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The effect of maternal exposure to contaminated sediment on the growth and condition of larval *Fundulus heteroclitus*

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Abstract

We employed a factorial laboratory experiment to determine the single and combined effect of maternal and larval exposure to contaminated sediment from Elizabeth River, Virginia, a site contaminated with high concentrations of multiple pollutants. Females were exposed to either reference or contaminated sediment and the larvae from both groups of mothers were in turn transferred to either reference or contaminated sediment. We found a strong maternal influence on yolk area, length and RNA:DNA ratio at hatch. Further, the maternal exposure significantly influenced growth rate and RNA:DNA ratios of larvae 14 days after hatch and was a more important factor in determining these endpoints than larval exposure. We found that after 14 days larvae were larger and had higher survivorship when the maternal and larval exposures were the same. There also was no statistical difference with respect to growth and condition between larvae that had hatched from exposed mothers and remained in contaminated water and larvae that had hatched from reference mothers and were placed in either reference or contaminated sediment. However, larvae that hatched from exposed mothers and then were switched to reference sediment had significantly lower growth, lower RNA:DNA ratios, and were smaller despite being large at hatch size, indicating that there are fitness trade-offs in exchange for apparent resistance to contaminants which are provided by the mother. Maternal effects add complexity to ecotoxicological research and should be incorporated into studies to predict population level responses more realistically.

Keywords: Fish early life history; Maternal effects; Contaminants; PAH; RNA:DNA ratios; Egg volume; Growth

1. Introduction

Maternal effects are those phenotypic traits observed in offspring that are independent of offspring genotype, but a direct result of maternal phenotype (Bernardo, 1996a). The correlations between phenotypic traits in the mother and offspring have been studied extensively in ecology and much of the maternal effects literature has focused on the influence of maternal phenotype on egg and offspring size because of the importance of size on survival in early life history (Bernardo, 1996b). For example, many studies have documented a positive relationship between female size and egg size in fish (Hislop, 1988; Chambers and Leggett, 1996; Chambers and Waiwood, 1996; Chambers, 1997). Some studies have linked maternal size or age not only with egg size and/or quality, but also with the subsequent size, growth, and survival of offspring (e.g., Benoit and Pepin, 1999; Heyer et al., 2001; Berkeley et al., 2004). These studies support the hypothesis that large, old females in better nutritional condition produce larvae in better condition which in turn may have higher survivorship and may result in higher recruitment to the population (Solemdal, 1997; Secor, 2000).

Environmental conditions and diet influence maternal nutritional condition and health, hereafter termed condition (Reznick, 1991; Yaragina and Marshall, 2000). In toxicology studies for example, fish exposed to sublethal levels of contaminants often have reduced growth and condition (Weis and Kahn, 1991; Ferraro et al., 2001; Rowe, 2003). It is logical to hypothesize that the offspring of females exposed to contaminants would be smaller and in poorer condition because maternal condition is

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low. In addition to such nutritionally mediated maternal effects, mothers may transfer contaminants to eggs and/or offspring directly. Maternal transfer of contaminants has been documented in many aquatic invertebrates (Enserink et al., 1990; Schweitzer et al., 2000), freshwater fish (Guiney et al., 1979; Vodicnik and Peterson, 1985; Hall and Oris, 1991; Miller, 1993; Walker et al., 1994; Latif et al., 2001), marine fish (von Westernhagen et al., 1981; Monteverdi and DiGiulo, 2000) and reptiles (Nagle et al., 2001; Roe et al., 2004). The presence of maternally transferred contaminants in eggs has been shown to directly or indirectly reduce survivorship of the offspring (Hall and Oris, 1991; Westerlund et al., 2000; Nagle et al., 2001). In contrast, examples of maternal influence on tolerance or resistance to metals have also been documented (Lin et al., 2000; Peake et al., 2004). Thus, maternal phenotype could influence offspring phenotype in two ways: (1) by differences in nutritional supplements to the egg and (2) transfer of contaminants or other substances as a result of the mother's contaminant exposure.

Several areas within the Chesapeake Bay are considered to be "of concern" because organisms living in these areas are exposed to high concentrations of multiple organic and inorganic contaminants (Dauer et al., 2000). One of these areas, the Elizabeth River, Virginia is influenced by highly industrialized activities and by the densely populated urban areas of the Hampton Roads region. Polycyclic aromatic hydrocarbons (PAHs) occur in sediments in moderate concentrations throughout most of the Elizabeth River and are more than 2500 times higher in the sediments near the Atlantic Woods Superfund site where creosote was historically used to treat wood for use in the marine environment (Walker and Dickhut, 2001). Trace elements (Hg, Cd, Cu, etc.) and tributyl tin (TBT), an antifoulant used on ship hulls, are also present (Bieri et al., 1986; Padma et al., 1998; Conrad and Chisholm-Brause, 2004). The macrobenthic communities of the Elizabeth River are considered to be highly impacted, exhibiting lower species diversity, lower abundances of individual species, lower biomass, and dominance by pollution tolerant taxa (Dauer, 1997).

Although the levels of multiple toxic contaminants are high, the killifish or mummichog, *Fundulus heteroclitus*, is found throughout the Elizabeth River. The Elizabeth River mummichog population exhibits heritable resistance to contaminants (Ownby et al., 2002). The persistence of mummichog populations within this contaminated area necessitates an understanding if factors that affects its population dynamics, including the role of maternal effects. We seek to understand how females exposed to a suite of contaminants in sediment may influence the growth and survival of their offspring when they in turn are exposed to either reference or contaminated sediments. We hypothesized that exposed mothers would produce smaller eggs and that their larvae would be smaller, have reduced condition, exhibit lower growth rates, and lower survivorship than those produced by unexposed mothers. We also hypothesized that the larval sediment exposure would have significant effects on larval condition and growth and that larvae exposed to contaminated sediments would have lower survival, growth and condition than those exposed to reference sediment.

2. Methods

The mummichog is one of the most abundant and ubiquitous estuarine species along the Atlantic coast making it a standard test species for contaminants in the environment. Because of the existence of locally adapted populations in this species, we used individuals from a naive population in our experiments. Although we did not determine specifically that these fish were resistant to contaminants, they were collected from the Patuxent River, MD (Fig. 1), a relatively low-impacted system (Hartwell et al., 1997). Adult *F. heteroclitus* were collected from the Patuxent River in the spring of 2002. Sediment for use in the experiment was collected from two sites, the reference site (REF), Fishing Bay, MD and a site known to be highly contaminated with multiple contaminants (TOX) within the Elizabeth River, VA (Fig. 1). We made a 1:4 dilution of Elizabeth River mud with Fishing Bay mud by volume to yield sediment approximately 25% the strength of contaminated Elizabeth River sediment. This mixture of sediments was contaminated primarily with PAHs (Fig. 2) and had been shown in previous experiments to induce statistically significant sub-lethal effects

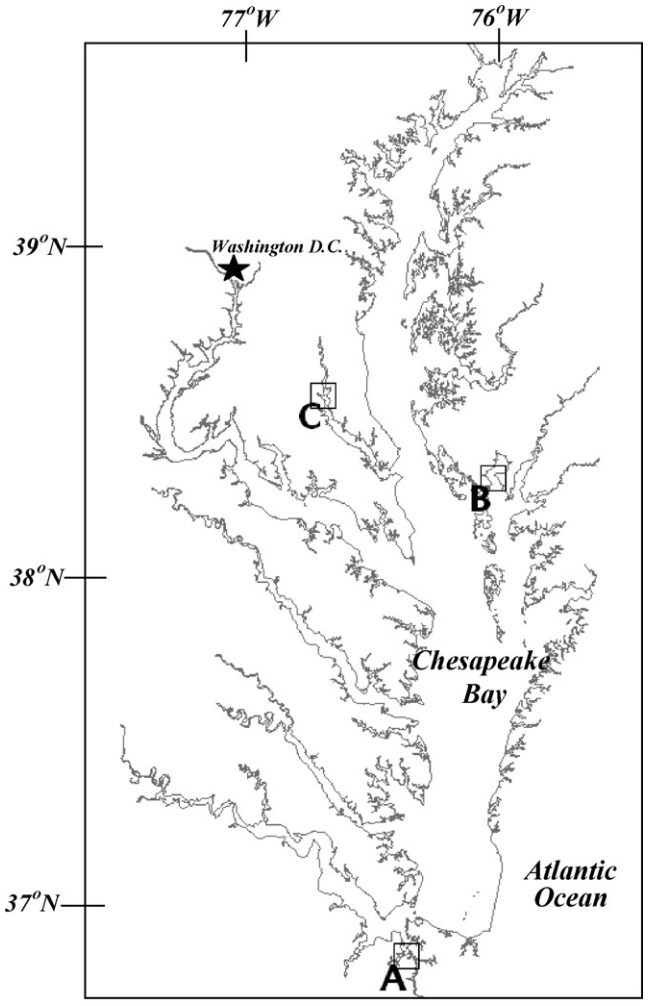

Fig. 1. Map of the Chesapeake Bay with the sources of (A) contaminated sediment from Elizabeth River, VA (TOX), (B) reference sediment from Fishing Bay, MD (REF), and (C) adult mummichogs from the Patuxent River, MD.

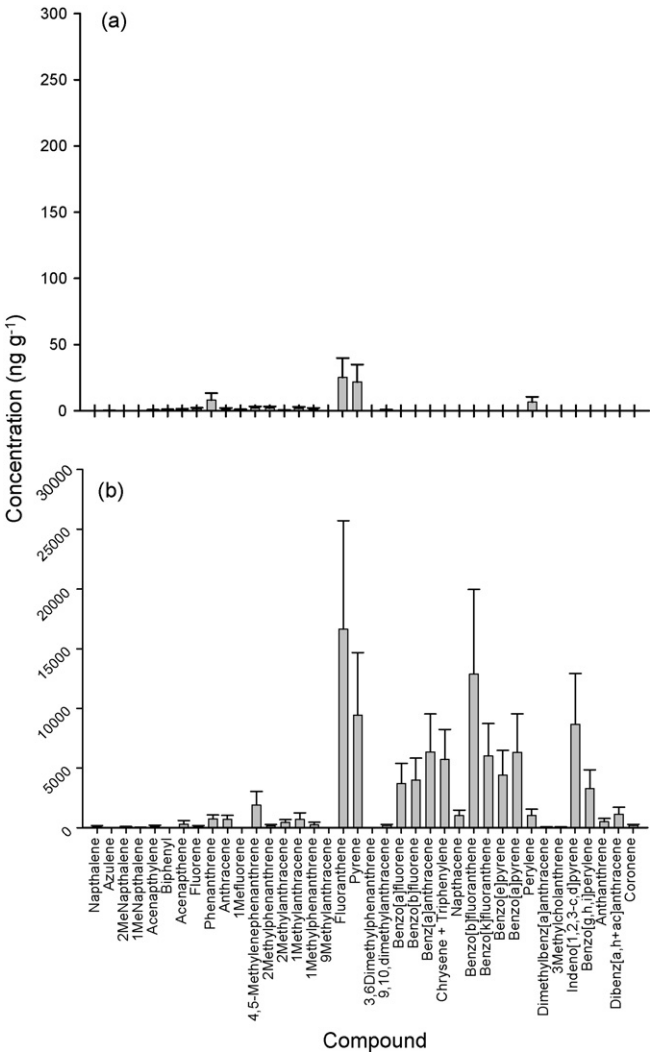

Fig. 2. Concentration of PAHs in the (a) reference sediment from Fishing Bay (REF, $n = 2$) and (b) 25% Elizabeth River sediment (TOX, $n = 7$). Each bar represents the mean concentration (±standard deviation) from tanks used in a complimentary experiment started at the same time as this study. Note that the scale of the y-axis is two orders of magnitude higher in the TOX sediment than the REF sediment. Compounds for which no bar exists indicate values below detection limits.

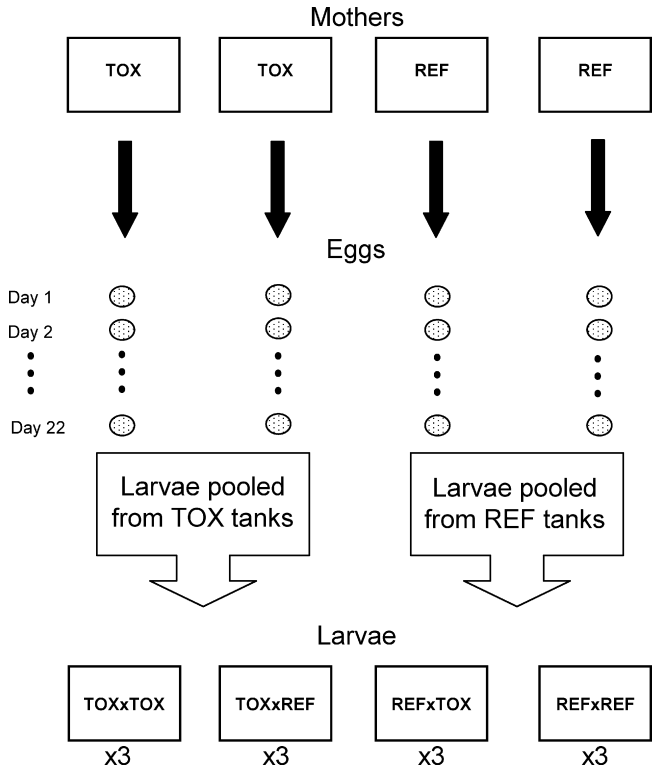

Fig. 3. Flow chart of the experiment from maternal treatment (REF or TOX) to egg stage to larval factorial design (TOX/TOX, TOX/REF, REF/TOX and REF/REF).

on growth in naïve adult mummichog, without inducing mortality (D. Davis, personal observation). Although PAHs were not measured for the experiment described herein, PAHs were quantified in a complementary experiment on the same REF and TOX sediment. Sediment samples were collected from nine tanks 2 weeks after sediment was introduced to flow through tanks.

We conducted experiments in which offspring of mothers held in either reference (REF) or contaminated sediments (TOX) were themselves exposed to either reference or contaminated sediment (Fig. 3). Thus, there were four possible treatment combinations: TOX/TOX, TOX/REF, REF/REF and REF/TOX. For the maternal exposures, four female and two male fish were placed into each of four 75-l, flow-through aquaria provided with ambient water from the Patuxent River. Two tanks served as the REF treatment and were filled with 15 l of reference sediment. For the TOX treatment, two tanks contained 15 l of contaminated sediment collected from Elizabeth River. Adults were held in these tanks at 20 °C for 40 days prior to egg collection.

Corner aquarium filters with filter floss were placed in each tank to serve as egg collectors. Filters were removed from the tank daily and examined for the presence of eggs. When eggs were present, they were counted and placed in petri dishes labeled with tank origin and collection date. All eggs were treated similarly. Petri dishes were filled with a fungicidal solution of methylene blue and filtered seawater. The concentration of this solution was 1.23×10^{-4} mg/l, well below the estimate of 0.4 mg/l suggested for safe usage (Rifici et al., 1996). Because eggs were not exposed to sediment during incubation, any negative impacts from contaminants were due to maternal transfer, not from contamination of the eggs directly. Eggs were held at 21 °C in a controlled-temperature room. The incubation solution was changed daily and any dead eggs or hatched larvae were removed from each petri dish. Eggs used in the experiments were spawned 12–27 days before hatch. Just prior to hatching, the volumes of three to ten eggs per petri dish were measured using an Olympus SZX9 dissecting microscope and imaging system (Optimas v.6. Media Cybernetics, Silver Spring, MD). Care was taken to measure only live eggs, defined as those in which a heartbeat or movement of the larva could be detected.

After measuring, eggs were pooled according to the maternal treatment (Fig. 3). Eggs were exposed to air for 2 h after which hatching was induced by flooding the shallow dishes containing the eggs with filtered seawater and aerating for another two hours. This process was repeated up to three times within one

day. After hatching, larvae were randomly selected from the two pools of eggs from each maternal treatment and assigned to a larval treatment. The total length (mm) and yolk sac area (mm^2) were measured for 120 larvae, 60 larvae from each maternal treatment, using the same imaging system described above. These larvae were immediately frozen at −80 °C for subsequent RNA:DNA analysis.

Twelve 38-l glass aquaria were established 14 days before hatching in a controlled temperature room held at 21 °C. There were three replicates tanks for each maternal/larval treatment combination (Fig. 3: TOX/TOX, TOX/REF, REF/TOX, REF/REF) with 30 larvae allocated to each treatment. Each aquarium was provided with 6.7 l of either REF or TOX sediment and filled with approximately 34.5 l of filtered ambient river water. Aquaria were gently aerated. A 50% water change was conducted on day 7 of the 14 days experiment.

Because egg production of the mothers exposed to TOX sediment was unexpectedly low (see Section 3), three TOX treatments were started 21 days and another three TOX treatments 33 days after the start of the FB experiments. Salinity, dissolved oxygen, and temperature were measured each day for both time periods and varied very little between time periods or tanks. Mean ± standard deviation for water temperature was maintained at 21.5 ± 0.14 °C, mean salinity at 14.5 ± 0.54, and mean dissolved oxygen at 6.5 ± 0.84 mg/l during the entire experimental period even though treatments were staggered temporally. Larvae were fed cultured *Artemia* twice daily *ad libitum*. They were not fed on day 14.

Larvae were sampled on the morning of day 15 of the experiment. All larvae were removed from the tanks and counted to calculate survivorship. The sediment was carefully filtered to make sure that no larvae were overlooked, but no dead larvae were found throughout the experiment. Ten larvae recovered from each tank were measured (TL in mm) using image analysis equipment and then immediately frozen for RNA:DNA analysis. Mean instantaneous growth rate, G, was calculated as $G = (\ln TL_2 - \ln TL_1)(t_2 - t_1)^{-1}$, where TL_1 and TL_2 are total length at times t_1 and t_2, the beginning and end of the experiment.

RNA:DNA ratios are considered a measure of nutritional status or growth potential in fish larvae (Ferron and Leggett, 1994; Buckley et al., 1999). The premise of RNA:DNA ratios as an indicator of nutritional status is that the amount of DNA per cell in an organism is constant, while an organism that is in good health will have more protein synthesis and thus, higher amounts of RNA per cell than an individual in poor condition. Therefore, RNA:DNA ratios were measured to quantify the health or condition of larvae at the start of the larval exposure and at 14 dph. Nucleic acids were extracted from each larva as described by Heyer et al. (2001) and Caldarone (2006), with one exception: prior to vortexing, each sample was partially homogenized using a small tissue homogenizer with a disposable tip, to ensure thorough homogenization of larger larvae. Some RNA:DNA ratios for large larvae that were sampled at 14 dph were clearly outliers and seem to be a result of incomplete digestion of tissue. To identify those samples that may not have been completely digested, we regressed DNA concentration on total length and constructed 95% upper and lower confidence intervals. This relationship is expected to be highly linear given that DNA concentration is dependent solely on cell number which should be closely correlated with length. Those observations that were outside the 95% confidence intervals of this relationship were excluded from analysis. All data points that were excluded were larvae sampled at the end of the experiment.

All data were analyzed for homogeneous variances and normality before analysis with parametric statistics. All statistical analyses were conducted in SAS Version 8.2 (SAS Corp., Cary, NC). A nested ANOVA was used to test for differences in egg volume due to maternal treatment. Tank means were nested within maternal treatment to account for variation due to individual females in each of the maternal tanks. Data were analyzed using one-way ANOVAs to test for differences between the maternal exposures (TOX and REF) in mean yolk area, total length at hatch, and RNA:DNA ratio at hatch. We used two-way ANOVAs to test for differences in means of the total length, instantaneous growth rate and RNA:DNA ratios at the end of the 14 days experiment. Post-hoc pairwise comparisons of means were made between the four mother/larval treatment combinations (TOX/TOX, TOX/REF, REF/TOX, REF/REF) and experiment-wide error was adjusted to $P_{experiment} = 0.05$ using the Tukey method.

3. Results

Egg production was an order of magnitude higher in females exposed to REF sediment than those exposed to TOX sediment. Over a 30-day period, females exposed to REF sediment produced 5762 eggs, whereas females exposed to TOX sediment produced 574 eggs (Table 1). Egg production was low in the TOX treatment because both batch fecundity and the number of spawns were lower than in the REF treatment (Table 1). Females spawned 18 and 22 times in the two REF treatment tanks, but only 8 and 17 times in the TOX treatment over a 30-day period.

Table 1
Egg and larval characteristics as a result of maternal exposure to reference (REF) and contaminated (CON) sediment

	Maternal exposure	
	REF	CON
Total egg production (number of eggs)	5762	574
Mean number of spawning events	20	12.5
Mean batch fecundity (number of eggs per female per spawn)	36.01 ± 30.68 (n = 40)	6.06 ± 5.90 (n = 25)
Mean egg volume (mm^3)	4.69 ± 0.87 (n = 88)	4.18 ± 0.53 (n = 258)

Mean measurements are shown ± standard deviation. Numbers in parentheses indicate sample size, n.

Mean batch fecundity, the number of eggs per female per spawning event, was six times higher in the REF treatment (Table 1). Less than 40% of the eggs from the TOX treatment hatched. Embryos appeared to be alive in the eggs hatched from TOX mothers, but failed to emerge after several attempts to induce hatching.

We detected no significant difference in egg volume between tanks nested within maternal treatments ($F_{1,1.91} = 0.77$, $P = 0.48$). However, without accounting for individual tank variance, mean egg volume was on average larger in the REF treatment than in the TOX treatment (Table 1). Logistically, we could not account for variation due to maternal line (tank effect) after eggs had hatched so variation in larval characteristics was related to maternal exposure only. Maternal exposure regime significantly influenced the size and condition of larvae at hatch. Total length of larvae at hatch was greater ($F_{1,118} = 25.39$, $P < 0.0001$), but yolk sac area was smaller in the TOX versus REF maternal treatment (Fig. 4, $F_{1,118} = 17.71$, $P < 0.0001$). RNA:DNA ratios were significantly higher at hatch in the larvae originating from mothers exposed to contaminated sediment (Fig. 4, $F_{1,118} = 107.77$, $P < 0.0001$).

Survivorship was high in all treatments and over 90% of larvae that were introduced to the experimental tanks survived the 14-day exposure. Average percent mortality was 2.2, 4.4, 6.7, and 1.1% in the TOX/TOX, TOX/REF, REF/TOX and REF/REF, respectively. Highest mortality occurred in tanks where the maternal and larval treatments were not the same i.e., the TOX/REF and REF/TOX treatments. At the end of the 14-day experiment, total length was significantly higher in the tanks where the maternal and larval exposure was the same, the REF/REF and TOX/TOX treatments (Fig. 5). Total length at 14 days post-hatch (dph) was significantly lower in the TOX/REF treatment compared to all other treatments. Total length at 14 dph was influenced significantly by the interaction of the mother and larval exposure ($F_{1,8,} = 7.40$, $P < 0.026$), but not by the larval exposure ($F_{1,8} = 4.84$, $P = 0.57$) or the maternal effect ($F_{1,8} = 4.34$, $P = 0.23$).

Mean instantaneous growth rates were highest in larvae originating from the REF maternal treatments. However, growth rate was significantly lower only in the TOX/REF treatment compared to all the other treatments (Fig. 5). There was a significant interaction between the maternal and larval treatment on growth rate ($F_{1,8} = 26.02$, $P = 0.0009$). There was no main effect of larval treatment on these growth rates ($F_{1,8} = 4.85$, $P < 0.059$), but maternal exposure was still significant in determining growth rates at the end of the 14-day experiment ($F_{1,8} = 33.48$, $P = 0.0004$).

At the end of the experiment, RNA:DNA ratios ranged from 0.35 to 0.934 and were much lower than they were at the start of the experiment at which time ratios ranged from 0.72 to 1.45. At 14 dph, RNA:DNA ratios were highest in offspring from reference mothers, the REF/TOX and REF/REF treatments, but were statistically the same in all treatments except for the TOX/REF treatment, for which low growth was also measured (Fig. 5). There was no interaction between the maternal and larval treatment affecting RNA:DNA ratios at the end of the experiment ($F_{1,8} = 2.64$, $P = 0.14$). Similar to the growth results, there was no significant effect of larval exposure ($F_{1,8} = 4.30$, $P = 0.072$), but there was a maternal effect at 14-day ($F_{1,8} = 11.42$, $P = 0.0096$) with RNA:DNA ratios higher in general in the offspring of REF mothers.

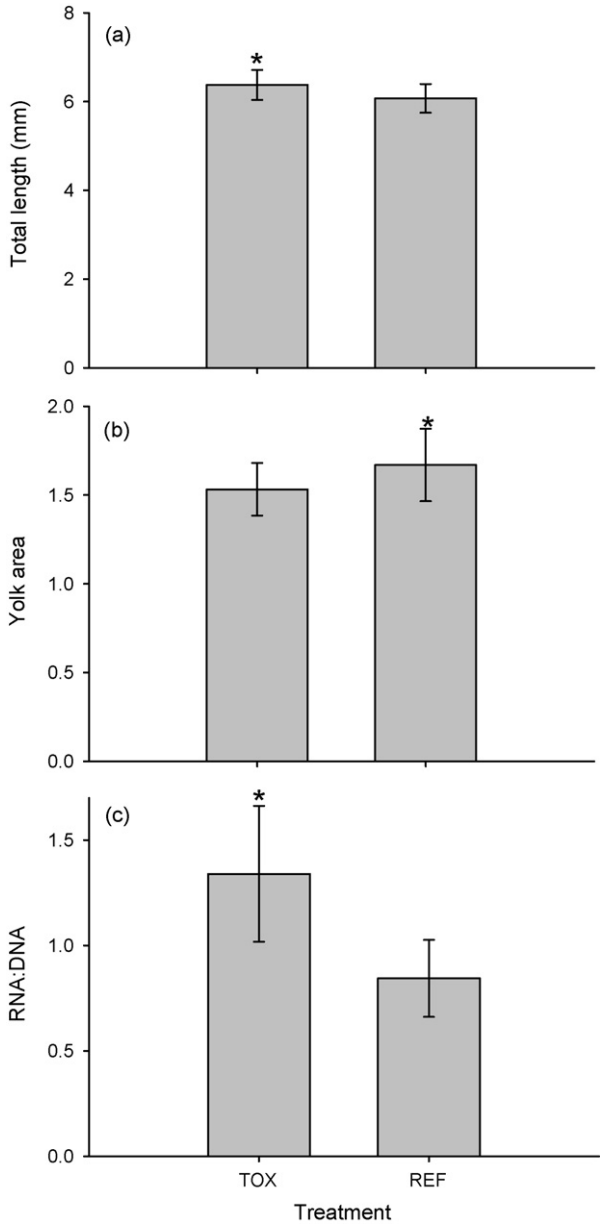

Fig. 4. Comparison of larvae from TOX and REF mothers in (a) mean total length at hatch ($n = 120$), (b) mean yolk sac area at hatch ($n = 120$) and (c) mean RNA:DNA ratio ($n = 120$). Error bars represent standard deviation and an asterisk (*) indicates a significantly higher result at $p \leq 0.05$ level.

4. Discussion

We observed strong maternal effects in *F. heteroclitus*, the existence of which had only been suggested in this species previously (Williams, 1994; Meyer et al., 2002; Meyer and DiGiulio, 2003). Maternal effects were manifested as differences the size, yolk sac area, growth and RNA:DNA ratios of larvae. Maternal effects on growth and RNA:DNA ratios were detectable at

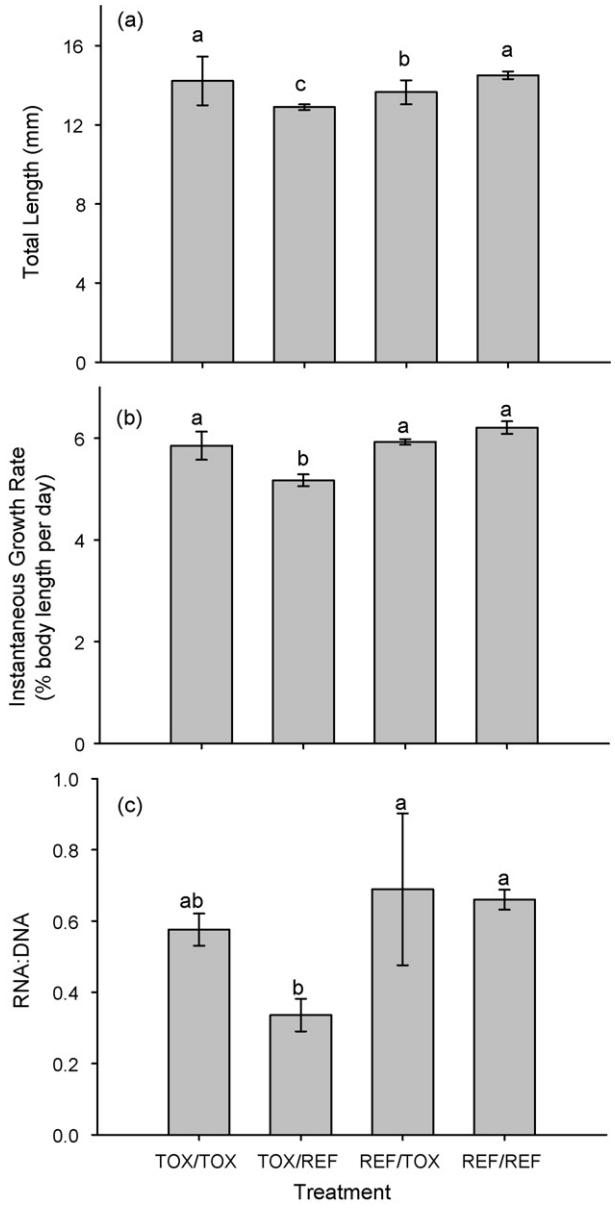

Fig. 5. Effect of maternal and larval exposures to TOX and REF sediment 14 dph in (a) mean total length, (b) mean instantaneous growth rate, and (c) mean RNA:DNA ratio. Error bars represent standard deviation and letters indicate significantly different treatment combinations (Tukey post hoc comparisons, $P_{\text{experiment}} = 0.05$).

14 dph. The maternal exposure was more important overall than larval exposure in explaining these differences.

Maternal differences were generated by exposing the mothers to either contaminated or uncontaminated sediment from Chesapeake Bay. However, the characteristics of the larvae arising from each of these treatments were unexpected. We hypothesized that the condition of the mother would be reflected in her offspring and that larvae from contaminated mothers would be small and in poor condition at hatch due to nutritionally-mediated maternal effects. In contrast, we found that in general, larvae hatched from contaminant-exposed mothers were longer with smaller yolk sacs and had higher RNA:DNA ratios than larvae produced by unexposed mothers. These results suggest that the embryos from contaminant-exposed females were spawned earlier, or developed faster and had used up more yolk reserves at the time of hatch than reference embryos. Egg production was so low that we had to use eggs hatched from the entire egg collection period to meet our sample size requirements (120 larvae for each mother/larva treatment). Therefore, the possibility that larvae from TOX mothers were larger, had smaller yolk sacs and higher RNA:DNA ratios because they were spawned earlier is unlikely. Rather, these results suggest that larvae from exposed mothers used the nutritional reserves from the yolk sac earlier or more quickly during embryogenesis to achieve a greater length at hatch. Similarly, Toppin et al. (1987) observed rapid development in mummichogs exposed to contaminated sediment.

An alternative explanation for our finding of larger larvae from the TOX mothers may be that only the fast-developing, large embryos were able to hatch in the TOX maternal treatment as hatching success is often inhibited in contaminant exposed larvae (Nagler and Cyr, 1997). The presence of contaminants, including PAHs, in eggs can reduce hatching success and survivorship of the embryo (Hall and Oris, 1991; Nelson et al., 1991; Perry et al., 1994; Nagler and Cyr, 1997; Nagle et al., 2001). Unfortunately, we did not directly quantify hatching success in this study. The fact that larvae from contaminated mothers were longer, but had smaller yolk sacs, suggests that only the largest and healthiest embryos could complete the hatching process, which is reflected in the RNA:DNA ratios. Accordingly, we hypothesize that RNA:DNA values were higher at hatch in the larvae from contaminated mothers because only the most fit embryos survived and hatched in this treatment.

Greater size and higher RNA:DNA ratios at hatch suggests an increased metabolic rate of developing contaminated larvae. Regardless of cause, larvae that are longer at hatch may be able to swim faster, which may confer a survival advantage once larvae have successfully switched to exogenous feeding (Miller et al., 1988; Heyer et al., 2001). These larvae may be able to travel a greater distance to obtain food or better habitat. In contrast, larvae that hatched from unexposed mothers were shorter, had large yolk sacs and had lower RNA:DNA ratios. Larvae with this phenotype may be better suited to survive periods of initial starvation prior to the onset of exogenous feeding (Miller et al., 1988; Heyer et al., 2001). One might expect such phenotypic differences to promote larval survival if (a) larvae that have hatched in contaminated areas would be stimulated to migrate to uncontaminated areas and (b) larval performance is higher in reference sediment regardless of maternal influence. However, the mummichog exhibits considerable site fidelity (Teo and Able, 2003) and probably would not move out of large contaminated areas such as the Elizabeth River estuary after hatch. Furthermore, we found that larval survivorship and growth were low when larvae from contaminant-exposed mothers were transferred to reference sediment. Counter to our hypothesis that larvae from reference mothers and exposed to reference sediment would perform better overall, we found that larvae were larger and had higher growth and survival when the maternal and larval exposures were the same. Therefore, differences in larval phenotype confer a survival advantage only in an environment similar to

the mother's and more importantly, a disadvantage if the environment is not the same even if that environment is high in contaminants.

These results suggest that if mothers are not exposed to contaminants, but deposit eggs in a contaminated area, these larvae are at a much greater disadvantage than those spawned from mothers that are exposed to contaminants. Less intuitively, larvae from exposed mothers may be at a greater disadvantage when exposed to reference sediment. This follows the prediction from evolutionary theory that there are fitness trade-offs in exchange for traits that afford resistance to contaminants and is supported by previous studies in mummichogs exposed to the same Elizabeth River sediment (Meyer and DiGiulio, 2003).

Our findings contribute to the idea that females transfer substances to eggs and larvae that acclimatize their offspring to the habitat in which the mothers themselves live. Mothers have been shown to transfer mRNA (Wang et al., 1998; Lin et al., 2000), acclimatory proteins, immunoglobins (Mor and Avtalion, 1990; Takemura and Takano, 1997), or the contaminants themselves to eggs that acclimatize larvae to toxins (Munkittrick and Dixon, 1989; Miller, 1993; Peterson et al., 1993; Peake et al., 2004). Although numerous studies have demonstrated the negative consequences of maternally transferred contaminants (Hall and Oris, 1991; Pelletier et al., 2000; Johnston et al., 2005), other studies have shown that maternally transferred metallothionein or mRNA provide larvae with resistance to metals such as cadmium and copper (Munkittrick and Dixon, 1989; Lin et al., 2000; Peake et al., 2004). Transfer of the contaminant itself may induce the larvae to produce enzymes that biotransform these contaminants into less toxic derivatives, but such preexposure may not be a benefit for all classes of contaminants. The derivatives of PAHs can sometimes be more deleterious than the parent compounds themselves and maternal transfer of PAHs has been shown to increase embryonic mortality (Pelletier et al., 2000). However, fish caught in Elizabeth River exhibit tolerance to PAHs and have reduced EROD activity, but higher survival compared to fish from reference habitats (Meyer et al., 2002).

In this study, we focused on the phenotype of the larvae in relation to the maternal phenotype. It should be noted that while the phenotype of the mother is a complex combination of her phenotype and genotype, the maternal effect itself is observed in the offspring and is independent of offspring genotype. In order to detect maternal effects in this study, we specifically used naïve fish to examine phenotypic maternal effects rather than heritable resistance. Previous studies have suggested a parental effect in the response of mummichog larvae to toxic effects of contaminants from the Elizabeth River site, but have simultaneously focused on the genetic component of heritable resistance (Williams, 1994; Meyer et al., 2002; Ownby et al., 2002; Meyer and DiGiulio, 2003). Williams (1994) found that offspring from resident Elizabeth River females and reference site males were more resistant to Elizabeth River sediment than offspring from reference site females and Elizabeth River males. Meyer et al. (2002) detected tolerance to Elizabeth River sediments in F1 generation offspring from Elizabeth River parents, but this tolerance was not evident in subsequent generations. They suggested that gene imprinting or some other physiological mechanism was more probable than transfer of mRNA or proteins in conferring resistance. However, they found no difference between offspring from Elizabeth River females and reference site males versus offspring from reference site females and Elizabeth River males even though both sets of hybrid offspring had higher survival than reference site offspring. In these studies and in our experiment we have assumed that maternal contribution is the main source of observed tolerance, but the paternal influence cannot be ruled out (Nagler and Cyr, 1997; Meyer et al., 2002). Further, the interaction of parental genotype and phenotype on offspring phenotype warrants further investigation.

RNA:DNA ratios were higher at hatch in the larvae of exposed mothers, suggesting that these larvae were in better condition than larvae of unexposed mothers. In many studies RNA:DNA ratios are a predictor of growth because RNA concentrations increase with the protein synthesis involved with growth (Wagner et al., 1998; Buckley et al., 1999; Caldarone, 2006). In a toxicological context, RNA:DNA ratios may not be an accurate reflection of growth, but may rather indicate induction of protein synthesis following exposure to contaminants. Exposure to PAHs may induce the production of cytochrome P450 1A (Roling et al., 2004), 7-ethoxyresorufin-*o*-deethylase (EROD) and metals can induce metallothionein production. An increase in RNA activity may also indicate a metabolic cost to repair damage from DNA methylation induced by a stressor. Therefore, elevated RNA:DNA ratios may indicate a metabolic stress of producing detoxifying proteins rather than proteins related to growth. The metabolic costs of producing detoxifying proteins would use energy that may otherwise be allocated to somatic growth, explaining the lower growth rate in some ER larvae, but high RNA:DNA ratios at hatch. These results agree with previous studies that question the use of RNA:DNA ratios as an indicator of growth in the presence of contaminants (DeBoeck et al., 1997).

In contrast to RNA:DNA ratios at hatch, RNA:DNA ratios were highest for the fish in the reference sediment 14 dph, as we expected. The TOX/REF treatment resulted in significantly lower growth and significantly lower RNA:DNA ratios, indicating that at this point RNA:DNA ratios reflect growth and condition as illustrated in larvae and juveniles of other fish (Malloy and Targett, 1994; Rooker et al., 1997; Caldarone, 2006). Overall, RNA:DNA ratios were lower at the end of 14 days than they were at the start of the experiment, which may be a result of the low nutritional quality of *Artemia* in relation to the size of these fish 14 dph. Interestingly, RNA:DNA ratios were still significantly influenced only by maternal exposure and not by larval exposure or the interaction between the maternal and larval exposure, indicating a persistent maternal effect on growth. Growth is one of the most important factors influencing survival of fish larvae (Houde, 1989). High growth rates decrease not only the amount of time that larvae are exposed to stressors, but also the number of stressors encountered that may cause death such as disease, starvation, and predation. Larvae grew equally well in all but the TOX/REF treatment where instantaneous growth rate was significantly lower even though these larvae were large at hatch. These results further emphasize that there is a survival advantage to the larvae when the maternal

and larval environment are the same, even if the environment is highly polluted. This maternal effect may help explain the persistence of small populations in contaminated areas and the speed with which populations exhibit genetic change.

Maternal effects add complexity to ecological processes as demonstrated by this study. If we had simply documented the effects of contaminated sediments on larval growth and survival we might have found what we expected, that larvae exposed to contaminants had reduced survivorship and growth. However, when considering maternal effects in the context of a stressor, we observed unexpected and counterintuitive results, but results that are more ecologically relevant. This study emphasizes that maternal phenotype influences contaminant resistance and vital rates throughout the early life stages of the mummichog. It also emphasizes the importance of examining the role of a stressor over the entire life cycle of an organism. In this study, we did not quantify the effects of contaminants on adult growth and condition, their fecundity, egg viability and hatching success, but ultimately, the sum of these processes will determine population growth rates and even evolution.

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References

Benoit, H.P., Pepin, P., 1999. Interaction of rearing temperature and maternal influence on egg development rates and larval size at hatch in yellowtail flounder (*Pleuronectes ferrugineus*). Can. J. Fish. Aquat. Sci. 56, 785–794.

Berkeley, S.A., Chapman, C., Sogard, S.M., 2004. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology 85, 1258–1264.

Bernardo, J., 1996a. Maternal effects in animal ecology. Am. Zool. 36, 83–105.

Bernardo, J., 1996b. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. Am. Zool. 36, 216–236.

Bieri, R.H., Hein, C., Huggett, R.J., Shou, P., Slone, H., Smith, C., Su, C.-W., 1986. Polycyclic aromatic hydrocarbons in surface sediments from the Elizabeth River subestuary. Int. J. Environ. Anal. Chem. 26, 97–113.

Buckley, L., Calderone, E., Ong, T.L., 1999. RNA-DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. Hydrobiologia 401, 265–277.

Caldarone, E.M., 2006. Estimating growth in haddock larvae *Melanogrammus aeglefinus* from RNA:DNA ratios and water temperature. Mar. Ecol. Prog. Ser. 293, 241–252.

Chambers, R.C., 1997. Environmental influences on egg and propagule sizes in marine fishes. In: Chambers, R.C., Trippel, E. (Eds.), Early Life History and Recruitment in Fish Populations. Chapman and Hall Ltd., London, pp. 63–102.

Chambers, R.C., Leggett, W.C., 1996. Maternal influences on variation in egg size in temperate marine fishes. Am. Zool. 36, 180–196.

Chambers, R.C., Waiwood, K.G., 1996. Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua*. Can. J. Fish. Aquat. Sci. 53, 1986–2003.

Conrad, C.F., Chisholm-Brause, C.J., 2004. Spatial survey of trace metal contaminants in the sediments of the Elizabeth River, Virginia. Mar. Pollut. Bull. 49, 319–324.

Dauer, D.M., 1997. Dynamics of an estuarine ecosystem: long-term trends in the macrobenthic communities of Chesapeake Bay (1985–1993). Oceanol. Acta 20, 291–298.

Dauer, D.M., Ranasinghe, J.A., Weisberg, S.B., 2000. Relationships between benthic community condition, water quality, sediment quality, nutrient loads, and land use patterns in Chesapeake Bay. Estuaries 23, 80–96.

DeBoeck, G., Vlaeminck, A., VBlust, R., 1997. Effects of sublethal copper exposure on copper accumulation, food consumption, growth, energy stores, and nucleic acid content in common carp. Arch. Environ. Con. Toxicol. 33, 415–422.

Enserink, L., Luttmer, W., Maas-Diepeveen, H., 1990. Reproductive strategy of *Daphnia magna* affects the sensitivity of its progeny in acute toxicity tests. Aquat. Toxicol. 17, 15–26.

Ferraro, M.L., Kaplan, L.A.E., Leamon, J., Crivello, J.F., 2001. Variation in physiological biomarkers among mummichogs collected from Connecticut salt marshes. J. Aquat. Anim. Health 13, 246–256.

Ferron, A., Leggett, W.C., 1994. An appraisal of condition measures for marine fish larvae. Adv. Mar. Biol. 30, 217–303.

Guiney, P., Melancon, M., Lech, J., Peterson, R., 1979. Effects of egg and sperm maturation and spawning on the distribution of a polychlorinated biphenyl in rainbow trout (*Salmo gairdneri*). Toxicol. Appl. Pharm. 47, 261–272.

Hall, A.T., Oris, J.T., 1991. Anthracene reduces reproductive potential and is maternally transferred during long-term exposure in fathead minnows. Aquat. Toxicol. 19, 249–264.

Hartwell, S.I., Dawson, C.E., Durell, E.Q., Alden, R.W., Adolphson, P.C., Wright, D.A., Coelho, G.M., Magee, J.A., Ailstock, S., Norman, M., 1997. Correlation of measures of ambient toxicity and fish community diversity in Chesapeake Bay, USA, tributaries-urbanizing watersheds. Environ. Toxicol. Chem. 16, 2556–2567.

Heyer, C.J., Miller, T.J., Binkowski, F.P., Caldarone, E.M., Rice, J.A., 2001. Maternal effects as a recruitment mechanism in Lake Michigan yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. 58, 1477–1487.

Hislop, J.R.G., 1988. The influence of maternal length and age on the size and weight of the eggs and the relative fecundity of the Haddock, *Melanogrammus-Aeglefinus*, in British waters. J. Fish Biol. 32, 923–930.

Houde, E.D., 1989. Subtleties and episodes in the early life stages of fishes. J. Fish Biol. 35, 29–38.

Johnston, T.A., Miller, L.M., Whittle, D.M., Brown, S.B., Wiegand, M.D., Kapuscinski, A.R., Leggett, W.C., 2005. Effects of maternally transferred organochlorine contaminants on early life survival in a freshwater fish. Environ. Toxicol. Chem. 24, 2594–2602.

Latif, M., Bodaly, R., Johnston, T.A., Fudge, R., 2001. Effects of environmental and maternally derived methylmercury on the embryonic and larval stages of walleye (*Stizostedion vitreum*). Environ. Pollut. 111, 139–148.

Lin, H., Hsu, S., Hwang, P., 2000. Maternal transfer of cadmium tolerance in larval *Oreochromis mossambicus*. J. Fish Biol. 57, 239–249.

Malloy, K.D., Targett, T.E., 1994. Effects of ration limitation and low temperature on growth, biochemical condition, and survival of juvenile summer flounder from two Atlantic coast nurseries. Trans. Am. Fish. Soc. 123, 182–193.

Meyer, J.N., DiGiulio, R.T., 2003. Heritable adaptation and fitness costs in killifish (*Fundulus heteroclitus*) inhabiting a polluted estuary. Ecol. Appl. 13, 490–503.

Meyer, J.N., Nacci, D.E., Di Giulio, R.T., 2002. Cytochrome P4501A (CYP1A) in killifish (*Fundulus heteroclitus*): heritability of altered expression and relationship to survival in contaminated sediments. Toxicol. Sci. 68, 69–81.

Miller, M.A., 1993. Maternal transfer of organochlorine compounds in Salmonines to their eggs. Can. J. Fish. Aquat. Sci. 50, 1405–1413.

Miller, T.J., Crowder, L.B., Rice, J.A., Marschall, E.A., 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. Can. J. Fish. Aquat. Sci. 45, 1657–1670.

Monteverdi, G.H., DiGiulo, R.T., 2000. Oocyte accumulation and tissue distribution of 2,3,7, 8-tetrachlorodibenzo-*p*-dioxin and benzo[*a*]pyrene in gravid *Fundulus heteroclitus*. Environ. Toxicol. Chem. 19, 2512–2518.

Mor, A., Avtalion, R.R., 1990. Transfer of antibody activity from immunized mother to embryo in tilapias. J. Fish Biol. 37, 249–255.

Munkittrick, K.R., Dixon, D.G., 1989. Effects of natural exposure to copper and zinc on egg size and larval copper tolerance in white sucker (*Catostomus commersoni*). Ecotoxicol. Environ. Safe 18, 15–26.

Nagle, R.D., Rowe, C.L., Congdon, J.D., 2001. Accumulation and selective maternal transfer of contaminants in the turtle *Trachemys scripta* associated with coal ash deposition. Arch. Environ. Con. Toxicol. 40, 531–536.

Nagler, D., Cyr, D., 1997. Exposure of male American plaice (*Hippoglossoides platessoides*) to contaminated marine sediments decreases the hatching success of their progeny. Environ. Toxicol. Chem. 16, 1733–1738.

Nelson, D., Miller, J., Rusanowsky, D., Greig, R., Sennefelder, G., Mercaldo-Allen, R., Kuropat, C., Gould, E., Thurberg, F., Calabrese, A., 1991. Comparative reproductive success of winter flounder in Long Island Sound: a three-year study (biology, biochemistry, and chemistry). Estuaries 14, 318–331.

Ownby, D.R., Newman, M.C., Mulvey, M., Vogelbein, W.K., Unger, M.A., Arzayus, L.F., 2002. Fish (*Fundulus heteroclitus*) populations with different exposure histories differ in tolerance of creosote-contaminated sediments. Environ. Toxicol. Chem. 21, 1897–1902.

Padma, T.V., Hale, R.C., Roberts, M.H.J., 1998. Toxicity of water-soluble fractions derived from whole creosote and creosote-contaminated sediments. Environ. Toxicol. Chem. 17, 1606–1610.

Peake, E.B., Locke, J.C., Tierney, L.L., Kolok, A.S., 2004. Copper tolerance in fathead minnows. II. Maternal transfer. Environ. Toxicol. Chem. 23, 208–211.

Pelletier, M.C., Burgess, R.M., Cantwell, M.G., Serbst, J.R., Ho, K.T., Ryba, S.A., 2000. Importance of maternal transfer of the photoreactive polycyclic aromatic hydrocarbon fluoranthene from benthic adult bivalves to their pelagic larvae. Environ. Toxicol. Chem. 19, 2691–2698.

Perry, D., Hughes, J., Hebert, A., 1994. Sublethal abnormalities in embryos of winter flounder (*Pleuronectes americanus*) from Long Island Sound. Estuaries 14, 306–317.

Peterson, R., Theobald, H.M., Kimmel, G.L., 1993. Developmental and reproductive toxicity of dioxins and related compounds: cross-species comparisons. Crit. Rev. Toxicol. 23, 283–335.

Reznick, D.N., 1991. Maternal effects in fish life histories. In: Dudley, E. (Ed.), The Unity of Evolutionary Biology: Proceedings of the Fourth ICSEB. Maternal Effects in Evolutionary Biology—Symposium, vol. 2. Dioscorides Press, Portland, Oregon, pp. 780–793.

Rifici, L.M., Cherry, D.S., Farris, J.L., Cairns Jr., J., 1996. Acute and subchronic toxicity of methylene blue to larval fathead minnows (*Pimephales promelas*): implications for aquatic toxicity testing. Environ. Toxicol. Chem. 15, 1304–1308.

Roe, J.H., Hopkins, W.A., Baionno, J.A., Staub, B.P., Rowe, C.L., Jackson, B.P., 2004. Maternal transfer of selenium in *Alligator mississippiensis* nesting downstream from a coal-burning power plant. Environ. Toxicol. Chem. 23, 1969–1972.

Roling, J.A., Bain, L.J., Baldwin, W.S., 2004. Differential gene expression in mummichogs (*Fundulus heteroclitus*) following treatment with pyrene: comparison to a creosote contaminated site. Mar. Environ. Res. 57, 377–395.

Rooker, J.R., Holt, G.J., Holt, S.A., 1997. Condition of larval and juvenile red drum (*Sciaenops ocellatus*) from estuarine nursery habitats. Mar. Biol. 127, 387–394.

Rowe, C.L., 2003. Growth responses of an estuarine fish exposed to mixed trave elements in sediments over a full life cycle. Ecotoxicol. Environ. Safe 54, 229–239.

Schweitzer, L.E., Bay, S.M., Suffet, I.H., 2000. Dietary assimilation of a polychlorinated biphenyl in adult sea urchins (*Lytechinus pictus*) and maternal transfer to their offspring. Environ. Toxicol. Chem. 19, 1919–1924.

Secor, D.H., 2000. Spawning in the nick of time: effect of adult demographics on spawning behaviour and recruitment in Chesapeake Bay striped bass. ICES J. Mar. Sci. 57, 403–411.

Solemdal, P., 1997. Maternal effects—a link between the past and the future. J. Sea Res. 37, 213–227.

Takemura, A., Takano, K., 1997. Transfer of maternally-derived immunoglobulin (IgM) to larvae in tilapia, *Oreochromis mosambicus*. Fish Shellfish Immunol. 7, 355–363.

Teo, S.L.H., Able, K.W., 2003. Habitat use and movement of the mummichog (*Fundulus heteroclitus*) in a restored salt marsh. Estuaries 26, 720–730.

Toppin, S.V., Heber, M., Weis, J.S., Weis, P., 1987. Changes in reproductive biology and life history in *Fundulus heteroclitus* in a polluted environment. In: Vernberg, W., Calabrese, A., Thurberg, F., Vernberg, F.J. (Eds.), Pollution Physiology of Estuarine Organisms. University of South Carolina Press, Columbia, pp. 171–184.

Vodicnik, M., Peterson, R., 1985. The enhancing effort of spawning on elimination of a persistent polychlorinated biphenys from yellow perch. Fund. Appl. Toxicol. 5, 770–776.

von Westernhagen, H., Rosenthal, H., Dethlefsen, V., Ernst, W., Harms, U., Hansen, P., 1981. Bioaccumulating substances and reproductive success in Baltic flounder *Platichthys flesus*. Aquat. Toxicol. 1, 85–99.

Wagner, M., Durbin, E., Buckley, L., 1998. RNA:DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchicus*. Mar. Ecol. Prog. Ser. 162, 173–181.

Walker, M., Cook, P., Batterman, A., Butterworth, B., Berini, C., Libal, J., Hufnagle, L., Peterson, R., 1994. Translocation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin from female lake trout (*Salvelinus namaycush*) to oocytes: effects on early life stage development and sac fry survival. Can. J. Fish. Aquat. Sci. 51, 1410–1419.

Walker, S.E., Dickhut, R.M., 2001. Sources of PAHs to sediments of the Elizabeth River, VA. Soil Sediment Contam. 10, 611–632.

Wang, W.D., Chen, Y.M., Hu, C.H., 1998. Detection of Ah receptor and Ah receptor nuclear translocator mRNAs in the oocytes and developing embryos of zebrafish (*Danio rerio*). Fish Physiol. Biochem. 18, 49–57.

Weis, J.S., Kahn, A.A., 1991. Reduction in prey capture ability and condition of mummichogs from a polluted habitat. Trans. Am. Fish. Soc. 120, 127–129.

Westerlund, L., Billsson, K., Andersson, P.L., Tysklind, M., Per-Erik, O., 2000. Early life-stage mortality in zebrafish (*Danio rerio*) following maternal exposure to polychlorinated biphenyls and estrogen. Environ. Toxicol. Chem. 19, 1582–1588.

Williams, C.A.H., 1994. Toxicity resistance in the mummichog (*Fundulus heteroclitus*) from a chemically contaminated environment. College of William and Mary.

Yaragina, N.A., Marshall, C.T., 2000. Trophic influences on interannual and seasonal variation in the liver condition index of Northeast Arctic cod. ICES J. Mar. Sci. 57, 42–55.