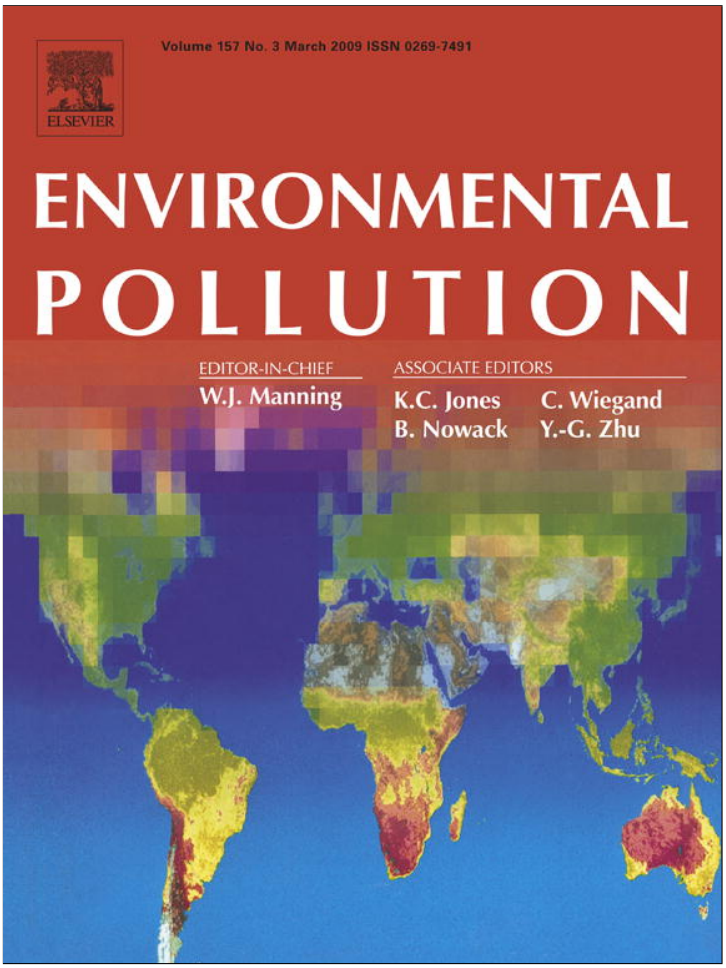

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Evolution of tolerance to PCBs and susceptibility to a bacterial pathogen (*Vibrio harveyi*) in Atlantic killifish (*Fundulus heteroclitus*) from New Bedford (MA, USA) harbor

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Killifish resident to a highly PCB-contaminated estuary survive pathogenic bacterial challenges well, suggesting their tolerance to PCB immunosuppression.

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ABSTRACT

A population of the non-migratory estuarine fish *Fundulus heteroclitus* (Atlantic killifish) resident to New Bedford (NB), Massachusetts, USA, an urban harbor highly contaminated with polychlorinated biphenyls (PCBs), demonstrates recently evolved tolerance to some aspects of PCB toxicity. PCB toxicology, ecological theory, and some precedence supported expectations of increased susceptibility to pathogens in NB killifish. However, laboratory bacterial challenges of the marine pathogen *Vibrio harveyi* to wild fish throughout the reproductive season and to their mature laboratory-raised progeny demonstrated comparable survival by NB and reference killifish, and improved survival by NB males. These results are inconsistent with hypothesized trade-offs of adaptation, and suggest that evolved tolerance in NB killifish may include mechanisms that minimize the immunosuppressive effects of PCBs. Compensatory strategies of populations persisting in highly contaminated environments provide a unique perspective for understanding the long-term ecological effects of toxic chemicals.

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1. Introduction

Populations that persist despite chronic, multi-generational exposures to chemical pollutants display a range of compensatory mechanisms that minimize toxic impacts. For example, many insect species display pesticide resistance or tolerance (e.g., McKenzie and Batterham, 1994), defined by Råberg et al. (2007) as mechanisms that minimize biological exposure or effects, respectively. Certain aquatic animal populations residing in highly contaminated sites also have evolved mechanisms that mitigate the adverse effects of toxic chemicals (Martinez and Levinton, 1996; Levinton et al., 1999; Weis, 2002; Wirgin and Waldman, 2004; Van Veld and Nacci, 2008). Despite some common, generalized mechanisms, each example illustrates unique biological and ecological compensatory responses that interact to produce condition-specific benefits, and, potentially, costs to population persistence.

The non-migratory estuarine fish, *Fundulus heteroclitus* (Atlantic killifish), has served as an important model species for the study of adaptation in the wild. Classic studies have characterized evolutionary responses of killifish to environmental conditions such as temperature across large geographic and geological time scales (e.g., reviewed in Mitton, 1994; Burnett et al., 2007). Contemporary evolution (e.g., Kinnison and Hairston, 2007) is also displayed in killifish populations whose residence sites vary widely in the nature and degree of their contamination by persistent, bioaccumulative, and toxic pollutants, such as polychlorinated biphenyls (PCBs) (Nacci et al., 1999, 2002a, b). This investigation focused on one population of killifish resident to an urban harbor, New Bedford (NB), Massachusetts (USA, Fig. 1), highly contaminated from industrial discharges of PCBs (e.g., Nelson et al., 1996). Although these discharges ceased in the 1970s (Nelson et al., 1996), NB killifish still contain tissue concentrations of PCBs that are toxic to many fish species (Black et al., 1998a; Monosson, 2000), and reduce reproductive output and early life stage survival in some

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Fig. 1. Map of Atlantic coast US study area showing collection sites for *Fundulus heteroclitus*.

populations of this fish species (Black et al., 1998b; Gutjahr-Gobell et al., 1999; Nacci et al., 1999). Yet, the NB killifish population appears robust: fish are abundant and display high condition indices (Nacci et al., 2001, 2002b).

A series of studies have explored demographic and toxicological compensatory mechanisms by which NB killifish persist (Nacci et al., 2002b, 2008). One that likely plays an important role is toxicological adaptation: an inherited or evolved tolerance to some of the toxic effects of PCBs (Hahn, 1998; Nacci et al., 1999, 2002a, 2008; Bello et al., 2001). Specifically, NB killifish and their uncontaminated progeny are protected from lethal effects of PCBs during embryonic development (Nacci et al., 1999, 2002a), a particularly susceptible period for PCB toxicity in fishes (e.g., Elonen et al., 1998; Tillitt et al., 2008). Because the exact biochemical and genetic mechanisms of developmental tolerance in NB killifish have yet to be identified (Hahn, 1998; Hahn et al., 2004; Van Veld and Nacci, 2008), it is not known to what extent and by what mechanism(s) other life stages and processes might be protected from the toxic effects of PCBs.

In this study, we were specifically concerned about pathogen susceptibility of NB killifish because immunological suppression is one of the most sensitive effects of PCBs on vertebrate species (Kerkvliet, 2002; Luebke et al., 2006). Since disease is an important regulator of wild populations (e.g., Acevedo-Whitehouse and Cunningham, 2006), protection from the immunosuppression effects of PCBs on adults, demographically important life stages, would be particularly beneficial for population persistence (e.g., Munns et al., 1997; Nacci et al., 2008). However, some empirical studies suggested that NB and other killifish resident to highly contaminated sites (but showing some aspects of tolerance) demonstrate more pathological lesions and increased incidence of parasites than reference killifish (e.g., Stegeman and Wolke, 1979; Cohen, 2002; Hicks and Steele, 2003; Cohen et al., 2006; Frederick et al., 2007; but, see Schmalz et al., 2002). In addition, one chemically-tolerant killifish population (resident to the Atlantic Wood site, Norfolk, VA, USA) shows increased susceptibility to opportunistic microbial infection when transferred to clean laboratory conditions (Rice, 2001; Meyer and Di Giulio, 2003; Frederick et al., 2007). These patterns of increased parasitism and disease could reflect poor immunological function, e.g., as a cost of evolved chemical tolerance (Meyer and Di Giulio, 2003).

Thus, existing information supported contradictory expectations concerning the vulnerability of NB killifish to infectious diseases. Tolerance of the immunosuppressive effects of PCBs might be an adaptive benefit, potentially related to mechanisms affording protection from developmental toxicity to PCBs. Alternatively,

increased susceptibility to infectious disease might be an adaptive cost. To evaluate pathogen susceptibility, we conducted acute laboratory challenges using a ubiquitous marine pathogen, *Vibrio harveyi* (Thompson et al., 2004; Austin and Zhang, 2006; Gauger and Gomez-Chiarri, 2002), comparing survival of killifish from NB and nearby, relatively uncontaminated reference sites. We tested highly PCB-contaminated field-collected killifish over the summer breeding season when the presumed cost of reproduction might increase disease susceptibility (e.g., Viney et al., 2005), and into the fall when reproduction had ceased. To evaluate whether potential differences in pathogen responses between wild fish populations were genetically based, and unrelated to differences in tissue PCB concentrations, we also tested mature, laboratory-reared uncontaminated individuals from these populations. These laboratory studies provide the first report of immune functionality of killifish with evolved PCB tolerance.

2. Materials and methods

2.1. Experimental animals

Adult fish were collected by trapping and held in the laboratory as described elsewhere (Nacci et al., 1999, 2005). Laboratory-reared fish (F_2 generation) were spawned from the progeny of field-collected fish and grown until about 2 years old, when they were similar in length to field-collected fish used in other bacterial challenges. Laboratory conditions supported high rates of survival and growth in young fish, and maintained adult fish in seasonally-appropriate reproductive condition. Fish were held at low densities in large tanks of flowing uncontaminated sea water, and fed a varied diet including commercial and live food (Nacci et al., 1999, 2002a, 2005). Two to three weeks prior to each bacteria challenge, fish were transferred to flowing sea water tanks where the holding temperature was adjusted to 25 °C at a rate no greater than 1 °C per day.

Fish were collected from the NB upper harbor (NB), nearby West Island (WI, Fairhaven, MA), and more distant to NB, Annisquam Inlet, Gloucester (GL, MA) (Fig. 1). Fish collected from another uncontaminated site, Block Island, RI (BI), were used to enhance bacterial virulence prior to bacterial challenges (described below). Collection site location, sediment PCB concentrations, and tolerance to PCB126 have been described previously for NB and WI (Nacci et al., 2002a), but are included here for comparison to the other reference sites, GL and BI (Table 1). For two sites, NB and WI, we also measured PCBs in the livers of female and male fish (Table 1). PCBs in sediments and tissues were measured using previously reported methods (Nacci et al., 2002a). Chemical tolerance was characterized using a standardized laboratory challenge as previously described (Nacci et al., 2005). Briefly, early life stage toxicity was assessed using exposures to a toxic PCB congener (3,3′,4,4′,5-pentachloro biphenyl, IUPAC congener number 126, PCB126), which contributes most of the toxicity to killifish from NB PCBs (Black et al., 1998b). Exposed embryos were monitored for development and survival until 7 days post-hatching (Nacci et al., 2005), and results were summarized as modeled estimates of exposure concentration producing 20% lethality, LC_{20} (Bruce and Versteeg, 1992).

2.2. Bacterial challenges

Bacterial challenges used similar proportions of male and female fish (treatment replicates ranged from 40 to 62% males) of similar size (mean length ranged from 62.5 to 78.2 mm). Challenges were conducted in early- (June), mid- (July), late- (September) or post- (October) spawning condition for field-collected fish or post-spawning condition (November) for lab-reared fish. Challenges with *Vibrio harveyi* strain DN01, (Soffientino et al., 1999) were conducted using reported methods (Gauger et al., 2006). Bacteria were cultured and passaged through BI killifish to increase virulence, then bacteria were re-isolated, cultured and used to challenge test fish. Challenge units consisted of four or five fish from a single population, held in static, aerated 20-l glass tanks at 25 °C, maintained by partial immersion in flowing water from a single source at constant temperature. On the day prior to challenge, fish were distributed into these tanks arrayed in a haphazard fashion, where three to five replicate tanks comprised each treatment × population combination. Challenges began by anesthetizing fish lightly with MS-222 (50 μg/ml 3-aminobenzoic acid ethyl ester or tricaine methane sulfonate, Sigma Chemical, St. Louis, MO, USA) then identified by sex, measured for total length, and inoculated by intraperitoneal injection with 100 μl of nine salts solution (NSS, Gauger et al., 2006) containing 0 (control) or 10^7–10^9 colony forming units (CFU) *Vibrio harveyi* per ml. After inoculation, fish were held unfed, and observed twice daily for 7 days during which time dead fish were removed, identified by sex and measured for length, and examined for signs of vibriosis. Bacteria were re-isolated from at least five fish using aseptic techniques to confirm *V. harveyi* as the cause of mortalities. At the termination of the experiment, all remaining fish were euthanized according to institutional animal care policies.

2.3. In vitro effects of PCBs on V. harveyi

An *in vitro* study was conducted to test for direct suppressive effects of PCB 126 on bacterial proliferation and production of proteases as a proxy for virulence factors (Denkin and Nelson, 1999). *Vibrio harveyi* DN01 was grown in LB20 overnight at 21 °C and then back-diluted to a concentration of 10^2 CFU/ml in 10 ml of LB20 containing acetone (0.1 ml, solvent control) or 36 μg/ml PCB 126 dissolved in acetone (0.1 ml). This concentration of PCB 126 was used to represent maximum tissue concentrations in NB killifish (36 μg/g liver, Black et al., 1998a). Each medium was assayed in triplicate, and the experiment was run twice. At each time-point (0, 0.5, 4, 8, and 72 h), the viable cell count (CFU) was determined by plating serial dilutions of *V. harveyi* cultures and counting colonies. Culture supernatants were also assayed for proteolytic activity using the azocasein assay (Denkin and Nelson, 1999). The endpoint for this assay is a colored reactant (442 nm), and specific proteolytic activity is calculated using the formula: $1000 \times OD_{442}/(\log(CFU/ml))$.

2.4. Statistical analysis

We used general linear models and Fisher's least-significant-difference test to test for differences between populations in their survival responses to *V. harveyi* challenges. Percent data (survival and males) per replicate were arc sine square root transformed prior to analysis to account for heterogeneity of variance. To evaluate differences among replicates that might be related to differences between sexes in mixed sex replicate tanks, responses were calculated using only males or females, then analyzed similarly to the unaltered, mixed sex data set. The effect of PCBs on the growth and protease production by *V. harveyi* was tested using two-way analysis of variance. All analyses were conducted using SAS© (SAS Institute, 2000).

3. Results

3.1. Fish populations tested

Killifish collected from NB were highly contaminated, with liver PCBs about 100 fold higher than those in WI fish. There were no significant differences between male and female NB fish ($p = 0.13$) (Table 1). PCB tolerance to developmental toxicity reflected differences in exposures at residence sites: the LC_{20} for NB was about 100–1000 fold higher than those for reference populations (Table 1).

3.2. Field-collected fish challenges

Vibrio harveyi had a significant effect on survival of field-collected fish for each challenge conducted throughout the reproductive season (Fig. 2, $p < 0.0002$). When tests were analyzed

Table 1
Characteristics of *Fundulus heteroclitus* populations and their residence sites, including average (standard error, SE) concentrations of polychlorinated biphenyls (PCBs) in sediments ($n = 3$ per site) and livers of male and female fish ($n = 6$ per site), and PCB sensitivity measured as developmental response to a toxic PCB congener (PCB126), estimated as concentrations producing lethalities to 20% of the population (LC_{20}) using methods described in the text

Fundulus heterclitus parental population	Collection location	Primary land use	Sediment average PCBs, ng/g dry wt	Male fish liver average PCBs, ng/g dry weight (SE)	Female fish average PCBs, ng/g dry wt (SE)	LC_{20}, ng PCB126/l
New Bedford	Fairhaven, MA	Industrial	22666[a]	534 (119)	367 (32)	23770[a]
West Island	Fairhaven, MA	Undeveloped	165[a]	3.1 (0.1)	2.7 (0.4)	304[a]
Gloucester	Gloucester, MA	Residential	14	nm	nm	84
Block Island	Block Island, RI	Undeveloped	3	nm	nm	24

nm, not measured.
[a] Nacci et al. (2002a).

individually, neither the source population ($p = 0.23, 0.80, 0.19$) nor the population by dose interaction ($p = 0.56, 0.16, 0.16$) significantly affected this response in the June, September, and October challenges, respectively. In the mid-reproduction (July) challenge, populations interacted differentially with dose to affect survival ($p = 0.0019$), with higher survival among the NB versus the WI fish. Similarly in the post-reproductive challenge (October), NB survival was significantly greater ($p < 0.05$) than both reference populations, WI and GL, for a single treatment (10^7 CFU per fish).

Data from NB and WI for a single treatment (10^7 CFU per fish) from multiple challenges were also analyzed together, excluding June data because treatment survivals were significantly lower for both NB and WI populations than for subsequent challenges. Overall, NB fish survived this treatment better ($p = 0.0001$) than did WI fish (Table 2). Across these tests, fish did not differ in size ($p = 0.60$), but there were more males in WI replicates ($p = 0.0042$), averaging 72.9% males (4.9% se) per replicate versus 51% males (4.3% se) per replicate for NB fish. Since sexes might respond differently, data were separated by sex and treatment responses reanalyzed (Table 2). Survival did not differ between sexes within WI or when populations were combined; however, survival was higher in males from NB when compared with NB females or WI males (Table 2).

3.3. Laboratory-reared fish challenges

A single experiment was conducted after the reproductive season (November, Table 3) using mature NB and WI killifish reared in the laboratory for two generations, which retain their differential sensitivities to PCBs (Nacci et al., 2002a). The single concentration of injected *V. harveyi* (3×10^7 CFU/fish) affected survival ($p = 0.0005$), but did not affect populations differently when mixed-sex tanks were compared, nor when only males or females were compared (Table 3).

3.4. In vitro effects of PCBs on V. harveyi

A solution of PCB 126 formulated at a concentration estimated from tissue measurements of *F. heteroclitus* from NB (36 μg/ml, Black et al., 1998b) had no significant effect on bacterial growth rate or specific proteolytic activity in culture supernatants (data not shown).

4. Discussion

NB killifish provide a dramatic example of persistence under extreme conditions, even within a species remarkable for its hardiness (e.g., Nordlie, 2006; Burnett et al., 2007). This large population of non-migrating, estuarine fish resides in one of the most highly PCB-contaminated estuaries in the US (Long et al., 1995), bioaccumulating toxic concentrations of PCBs in adult fish (Table 1), which are distributed to their developing progeny (Nacci et al., 1999). Consistent with our expectations concerning adaptation, extraordinary tolerance to developmental PCB toxicity has been documented in NB killifish in this (Table 1) and in other studies (Nacci et al., 1999, 2002a). For example, the range of developmentally-toxic PCB concentrations (LC_{20}) between NB killifish and the most sensitive killifish populations exceeds the range of sensitivities to similar compounds across all fish species tested (Tillitt et al., 2008; Van Veld and Nacci, 2008). In combination with other independently evolving killifish populations persisting under similarly extreme but unique chemical conditions, this species provides an important system to explore intra-specific patterns and test hypotheses concerning genetic, biological, and ecological compensatory mechanisms (Cohen, 2002; Meyer and Di Guilio, 2003; Cohen et al., 2006; Fisher and Oleksiak, 2007; Burnett et al., 2007; Tirindelli, 2007; Van Veld and Nacci, 2008).

In this study, we were specifically concerned with the relationship between evolved chemical tolerance and disease vulnerability of adult NB killifish. As others have (e.g., Arkoosh et al., 2005; Carlson and Zelikoff, 2008), we used survival following acute bacterial challenges as an indicator of pathogen susceptibility in fish. Results of these tests reflect innate immunity: immediate, non-specific pathogen responses used by fish as a first line of defense (e.g., Rice, 2001; Neumann et al., 2001; Dautremepuits et al., 2006; Magnadottir, 2006; Carlson and Zelikoff, 2008). In addition to direct interaction with infectious agents, the innate immune system also mediates more generalized inflammatory

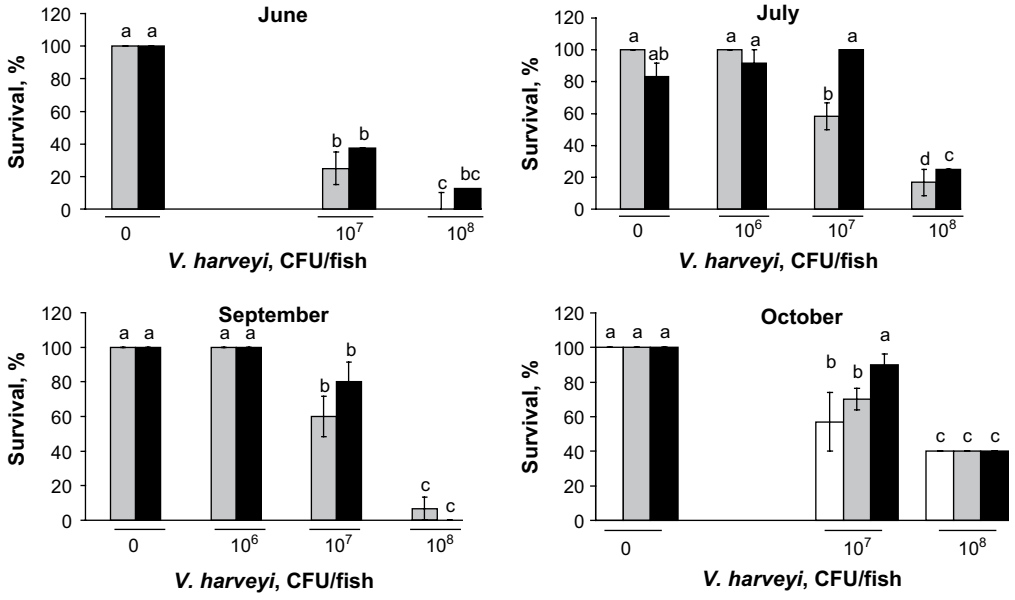

Fig. 2. Cumulative 7-day survival (means and standard errors of within-test replicates) of *Fundulus heteroclitus* from West Island (gray bar), or New Bedford (black bar) (MA, USA) following injections of bacteria (*Vibrio harveyi*) during tests conducted early- (June), mid- (July), late- (September) or post-(October) reproductive season; Gloucester (white bar), was also tested in October, only; letters indicate within-test statistical comparisons.

Table 2
Average (±standard error) survival for tests conducted in July, September, and October following exposure to the pathogen, *Vibrio harveyi* (10^7 colony forming units per fish) in (n) tanks of fish collected from West Island (WI), a relatively uncontaminated reference site, or New Bedford (NB), Massachusetts, USA, including probabilities (p) for rejecting differences within rows or columns

Population	Females, only	Males, only	Both sexes	p
WI	60.0 ± 12.5 (10)	65.2 ± 6.9 (12)	64.6 ± 4.1 (12)	0.831
NB	79.2 ± 7.7 (12)	100.0 ± 0.0 (12)	90.0 ± 3.9 (12)	0.011
Both populations	70.5 ± 7.2 (22)	82.6 ± 5.0 (24)	77.3 ± 3.8 (24)	0.0001
p	0.1607	<0.0001		

responses and acquired immune responses, such as the production of specific antibodies which may begin weeks after infection in fish (e.g., Rice and Xiang, 2000; Yada and Nakanishi, 2002; Dautremepuits et al., 2006). Based on its critical role in early as well as later responses, the innate immune system in fishes has been described as even more important than acquired immunity in infectious disease susceptibility and outcome (Maule et al., 1996; Camp et al., 2000; Palm et al., 2003). Because innate immunity is important, short-term challenges such as ours have been used by others to assess one aspect of disease vulnerability in fish exposed to chemical pollutants (e.g., Arkoosh et al., 1998, 2005; Palm et al., 2003; Carlson and Zelikoff, 2008). Results from these tests are also useful because innate immunity provides primary protection against infection without depending upon prior exposure to any particular agent. Therefore, our tests using a single bacterial pathogen may be representative of short-term responses to typically encountered bacterial pathogens.

Our studies showing that NB killifish respond acutely to a common marine pathogen at least as vigorously as reference populations suggest that some important, short-term immune responses are not compromised in NB killifish, and in fact, may be enhanced. In addition, our *in vitro* tests suggested that PCBs did not directly suppress bacterial virulence, which might have masked poor immunological responses in heavily contaminated NB fish. However, field studies of chemically-tolerant Atlantic Wood killifish, which appear to be immunocompromised (Frederick et al., 2007), show alterations in innate and acquired immunity. Although it has been proposed that innate immunity may compensate for acquired immunity deficiencies (e.g., Kurtz et al., 2003), it may be important to account for responses of the acquired immune system in NB killifish before making inferences concerning disease susceptibility of NB killifish in the wild (although see below and, e.g., Cohen et al., 2006).

While our results did not show a strong seasonal pattern, NB killifish had improved survival during peak reproduction (Fig. 2), and a consistent pattern of improved survival throughout the reproductive season (Table 2). Due to increased energetic demands, e.g., from gamete production and mating behavior, and a complex interplay between stress and reproductive hormones and the immune system, spawning is often considered a period of increased vulnerability to disease and other stressors in fishes (e.g., Luebke et al., 1997; Schreck, 1996; Tatner, 1996; Harris and Bird, 2000; Maule et al., 1996; Yada and Nakanishi, 2002). Other endocrine factors that may also vary seasonally, such as thyroid hormone, have been shown to affect immune response in killifish and other fish (Yada and Nakanishi, 2002). Unexpectedly, there were differential effects of sex between populations, with male fish from NB surviving better than other groups (Table 2), which could not be explained by differential levels of PCB contamination between male and female fish (Table 1).

Although NB killifish show apparently normal reproductive output (Black et al., 1998a; Nacci et al., 2002b), male killifish collected from NB show evidence of endocrine disruption, including markers of estrogenic exposures (Greytak et al., 2005) and altered levels of estrogen receptors (Greytak and Callard, 2007). This feminization of NB fish could contribute through direct or indirect mechanisms to the apparently enhanced immune responses in male fish collected from NB. However in a single bacterial challenge, uncontaminated (laboratory-raised) progeny from NB performed similarly to field-collected fish. These results suggest that tissue concentrations of PCBs that are toxic to killifish from reference sites (e.g., Black et al., 1998b) are not immunotoxic to NB killifish. However, these limited findings do not establish definitely the heritability of enhanced male performance in bacterial challenges by NB killifish. Therefore, additional studies to clarify the mechanistic basis for the sex-specific improved survival following bacterial challenge in NB fish are needed. Such studies may also provide information more generally relevant to understanding interactions between reproductive hormones/antagonists, immunological responses, and altered disease incidence in fishes (e.g., Maule et al., 1996; Kurtz et al., 2007).

Increased disease susceptibility, which has been proposed as an adaptive trade-off of chemical tolerance in Atlantic Wood killifish (Meyer and Di Giulio, 2003), might also be expected for NB killifish. Several types of fitness costs have been associated with the evolution of chemical tolerance. For example, rapid adaptation is often accompanied by decreased genetic variation, which is strongly associated with increased susceptibility to infectious diseases and parasites in many species (e.g., Coltman et al., 1999; Acevedo-Whitehouse et al., 2003; Reid et al., 2003; Spielman et al., 2004; Hale and Briskie, 2007). However, reduced genetic diversity is unlikely in killifish populations (e.g., Mitton, 1994). Even those killifish populations resident to highly contaminated sites, including NB, show no evidence of genetic bottlenecks (Cohen, 2002; Mulvey et al., 2003; Roark et al., 2005; McMillan et al., 2006; Adams et al., 2006; Tirindelli, 2007; Duvernell et al., 2008). More specific to immunological function, killifish populations resident to sites that vary widely in chemical contamination, including NB, show high diversity and unique amino acid substitutions in the peptide binding site of major histocompatibility complex (MH in fishes, known as MHC in mammals), suggesting that this component of acquired immunity is not compromised (Cohen, 2002; Cohen et al., 2006; Tirindelli, 2007).

However, other types of trade-offs or conditional fitness costs, specific genetic "pleiotropic by-products" (Futuyma, 1986) or more general energetic costs of adaption, have sometimes been associated with chemical tolerance in examples from the laboratory and the field. For example, lines of the least killifish (*Heterandria formosa*) selected in the laboratory for resistance to cadmium had reduced fecundity under uncontaminated conditions relative to unselected lines (Xie and Klerks, 2004a), and pesticide-tolerant versus sensitive mosquitoes respond more poorly to bacterial infection (Duron et al., 2006). Yet, trade-off costs have not always been found (e.g., Roush and McKenzie, 1987), and should, perhaps, be expected only when consistent with adaptive mechanisms (e.g., Taylor and Feyereisen, 1996; Coustau et al., 2000). For example, common mechanisms of tolerance to metals in fish (Xie and Klerks, 2004b) and pesticides in insects (e.g., Roush and McKenzie, 1987;

Table 3
Average survival (±standard error) following exposure to *Vibrio harveyi* (3×10^7 colony forming units per fish) in (n) tanks of laboratory-bred fish from populations resident to West Island (WI), a relatively uncontaminated reference site, or New Bedford (NB), Massachusetts, USA, including probabilities (p) for rejecting differences within rows or columns

Population	Females, only	Males, only	Both sexes	p
WI	11 ± 11 (3)	0 ± 0 (3)	6.7 ± 6.7 (3)	0.374
NB	22.3 ± 22.3 (3)	33.3 ± 16.7 (3)	26.7 ± 17.6 (3)	0.627
Both populations	16.7 ± 9.5 (6)	16.7 ± 11.4 (6)	16.7 ± 9.5 (6)	0.388
p	0.802	0.091		

Raymond et al., 2001) involve costly production (up regulation) of protective proteins.

Unlike these common mechanisms of tolerance, poor responsiveness or down regulation of the aryl hydrocarbon receptor (AHR) signal transduction pathway is the hallmark of tolerance in killifish populations studied to date (e.g., Prince and Cooper, 1995; Hahn, 1998; Elskus et al., 1999; Bello et al., 2001; Wirgin and Waldman, 2004; Meyer et al., 2003b; Van Veld and Nacci, 2008) and, most recently, in Hudson River tomcod (Yuan et al., 2006). While perhaps an overly simplistic generalization of the mechanisms that confer chemical tolerance in killifish (e.g., Hahn, 1998; Bard et al., 2002; Weis, 2002; Meyer et al., 2002, 2003a, 2005; VanVeld and Nacci, 2008), down regulation draws few resources away from expensive biological processes such as reproduction and immunity. Therefore, this energetically thrifty strategy employed by killifish may not engender the energetic costs associated with chemical tolerance in some other examples.

Because PCBs are known to be highly immunosuppressive to fish species (e.g., Duffy et al., 2002; Carlson and Zelikoff, 2008), including killifish (Fries, 1986), tolerance to this aspect of PCB toxicity would be very beneficial to NB killifish. It is known that NB killifish are developmentally tolerant to the class of PCB congeners that are most toxic to vertebrates (Nacci et al., 1999, 2002a), and are mechanistically related to the highly toxic compound, dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD) (e.g., Safe, 1994). Most, if not all, of the toxic effects of these compounds to fish are mediated through the AHR pathway (e.g., Tillitt et al., 2008). Furthermore, it is known that the immunosuppressive action of dioxin-like compounds is at least partially mediated via the AHR in the vertebrate species tested (Silkworth et al., 1984; Kerkvliet et al., 1990; Kerkvliet, 2002). Since dioxin-like PCB congeners are immunotoxic to fishes (e.g., Rice and Schlenk, 1995; Regala et al., 2001; Duffy et al., 2005), AHR-mediated tolerance in NB killifish may mitigate some of the effects of PCBs on disease susceptibility. However, nondioxin-like PCBs also affect fish immune responses (e.g., Maule et al., 2005; Duffy and Zelikoff, 2006), and PCB congeners that vary toxicologically probably affect pathogen responses differently (e.g., Rice and Schlenk, 1995; Regala et al., 2001; Arkoosh et al., 2001; Maule et al., 2005; Duffy et al., 2002, 2005; Duffy and Zelikoff, 2006; Carlson and Zelikoff, 2008). While the relative importance of AHR-mediated tolerance to disease susceptibility in PCB-exposed fish is difficult to predict, out results suggest that high tissue concentrations of PCBs are not immunosuppressive to NB killifish.

It is reasonable that the relative success of NB killifish in response to acute infectious challenges may reflect adaptive benefits of alterations of the AHR pathway. But, adaptive strategies indirectly related or unrelated to chemical pollution may also be involved in the persistence of NB killifish in their highly contaminated and ecologically disturbed residence site. A diversity of primary and (potentially) secondary adaptations may be revealed by ongoing research into the biochemical and genetic mechanisms of chemical tolerance in killifish, which complement empirical studies such as this one that explore the realized benefits and costs of contemporary evolution.

5. Conclusion

A population of the non-migratory estuarine Atlantic killifish (*Fundulus heteroclitus*) that persists despite toxic pollutants contaminating their residence site, PCB-contaminated NB harbor, serves as a unique example of contemporary evolution. In this study, we were specifically concerned with the relationship between evolved chemical tolerance and disease vulnerability of adult fish: demographically-important life stages. Some theoretical and empirical considerations supported expectations that NB killifish would be at increased risk for infectious disease, especially during the stressful, energetically-expensive reproductive period. However, our results showed that field-collected, contaminated NB killifish and their laboratory-raised uncontaminated progeny survived acute bacterial challenges as well as or better (NB males) than did fish from less-contaminated reference sites. Although our studies were not mechanistic by design, a parsimonious explanation of our results consistent with other studies using this species and other vertebrates, is that the biochemical mechanism of developmental PCB tolerance in NB killifish may also mitigate some aspects of immunosuppression. While we interpret our results cautiously with respect to the complex factors associated with disease susceptibility in the wild, our findings challenge expectations of generalized adaptive costs and suggest realized adaptive benefits associated with this example of contemporary evolution to toxic chemicals. Independently evolving populations of killifish, such as those resident to chemically-contaminated sites, provide a unique example of intra-specific compensatory strategies in response to human-mediated stressors.

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References

Acevedo-Whitehouse, K., Cunningham, A.A., 2006. Is MHC enough for understanding wildlife immunogenetics? Trends in Ecology and Evolution 21, 433–438.

Acevedo-Whitehouse, K., Gulland, F., Greig, D., Amos, W., 2003. Inbreeding: disease susceptibility in California sea lions. Nature 422, 35.

Adams, S.M., Lindmeier, J.B., Duvernell, D.D., 2006. Microsatellite analysis of the phylogeography and secondary contact hypotheses for the killifish, *Fundulus heteroclitus*. Molecular Ecology 15, 1109–1123.

Arkoosh, M.R., Casillas, E., Huffman, P., Clemons, E., Evered, J., Stein, J.E., Varanasi, U., 1998. Increased susceptibility of juvenile chinook salmon (*Oncorhynchus tshawytscha*) from a contaminated estuary to the pathogen *Vibrio anguillarum*. Transactions of American Fisheries Society 127, 360–374.

Arkoosh, M.R., Clemons, E., Huffman, P., Kagley, A.N., 2001. Increased susceptibility of juvenile Chinook salmon to vibriosis after exposure to chlorinated and aromatic compounds found in contaminated urban estuaries. Journal of Aquatic Animal Health 13, 257–268.

Arkoosh, M.R., Boylen, D., Stafford, C.L., Johnson, L.L., Collier, T.K., 2005. Use of disease challenge assay to assess immunotoxicity of xenobiotics in fish. In: Ostrander, G.K. (Ed.), Techniques in Aquatic Toxicology, Vol. 2. CRC Press, Boca Raton, FL, pp. 19–38.

Austin, B., Zhang, X.-H., 2006. *Vibrio harveyi*: a significant pathogen of marine vertebrates and invertebrates. Letters in Applied Microbiology 43, 119–124.

Bard, S.M., Bello, S.M., Hahn, M.E., Stegeman, J.J., 2002. Expression of P-glycoprotein in killifish (*Fundulus heteroclitus*) exposed to environmental xenobiotics. Aquatic Toxicology 59, 237–251.

Bello, S.M., Franks, D.G., Stegeman, J.J., Hahn, M.E., 2001. Acquired resistance to Ah receptor agonists in a population of Atlantic killifish (*Fundulus heteroclitus*) inhabiting a marine Superfund site: *in vivo* and *in vitro* studies on the inducibility of xenobiotic metabolizing enzymes. Toxicological Sciences 60, 77–91.

Black, D.E., Gutjahr-Gobell, R., Pruell, R.J., Bergen, B., Mills, L., McElroy, A.E., 1998a. Reproduction and polychlorinated biphenyls in *Fundulus heteroclitus* (Linnaeus)

from New Bedford Harbor, Massachusetts. USA. Environmental Toxicology and Chemistry 17, 1405–1414.
Black, D.E., Gutjahr-Gobell, R., Pruell, R.J., Bergen, B., McElroy, A.E., 1998b. Effects of a mixture of non-*ortho*- and mono-*ortho*-polychorinated biphenyls on reproduction in *Fundulus heteroclitus* (Linnaeus). Environmental Toxicology and Chemistry 17, 1396–1404.
Bruce, R.D., Versteeg, D.J., 1992. A statistical procedure for modeling continuous toxicity data. Environmental Toxicology and Chemistry 11, 1485–1494.
Burnett, K.G., Bain, L.J., Baldwin, W.S., Callard, G.V., Cohen, S., Di Giulio, R.T., Evans, D.H., Gomez-Chiarri, M., Hahn, M.E., Hoover, C.A., Karchner, S.I., Katoh, F., MacLatchy, D.L., Marshall, W.S., Meyer, J.N., Nacci, D.E., Oleksiak, M.F., Rees, B.B., Singer, T.P., Stegeman, J.J., Towle, D.W., Van Veld, P.A., Vogelbein, W.K., Whitehead, A., Winn, R., Crawford, D.L., 2007. *Fundulus* as the premier teleost model in environmental biology: opportunities for new insights using genomics. Comparative Biochemistry and Physiology Part D 2, 257–286.
Camp, K.I., Wolters, W.R., Rice, C.D., 2000. Survivability and immune responses after challenge with *Edwardsiella ictaluri* in susceptible and resistant families of channel catfish, *Ictalurus punctatus*. Fish and Shellfish Immunology 10, 475–487.
Carlson, E., Zelikoff, J.T., 2008. The immune system of fish: a target organ of toxicity. In: Di Giulio, R.T., Hinton, D.E. (Eds.), The Toxicology of Fishes. Taylor & Francis, Washington, DC, pp. 489–530.
Cohen, S., 2002. Strong positive selection and habitat-specific amino acid substitution patterns in *Mhc* from an estuarine fish under intense pollution stress. Molecular Biology and Evolution 19, 1870–1880.
Cohen, C.S., Tirindelli, J., Gomez-Chiarri, M., Nacci, D., 2006. Functional implications of Major Histocompatibility variation using estuarine fish populations. Integrative and Comparative Biology 46 (6), 1016–1029.
Coltman, D.W., Pilkington, J.G., Smith, J.A., Pemberton, J., 1999. Parasite mediated selection against inbred Soay sheep in a free-living, island population. Evolution 53, 1259–1267.
Coustau, C., Chevillon, C., French-Constant, R., 2000. Resistance to xenobiotics and parasites: can we count the costs. Trends in Ecology and Evolution 15, 378–383.
Dautremepuits, C., Fortier, M., Croisetiere, S., Belhumeur, P., Fournier, M., 2006. Modulation of juvenile brook trout (*Salvelinus fontalinalis*) cellular immune system after *Aeromonas salmonicida* challenge. Veterinary Immunology and Immunopathology 110, 27–36.
Denkin, S.M., Nelson, D.R., 1999. Induction of protease activity in *Vibrio anguillarum* by gastrointestinal mucus. Applied and Environmental Microbiology 65 (8), 3555–3560.
Duffy, J.E., Zelikoff, J.T., 2006. The relationship between noncoplanar PCB-induced immunotoxicity and hepatic CYP1A induction in a fish model. Journal of Immunotoxicology 3, 39–47.
Duffy, J.E., Carlsen, E., Li, Y., Prophete, C., Zelikoff, J.T., 2002. Impact of polychlorinated biphenyls (PCBs) on the immune function of fish: age as a variable in determining outcome. Marine Environmental Research 54, 559–563.
Duffy, J.E., Li, Y., Zelikoff, J.T., 2005. PCB-induced hepatic CYP1A induction is associated with innate immune dysfunction in a feral teleost fish. Bulletin of Environmental Contamination and Toxicology 74, 107–113.
Duron, O., Labbe, P., Berticat, C., Rousset, F., Guillot, S., Raymond, M., Weill, M., 2006. High *Wolbachia* density correlates with cost of infection for insecticide resistant *Culex pipiens* mosquitoes. Evolution 60 (2), 303–314.
Duvernell, D.D., Lindmeier, J.B., Faust, K.E., Whitehead, A., 2008. Relative influences of historical and contemporary forces shaping the distribution of genetic variation in the Atlantic killifish, *Fundulus heteroclitus*. Molecular Ecology 17, 1344–1360.
Elonen, G.E., Spehar, R.L., Holcombe, G.W., Johnson, R.D., Fernandez, J.D., Erickson, E.J., Tietge, J.E., Cook, P.M., 1998. Comparative toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to seven freshwater fish species during early life-stage development. Environmental Toxicology and Chemistry 17 (3), 472–483.
Elskus, A.A., Monosson, E., McElroy, A.E., Stegeman, J.J., Woltering, D.S., 1999. Altered CYP1A expression in *Fundulus heteroclitus* adults and larvae: a sign of pollutant resistance? Aquatic Toxicology 45, 99–113.
Fisher, M.A., Oleksiak, M.F., 2007. Convergence and divergence in gene expression among natural populations exposed to pollution. BMC Genomics 8, 108.
Frederick, L.A., Van Veld, P.A., Rice, C.D., 2007. Bioindicators of immune function in creosote-adapted estuarine killifish, Fundulus heteroclitus. Journal of Toxicology and Environmental Health Part A 70 (17), 1433–1442.
Fries, C.R., 1986. Effects of environmental stressors and immunosuppressants on immunity in *Fundulus heteroclitus*. American Zoologist 26, 271–282.
Futuyma, D.J., 1986. Evolutionary Biology. Sinauer Associates, Sunderland, MA.
Gauger, E.J., Gomez-Chiarri, M., 2002. 16S ribosomal DNA sequencing confirms synonymy of *Vibrio harveyi* and *V. carchariae*. Diseases of Aquatic Organisms 52, 39–46.
Gauger, E.J., Smolowitz, R., Uhlinger, K., Casey, J., Gomez-Chiarri, M., 2006. *Vibrio harveyi* and other bacterial pathogens in cultured summer flounder, *Paralichthys dentatus*. Aquaculture 260, 10–20.
Greytak, S.R., Callard, G.V., 2007. Cloning of three estrogen receptors (ER) from killifish (*Fundulus heteroclitus*): differences in expression in populations from polluted and reference environments. General and Comparative Endocrinology 150 (1), 174–188.
Greytak, S.R., Champlin, D., Callard, G.V., 2005. Isolation and characterization of two cytochrome P450 aromatase forms in killifish (*Fundulus heteroclitus*): differences in expression in populations from polluted and reference environments. Aquatic Toxicology 71, 371–389.
Gutjahr-Gobell, R.E., Black, D.E., Mills, L., Pruell, R., Taplin, B.K., Jayaraman, S., 1999. Feeding the killifish (*Fundulus heteroclitus*) a diet spiked with non-*ortho*- and mono-*ortho*-substituted polychlorinated biphenyls: accumulation and effects. Environmental Toxicology and Chemistry 18, 699–707.
Hahn, M.E., 1998. Mechanisms of innate and acquired resistance to dioxin-like compounds. Reviews in Toxicology 2, 395–443.
Hahn, M.E., Karchner, S.I., Franks, D.G., Merson, R.R., 2004. Aryl hydrocarbon receptor polymorphisms and dioxin resistance in Atlantic killifish (*Fundulus heteroclitus*). Pharmacogenetics 14, 131–143.
Hale, K.A., Briskie, J.V., 2007. Decreased immunocompetence is a severely bottlenecked population of endemic New Zealand bird. Animal Conservation 10, 2–10.
Harris, J., Bird, D.J., 2000. Modulation of the fish immune system by hormones. Veterinary Immunology and Immunopathology 77, 163–176.
Hicks, T., Steele, E., 2003. Histological effect of *Ascocotyle tenuicollis* (Digenea: Heterophyidae) metacercarial infection on the heart of *Fundulus heteroclitus* (Teleostei: Cyprinodontidae). Journal of the South Carolina Academy 1, 10–18.
Kerkvliet, N., 2002. Recent advances in understanding the mechanisms of TCDD immunotoxicity. International Journal of Immunopharmacology 2, 277–291.
Kerkvliet, N.I., Baecher-Steppan, L., Smith, B.B., Youngerberg, J.A., Henderson, M.C., Buhler, D.R., 1990. Role of the Ah locus in suppression of cytotoxic T-lymphocyte activity by halogenated aromatic hydrocarbons (PCBs and TCDD): structure-activity relationships and effects in C57B1/6 mice congenic at the Ah locus. Fundamental and Applied Toxicology 14, 532–541.
Kinnison, M.T., Hairston Jr., N.G., 2007. Eco-evolutionary conservation biology: evolution and the dynamics of persistence. Functional Ecology 21, 444–454.
Kurtz, J., Kalbe, M., Aeschlimann, P.B., Haberli, M.A., Wegner, K.M., Reusch, B.H., Milinshki, M., 2003. Major histocompatibility complex diversity influences parasite resistance and innate immunity in sticklebacks. Proceeding of the Royal Society of London B 271, 197–204.
Kurtz, J., Kalbe, M., Langefors, A., Mayer, I., Milinski, M., Hasselquist, D., 2007. An experimental test of the immunocompetence handicap hypothesis: 11-ketotestosterone suppresses innate immunity in three-spined sticklebacks. The American Naturalist 170 (4), 509–519.
Levinton, J.S., Klerks, P., Martinez, D.E., Montero, C., Sturmbauer, C., Suatoni, L., Wallace, W., 1999. Running the gauntlet: pollution, evolution and reclamation of an estuarine bay and its significance in understanding the population biology of toxicology and food web transfer, in: Whitfield, M. (Ed.), Aquatic Life Cycle Strategies. Marine Biological Association of the United Kingdom.
Long, E.R., MacDonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environmental Management 19, 81–97.
Luebke, R.W., Hodson, P.V., Faisal, M., Ross, P.S., Grasman, K.A., Zelikoff, J., 1997. Aquatic pollution-induced immunotoxicity in wildlife species. Fundamental and Applied Toxicology 37, 1–15.
Luebke, R.W., Chen, D.H., Dietert, R., Yang, Y., King, M., Luster, M.I., 2006. The comparative immunotoxicity of five selected compounds following developmental or adult exposure. Journal of Toxicology and Environmental Health Part B 9, 1–26.
Magnadottir, B., 2006. Innate immunity of fish (overview). Fish and Shellfish Immunology 20 (2), 137–151.
Martinez, D.E., Levinton, J., 1996. Adaptation to heavy metals in the aquatic oligochaete *Limnodrilus hoffmeisteri*: evidence for control by one gene. Evolution 50, 1339–1343.
Maule, A.G., Schrock, R., Slater, C., Fitzpatrick, M.S., Schreck, C.B., 1996. Immune and endocrine responses of adult Chinook salmon during freshwater immigration and sexual maturation. Fish and Shellfish Immunology 6, 221–233.
Maule, A.G., Jorgensen, E.H., Vijayan, M.M., Killie, J.A., 2005. Arochlor 1254 exposure reduced disease resistance and innate immune responses in fasted arctic char. Environmental Toxicology and Chemistry 24, 117–124.
McKenzie, J.A., Batterham, P., 1994. The genetic, molecular, and phenotypic consequences of selection for pesticide resistance. Trends in Ecology and Evolution 9, 166–169.
McMillan, A.M., Bagley, M.J., Jackson, S.A., Nacci, D.E., 2006. Genetic diversity and structure of an estuarine fish (*Fundulus heteroclitus*) indigenous to sites associated with a highly contaminated urban harbor. Ecotoxicology 15, 539–548.
Meyer, J.N., Di Giulio, R.T., 2003. Heritable adaptation and associated fitness tradeoffs in killifish (*Fundulus heteroclitus*) inhabiting a contaminated estuary. Ecological Applications 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 activity and relationship to survival in contaminated sediments. Toxicological Sciences 68, 69–81.
Meyer, J.N., Smith, J.D., Winston, G.D., Di Giulio, R.T., 2003a. Antioxidant defenses in killifish (*Fundulus heteroclitus*) exposed to Superfund sediments and model prooxidants: short-term and heritable responses. Aquatic Toxicology 65, 377–395.
Meyer, J.N., Wassenberg, D.M., Karchner, S.I., Hahn, M.E., Di Giulio, R.T., 2003b. Expression and inducibility of aryl hydrocarbon receptor pathway genes in wildcaught killifish (*Fundulus heteroclitus*) with different contaminant-exposure histories. Environmental Toxicology and Chemistry 22, 2337–2343.
Meyer, J.N., Volz, D.C., Freedman, J.H., Di Giulio, R.T., 2005. Differential display of hepatic mRNA from killifish (*Fundulus heteroclitus*) inhabiting a Superfund estuary. Aquatic Toxicology 73, 327–341.
Mitton, J.B., 1994. Selection in Natural Populations. Oxford University Press, New York.
Monosson, E., 2000. Reproductive and developmental effects of PCBs in fish: a synthesis of laboratory and field studies. Reviews in Toxicology 3, 25–42.

Mulvey, M., Newman, M.C., Vogelbein, W., Unger, M.A., Ownby, D.R., 2003. Genetic structure and mtDNA diversity of *Fundulus heteroclitus* populations from polycyclic aromatic hydrocarbon-contaminated sites. Environmental Toxicology and Chemistry 22, 671–677.

Munns Jr., W.R., Black, D.E., Gleason, T.R., Salomon, K., Bengtson, D., Gutjahr-Gobell, R., 1997. Evaluation of the effects of dioxin and PCBs on *Fundulus heteroclitus* populations using a modeling approach. Environmental Toxicology and Chemistry 16, 1074–1081.

Nacci, D., Coiro, L., Champlin, D., Jayaraman, S., McKinney, R., Gleason, T., Munns Jr., W.R., Specker, J., Cooper, K., 1999. Adaptation of wild fish populations to dioxin-like environmental contamination. Marine Biology 134, 9–17.

Nacci, D., Jayaraman, S., Specker, J., 2001. Stored retinoids in populations of an estuarine fish, *Fundulus heteroclitus*, indigenous to highly PCB-contaminated and reference sites. Archives Environmental Contamination and Toxicology 40 (4), 511–518.

Nacci, D., Coiro, L., Champlin, D., Jayaraman, S., McKinney, R., 2002a. Predicting the occurrence of genetic adaptation to dioxin-like compounds in populations of the estuarine fish *Fundulus heteroclitus*. Environmental Toxicology and Chemistry 21 (7), 1525–1532.

Nacci, D., Gleason, T., Gutjahr-Gobell, R., Huber, M., Munns Jr., W.R., 2002b. Effects of environmental stressors on wildlife populations. In: Newman, M.C. (Ed.), Coastal and Estuarine Risk Assessment. Risk on the Edge. CRC Press/Lewis Publishers, Washington, DC.

Nacci, D., Coiro, L., Wasserman, D., Di Giuilio, R., 2005. A non-destructive technique to measure cytochrome P4501A enzyme activity in living embryos of the estuarine fish *Fundulus heteroclitus*. In: Ostrander, G.K. (Ed.), Techniques in Aquatic Toxicology, Vol. 2. Taylor & Francis, Boca Raton, FL, pp. 209–225.

Nacci, D., Walters, S., Gleason, T., Munns Jr., W.R., 2008. Using a spatial modeling approach to explore ecological factors relevant to the persistence of estuarine fish (*Fundulus heterclitus*) in a PCB-contaminated estuary. In: Akcakaya, R., Stark, J. (Eds.), Population-level Ecotoxicology. Oxford University Press, North Carolina.

Nelson, W.G., Bergen, B.J., Benyi, S.J., Morrison, G., Voyer, R.A., Strobel, C.J., Rego, S., Thursby, G., Pesch, C.E., 1996. New Bedford harbor long-term monitoring assessment report: baseline sampling. US Environmental Protection Agency. National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, Narragansett, RI. EPA/600/R-96/097.

Neumann, N.F., Stafford, J.L., Barreda, D., Ainsworth, A.J., Belosevic, M., 2001. Antimicrobial mechanisms of fish phagocytes and their role in host defense. Developmental and Comparative Immunology 25, 607–825.

Nordlie, F.G., 2006. Physicochemical environments and tolerances of cyprinodontoid fishes found in estuaries and salt marshes of eastern North America. Reviews in Fish Biology and Fisheries 16, 51–106.

Palm Jr., R.C., Powell, D.B., Skillman, A., Godtfredsen, K., 2003. Immunocompetence of juvenile chinook salmon against *Listonella anguillarum* following dietary exposure to polycyclic aromatic hydrocarbons. Environmental Toxicology and Chemistry 22, 2986–2994.

Prince, R., Cooper, K.R., 1995. Comparisons of the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on chemically-impacted and non-impacted subpopulations of *Fundulus heteroclitus*: I. TCDD Toxicity. Environmental Toxicology and Chemistry 14, 579–588.

Råberg, L., Sim, D., Read, A.F., 2007. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. Science 318, 812–814.

Raymond, M., Berticat, C., Weill, M., Pasteur, N., Chevillon, C., 2001. Insecticide resistance in the mosquito (*Culex pipiens*): what have we learned about the adaptation? Genetica 112, 287–296.

Regala, R.P., Rice, C.D., Schwedler, T.E., Dorociak, I.R., 2001. The effects of tributyltin (TBT) and 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) mixtures on antibody responses and phagocyte oxidative burst activity in channel catfish, *Ictalurus punctatus*. Archives of Environmental Contamination and Toxicology 40, 386–391.

Reid, J.M., Arcese, P., Keller, L.F., 2003. Inbreeding depresses immune response in song sparrows (*Melospiza melodia*): direct and intergenerational effects. Proceedings of the Royal Society of London Series B 270, 2151–2157.

Rice, C.D., 2001. Fish immunotoxicology: understanding mechanisms of action. In: Schlenk, D., Benson, W.H. (Eds.), Target Organ Toxicity in Marine and Freshwater Teleosts, Vol. 2. Taylor & Francis, New York, pp. 96–138. Systems.

Rice, C.D., Schlenck, D., 1995. Immune function and cytochrome P4501A activity after acute exposure to 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) in channel catfish, *Ictalurus punctatus*. Aquatic Animal Health 7, 195–204.

Rice, C.D., Xiang, Y., 2000. Immune function, hepatic CYP1A, and reproductive biomarker responses in the gulf killifish, *Fundulus grandis*, during dietary exposures to endocrine disrupters. Marine Environmental Research 50, 163–168.

Roark, S.A., Nacci, D., Coiro, L., Champlin, D., Guttman, S.I., 2005. Population genetic structure of a non-migratory marine fish *Fundulus heteroclitus* across a strong gradient of PCB contamination. Environmental Toxicology and Chemistry 24 (3), 717–725.

Roush, R.T., McKenzie, J.A., 1987. Ecological genetics of insecticide and acaricide resistance. Annual Review of Entomology 32, 361–380.

SAS Institute, 2000. SAS® User's Guide. SAS Institute, Cary, NC, 1686 pp.

Safe, S., 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assess. CRC Critical Reviews in Toxicology 24, 87–149.

Schmalz Jr., W.F., Hernandez, A.D., Weis, P., 2002. Hepatic histopathology in two populations of the killifish, *Fundulus heteroclitus*. Marine Environmental Research 54, 539–542.

Schreck, C.B., 1996. Immunomodulation: endogenous factors. In: Iwama, G., Nakanishi, T. (Eds.), The Fish Immune System: Organism, Pathogen, and Environment. Academic Press, San Diego, CA, pp. 311–337.

Silkworth, J.B., Antrim, L., Kaminsky, L.S., 1984. Correlations between polychlorinated biphenyl immunotoxicity, the aromatic hydrocarbon locus, and liver microsomal enzyme induction in C57BL/6 and DBA/2 mice. Toxicology and Applied Pharmacology 75, 156–165.

Soffientino, B., Gwaltney, T., Nelson, D.R., Specker, J.L., Mauel, M., Gomez-Chiarri, M., 1999. Infectious necrotizing enteritis and mortality caused by Vibrio carchariae in summer flounder *Paralichthys dentatus* during intensive culture. Diseases of Aquatic Organisms 38 (3), 201–210.

Spielman, D., Brook, B.W., Briscoe, D.A., Frankham, R., 2004. Does inbreeding and loss of genetic diversity decrease disease resistance? Conservation Genetics 5, 439–448.

Stegeman, J.J., Wolke, R.E., 1979. Sea Grant Annual Report 1978–1979. Woods Hole Oceanographic Institute, p. 18.

Tatner, M.F., 1996. Natural changes in the immune system. In: Iwama, G., Nakanishi, T. (Eds.), The Fish Immune System. Organism, Pathogen, and Environment. Academic Press, San Diego, CA, pp. 255–287.

Taylor, M., Feyereisen, R., 1996. Molecular biology and evolution of resistance to toxicants. Molecular Biology and Evolution 13, 719–734.

Thompson, J.R., Randa, M.A., Marcelino, L.A., Tomita-Mitchell, A., Lim, E., Polz, M.F., 2004. Diversity and dynamics of a North Atlantic coastal *Vibrio* community. Applied Environmental Microbiology 70, 4103–4110.

Tillitt, D.E., Cook, P.M., Giesy, J.P., Heideman, W., Peterson, R.E., 2008. Reproductive impairment of Great Lakes lake trout by dioxin-like chemicals. In: Di Giulio, R.T., Hinton, D.E. (Eds.), The Toxicology of Fishes. Taylor and Francis, Washington, DC, pp. 819–876.

Tirindelli, J., 2007. A comparison of immunogenetic profiles between PCB contaminated and reference killifish populations. MSc thesis, San Francisco State University, San Francisco, CA.

Van Veld, P.A., Nacci, D., 2008. Toxicity resistance. In: Di Giulio, R.T., Hinton, D.E. (Eds.), The Toxicology of Fishes. Taylor & Francis, Washington, DC 697–641.

Viney, M.E., Riley, E.M., Buchanan, K.L., 2005. Optimal immune responses: immunocompetence revisited. Trends in Ecology and Evolution 20 (12), 665–669.

Weis, J.S., 2002. Tolerance to environmental contaminants in the killifish, *Fundulus heteroclitus*. Human and Ecological Risk Assessment 8 (5), 933–953.

Wirgin, I., Waldman, J.R., 2004. Resistance to contaminants in North American fish populations. Mutation Research 552, 73–100.

Xie, L., Klerks, P.L., 2004a. Fitness cost of resistance to cadmium in the least killifish (*Heterandria Formosa*). Environmental Toxicology and Chemistry 23 (6), 1499–1503.

Xie, L., Klerks, P.L., 2004b. Metallothionein-like protein in the least killifish (*Heterandria Formosa*) and its role in cadmium resistance. Environmental Toxicology and Chemistry 23 (1), 173–177.

Yada, T., Nakanishi, T., 2002. Interaction between endocrine and immune systems in fish. International Review of Cytology 220, 35–92.

Yuan, Z., Courtenay, S., Wirgin, I., 2006. Comparison of hepatic and extra hepatic induction of cytochrome P4501A by graded doses of aryl hydrocarbon receptor agonists in Atlantic tomcod from two populations. Aquatic Toxicology 76, 306–320.