point. Overall incidence of the other teratogenic deformities (i.e., excluding edemas) were also higher on average at Blueberry Point than at Fallis Point, but these increases were nonsignificant and generally <10%. Incidences of edemas did not differ significantly between areas, and in fact incidence of yolk sac edema was on average 19% lower at Blueberry Point than Fallis Point; this may indicate no effect of the oil on incidence of edemas, or it may indicate higher mortality of edematous larvae at Blueberry Point.

An additional finding was that whitefish larvae at both Fallis Point and Blueberry Point (including stations with no detectable PAHs) exhibited much higher incidence of deformity than commonly observed in laboratory studies or field observations of freshwater fish. Laboratory negative controls often exhibit deformity incidence of 2% or less (*19*, *20*). Field studies of salmonids in reference areas report highly variable and sometimes much higher deformity incidence than laboratory studies, ranging from <3% (*21*) to >40% in some species (*22*). In both of our study areas in Wabamun Lake, the mean incidence of skeletal deformities was >50%, and the mean incidence of any deformity (including edemas) was >85%. These incidences are higher than have been reported for areas considered to be impacted by selenium contamination (e.g., 25−33% (*23*), 40% (*22*)). There are several possible explanations. The most likely is that Wabamun Lake has atypically high deformity rates, as a result of either long-term, widespread contamination with PAHs and other contaminants (*15*) or unfavorable conditions such as near-bottom hypoxia (*24*). Alternatively (or additionally), some element of our experimental procedure may have increased deformity rates. We designed our study to approximate real incubation conditions: embryos were exposed to actual near-bottom water quality, temperature, and sedimentation with native particulate matter. It was impractical to monitor water quality variables such as ammonia and dissolved oxygen for 6 months under ice, so it was necessary to assume that conditions in the trays were similar to those in the underlying cobble and similar between Fallis Point and Blueberry Point, with the exception of potential oil exposure. If there was a systematic difference between the trays and the cobble, this would likely be the case in both study areas, and possible experimental artifacts would represent only a source of error,

TABLE 1. Mean (±SD) Deformity Incidence in Lake Whitefish Larvae Incubated at Fallis Point (F; Reference, $n = 11$) and Blueberry Point (B; Oil-Exposed, $n = 10$)[a]

		slight (GSI = 1)			moderate to severe (GSI = 2−3)			any severity		
endpoint	site	incidence	*U*	*P*	incidence	*U*	*P*	incidence	*U*	*P*
lordosis	F	0.045 ± 0.033	49.5	0.69	0	77	**0.024**	0.045 ± 0.033	68	0.36
	B	0.046 ± 0.055			0.048 ± 0.086			0.094 ± 0.11		
kyphosis	F	0.32 ± 0.12	78.5	0.098	0.020 ± 0.040	82.5	**0.036**	0.34 ± 0.12	86	**0.029**
	B	0.42 ± 0.18			0.069 ± 0.066			0.49 ± 0.15		
scoliosis	F	0.32 ± 0.13	47	0.57	0.065 ± 0.059	79	0.091	0.38 ± 0.16	71	0.26
	B	0.29 ± 0.16			0.20 ± 0.22			0.49 ± 0.23		
stunting	F	0.0017 ± 0.0055	67	0.22	0	55	1	0.0017 ± 0.0055	67	0.22
	B	0.014 ± 0.028			0			0.014 ± 0.028		
skeletal overall	F	0.35 ± 0.11	65	0.48	0.23 ± 0.15	79.5	0.084	0.58 ± 0.15	84.5	**0.038**
	B	0.37 ± 0.13			0.36 ± 0.18			0.73 ± 0.12		
fin presence	F	0	55	1	0	82.5	**0.0096**	0	82.5	**0.0096**
	B	0			0.048 ± 0.098			0.048 ± 0.098		
fin width/shape	F	0.24 ± 0.18	57	0.89	0.0092 ± 0.017	66	0.36	0.25 ± 0.17	60.5	0.70
	B	0.21 ± 0.12			0.045 ± 0.080			0.26 ± 0.13		
fin overall	F	0.24 ± 0.18	55	1	0.0092 ± 0.017	77	0.086	0.25 ± 0.17	62.5	0.60
	B	0.20 ± 0.12			0.067 ± 0.11			0.27 ± 0.14		
jaw	F	0.14 ± 0.078	57	0.89	0.018 ± 0.030	79.5	0.068	0.15 ± 0.075	70	0.29
	B	0.14 ± 0.077			0.086 ± 0.12			0.23 ± 0.16		
ocular	F	0.055 ± 0.056	56.5	0.91	0.071 ± 0.061	66	0.44	0.13 ± 0.10	59.5	0.75
	B	0.05 ± 0.047			0.12 ± 0.13			0.17 ± 0.17		
head	F	0.014 ± 0.023	48	0.57	0.028 ± 0.041	54	0.94	0.042 ± 0.044	52	0.83
	B	0.030 ± 0.071			0.030 ± 0.053			0.059 ± 0.097		
craniofacial overall	F	0.16 ± 0.065	41.5	0.34	0.089 ± 0.074	68	0.36	0.25 ± 0.12	64	0.53
	B	0.14 ± 0.070			0.18 ± 0.21			0.32 ± 0.20		
yolk sac edema	F	0.39 ± 0.20	37.5	0.22	0.17 ± 0.21	47	0.56	0.56 ± 0.33	36	0.18
	B	0.28 ± 0.22			0.096 ± 0.11			0.37 ± 0.31		
head edema	F	0.0070 ± 0.023	61	0.49	0	55	1	0.0070 ± 0.023	61	0.49
	B	0.013 ± 0.029			0			0.013 ± 0.029		
pericardial edema	F	0.020 ± 0.054	65	0.40	0	55	1	0.020 ± 0.054	65	0.40
	B	0.071 ± 0.15			0			0.072 ± 0.15		
edema overall	F	0.40 ± 0.20	39.5	0.27	0.18 ± 0.20	48	0.62	0.57 ± 0.34	34	0.14
	B	0.29 ± 0.24			0.11 ± 0.12			0.40 ± 0.32		

[a] Mann−Whitney *U* statistics and *p* values for comparison of means between sites are also shown. Bold values are significant at an α of 0.05.

TABLE 2. Spearman Rank Correlations between PAH Exposure and Hatching Success, Larval Length, and Incidence of Deformities[a]

endpoint	slight	moderate to severe	any severity
lordosis	−0.018	**0.679**	0.366
kyphosis	0.027	**0.883**	0.324
scoliosis	−0.060	**0.526**	0.393
stunting	0.054	NA	0.054
skeletal overall	0.041	**0.526**	**0.510**
fin presence	NA	**0.745**	**0.745**
fin width/shape	−0.320	0.181	−0.168
fin overall	−0.359	0.375	−0.123
jaw	0.131	**0.445**	0.335
ocular	0.342	**0.438**	0.403
head	−0.074	0.372	0.246
craniofacial overall	−0.134	**0.440**	0.385
yolk sac edema	−0.039	0.132	0.001
head edema	−0.338	NA	−0.338
pericardial edema	0.178	NA	0.178
edema overall	0.023	0.087	−0.023
hatching success			−0.071
mean larval length			−0.008

[a] Incidences of slight (GSI = 1), moderate to severe (GSI = 2−3), and any severity of deformities were analyzed separately for each deformity type. Bold values are significant at a two-tailed α of 0.05 ($n = 21$, critical $R = 0.435$). NA indicates no deformities of this severity observed.

not a bias in the test for a potential effect of oil exposure. The low number of adults that we were able to capture for broodstock may have increased the potential for inbreeding effects, but again this would be the case for both study areas. Finally, it is possible that previous laboratory studies and field observations may underestimate true deformity rates in wild larvae. Field-collected larvae in particular could be a biased subsample, failing to sample larvae so deformed that they died or were depredated prior to sampling. Conversely, laboratory studies clearly isolate the effect of single toxicants or mixtures, but cannot replicate background deformity sources (with the exception of mutational load, if wild-collected eggs are used) or exacerbating factors in the field.

In addition to high deformity rates, we observed high egg mortality (60−70% on average) in both study areas. This may reflect stress from the experimental procedure (e.g., electrofishing, transferring eggs to incubation trays) or incubation conditions (e.g., sedimentation), although previous studies using this type of incubation tray have not reported elevated egg mortality or larval deformities (*15*). Alternatively, these high mortality rates may simply reflect the high early life stage mortality typical of coregonids (e.g., refs *25*−*27*) or may be symptomatic of an impaired fish population: Schindler et al. (*14*) report that lake whitefish had not reproduced successfully in Wabamun Lake for several years prior to the derailment. We found no significant difference in egg mortality between reference and oil-exposed areas, however, and no correlation between egg mortality and PAH exposure (cf. ref *28*).

Studies of Pacific herring following the *Exxon Valdez* oil spill also listed the characteristic types of deformities that are associated with chronic PAH exposure in fish early life stages, including increased spinal defects, optic, maxillary, and mandibular malformations, and enlargement of the

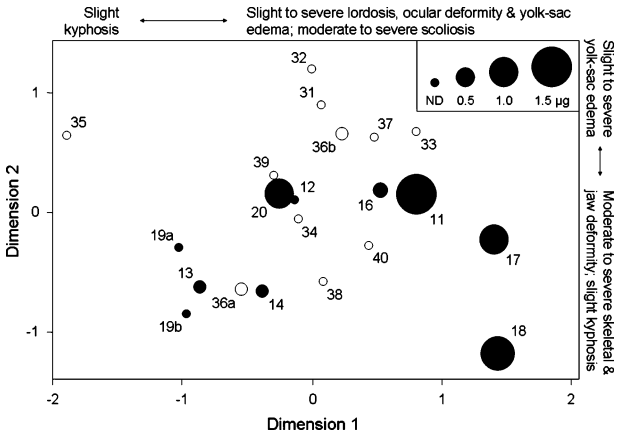

FIGURE 3. NMDS ordination of study stations at Fallis Point (reference area, open circles) and Blueberry Point (oil-exposed area, filled circles) on the basis of incidence and severity of deformities in larval lake whitefish. The size of the symbols reflects the total concentration (μg/SPMD) of detectable PAHs in SPMDs deployed near the egg trays. Tray numbers are shown for comparison with other figures. Interpretation of NMDS dimensions is based on bivariate correlations with individual variables (Table S2, Supporting Information).

pericardial region (*8*, *29*), with skeletal deformities showing the strongest correlation with PAH exposure (*7*). We found a similar pattern of response for teratogenicities, but not for edemas. NMDS and correlation analysis revealed that the main axes of response to oil exposure represented an increase in moderate to severe lordosis, scoliosis, and jaw and ocular deformity. Pericardial edema did not correlate with either dimension score, did not differ between areas, and was not correlated with PAH exposure.

The implications of the observed increase in deformity for population-level processes and the long-term persistence of lake whitefish in Wabamun Lake are difficult to assess. Coregonids are well-known to have naturally high early life stage mortality (e.g., <0.005% survival to maturity (ref *26* and references therein)). If some of this mortality is density-dependent, a modest increase in deformity severe enough to result in mortality may have a negligible impact on recruitment of the affected year class (*30*). However, lake whitefish in Wabamun Lake have already experienced declines as a result of overharvesting and other stressors, prompting recommendations to close the commercial fishery and restrict recreational fishing to catch-and-release (*14*); it is possible that the additional stress of oil exposure may contribute to the need for a protracted period of recovery for the population. Monitoring activities in Wabamun Lake are ongoing to assess the potential contribution of residual oil to reproductive effects in lake whitefish.

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Supporting Information Available

Graduated severity index for assessing larval deformities, assessment of embryos in incubation trays retrieved in February 2006 (midincubation), concentrations of parent and alkylated PAHs detected in SPMDs, composition of bunker C oil, correlations of individual deformity variables with NMDS dimensions, scatter plots of relationships between PAH exposure and hatching success, larval length, and incidence of deformities, and scatter plots of PAH composition between PAHs and bunker C oil. This material is available free of charge via the Internet at http://pubs.acs.org.

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