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Diane Nacci

Marina Huber

See next page for additional authors

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Authors

Diane Nacci, Marina Huber, Denise Champlin, Saro Jayaraman, Sarah Cohen, Eric Gauger, Allison Fong, and Marta Gomez-Chiarri



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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

Diane Nacci¹, Marina Huber¹, Denise Champlin¹, Saro Jayaraman¹, Sarah Cohen², Eric Gauger³, Allison Fong³, and Marta Gomez-Chiarri³

¹ US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, Narragansett RI

² San Francisco State University, Department of Biology, Romberg Tiburon Center, San Francisco, CA

³ University of Rhode Island, Department of Fisheries, Aquaculture, and Veterinary Sciences, Kingston RI

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 tradeoffs 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.

Keywords

PCB tolerance; Genetic adaptation; Fitness costs; Infectious disease

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

nacci.diane@epa.gov, akualtzin@yahoo.com, champlin.denise@epa.gov, jayaraman.saro@epa.gov, sarahcoh@sfsu.edu, ejgauger@yahoo.com, fonga@hawaii.edu, gomezchi@uri.edu.

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

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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; Nacci et al. 2002a; Nacci et al. 2002b). This investigation focused on one population of killifish resident to an urban harbor, New Bedford (NB), Massachusetts (USA, Figure 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; Monnosson 1999/2000), and reduce reproductive output and early life stage survival in some 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; Nacci et al. 2002b).

A series of studies have explored demographic and toxicological compensatory mechanisms by which NB killifish persist (Nacci et al. 2002b; Nacci et al. 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; Bello et al. 2001; Nacci et al. 2002a; Nacci et al. 2008). Specifically, NB killifish and their uncontaminated progeny are protected from lethal effects of PCBs during embryonic development (Nacci et al. 1999; Nacci et al. 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-Chiari 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.

Materials and Methods

Experimental animals

Adult fish were collected by trapping and held in the laboratory as described elsewhere (Nacci et al. 1999; Nacci et al. 2005). Laboratory-reared fish (F₂ generation) were spawned from the progeny of field-collected fish and grown until about two 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; Nacci et al. 2002a; Nacci et al. 2005). Two to three weeks prior to each bacteria challenge, fish were transferred to flowing sea water tanks where 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) (Figure 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 seven-days post-hatching (Nacci et al. 2005), and results were summarized as modeled estimates of exposure concentration producing 20% lethality, LC₂₀ (Bruce and Versteeg 1992).

Bacterial Challenges

Bacterial challenges used similar proportions of male and female fish (treatment replicates ranged from 40 – 62% males) of similar size (mean length ranged from 62.5 – 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 sulphonate, 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 seven 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.

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 per ml in 10 ml 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 hours), 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: $1000 \text{ OD}_{442}/(\text{Log}(\text{CFU/ml}))$.

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. Per cent 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).

Results

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).

Field-collected fish challenges

Vibrio harveyi had a significant effect on survival of field-collected fish for each challenge conducted throughout the reproductive season (Figure 2, $p < 0.0002$). When tests were analyzed 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 (107 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).

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).

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 $\mu\text{g/ml}$, Black et al. 1999b) had no significant effect on bacterial growth rate nor specific proteolytic activity in culture supernatants (data not shown).

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; Nacci et al. 2002a). For example, the range of developmentally - toxic PCB concentrations (LC₂₀) 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 Giulio 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 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, Arkoosh et al. 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 (Figure 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. 1999b) 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; Whitman et al. 2006; 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 specifically 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 adaptation, 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; 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 2002; 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; Meyer et al. 2003a; Meyer et al. 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; Nacci et al. 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; Duffy et al. 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, our 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.

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 examples of intra-specific compensatory strategies in response to human-mediated stressors.

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

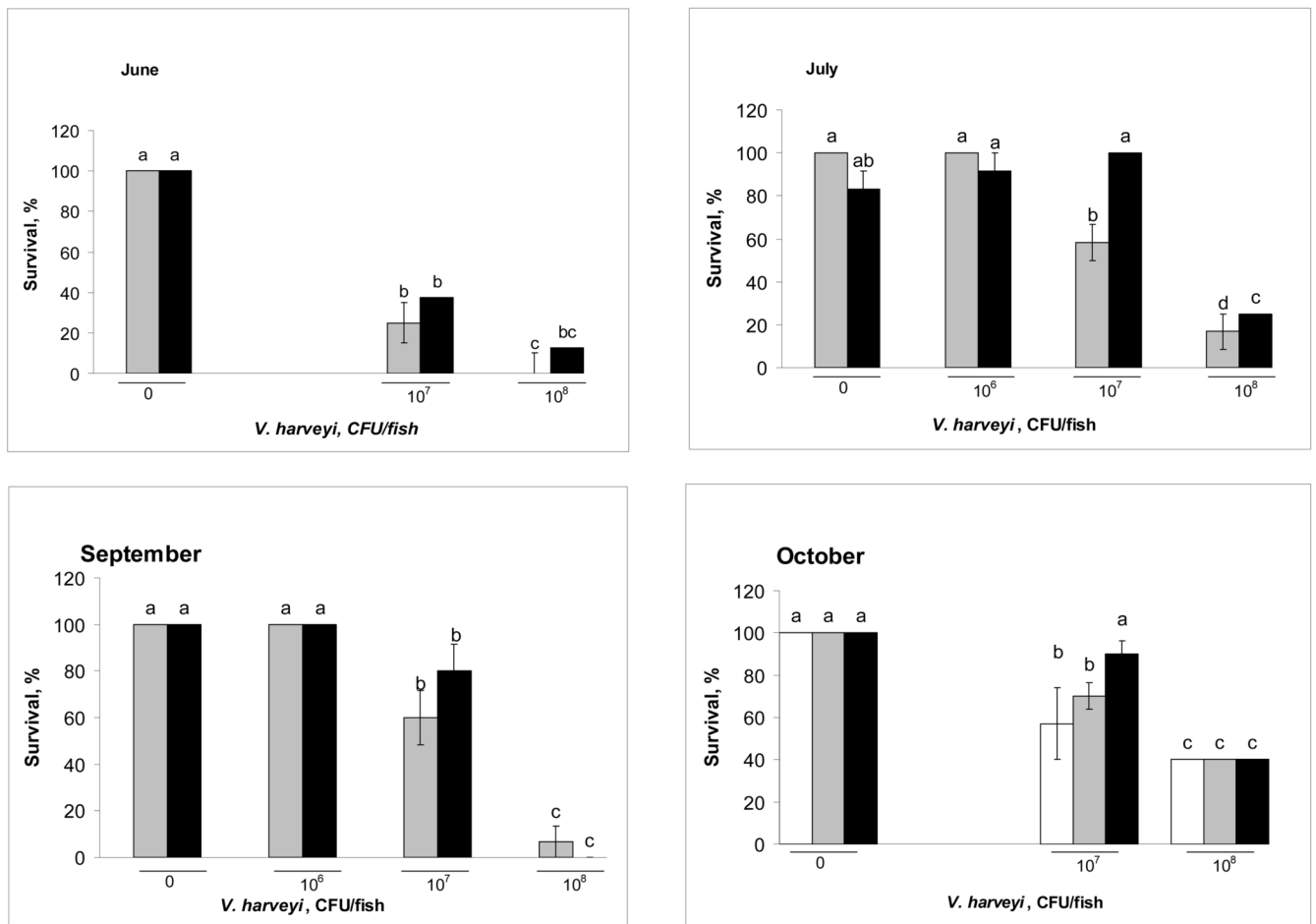


Figure 2. Cumulative seven-day survival (means and standard errors of within-test replicates) of *Fundulus heteroclitus* from West Island (gray bar), or New Bedford (black bar) (MA, USA) following injections of bacteria (*Vibrio harveyi*) during tests conducted early- (June), mid- (July), late- (September)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

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 lethality to 20% of the population (LC₂₀) using methods described in the text.

Table 1

<i>Fundulus heteroclitus</i> Parental Population	Collection Location	Primary Land Use	Sediment average PCBs, ng/g dry wt	Male Fish Liver average PCBs, ng/g dry wt (se)	Female Fish average PCBs, ng/g dry wt (se)	LC ₂₀ , ng PCB126/L
New Bedford	Fairhaven, MA	Industrial	22666 ^d	534 (119)	367 (32)	23770 ^d
West Island	Fairhaven, MA	Undeveloped	165 ^d	3.1 (0.1)	2.7 (0.4)	304 ^d
Gloucester	Gloucester, MA	Residential	14	nm	nm	84
Block Island	Block Island, RI	Undeveloped	3	nm	nm	24

^dNacci et al. 2002a

nm: not measured

Table 2

Average (\pm 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 \pm 12.5 (10)	65.2 \pm 6.9 (12)	64.6 \pm 4.1 (12)	0.831
NB	79.2 \pm 7.7 (12)	100.0 \pm 0.0 (12)	90.0 \pm 3.9 (12)	0.011
Both populations	70.5 \pm 7.2 (22)	82.6 \pm 5.0 (24)	77.3 \pm 3.8 (24)	0.0001
p	0.1607	<0.0001		

Table 3

Average survival (\pm 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 \pm 11 (3)	0 \pm 0 (3)	6.7 \pm 6.7 (3)	0.374
NB	22.3 \pm 22.3 (3)	33.3 \pm 16.7 (3)	26.7 \pm 17.6 (3)	0.627
Both populations	16.7 \pm 9.5 (6)	16.7 \pm 11.4 (6)	16.7 \pm 9.5 (6)	0.388
p	0.802	0.091		