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ACCLIMATION OF LIFE-HISTORY TRAITS TO EXPERIMENTAL CHANGES IN ENVIRONMENTAL CONTAMINANT CONCENTRATIONS IN BROWN BULLHEAD (*AMEIURUS NEBULOSUS*)

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Abstract—One adaptive mechanism aquatic populations use to facilitate tolerance to environmental contaminants is acclimation. Polychlorinated biphenyls (PCBs) are a globally ubiquitous class of persistent organic contaminants that have been linked to reproductive impairments in fish. The authors used female brown bullhead (*Ameiurus nebulosus*) to test whether acclimation of reproductive life-history traits occurs in response to changes in sum PCB exposure. They compared egg diameter, gonadosomatic index (GSI), and fecundity of fish directly caught from wild populations exposed to a range of contaminant concentrations (acute), to those collected from the same populations a year before, which were placed in a clean environment to clear their contaminants throughout that year (cleared). Sum PCB concentrations were also determined for each individual. Brown bullhead from acute treatments had significantly greater sum PCB concentrations compared with cleared treatments. Egg diameter and GSI metrics were greater in cleared treatments compared with acute treatments (by 6 and 14%, respectively). Treatment effect (i.e., acute or cleared), as opposed to where the fish were collected from, accounts for 72 to 89% of the variation in the reproductive life-history trait variables. No difference in fecundity was found between acute and cleared treatments. The authors found support that acclimation of reproductive life-history traits occurs to changes in sum PCB concentration. To their knowledge, the present study is the first experimental test of acclimation responses of female life-history traits to contaminants in wild populations. Environ. Toxicol. Chem. 2012;31:863–869. © 2012 SETAC

Keywords—Acclimation   Life-history traits   Polychlorinated biphenyls   Wild population   Adaptation

INTRODUCTION

Aquatic ecosystems are known to be exposed to a variety of environmental stressors, including contaminants, which have the potential to disrupt population dynamics [1]. Regions such as Newark Bay–Hudson River [2] and several Great Lakes tributaries [3] have become well-established study systems for investigating the adaptive mechanisms of population-level tolerance to contaminants. Populations in aquatic ecosystems such as these are able to survive and reproduce in environments where they are chronically exposed to contaminant concentrations well above those known to elicit lethal toxic effects in acute laboratory experiments [4]. Adaptive mechanisms that elicit tolerance to environmental contaminants can be classified into two general categories: genetic adaptation and acclimation [4]. Genetic adaptation describes genetically based changes in phenotype at a population level with selection occurring at the individual level, thus facilitating phenotypes that maximize an individual's fitness to be passed on to future generations [5]. Genetic adaptations can only be observed over multiple generations once selection has occurred. A recent study by Wirgin et al. [2] found evidence in Atlantic tomcod (*Microgadus tomcod*) of rapid evolutionary change at the locus of a functionally active aryl hydrocarbon receptor (AHR), AHR2, which is involved in the mediation of contaminant-metabolizing enzymes. A six-base deletion in the sequence of the AHR2 locus in tomcod living in contaminated regions (Hudson River and Hackensack River), compared with reference locations (including St. Lawrence River and Miramichi River), is suggested to be a mechanism of polychlorinated biphenyl (PCB) tolerance and to have resulted from selection against embryos with a high incidence of mortality and malformations.

Individuals also may express tolerance to contaminants via acclimation. Acclimation is a plastic change in phenotype at an individual level in response to an environmental change [5]. Such changes in phenotype are largely based on the plasticity of a particular trait and the spectrum of alternate phenotypes an individual has the potential to express [6]. Acclimation responses resulting in similar phenotypes across individuals can occur both within and across generations and can only be observed in response to change in a particular stressor of interest [4]. For example, mosquitofish from an industrially contaminated habitat (Bayou Trepangier, Los Angeles, California, USA), with tissue concentrations of lead 550% greater than those from a reference habitat, showed decreased mortality after laboratory lead exposure compared with reference fish, yet showed no difference in mortality after 34 d of captivity in clean water [7].

Life-history traits (i.e., those linked to growth or reproduction) have a complex and integrated genetic basis and thus, although having some degree of heritability, tend to express relatively higher plasticity across environmental gradients than traits that do not directly impact fitness [8]. This plasticity and direct link to individual fitness explains why variation in life-history traits corresponding to differences in contaminant exposure has been a predominant focus in the literature for decades [9,10]. Despite the extensive laboratory research that characterizes variation in life-history traits, little evidence has been found for acclimation in life-history traits in response to contaminant exposure using field experiments and wild organisms [9]. This

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lack of evidence is most likely a consequence of complex population dynamics and interannual variation in life-history trait expression [11], complex mixtures of contaminants [12], seasonal and annual environmental variation, and logistical difficulties in experimental design [13]. One must understand the mechanisms underlying patterns in life-history trait variation in wild populations for the design and implication of remediation processes and population-level management.

Life-history data collected from wild populations with respect to contaminants often compare traits between relatively clean and contaminated habitats to account for confounding environmental effects. Any observed differences in life-history traits should reflect their environment as a whole. However, focusing on a single class of contaminants, relevant to the traits in question, may be useful to quantify differences in contamination between habitats. Polychlorinated biphenyls are a globally ubiquitous class of persistent organic contaminants and have been linked to reproductive impairments in fish [14]. Declines in hatching success and fecundity have been reported for both increases in laboratory PCB-fed minnows [15] and increases of PCB concentration in egg tissue of wild-caught salmon [16]. In addition, exposures of many other organic contaminants often exhibit correlations with PCBs attributed to common exposure routes at contaminated locations. Thus, PCB concentrations often provide a good set of readily detectable reference compounds for denoting gradients of highly exposed populations from reference populations.

Readily abundant and native to North America, brown bullhead (*Ameiurus nebulosus*) are a philopatric [17] warm-water species that are tolerant of a variety of environmental stressors, including contaminants. They are also benthic throughout most of their life and are therefore exposed to contaminants that accumulate in sediments. Because of these inherent qualities, brown bullhead have been a model study system for investigating nonlethal effects of aquatic contaminant exposure for decades. Much of the current research has focused on the prevalence and pathologies of tumors, genotoxic responses, and their importance as indicators of environmental toxicity [18], whereas little is known about their reproduction and life history.

We used female brown bullhead to test whether acclimation of life-history traits occurs in response to changes in sum PCB exposure. For brevity, we call this the "acclimation hypothesis." To test the acclimation hypothesis, we compared life-history traits of fish directly caught from wild populations exposed to a range of contaminant concentrations (acute) with those collected from the same populations a year prior, which were placed in a clean environment to clear their contaminants throughout that year (cleared). The acclimation hypothesis assumes that sum PCB concentrations will be lower in cleared treatments compared with acute treatments, demonstrating an environmental change through which acclimation may occur. After this assumption, the acclimation hypothesis predicts that reproductive life-history traits (egg diameter, gonadosomatic index [GSI], fecundity) from cleared treatments will be significantly greater than those from acute treatments, in accordance with changes in PCB concentration. Polychlorinated biphenyl analyses were conducted on egg tissue, as opposed to muscle or sediment, to reflect maternal offloading concentrations during egg development and to address concentrations that directly affect fitness via embryo survival and egg development [19]. To our knowledge, the present study is the first experimental test of acclimation responses of female life-history traits to contaminants in wild populations.

MATERIALS AND METHODS

Sample collection

Female brown bullhead were collected using boat electroshocking from April 20 to June 19, 2008 (cleared treatment) and April 14 to June 16, 2009 (acute treatment) from four distinct locations in the lower Great Lakes region (Fig. 1): Peche Island (PI; 42°34′N, 82°92′W), Belle River (BR; 42°28′N, 82°71′W), Belle Island (BI; 42°20′N, 82°59′W), and Trenton Channel (TC; 42°51′N, 82°55′W). This region was chosen because it has been used in previous ecotoxicological studies [20], it is a Great Lakes area of concern [21], and it has areas of localized contaminant exposure, concentrations of which vary geographically depending on hydrodynamics and local source inputs, but not on a small temporal scale (i.e., year to year) [22].

In 2008, field-collected female brown bullhead were transported immediately after capture to Leadley Environmental Ltd. (42°6′N, 82°55′W) and placed in one of four semi-natural, aerated ponds, 6 m × 12 m × 3 m (L × W × D), corresponding to their original location, for a period of 1 y, until the next reproductive period. This translocation removed bullhead from their original sources of contaminant exposure and allowed contaminants in the fish to be depurated [23]. The transplanted brown bullhead are hereafter referred to as "cleared," because their life history traits were assessed after this clearing time period. Ponds were constructed 1 y before the experiment and had no prior fish in them. Each pond also had similar sun exposure, aeration, water temperature, insect communities, and vegetation growth, suggesting that each pond experienced similar environmental conditions. Once brown bullhead were introduced, fish density was also similar between the ponds (approximately 40–50 g/m^3 on average). Fish were fed floating pellets daily (Martin Mills Inc.), ad libitum, until

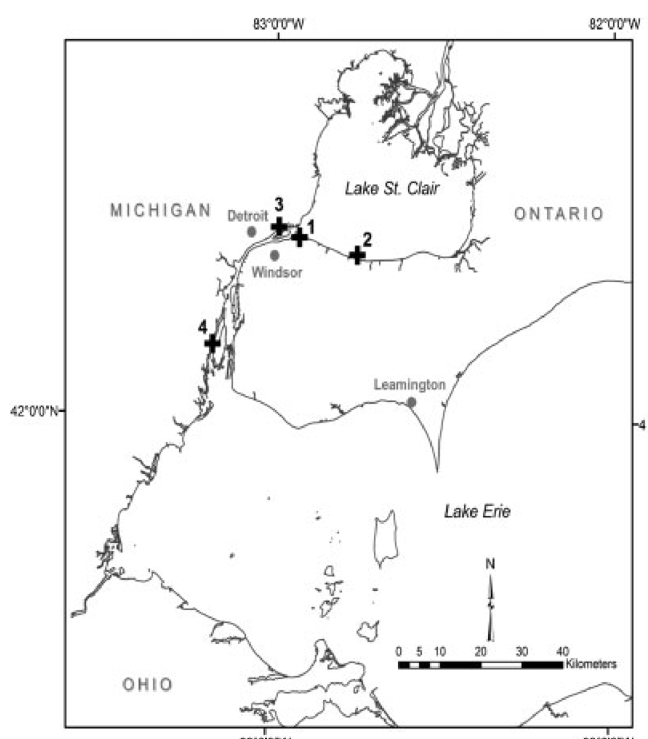

Fig. 1. Map of the four brown bullhead (*Ameiurus nebulosus*) collection sites in the lower Great Lakes region: 1 = Peche Island (PI); 2 = Belle River (BR); 3 = Belle Island (BI); 4 = Trenton Channel (TC).

November 2008, when the ponds began to ice over. Fish were not fed pellets in 2009, with naturally occurring insects and aquatic vegetation in the ponds available as food, similar to that in a wild habitat.

In 2009, female brown bullhead were immediately transported to Leadley Environmental Ltd., after capture. These brown bullhead are hereafter referred to as "acute," because their life history traits were assessed after exposure to environmental contaminants present in their respective wild locations. Females that were sexually mature at the time of collection from two of the populations (PI, BI) were assessed immediately. Females collected from the other two populations (BR, TC) that were collected before vitellogenesis (i.e., large, yellow eggs) were held in covered, rubber-lined, aerated ponds 2 m × 2 m × 0.75 m (L × W × D) until distended bellies and swollen genital pores were observed. Fish were held no longer than 2 weeks before processing. Elimination rates for most PCBs were longer than 2 weeks [24], and egg development would have begun before collection; therefore, potential clearing of PCBs during this time and its effect on egg development were considered to be minimal. Also at this time, sexually mature female brown bullhead from the cleared treatment were collected from their respective ponds, and their life-history traits were assessed. In both cleared and acute treatments, females with eggs that had not undergone vitellogenesis at this time were not included in the present study.

Life-history trait assessment

Cleared and acute females were euthanized using a lethal dose of MS-222, and total length (mm) and mass (g) were recorded. Ovaries were removed and patted dry with a paper towel, and their mass (g) was recorded to estimate GSI = ovary mass/(total mass − ovary mass). A subsample of eggs of a known mass was taken to estimate fecundity, 50 of which were used to calculate average egg diameter (mm). The remaining eggs were divided into subsamples of a known mass and preserved in 1.5 ml Cryovials at −20°C for PCB analyses (see later discussion). All egg samples were obtained and stored within 5 min after euthanasia. A pectoral spine was removed for age determination following methods in Blouin and Hall [25]. Briefly, spines were cut into 0.75-mm sections using a low-speed saw (Isomet, Buehler Inc.), mounted onto a slide using mounting medium (Flo-Texx, Lerner Laboratories), and annual rings were counted under a zoom stereomicroscope (SZX7 Olympus; www.olympusamerica.com).

Polychlorinated biphenyl extraction and analyses

Thawed egg samples 0.2 to 0.5 g (20–50 eggs) from each individual were homogenized and extracted using a micro-extraction technique following the protocol outlined by Daley et al. [19]. The extraction equipment consisted of eight 20-ml glass syringes, each connected to 1 μm glass fiber syringe filter, fitted to a solid phase extraction manifold (Phenomenex). Egg samples were ground with 15 g activated sodium sulfate and placed into the syringes containing 15 ml dichloromethane:hexane (1:1). An additional 15 ml of dichloromethane:hexane was used to rinse the glass mortar and pestle used to homogenize the egg samples and was added to the syringes. Each syringe was then spiked with an internal recovery standard of 200 ng PCB 30/ml. Six samples were run concurrently with each set, also including a method blank and an in-house homogenate fish sample (Detroit River common carp, *Cyprinus carpio*) as an inter-assay control. Extracts were then concentrated by rotary-evaporator to approximately 2 ml, and Florisil chromatography was used for sample cleanup [26]. Fractions 1 (50 ml hexane) and 2 (50 ml dichloromethane:hexane; 15:85) were collected. Extracts were then concentrated to 1 ml and placed in glass vials for analysis by gas chromatography electron capture detection. The PCB congeners in each sample were identified by retention time and referenced against an external PCB standard (Quebec Ministry of Environment Congener Mix; AccuStandard). The following congeners were present in the external standard and analyzed for detection in egg samples (International Union of Pure and Applied Chemistry numbers, coeluting congeners separated by slash): 18/17, 31/28, 33, 52, 49, 44, 74, 70, 95, 101, 99, 87, 110, 151/82, 149, 118, 153, 105/132, 138, 158, 183, 128, 177, 156/171, 180, 191, 170, 201, 195/208, 194, 205, 206, and 209. Sum PCB concentrations were calculated for each individual as the sum of each congener in μg/kg wet weight. Mean percentage of recovery of the PCB 30 spike was $78.45 \pm 0.53\%$ (mean ± S.E.). Four of 72 samples had recoveries less than the 70% threshold generally used for quality assurance and quality control (range = 67.1–69.7%); however, all sum PCB in-house homogenate samples were in compliance (mean ± 2 SD) with the Great Lakes Institute for Environmental Research analytical laboratory quality assurance guidelines (Canadian Association for Environmental Analytical Laboratories Accreditation and ISO17025 certified). Samples were therefore not corrected for recovery.

Statistical analyses

Site-specific differences in sum PCB concentration between acute and cleared treatments were determined using independent t tests. Variation in mass and Fulton's condition factor (C = body mass/[total length]^3) across the four locations, and between acute and cleared treatments, were determined using an analysis of variance followed by a post-hoc Tukey test where applicable. This determination was made because PCB distribution and accumulation can be dependent on body condition [27].

Variation in life-history traits between acute and cleared fish was analyzed using linear mixed-effects (or multilevel) models fitted via restricted maximum likelihood (*glht* package multcomp, R Development Core Team, 2009). In these analyses, life-history traits (egg diameter, GSI, total lipids, fecundity) were entered as response variables. Because negative relationships were found between body mass and GSI and between body mass and egg diameter, body mass was used as a covariate for both traits. Whether a population was acute or cleared was entered as a fixed factor and location entered as a random effect (treated as random intercepts). Random slopes were not applied as slopes between acute and cleared treatments, within locations, and were not statistically different when models were fitted. Differences in the means of the dependent variables between acute and cleared treatments were evaluated using z tests [28]. To examine the between-group effect of locations, we calculated intraclass correlation coefficients, reflecting the proportion of variance of dependent variables occurring among locations. The remaining proportion of variance is then assumed to occur within locations, including that of acute and cleared treatments [29].

RESULTS

Within locations, acute and cleared treatments differed in average sum PCB concentration, with cleared treatments having lower concentrations (PI: $t = -15.15$, $p < 0.001$, $df = 13$; BR:

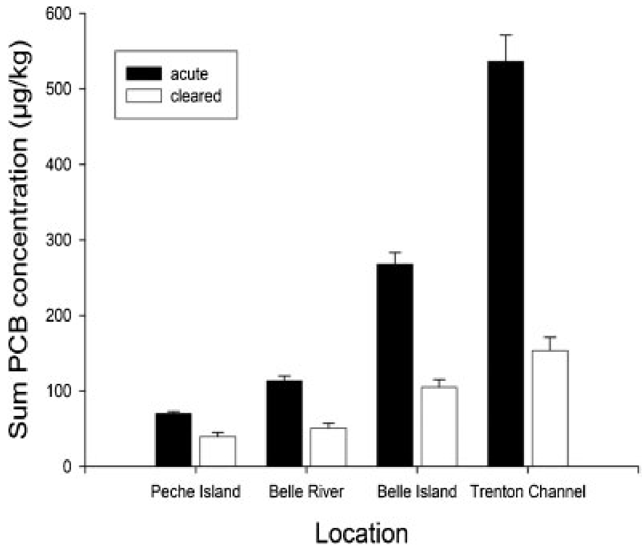

Fig. 2. Sum polychlorinated biphenyl (PCB) concentrations in female brown bullhead (*Ameiurus nebulosus*) eggs for the eight populations across the four locations. Black bars represent acute populations, and white bars represent cleared populations.

$t = -21.49$, $p < 0.0001$, $df = 16$; BI: $t = -26.64$, $p < 0.0001$, $df = 15$; TC: $t = -31.82$, $p < 0.0001$, $df = 18$). Compared with acute treatments, average sum PCB concentrations in cleared treatments declined by 43% in PI, 55% in BR, 61% in BI, and 71% in TC (Fig. 2).

No differences were seen in mean body mass among locations within acute ($F = 2.73$, $p = 0.06$, $df = 33$) and cleared ($F = 2.71$, $p = 0.06$, $df = 35$) treatments. Within locations, there was no difference in mean body mass between acute and cleared treatments for PI ($F = 0.12$, $p = 0.74$, $df = 14$), BR ($F = 2.13$, $p = 0.16$, $df = 17$), and BI ($F = 3.85$, $p = 0.07$, $df = 16$). However, in TC, cleared fish had greater masses than acute fish ($F = 7.49$, $p = 0.01$, $df = 19$).

We also examined differences in Fulton's condition factor, which may better reflect overall condition than mass [30]. No difference was found in body condition within three of four locations between acute and cleared treatments (PI: $F = 1.02$, $p = 0.33$, $df = 14$; BI: $F = 2.0$, $p = 0.18$, $df = 16$; TC: $F = 3.77$, $p = 0.07$, $df = 19$). In BR, cleared fish had greater body condition than acute fish ($F = 8.57$, $p = 0.01$, $df = 17$). Within acute treatments, a difference in body condition occurred between locations, with BI having a greater body condition than BR ($F = 3.49$, $p = 0.03$, $df = 33$). No difference was seen within cleared treatments between locations ($F = 1.94$, $p = 0.14$, $df = 35$). No apparent difference was seen in mean age between locations or treatments, which can be related to condition (Table 1).

Differences in female reproductive life-history traits occurred among locations, which were attributed to acute and cleared treatments. Combining the location treatments together, acute populations had significantly smaller egg diameters ($t = 4.87$, $p < 0.001$, $df = 68$; t test) by an average of 6% and lower GSIs ($t = 2.07$, $p < 0.001$, $df = 68$) by 17% compared with cleared populations (Fig. 3A, B). Although both egg diameter and GSI have a significant response to clearing, this response is more apparent and variable for GSI. No observable difference was seen in the magnitude of change in these traits between acute and cleared populations and within locations. Combining location treatments together, no difference in fecundity was observed between acute and cleared fish. Intraclass correlation coefficient values (11–28%) also indicate that 72 to 89% of variation in the dependent variables occur within locations (e.g., between acute and cleared treatments), rather than among locations (Table 2). No differences in fecundity were observed between acute and cleared treatments, both combined and across locations (Fig. 3C).

DISCUSSION

Our field experiment is, to our knowledge, the first to examine changes in life-history traits in response to contaminant clearing in a field setting. Our results support the hypothesis that acclimation of life-history traits in female brown bullhead occurs in response to experimental changes in contaminant exposure. We found that females from cleared treatments had lower contaminant concentrations and had greater egg diameters and GSIs than fish from acute treatments.

A major assumption of the acclimation hypothesis is that fish from cleared treatments will have lower sum PCB concentrations in their eggs than those from acute treatments. Our data supported this assumption; for each of the four locations, sum PCB concentrations in eggs from cleared treatments were found to be significantly lower than those from acute treatments. An overall clearing effect was predicted given that uptake of PCBs via ingestion and respiratory surfaces [31] would have been limited in the aquaculture pond environment relative to the Detroit River, and elimination routes of PCBs, because of loss by gills or fecal egestion, would be maximized [32]. We also found that fish from more contaminated locations (e.g., TC) had cleared relatively more of their sum PCBs than fish from less contaminated locations (e.g., PI). This was not expected, because metabolic rate, which strongly mediates both respiration and fecal egestion rates of fish, would not have been expected to differ across cleared treatments, given that similar temperature and feeding conditions were provided. Indeed, metabolic rate has been demonstrated to be lower in fish exposed to contaminants in both laboratory and field studies [33,34], which should have produced an opposite pattern to that

Table 1. Means ± standard deviation and sample sizes for body length, body mass, and age of brown bullhead (*Ameiurus nebulosus*) collected from four locations in the Detroit River

Location	Status	*n*	Total length (mm)	Total mass (g)	Age (years)
PI	Acute	9	266 ± 10.0	252.1 ± 18.67	3 ± 0.2
	Cleared	6	279 ± 10.6	260.8 ± 34.36	3 ± 0.3
BR	Acute	9	271 ± 9.5	227.8 ± 19.85	3 ± 0.2
	Cleared	9	272 ± 10.7	294.2 ± 40.97	3 ± 0.2
BI	Acute	7	272 ± 16.0	343.6 ± 52.13	4 ± 0.5
	Cleared	10	260 ± 5.2	248.4 ± 18.66	3 ± 0.3
TC	Acute	9	266 ± 7.7	253.0 ± 25.31	3 ± 0.2
	Cleared	11	289 ± 5.7	342.3 ± 21.06	3 ± 0.1

PI = Peche Island; BR = Belle River; BI = Belle Island; TC = Trenton Channel.

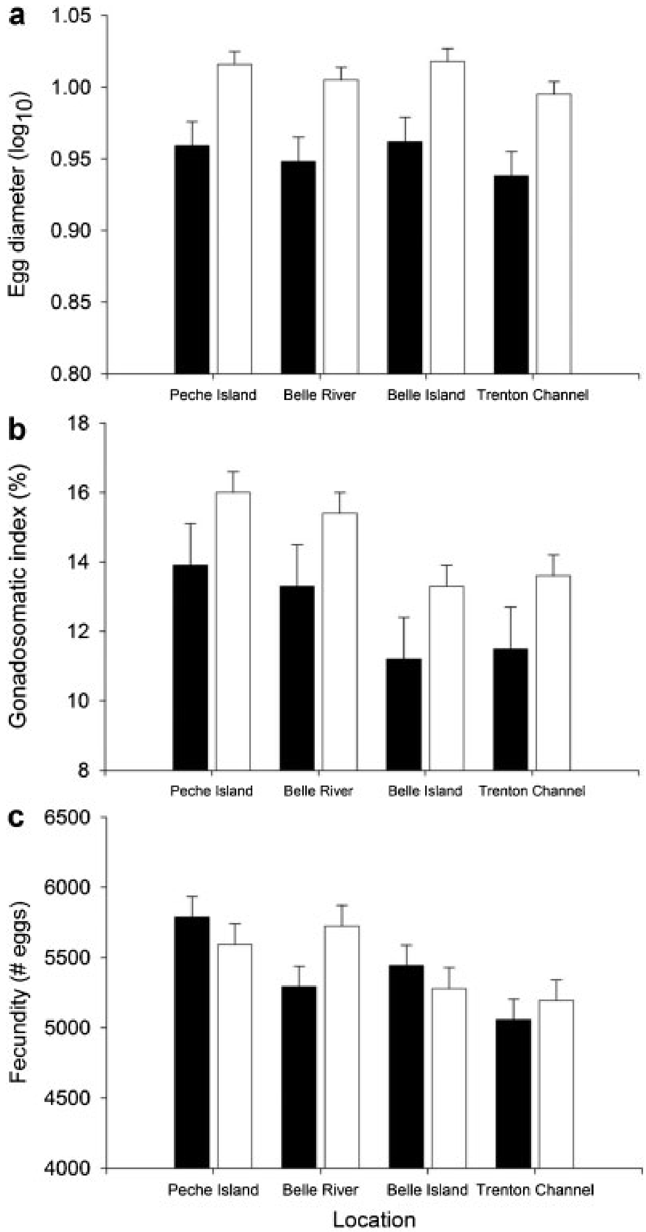

Fig. 3. (**A**) Mean (± standard error) egg diameter across eight populations of brown bullhead, paired by sampling location, comparing acute (black) and cleared (white) treatments. (**B**) Mean (± standard error) gonadosomatic index across eight populations of brown bullhead, paired by sampling location, comparing acute (black) and cleared (white) treatments. (**C**) Mean (± standard error) fecundity across eight populations of brown bullhead, paired by sampling location, comparing acute (black) and cleared (white) treatments.

observed, with higher relative clearance of PCBs from the least contaminated (Peche Island) site. Our study suggests that clearing efficiency may be species or population specific, or that local genetic adaptation of PCB clearing mechanisms may have occurred [2].

The acclimation hypothesis predicts that females from cleared treatments will have a greater output of reproductive life history traits compared with those from acute treatments. Our data supported this prediction in that egg diameter and GSI were significantly greater in cleared treatments than in acute treatments, with GSI showing greater variability. However, our data showed no difference in fecundity between treatments. Although some evidence has been found for a response to contaminant exposure in egg size in fishes [10], declines in fecundity and GSI have been observed for several species in contaminated habitats [10,35]. Contaminant-exposed populations have also exhibited fitness costs linked to changes in life-history traits, thus providing biological relevance. Munkittrick and Dixon [36] found that white suckers (*Catostomus commersoni*) collected from lakes elevated in Zn and Cu had smaller growth, egg size, fecundity, and greater spawning failure compared with white suckers collected from reference lakes. Hose et al. [37] found that white croakers from contaminated San Pedro Bay, California, had a smaller fecundity of 32% and an associated reduction in fertility of 14% compared with a reference location. Similar results were reported by Spies and Rice [38] in starry flounder from San Francisco Bay, where an increase in sum PCBs in eggs from 5 to 30 μg/g resulted in a decline in fecundity, fertilization success, and in embryo survival, which dropped from an approximate average of 70 to 30%. Lesko et al. [39] reported results contrary to ours, in which brown bullhead from more contaminated Lake Erie tributaries had greater fecundities and egg diameters than those from less contaminated tributaries. These differences corresponded with greater total lengths and ages and were explained because of greater food availability and decreased predation in more contaminated tributaries; thus, an indirect effect of contaminant exposure.

In the present study, overall increases in egg diameter and GSI of cleared fish may have been responding to changes in environmental quality characteristics (e.g., food and habitat of aquaculture ponds relative to the Detroit River) as well as contamination. Egg diameter has been shown to be affected by food availability and the presence of predators [40,41]. However, the condition indices did not show consistent differences among locations in acute versus cleared fish, which would have been expected had food quality effects strongly impacted life history traits. Gonadosomatic index metrics also appeared to retain an overall correlation to PCB contamination

Table 2. Acute and cleared treatment comparisons of female bullhead (*Ameiurus nebulosus*) life-history traits (mean ± standard error)[a]

Life-history trait	Treatment	*n*	Mean ± SE	*z*	*p* value	ICC %
Egg diameter (mm)	Acute	34	2.59 ± 1.01	6.08	< 0.001	11
	Cleared	36	2.74 ± 1.01			
GSI (%)	Acute	34	12.50 ± 1.20	3.8	< 0.001	28
	Cleared	36	14.60 ± 1.20			
Fecundity (no. of eggs)	Acute	34	5390 ± 145.6	0.36	0.72	13
	Cleared	36	5444 ± 148.7			

[a] Samples sizes are those combined from all four locations for each treatment. The *z* scores and associated *p* values (*z* tests) describe whether statistical differences occur between treatment means of each life-history trait. Intraclass correlation coefficients (ICC) were calculated to examine the between-group effect of locations, reflecting the proportion of variance of life-history traits occurring among locations. The remaining proportion of variance (1–ICC) is then assumed to occur within locations, including that of acute and cleared treatments.
GSI = gonadosomatic index.

both before and after clearance, given that fish did not depurate all of their contaminant burdens. Although predation rates were not investigated, brown bullhead were subject to aerial predation in both wild and pond environments. Although the exact mechanisms have yet to be discerned, a link between environmental contamination, life-history traits, and reproductive fitness appears to exist.

Our results have caveats that need to be acknowledged. First, we compared life-history traits using a quantified measure of environmental contamination (sum PCB concentration in eggs) instead of the more common method of comparing traits between two habitats known to differ in overall contamination, with one relatively low in overall contaminants and one relatively high in overall contaminants. Despite benefits to quantifying our environmental contaminant variable using a metric suitable to explain differences in life-history traits, dismissing potential impacts of the multitude of additional environmental contaminants, and their interactions, that are present in the present study region (for examples, see Bustness [42]) would be naive. Second, the PCB clearing rates appear to be different among the four locations; however, no corresponding proportional difference in life-history traits was observed. This may be because of differences in maternal allocation of PCBs [43], life-history traits responding to environmental variables other than sum PCBs [44], or that differences in life-history traits cannot be observed beyond a certain PCB concentration threshold. Finally, other contaminants, which may have had more direct impacts on the life-history traits measured here, may have exhibited different clearance rates in aquaculture ponds relative to PCBs.

In summary, our field-based experiment provides support for the acclimation hypothesis in that egg diameter and GSI can increase over the period of one reproductive season in response to a decrease in PCB exposure. Our experiment also examines changes in life-history traits in response to contaminant clearing in a field setting, thus giving our findings particular relevance to natural populations. This aspect of our study is especially important for habitat remediation programs and ecosystem managers, because changes in life-history traits, with the potential to positively impact population dynamics, can occur with the removal of environmental contaminants. Even more so, these changes may occur within an individual's life span, resulting in measurable changes in a relatively short period. Our results also highlight the potential benefits of using multiple life-history traits, including those that are egg specific, as biological indicators of environmental change. Life-history traits can be relatively easy to measure, reflect an overall environmental condition, and have population-level implications. Environmental contamination and remediation are issues of global concern and interest, and thus more experimental research investigating individual and population-level responses to contaminant exposure and clearing, which is relevant to natural populations, is needed to aid managers in decision-making processes and predictions of future ecosystem health.

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