2009

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 Nacci

Marina Huber

See next page for additional authors

Citation/Publisher Attribution

Nacci, D., Huber, M., Champlin, D., Jayaraman, S., Cohen, S., Gauger, E., Fong, A., & Gomez-Chiarri, M. (2009). Evolution of tolerance to PCBs and susceptibility to a bacterial pathogen (*Vibrio harveyi*) in Atlantic killifish (*Fundulus heteroclitus*) from New Bedford (MA, USA) harbor. *Environmental Pollution, 157*(3), 857-864. doi: 10.1016/j.envpol.2008.11.016
Available at: https://doi.org/10.1016/j.envpol.2008.11.016

Published in final edited form as:
Environ Pollut. 2009 March ; 157(3): 857–864. doi:10.1016/j.envpol.2008.11.016.

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 Nacci[1], **Marina Huber**[1], **Denise Champlin**[1], **Saro Jayaraman**[1], **Sarah Cohen**[2], **Eric Gauger**[3], **Allison Fong**[3], and **Marta Gomez-Chiarri**[3]

[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.

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-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.

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 (F_2 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, LC_{20} (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 OD_{442}/(Log (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 μg/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 (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 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 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; 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, 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.

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.

Acknowledgments

We appreciate the technical assistance of Jane Copeland, and James Heltshe, and the helpful advice from reviewers of early drafts, Glen Thursby, Jason Grear, and Anne Kuhn, as well as that of anonymous reviewers contributing to the final version of this manuscript. This is contribution number AED-05-079 of the U.S. EPA ORD NHEERL Atlantic Ecology Division. Although the research described in this contribution has been funded partially by the U.S EPA, it has not been subjected to Agency-level review. Therefore, it does not necessarily reflect the views of the agency. Mention of trade names, products, or services does not constitute endorsement or recommendation for use. E. Gauger received support from the Rhode Island Agricultural Experiment Station. Funding for A. Fong was provided by the University of Rhode Island Coastal Fellowship program. Some aspects of this research were also made possible by the use of the Rhode Island-INBRE Research Core facility, supported by Grant #P20 RR16457 from NCRR, NIH.

Citations

Acevedo-Whitehouse K, Cunningham AA. Is MHC enough for understanding wildlife immunogenetics? Trends in Ecology and Evolution 2006;21:433–438. [PubMed: 16764966]

Acevedo-Whitehouse K, Gulland F, Greig D, Amos W. Inbreeding: disease susceptibility in California sea lions. Nature 2003;422:35. [PubMed: 12621424]

Adams SM, Lindmeier JB, Duvernell DD. Microsatellite analysis of the phylogeography and secondary contact hypotheses for the killifish, *Fundulus heteroclitus*. Molecular Ecology 2006;15:1109–1123. [PubMed: 16599970]

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

Arkoosh MR, Clemons E, Huffman P, Kagley AN. 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 2001;13:257–268.

Arkoosh, MR.; Boylen, D.; Stafford, CL.; Johnson, LL.; Collier, TK. Use of disesase challenge assay to assess immunotoxicity of xenobiotics in fish. In: Ostrander, GK., editor. Techniques in Aquatic Toxicology. Vol. 2. CRC Press; Boca Raton, FL, USA: 2005. p. 19-38.

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

Bard SM, Bello SM, Hahn ME, Stegeman JJ. Expression of P-glycoprotein in killifish (*Fundulus heteroclitus*) exposed to environmental xenobiotics. Aquatic Toxicology 2002;59:237 – 251. [PubMed: 12127740]

Bello SM, Franks DG, Stegeman JJ, Hahn ME. 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 2001;60:77–91. [PubMed: 11222875]

Black DE, Gutjahr-Gobell R, Pruell RJ, Bergen B, Mills L, McElroy AE. Reproduction and polychlorinated biphenyls in *Fundulus heteroclitus* (Linnaeus) from New Bedford Harbor, Massachusetts, USA. Environmental Toxicology and Chemistry 1998a;17:1405–1414.

Black DE, Gutjahr-Gobell R, Pruell RJ, Bergen B, McElroy AE. Effects of a mixture of non-*ortho*- and mono-*ortho*-polychorinated biphenyls on reproduction in *Fundulus heteroclitus* (Linnaeus). Environmental Toxicology and Chemistry 1998b;17:1396–1404.

Bruce RD, Versteeg DJ. A statistical procedure for modeling continuous toxicity data. Environmental Toxicology and Chemistry 1992;11:1485 – 1494.

Burnett KG, Bain LJ, Baldwin WS, Callard GV, Cohen S, Di Giulio RT, Evans DH, Gomez-Chiarri M, Hahn ME, Hoover CA, Karchner SI, Katoh F, MacLatchy DL, Marshall WS, Meyer JN, Nacci DE, Oleksiak MF, Rees BB, Singer TP, Stegeman JJ, Towle DW, Van Veld PA, Vogelbein WK, Whitehead A, Winn R, Crawford DL. *Fundulus* as the premier teleost model in environmental biology: opportunities for new insights using genomics. Comparative Biochemistry and Physiology, Part D 2007;2:257–286.

Camp, Kl; Wolters, WR.; Rice, CD. Survivability and immune responses after challenge with *Edwardsiella ictaluri* in susceptible and resistant families of channel catfish, *Ictalurus punctatus*. Fish and Shellfish Immunology 2000;10:475 –487. [PubMed: 11016583]

Carlson, E.; Zelikoff, JT. The immune system of fish: a target organ of toxicity. In: Di Giulio, RT.; Hinton, DE., editors. The Toxicology of Fishes. Taylor and Francis; Washington DC: 2008. p. 489-530.

Cohen S. Strong positive selection and habitat-specific amino acid substitution patterns in *Mhc* from an estuarine fish under intense pollution stress. Molecular Biology and Evolution 2002;19:1870–1880. [PubMed: 12411596]

Cohen CS, Tirindelli J, Gomez-Chiarri M, Nacci D. Functional implications of Major Histocompatibility variation using estuarine fish populations. Integrative and Comparative Biology 2006;46(6):1016–1029.

Coltman DW, Pilkington JG, Smith JA, Pemberton J. Parasite mediated selection against inbred Soay sheep in a free-living, island population. Evolution 1999;53:1259 – 1267.

Coustau C, Chevillon C, French-Constant R. Resistance to xenobiotics and parasites: can we count the costs. Trends in Ecology and Evolution 2000;15:378 –383. [PubMed: 10931680]

Dautremepuits C, Fortier M, Croisetiere S, Belhumeur P, Fournier M. Modulation of juvenile brook trout (*Salvelinus fontalinalis*) cellular immune system after Aeromonas salmonicida challenge. Veterinary Immunology and Immunopathology 2006;110:27 –36. [PubMed: 16263179]

Denkin SM, Nelson DR. Induction of protease activity in *Vibrio anguillarum* by gastrointestinal mucus. Applied and Environmental Microbiology 1999;65(8):3555 –3560. [PubMed: 10427048]

Duffy JE, Zelikoff JT. The relationship between noncoplanar PCB-induced immunotoxicity and hepatic CYP1A induction in a fish model. Journal of Immunotoxicology 2006;3:39 – 47. [PubMed: 18958684]

Duffy JE, Carlsen E, Li Y, Prophete C, Zelikoff JT. Impact of polychlorinated biphenyls (PCBs) on the immune function of fish: age as a variable in determining outcome. Marine Environmental Research 2002;54:559– 563. [PubMed: 12408617]

Duffy JE, Li Y, Zelikoff JT. PCB-induced hepatic CYP1A induction is associated with innate immune dysfunction in a feral teleost fish. Bulletin of Environmental Contamination and Toxicology 2005;74:107 – 113. [PubMed: 15768506]

Duron O, Labbe P, Berticat C, Rousset F, Guillot S, Raymond M, Weill M. High *Wolbachia* density correlates with cost of infection for insecticide resistant *Culex pipiens* mosquitoes. Evolution 2006;60 (2):303 – 314. [PubMed: 16610322]

Duvernelle DD, Lindmeier JB, Faust KE, Whitehead A. Relative influences of historical and contemporary forces shaping the distribution of genetic variation in the Atlantic killifish, *Fundulus heteroclitus*. Molecular Ecology 2008;17:1344 – 1360. [PubMed: 18302693]

Elonen GE, Spehar RL, Holcombe GW, Johnson RD, Fernandez JD, Erickson EJ, Tietge JE, Cook PM. Comparative toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin to seven freshwater fish species during early life-stage development. Environmental Toxicology and Chemistry 1998;17(3):472–483.

Elskus AA, Monosson E, McElroy AE, Stegeman JJ, Woltering DS. Altered CYP1A expression in *Fundulus heteroclitus* adults and larvae: a sign of pollutant resistance? Aquatic Toxicology 1999;45:99 – 113.

Fisher MA, Oleksiak MF. Convergence and divergence in gene expression among natural populations exposed to pollution. BMC Genomics 2007;8:108. [PubMed: 17459166]

Frederick Lee A, Van Veld, Peter A, Rice Charles D. Bioindicators of Immune Function in Creosote-adapted Estuarine Killifish, *Fundulus heteroclitus*. Journal of Toxicology and Environmental Health, Part A 2007;70(17):1433–1442. [PubMed: 17687729]

Fries CR. Effects of environmental stressors and immunosuppressants on immunity in *Fundulus heteroclitus*. American Zoologist 1986;26:271 – 282.

Futuyma, DJ. Evolutionary Biology. Sinauer Associates; Sunderland, MA: 1986.

Gauger EJ, Smolowitz R, Uhlinger K, Casey J, Gomez-Chiarri M. *Vibrio harveyi* and other bacterial pathogens in cultured summer flounder, *Paralichthys dentatus*. Aquaculture 2006;260:10 – 20.

Gauger EJ, Gomez-Chiarri M. 16S ribosomal DNA sequencing confirms synonymy of *Vibrio harveyi* and *V. carchariae*. Diseases of Aquatic Organisms 2002;52:39 – 46. [PubMed: 12517004]

Greytak SR, Callard GV. Cloning of three estrogen receptors (ER) from killifish (*Fundulus heteroclitus*): differences in expression in populations from polluted and reference environments. General and Comparative Endocrinology 2007;150(1):174 – 188. [PubMed: 16962596]

Greytak SR, Champlin D, Callard GV. 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 2005;71:371 – 389. [PubMed: 15710484]

Gutjahr-Gobell RE, Black DE, Mills L, Pruell R, Taplin BK, Jayaraman S. Feeding the killifish (*Fundulus heteroclitus*) a diet spiked with non-*ortho*-and mono-*ortho*-substituted polychlorinated biphenyls: accumulation and effects. Environmental Toxicology and Chemistry 1999;18:699–707.

Hahn ME. Mechanisms of innate and acquired resistance to dioxin-like compounds. Reviews in Toxicology 1998;2:395–443.

Hahn ME, Karchner SI, Franks DG, Merson RR. Aryl hydrocarbon receptor polymorphisms and dioxin resistance in Atlantic killifish (*Fundulus heteroclitus*). Pharmacogenetics 2004;14:131–143. [PubMed: 15077014]

Hale KA, Briskie JV. Decreased immunocompetence is a severely bottlenecked population of endemic New Zealand bird. Animal Conservation 2007;10:2 – 10.

Harris J, Bird DJ. Modulation of the fish immune system by hormones. Veterinary Immunology and Immunopathology 2000;77:163 – 176. [PubMed: 11137116]

Harris CE, Vogelbein WK. Parasites of killifish, *Fundulus heteroclitus*, from the York River, Virginia, USA, with a checklist of parasites of Atlantic Coast *Fundulus* spp. Comparative Parasitology 2006;73 (1):72 – 110.

Hicks T, Steele E. Histological effect of *Ascocotyle tenuicollis* (Digenea: Heterophyidae) metacercarial infection on the heart of *Fundulus heteroclitus* (Teleostei: Cyprinodontidae). Journal of the South Carolina Academy 2003;1:10 –18.

Kerkvliet N. Recent advances in understanding the mechanisms of TCDD immunotoxicity. International Journal of Immunopharmacology 2002;2:277 – 291.

Kerkvliet NI, Baecher-Steppan L, Smith BB, Youngerberg JA, Henderson MC, Buhler DR. 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 1990;14:532 – 541. [PubMed: 2111257]

Kinnison MT, Hairston NG Jr. Eco-evolutionary conservation biology: evolution and the dynamics of persistence. Functional Ecology 2007;21:444 – 454.

Kurtz J, Kalbe M, Aeschlimann PB, Haberli MA, Wegner KM, Reusch BH, Milinshki M. Major histocompatibility complex diversity influences parasite resistance and innate immunity in sticklebacks. Proceeding of the Royal Society of London B 2003;271:197 – 204.

Kurtz J, Kalbe M, Langefors A, Mayer I, Milinski M, Hasselquist D. An experimental test of the immunocompetence handicap hypothesis: 11-ketotestosterone suppresses innate immunity in three-spined sticklebacks. The American Naturalist 2007;170(4):509 – 519.

Levinton, JS.; Klerks, P.; Martinez, DE.; Montero, C.; Sturmbauer, C.; Suatoni, L.; Wallace, W. 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., editor. Aquatic Life Cycle Strategies. Marine Biological Association of the United Kingdom; 1999.

Long ER, MacDonald DD, Smith SL, Calder FD. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environmental Management 1995;19:81 – 97.

Luebke RW, Hodson PV, Faisal M, Ross PS, Grasman KA, Zelikoff J. Aquatic pollution-induced immunotoxicity in wildlife species. Fundamental and Applied Toxicology 1997;37:1 – 15. [PubMed: 9193919]

Luebke RW, Chen DH, Dietert R, Yang Y, King M, Luster MI. The comparative immunotoxicity of five selected compounds following developmental or adult exposure. Journal of Toxicology and Environmental Health, Part B 2006;9:1 – 26.

Magnadottir B. Innate immunity of fish (overview). Fish and Shellfish Immunology 2006;20(2):137 – 151. [PubMed: 15950491]

Martinez DE, Levinton J. Adaptation to heavy metals in the aquatic oligochaete *Limnodrilus hoffmeisteri*: evidence for control by one gene. Evolution 1996;50:1339 – 1343.

Maule AG, Schrock R, Slater C, Fitzpatrick MS, Schreck CB. Immune and endocrine responses of adult Chinook salmon during freshwater immigration and sexual maturation. Fish and Shellfish Immunology 1996;6:221 – 233.

Maule AG, Jorgensen EH, Vijayan MM, Killie JA. Arochlor 1254 exposure reduced disease resistance and innate immune responses in fasted arctic char. Environmental Toxicology and Chemistry 2005;24:117 – 124. [PubMed: 15683174]

McKenzie JA, Batterham P. The genetic, molecular, and phenotypic consequences of selection for pesticide resistance. Trends in Ecology and Evolution 1994;9:166 – 169.

McMillan AM, Bagley MJ, Jackson SA, Nacci DE. Genetic diversity and structure of an estuarine fish (*Fundulus heteroclitus*) indigenous to sites associated with a highly contaminated urban harbor. Ecotoxicology 2006;15:539–548. [PubMed: 16988885]

Meyer JN, Di Giulio RT. Heritable adaptation and associated fitness tradeoffs in killifish (*Fundulus heteroclitus*) inhabiting a contaminated estuary. Ecological Applications 2003;13:490–503.

Meyer JN, Smith JD, Winston GD, Di Giulio RT. Antioxidant defenses in killifish (*Fundulus heteroclitus*) exposed to Superfund sediments and model prooxidants: short-term and heritable responses. Aquatic Toxicology 2003a;65:377 – 395. [PubMed: 14568353]

Meyer JN, Wassenberg DM, Karchner SI, Hahn ME, Di Giulio RT. Expression and inducibility of aryl hydrocarbon receptor pathway genes in wildcaught killifish (*Fundulus heteroclitus*) with different contaminant-exposure histories. Environmental Toxicology and Chemistry 2003b;22:2337 – 2343. [PubMed: 14551997]

Meyer JN, Nacci DE, Di Giulio RT. Cytochrome P4501A (CYP1A) in killifish (*Fundulus heteroclitus*): heritability of altered expression and activity and relationship to survival in contaminated sediments. Toxicological Sciences 2002;68:69–81. [PubMed: 12075112]

Meyer, Jn; Volz, DC.; Freedman, JH.; Di Giulio, RT. Differential display of hepatic mRNA from killifish (*Fundulus heteroclitus*) inhabiting a Superfund estuary. Aquatic Toxicology 2005;73:327 – 341. [PubMed: 15916819]

Mitton, JB. Selection in natural populations. Oxford University Press; New York: 1994.

Monnosson E. Reproductive and developmental effects of PCBs in fish: a synthesis of laboratory and field studies. Reviews in Toxicology 19992000;3:25 – 42.

Mulvey M, Newman MC, Vogelbein W, Unger MA, Ownby DR. Genetic structure and mtDNA diversity of *Fundulus heteroclitus* populations from polycyclic aromatic hydrocarbon-contaminated sites. Environmental Toxicology and Chemistry 2003;22:671 – 677. [PubMed: 12627657]

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

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

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

Nacci D, Coiro L, Champlin D, Jayaraman S, McKinney R. Predicting the occurrence of genetic adaptation to dioxin-like compounds in populations of the estuarine fish *Fundulus heteroclitus*. Environmental Toxicology and Chemistry 2002a;21(7):1525–1532. [PubMed: 12109755]

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

Nacci D, Kohan M, Coiro L, George E. Effects of Benzo(a)pyrene Exposure on a PCB-adapted Fish Population. Aquatic Toxicology 2002;57:203–215. [PubMed: 11932001]

Nacci, D.; Coiro, L.; Wasserman, D.; Di Giuilio, R. A non-destructive technique to measure Cytochrome P4501A enzyme activity in living embryos of the estuarine fish *Fundulus heteroclitus*. In: Ostrander, GK., editor. Techniques in Aquatic Toxicology. Vol. 2. Taylor and Francis; Boca Raton, FL: 2005. p. 209-225.

Nacci, D.; Walters, S.; Gleason, T.; Munns, WR, Jr. 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., editors. Population-level Ecotoxicology. Oxford University Press; North Carolina: 2008.

Nelson, WG.; Bergen, BJ.; Benyi, SJ.; Morrison, G.; Voyer, RA.; Strobel, CJ.; Rego, S.; Thursby, G.; Pesch, CE. New Bedford harbor long-term monitoring assessment report: baseline sampling. U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division; Narragansett, RI: 1996. EPA/600/R-96/097

Neumann NF, Stafford JL, Barreda D, Ainsworth AJ, Belosevic M. Antimicrobial mechanisms of fish phagocytes and their role in host defense. Developmental and Comparative Immunology 2001;25:607 –825. [PubMed: 11472782]

Nordlie FG. Physicochemical environments and tolerances of cyprinodontoid fishes found in estuaries and salt marshes of eastern Northe America. Reviews in Fish Biology and Fisheries 2006;16:51 –106.

Palm RC Jr, Powell DB, Skillman A, Godtfredsen K. Immunocompetence of juvenile chinook salmon against *Listonella anguillarum* following dietary exposure to polycyclic aromatic hydrocarbons. Environmental Toxicology and Chemistry 2003;22:2986–2994. [PubMed: 14713040]

Prince R, Cooper KR. 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 1995;14:579 – 588.

Råberg L, Sim D, Read AF. Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals. Science 2007;318:812 –814. [PubMed: 17975068]

Raymond M, Berticat C, Weill M, Pasteur N, Chevillon C. Insecticide resistance in the mosquito (*Culex pipiens*): what have we learned about the adaptation? Genetica 2001;112:287–296. [PubMed: 11838771]

Regala RP, Rice CD, Schwedler TE, Dorociak IR. 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 Environmental Contamination and Toxicology 2001;40:386 – 391.

Reid JM, Arcese P, Keller LF. Inbreeding depresses immune response in song sparrows (*Melospiza melodia*): direct and intergenerational effects. Proceedings Royal Society London Series B 2003;270:2151 – 2157.

Rice, CD. Fish immunotoxicology: understanding mechanisms of action. In: Schlenk, D.; Benson, WH., editors. Target Organ Toxicity in Marine and Freshwater Teleosts. Vol. 2. Taylor & Francis; New York: 2001. p. 96-138.Systems

Rice CD, Schlenck D. 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 1995;7:195 – 204.

Rice CD, Xiang Y. Immune function, hepatic CYP1A, and reproductive biomarker responses in the gulf killifish, *Fundulus grandis*, during dietary exposures to endocrine disrupters. Marine Environmental Research 2000;50:163 –168. [PubMed: 11460684]

Roark SA, Nacci D, Coiro L, Champlin D, Guttman SI. Population genetic structure of a non-migratory marine fish *Fundulus hetero*clitus across a strong gradient of PCB contamination. Environmental Toxicology and Chemistry 2005;24(3):717 – 725. [PubMed: 15779774]

Roush RT, McKenzie JA. Ecological genetics of insecticide and acaricide resistance. Annual Review Entomology 1987;32:361 –380.

SAS Institute. SAS® User's Guide. Cary, NC, USA: 2000. p. 1686

Safe S. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assess. CRC Critical Reviews in Toxicology 1994;24:87 – 149. [PubMed: 8037844]

Schmalz WF Jr, Hernandez AD, Weis P. Hepatic histopathology in two populations of the killifish, *Fundulus heteroclitus*. Marine Environmental Research 2002;54:539 – 542. [PubMed: 12408613]

Schreck, CB. Immunomodulation: endogenous factors. In: Iwama, G.; Nakanishi, T., editors. The Fish Immune System: Organism, Pathogen, and Environment. Academic Press; San Diego: 1996. p. 311-337.

Silkworth JB, Antrim L, Kaminsky LS. 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 1984;75:156 – 165. [PubMed: 6431639]

Soffientino B, Gwaltney T, Nelson DR, Specker JL, Mauel M, Gomez-Chiarri M. Infectious necrotizing enteritis and mortality caused by Vibrio carchariae in summer flounder *Paralichthys dentatus* during intensive culture. Diseases of Aquatic Organisms 1999;38(3):201– 210. [PubMed: 10686671]

Spielman D, Brook BW, Briscoe DA, Frankham R. Does inbreeding and loss of genetic diversity decrease disease resistance? Conservation Genetics 2004;5:439 – 448.

Stegeman JJ, Wolke RE. Woods Hole Oceanographic Institute, Sea Grant Annual Report 1978 – 1979 1979:18.

Tatner, MF. Natural changes in the immune system. In: Iwama, G.; Nakanishi, T., editors. The Fish Immune System: Organism, Pathogen, and Environment. Academic Press; San Diego: 1996. p. 255-287.

Taylor M, Feyereisen R. Molecular biology and evolution of resistance to toxicants. Molecular Biology and Evolution 1996;13:719 – 734. [PubMed: 8754209]

Thompson JR, Randa MA, Marcelino LA, Tomita-Mitchell A, Lim E, Polz MF. Diversity and dynamics of a North Atlantic coastal *Vibrio* community. Applied. Environmental. Microbiology 2004;70:4103 – 4110.

Tillit, DE.; Cook, PM.; Giesy, JP.; Heideman, W.; Peterson, RE. Reproductive impairment of Great Lakes lake trout by dioxin-like chemicals. In: Di Giulio, RT.; Hinton, DE., editors. The Toxicology of Fishes. Taylor and Francis; Washington DC: 2008. p. 819-876.

Tirindelli, J. Master of Science. San Francisco State University; San Francisco, CA: 2007. A comparison of immunogenetic profiles between PCB contaminated and reference killifish populations.

Van Veld, PA.; Nacci, D. Toxicity resistance. In: Di Giulio, RT.; Hinton, DE., editors. The Toxicology of Fishes. Taylor and Francis; Washington DC: 2008. p. 697-641.

Viney ME, Riley EM, Buchanan KL. Optimal immune responses: immunocompetence revisited. Trends in Ecology and Evolution 2005;20(12):665 –669. [PubMed: 16701455]

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

Wirgin I, Waldman JR. Resistance to contaminants in North American fish populations. Mutation Research 2004;552:73 – 100. [PubMed: 15288543]

Xie L, Klerks PL. Fitness cost of resistance to cadmium in the least killifish (*Heterandria Formosa*). Environmental Toxicology and Chemistry 2004a;23(6):1499 – 1503. [PubMed: 15376535]

Xie L, Klerks PL. Metallothionein-like protein in the least killifish (*Heterandria Formosa*) and its role in cadmium resistance. Environmental Toxicology and Chemistry 2004b;23(1):173 – 177. [PubMed: 14768882]

Yada T, Nakanishi T. Interaction between endocrine and immune systems in fish. International Review of Cytology 2002;220:35 – 92. [PubMed: 12224552]

Yuan Z, Courtenay S, Wirgin I. 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 2006;76:306–320. [PubMed: 16313980]

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

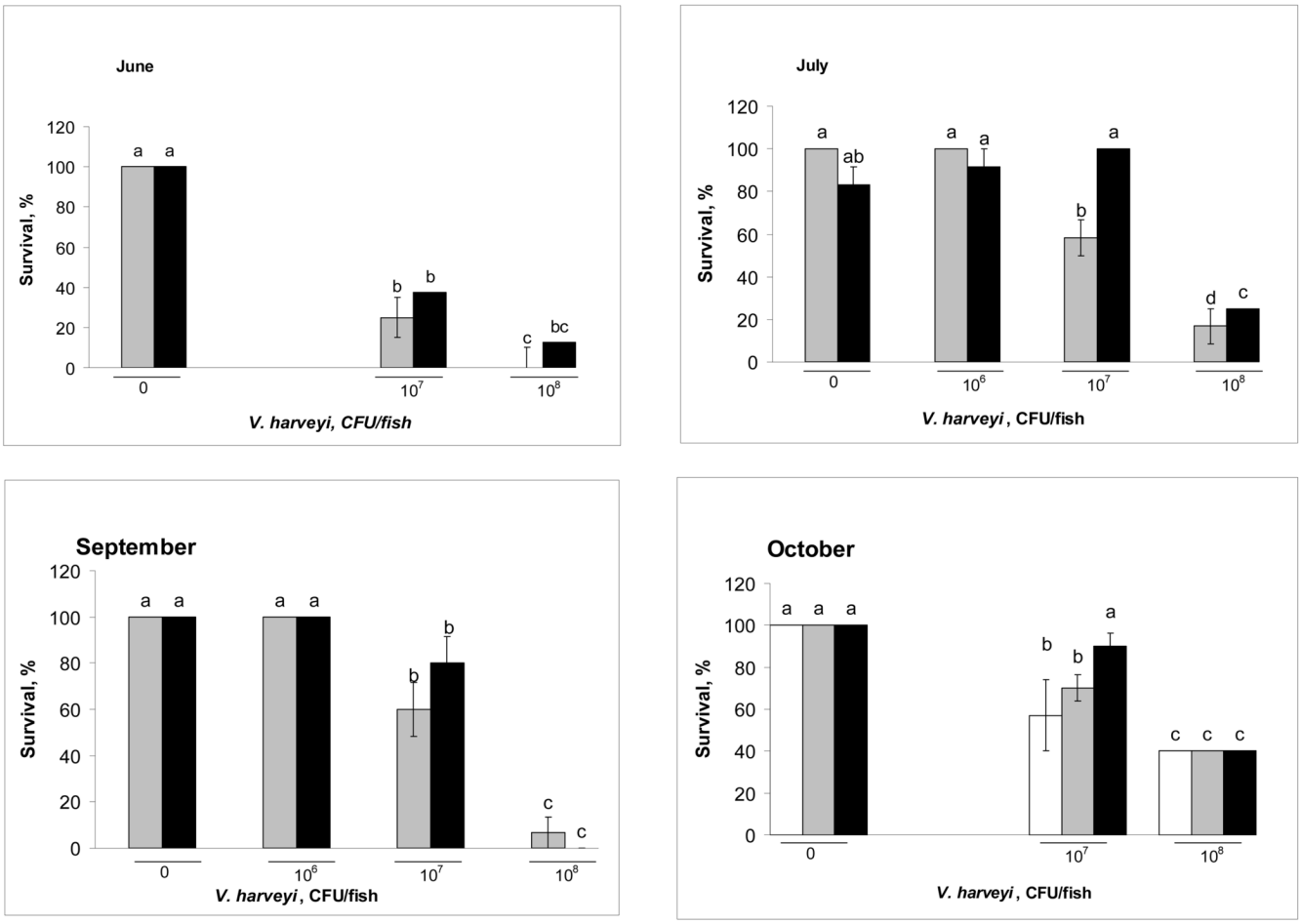

Figure 2.

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.

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

[a] Nacci et al. 2002a

nm: not measured

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), Massachussetts, 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		

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), Massachussetts, 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		