Evolutionary Toxicology: Population Adaptation in Response to Anthropogenic Pollution

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Abstract Anthropogenic activity has affected nearly every environment on the planet. The changes that have occurred as a consequence of human activities have altered aquatic habitats by exacerbating already existing extreme environments and by introducing novel stressors. In some cases, particularly adjacent to heavily industrialized areas, these changes have introduced sufficient novel selective pressures to drive resident populations to genetically adapt in order to survive in the altered habitats, while species that were unable to adapt have been extirpated from these extreme environments. In this chapter, we aim to explore the effects of natural and novel stressors, resulting from anthropogenic activity, on fish populations. We will provide an overview of the possible multi-generational outcomes of anthropogenic contamination, as well as explore documented examples of population-wide changes that have occurred. We present case studies, including population responses to UV light, radionuclides, and metals contamination, as well as adaptational responses to persistent organic pollutants. Through this examination, we aim to not only give an overview of the existing evolutionary changes in fish populations in response to anthropogenic contamination, but also identify future areas of research on the impacts, long-term persistence, and ecological significance of these effects.

1 Anthropogenic Changes to Aquatic Environments

There is great natural variability in aquatic environments, which has provided the grounds for the phenomenal diversification and adaptation of fish species to fill available ecological niches. In addition to adaptation along natural environmental gradients, some fish possess unique phenotypes, which allow them to survive under the most extreme environmental conditions. Modern extreme environments include many aquatic habitats that have been so thoroughly contaminated by human activities that many aquatic species are unable to survive or reproduce in them.

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R. Riesch et al. (eds.), *Extremophile Fishes*, DOI 10.1007/978-3-319-13362-1_11

These are generally located in heavily industrialized areas or in waterways downstream of industrial activity, and are characterized by dramatic reductions in species diversity. Anthropogenic changes to the climate and environment are increasingly altering freshwater and marine habitats, and will require adaptive responses from resident fish populations to allow for their persistence (Moe et al. 2013; Rosenzweig and Neofotis 2013; Anderegg et al. 2010). As adaptation involves allele frequency changes over many generations, species with shorter generation times are likely to adapt more rapidly to ongoing anthropogenic environmental alterations. Thus, human-induced evolutionary change has been observed more often in simple unicellular or invertebrate species (Palumbi 2001; Medina et al. 2007).

Human-induced environmental changes can act to exacerbate preexisting extreme environments by increasing natural stressors, which include, but are not limited to, salinity/conductivity, temperature, metals, UV radiation, and pH balance (Kaushal et al. 2005; Poloczanska et al. 2013; Core Writing Team et al. 2007; Dijkstra et al. 2013; Veron 2008). In addition to augmenting existing extreme environments, anthropogenic activities have caused compounds with low natural occurrences to reach concentrations high enough to cause toxicity, thus creating new selective pressures shaping evolutionary change in natural populations. These new selective pressures will be referred to hereafter as novel stressors. Some pollutants, such as synthetic estrogenic compounds, pharmaceuticals, polychlorinated dibenzo dioxins/furans (PCDD/Fs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs), have known modes of action affecting the behavior, reproduction, and survival of fish (Knecht et al. 2013; Incardona et al. 2004, 2011; Clark et al. 2010; Matson et al. 2009; Wassenberg and Di Giulio 2004a; Nebert et al. 2004; Mennigen et al. 2011). Sources for these contaminants include heavy industrial activity, which leads to the production of many persistent organic pollutants (POPs) (Wenning et al. 1993; Walker et al. 2005), and urban water use, including the release of pharmaceuticals into freshwater and marine environments (Camacho-Munoz et al. 2014). Many of these compounds have been shown to adversely affect developmental processes (Wassenberg et al. 2002), survival (Nacci et al. 2010), and reproduction (Valenti et al. 2012) at environmentally relevant concentrations. As a result, environments with high concentrations of contamination pose a threat to resident aquatic biota (Wassenberg et al. 2002). Environmentally relevant concentrations of estrogenic compounds have been tested under realistic exposure conditions and have been shown to be detrimental to populations of fish (Kidd et al. 2007), whereas some persistent pollutants have already led to evolutionary adaptation in fish populations in the wild (Wirgin et al. 2011; Nacci et al. 2010). By focusing on the population-level responses of aquatic organisms to anthropogenic pollution, we account for the cumulative impacts of these novel extreme environments on organismal survival and reproduction. As discovery of human-induced phenotypic and genetic change has increased, attention to the developing field of evolutionary toxicology has also expanded and is likely to continue to grow, as new anthropogenic stressors are unleashed upon the environment (Bickham et al. 2000).

The effects of environmental stressors on populations of fish depend largely on the magnitude, duration, and frequency of the exposure. Even short-term exposures, such as transient oil spills or temporary encounters of a contaminated environment by a migratory species, can lead to trans- or multi-generational effects in organisms (Dubansky et al. 2013; Everett et al. 2012; Gao et al. 2011). Chronic exposure of sufficient magnitude to affect reproductive success or the integrity of genetic material in individuals can lead to evolutionary adaptation, making individuals more fit for those environments (Bickham 2011). Evolutionary theory would predict rapid spread and fixation of preexisting alleles (even when initially rare), if they conferred a selective advantage in responding to a new anthropogenic stressor (Bickham 2011; Bickham et al. 2000), while alternatively, the likelihood of a beneficial de novo mutation becoming common within a fish population in a century or less is less likely. Either way, we can only evaluate the adaptive changes after many generations, which makes evolutionary studies difficult when we are considering anthropogenic stressors introduced into the environment generally less than a century ago.

2 Organismal Responses to Stressors

When attempting to observe the effects of a stressor on populations of fish, we need to consider not only the characteristics of the event itself but also the various ways by which organisms are able to respond. Acclimation refers to a response in which exposed individuals are capable of dealing with the stressor by altering gene expression, protein levels or activity, or other physiological processes. In this case, the organisms can tolerate the stressful event depending on their genetic makeup and the degree of plasticity of relevant traits. Such changes in expression or activity can have trans-generational effects, but they are not genetically heritable. Thus, in the absence of the stressor, the changes in gene expression or physiology are likely to disappear within two generations. Mounting a physiological response can have various consequences, including higher energy expenditure, and long-term costs often depend on the severity and duration of the response.

There exists a middle ground between acclimation and adaptation that may result in long-term or multi-generational effects of stressors on populations of fish. The group of responses that encompass non-genetic, yet heritable, changes that affect the expression or behavior of genes are called epigenetic alterations (Laird 2003). Epigenetic events are often environmentally driven and include, but are not limited to, DNA methylation on cytosine nucleotides, acetylation on lysine residues of histones, and differential microRNA expression (Wolffe and Matzke 1999). Events such as the methylation of cytosine have been observed to increase mutation rates in those specific genomic regions and provide one of the connections between short-term exposures and resulting DNA alterations (Fryxell and Moon 2005; Gonzalgo and Jones 1997). Thus, with epigenetics in mind, even short-term environmental events can result in heritable multi-generational outcomes, including

altered phenotypes resulting from differential gene expression (Guerrero-Bosagna and Skinner 2012; Pfennig and Servedio 2013). The field of epigenetics is very young, but researchers are making major strides in the understanding of this bridge between acclimation and adaptation. Even though we have a limited understanding of the impacts of these heritable non-genetic changes, they provide a potentially very important mechanism by which populations can respond to new stressors more rapidly than through more traditional evolutionary processes. Including epigenetic processes within the field of evolutionary toxicology or human-induced evolution radically expands the number of populations that could potentially be studied.

To study adaptive responses to anthropogenic contaminants, a model organism is needed that has sufficient genetic variability to provide the capacity for rapid adaptation to varying selective pressures. Teleost fishes are a unique and rich system to study novel gene function, partially because of the multiple gene duplications that have occurred in some lineages, resulting in as many as eight gene copies of some genes (Pittman et al. 2013). The availability of multiple gene copies reduces the selective disadvantage normally associated with new mutations. It also increases the opportunity for differential gene regulation, alternative splicing, or other post-translational modifications (Pittman et al. 2013). In addition to gene duplications, the potential for rapid environmental changes in aquatic systems has also selected for species with the capacity to acclimate and adapt to fluctuating environmental conditions. One example of this would be the highly variable nature of estuarine environments favoring euryhaline fish species, which are able to survive dramatic swings in salinity. Such conditions seem to produce robust species that have the genetic capability to respond relatively quickly to changes in environmental stressors (Pittman et al. 2013). Some euryhaline teleosts have previously been studied for their capability to tolerate high variability in temperature, salinity, and pollutants (Crawford and Powers 1992; Whitehead et al. 2011; Nacci et al. 1999). In the case of thermal adaptation, it has been shown that both acclimation and genetic alterations contribute to the physiological adaptation in Atlantic killifish (*Fundulus heteroclitus*) from different sites (Crawford and Powers 1989). Multiple similar studies suggest that estuarine teleosts may be valuable model organisms for identifying genetic and physiological responses to novel stressors. Their adaptability, ubiquitous presence, and short generation times allow for the investigation of the evolutionary effects of contaminants over relatively short time frames.

A previous summary focused on the mechanistic details of specific examples of adaptation to contaminants in North American fish populations (Wirgin and Waldman 2004). In this chapter, we aim to give an overview of anthropogenic alterations to the environment that are likely to or have already driven evolutionary adaptations in fish. We will discuss two major categories of anthropogenic environmental alterations: alteration of existing natural stressors and the introduction of novel stressors. In order to more comprehensively cover the possible genetic influences, we will discuss both short-term responses with multi-generational effects as well as mutations and shifts in allele frequencies that have led to evolutionary adaptation in fish species.

3 Altered Natural Stressors

3.1 UV Light

One of the many natural stressors that has been altered by anthropogenic activity is UV light penetration into aquatic environments. Factors such as the thickness of the ozone layer, amount of colored dissolved organic matter, and cloud density dictate the intensity and the amount of UV light that reaches aquatic organisms (Hader et al. 2011). Among the well-studied anthropogenic contributions to the increase of both UV-A (315–400 nm) and UV-B (280–315 nm) radiation is ozone depletion (Hader et al. 2011; Core Writing Team et al. 2007). The mechanisms of UV toxicity have been well documented to include dimerizations of thymine DNA bases, increase of reactive oxygen species (ROS) production, and an increased production of the adduct 8-hydroxyguanosine—a biomarker of oxidative stress (Durbeej and Eriksson 2002; Zhang et al. 2004). In addition, UV light has been known to exasperate the toxicity of some PAHs in various organisms because of common modes of toxicity or photoactivation of these compounds (Marquis et al. 2009; Nikkila et al. 1999; Shemer and Linden 2007; Wei et al. 2007). The synergistic increase in toxicity, however, is also accompanied by higher rates of degradation of PAHs due to UV light (Xu and Li 2014; Buth et al. 2010; Peachey 2005). Given these possible adverse effects of UV light, aquatic organisms that are dependent on light, such as algae, have developed methods to minimize UV exposure via mycosporine-like amino acids or absorptive surface pigments (Hader et al. 2011). On the other hand, some fish spend sensitive portions of their development exposed to sunlight in shallow waters, which leaves them vulnerable to the harmful effects of UV radiation (Hakkinen et al. 2004).

Since UV exposure can alter diverse molecular pathways, the effects of this stressor have been studied in various organisms. Increased mortality in aquatic invertebrates and fish has been observed as an effect of UV exposure, which suggests that it may be the driver behind evolutionary change (Nikkila et al. 1999; Jokinen et al. 2011). An example is the intertidal fish *Girella laevifrons*, which has been observed to spend large portions of its development in waters exposed to high levels of UV radiation (Carrasco-Malio et al. 2014). Since Chilean waters accommodate a multitude of migratory and resident species, biomonitoring efforts are in place to follow the effects of increasing UV. However, recent investigations suggest that, despite being one of the few resident species, *G. laevifrons* may be an unsuitable species for biomonitoring, due to an apparent resistance to UV-induced mortality (Carrasco-Malio et al. 2014). Even though increased enzymatic activity against ROS was uncovered (including superoxide dismutase, catalase, and lipid peroxidation) along with an increase in DNA damage in the liver, the researchers did not observe the previously reported mortality in *G. laevifrons* in response to larval UV exposure in the laboratory (Carrasco-Malio et al. 2014). Unfortunately, the results of this experiment are not enough to allow discrimination between acclimation, epigenetic mechanisms, or genetic causes of

this apparent protection. A mechanistic explanation or a comparison with a similar species could allow an in-depth interpretation of the results, but the current evidence is promising in terms of documenting adaptation to UV stress. The hypothesized explanation for the lack of mortality in *G. laevifrons* is that increasing UV radiation in this southern hemisphere intertidal zone acted as a selective pressure to drive this species to adapt to the potentially lethal effects of high UV exposures (Carrasco-Malio et al. 2014).

3.2 Radionuclides

Another source of radiation pollution, which has increased dramatically through human activity, is radionuclide contamination. Natural sources of radioactivity exist and have been shown to have genotoxic effects on human populations resident in naturally radioactive areas (Forster et al. 2002). On the other hand, anthropogenic radionuclide contamination has been widely studied and often stems from nuclear power plant waste or in a few cases from significant disasters, like the Chernobyl and Fukushima nuclear disasters from 1986 and 2011, respectively (Steinhauser et al. 2014). As with radiation-exposed human populations, radionuclides have genotoxic effects on contaminated aquatic biota, with the study focus mainly being DNA damage (Theodorakis et al. 1997).

An investigation of channel catfish (*Ictalurus punctatus*) populations from radiocesium-contaminated nuclear reactor cooling ponds at Chernobyl showed that fish appear to exhibit higher levels of double-stranded DNA damage, but not an increase in micronuclei (Sugg et al. 1996). The study was unable to determine whether adaptation or acclimation was the cause of the lower apparent micronucleus formation, but it confirmed that radiation contamination could have effects on fish populations at the genetic level (Sugg et al. 1996). A second study focused on the decades-old radionuclide contamination of ponds at the U.S. Department of Energy's Savannah River Site. Theodorakis and Shugart (1998) investigated populations of mosquitofishes, *Gambusia affinis* and *G. holbrooki*, to determine whether long-term exposure in contaminated ponds had affected the genetic integrity of resident populations. These species have short generation times, and their populations were restricted to the study ponds. Researchers found that after multiple generations of exposure, populations of exposed mosquitofishes exhibit higher levels of DNA strand breakage as well as reduced fertility (Theodorakis and Shugart 1998). On the other hand, some individuals in the contaminated sites exhibited lower DNA strand breakage and their genotypes, as determined by random amplified polymorphic DNA (RAPD) assay, were different from the rest of the sampled fish, which suggested a genetic basis for the phenotypic differences (Theodorakis et al. 1999). These studies imply that there may be a possibility of different genotypes providing different protection from radionuclide contamination in mosquitofishes and that human-induced evolution has occurred (Theodorakis et al. 1999).

There are a multitude of existing selective pressures on the planet and anthropogenic events are intentionally or unintentionally contributing to their magnitude. The example studies included in this chapter suggest that the increases of some stressors have led to emerging selective pressures or to the amplification of existing pressures through human activity. Aquatic organisms faced with such challenges may or may not have the capacity to adapt, depending on the natural history of the organism, the availability of genetic resources, and/or the complexity of altered selective pressure. Anthropogenic activities have led to significant alterations in the selection landscape including the increased release of estrogenic compounds, changes in temperature, UV permeability, radionuclide contamination, salinity, dissolved oxygen, and pH. Unfortunately, it is currently not possible to accurately predict the evolutionary impacts of anthropogenic alterations to the existing stressors. However, we are rapidly gaining insight into the mechanisms through which selection may act. This process is even more difficult when considering the potential evolutionary impacts of emerging contaminants.

4 Novel Stressors

This section considers stressors that prior to anthropogenic influences may have been present in the environment, but not at levels likely to have had significant toxicological effects on fish. These are often chemicals that are persistent in the environment and are highly toxic to fish, allowing those pollutants to act as selective pressures for extended periods of time. We will discuss most of these compounds in terms of putative evolutionary adaptations in fish. While there is certainly interest in the short-term impacts of many of these classes of contaminants, we will only focus on observed and potential adaptation in fish populations in response to chronic exposures.

4.1 Metals

The concentrations and bioavailability of many toxic metals have increased dramatically in aquatic environments as a result of human activities. Various metals have toxic properties due to interference with biological pathways in fish (Strydom et al. 2006). There are multiple sources of metals, both natural and more importantly anthropogenic, and each of them produces unique compositions of metals pollution that aquatic organisms experience. Mining for coal and minerals is an environmental concern in many parts of the world. For example, acid mine drainage often leaves environments heavily contaminated with metals (Griffith et al. 2012; Pumure et al. 2010). Concern for the integrity of the impacted environments is substantial, which often leads to wetlands being constructed to attempt to contain the considerable contaminant loads being released, and reduce impacts on

downstream habitats (Turker et al. 2014; Guittonny-Philippe et al. 2014). The biological implications of high metals concentrations have been investigated for many years, and some studies have suggested that adaptational processes may have led to lower retention rates of metals in fish populations chronically exposed to high metals concentrations for over 50 years (Jeffree et al. 2014). These studies are not conclusive regarding the causality or mechanisms of this adaptation, but the increasing contamination and frequency of acid mine drainage and mountaintop removal beget the study of the biological impacts of observed adaptation.

A large portion of evolutionary toxicology studies focus on finding adaptive responses in the field by comparing populations of contaminant-exposed fish to reference populations, assuming that reference sites represent ancestral populations prior to the impact of contamination (Klerks et al. 2011). Such assays carry high environmental relevance, but sometimes lack the ability to establish causality. Another set of investigations focus on establishing and quantifying the causal links between contamination and adaptation through artificial selection experiments in the laboratory (Klerks et al. 2011). One significant advantage of these studies is that a true control can be included (i.e., a subset of individuals is not exposed while another subset is exposed to the contaminant for multiple generations). This allows for the derivation of true quantitative knowledge about the causality and heritability of adaptive traits (Klerks et al. 2011). On the other hand, long generation turnover times and a simplified exposure scenario are challenges inherent within such experiments, particularly when using vertebrates (Klerks et al. 2011). An artificial selection experiment exposed a population of least killifish, *Heterandria formosa*, to LC_{50} values of cadmium for six consecutive generations, continuing exposures of each generation until at least 50 % of the generation died from Cd exposures (Xie and Klerks 2003). The surviving individuals (generally 15–25 %) were used to produce consecutive generations. The final generation (sixth) produced in this long-term selection study exhibited a threefold increase in Cd resistance, relative to control fish (Xie and Klerks 2003). The realized heritability of the resistance was relatively high ($h^2 = 0.5$), allowing it to be carried to further generations quite rapidly (Xie and Klerks 2003).

When observing evolutionary events, one also needs to consider the costs that may be associated with the newly acquired phenotype. In the case of least killifish, the cadmium-resistant populations had lower heat tolerance, smaller size, as well as lower lifetime fecundity, brood size, and female life span (Xie and Klerks 2003, 2004). These results demonstrate that the effects of adaptation can be complex and often unpredictable. Populations of adapted fish may be compromised unexpectedly, without visible mortality from the selective stressor, leading to a multitude of possible susceptibilities. In this case, an artificial selection experiment was able to quantify and characterize the ability of organisms to adapt to a single contaminant, while other environmental studies have to deal with complex mixtures and their possible adaptational effects.

A separate study identified populations of Atlantic killifish (*F. heteroclitus*), which were present at a site with high concentrations of dioxin and metals, to have developed resistance to methylmercury toxicity (Weis et al. 1981a, b). In addition

to the protection from the early teratogenic effects of methylmercury, F_1-larvae from contaminated site fish also retained higher prey capture efficiency when dosed with meHg, compared to larvae from reference sites (Zhou et al. 1996). These results suggest that adaptive responses to metals can be observed in field-exposed populations, supporting the idea that metals contamination can have evolutionary impacts on aquatic organisms.

4.2 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a class of widely distributed contaminants, formed by human activities as a by-product of incomplete combustion. They can also be found naturally in oil, natural gas, and coal, and can be produced by forest fires and volcanoes (Walker et al. 2005). These compounds are always found as complex mixtures, and several PAHs are known to be carcinogenic to a wide variety of organisms, including fish and mammals (Schneider et al. 2002; Hermann 1981; Karahalil et al. 1998). Mixtures of PAHs have also been found to have immunotoxic effects (Reynaud and Deschaux 2006; Carlson et al. 2004) and to cause DNA adducts (Jung et al. 2009) and cardiac deformities in aquatic organisms (Clark et al. 2010). Wastewater runoff, in addition to other anthropogenic activities, has been shown to move complex mixtures of PAHs, allowing them to deposit in coastal regions and estuaries (Walker et al. 2005; Menzie et al. 2002; Daskalakis and Oconnor 1995) and causing them to concentrate in surface sediment (Viguri et al. 2002). Thus, the highly teratogenic properties of PAH mixtures to fish embryos can introduce new selective pressures for estuarine fish (Wassenberg and Di Giulio 2004b).

The Atlantic Wood Industries Superfund site (AWI) on the Elizabeth River in Virginia has been on the National Priorities List since 1990 (Fig. 1) (Landers 2006). The contamination at the AWI mainly consists of PAHs (Mitra et al. 1999). PAHs are generally readily biotransformed by fish, but this process involves the activation of the aryl hydrocarbon receptor (AHR) pathway and has been linked to cardiovascular deformities in developing fish embryos, a shared mode of toxicity with some dioxins and co-planar PCBs (Heinrich et al. 1986; Ashurst et al. 1983; Wassenberg and Di Giulio 2004b). The source of the PAH contamination at the AWI was a wood treatment facility releasing creosote, primarily made up of heavy pyrogenic PAHs, into the river and contaminating aquatic life from 1926 to 1992 (Mitra et al. 1999). The incomplete remediation efforts to reduce PAH concentrations at the AWI included a capping of the old wood treatment facility location in 2002 and a dredging campaign in 2007 (Landers 2006). Additional dredging efforts have been planned for the contaminated sediment including the construction of a steel wall to contain the sediment, but these efforts have not yet been completed.

The first indication that fish were being impacted by contamination in the Elizabeth River was the discovery that mummichog (*F. heteroclitus*) from the AWI had a higher occurrence of hepatic neoplasms (Van Veld et al. 1991;

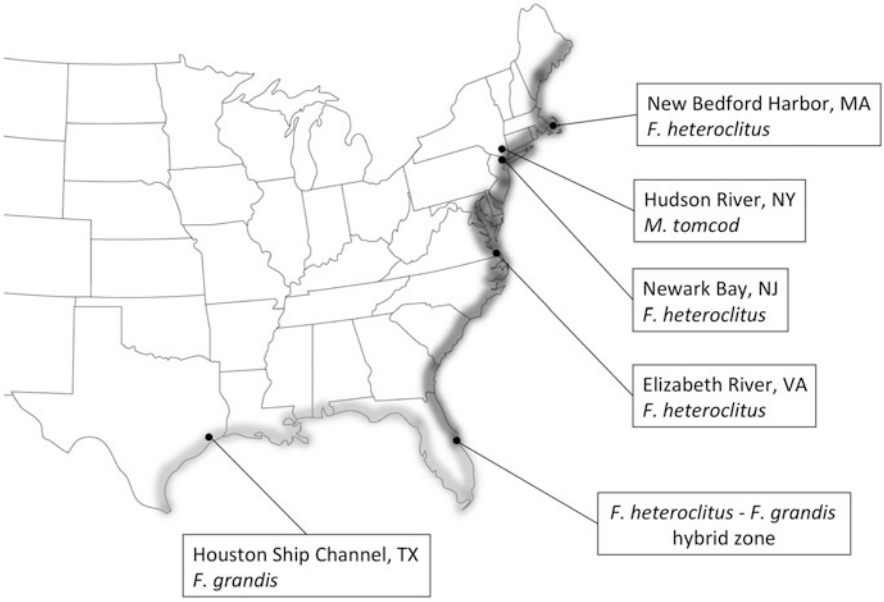

Fig. 1 Map of the locations where adaptations in response to anthropogenic events have been documented in natural populations of fish. *Darker shading* represents zones occupied mainly by *F. heteroclitus*, while *lighter shading* represents zones primarily occupied by *F. grandis*

Vogelbein et al. 1990). The cause of those lesions was not found, but was attributed to the PAH mixtures found throughout the Elizabeth River, particularly the high concentrations found at the AWI. Following this discovery, the same investigators tested the activity of the main enzyme involved in the biotransformation of large PAHs, cytochrome P4501A (CYP1A). The expected response following PAH exposure is a significant induction of CYP1A, which is why CYP1A is used as a biomarker of PAH exposure (Nacci et al. 1998; Matson et al. 2009). An example dataset with *Fundulus grandis* shows the induction of CYP1A activity, measured through a standard ethoxyresorufin-*O*-deethylase (EROD) assay, with a chosen PCB agonist (Fig. 2). This activity is commonly seen to diminish with the onset of cardiac deformities in embryos, while the causes of this relationship are not fully understood (Fig. 2). Despite the expectation that CYP1A should be elevated in AWI, *F. heteroclitus* from this highly contaminated site had depressed levels of CYP1A (Van Veld and Westbrook 1995). These results were confirmed in F_1-progeny (Wills et al. 2010). In addition, protection from cardiac, tail, and bladder abnormalities in embryos was discovered and linked to the reduced activity of CYP1A, and more specifically a recalcitrant AHR pathway (Meyer et al. 2002; Ownby et al. 2002; Clark et al. 2010). Further studies went on to confirm that altered AHR2 activity was the specific AHR gene alteration likely responsible for the observed resistance, and not simply reduced CYP1A activity (Clark et al. 2010; Matson et al. 2008). These findings were consistent with mechanistic studies

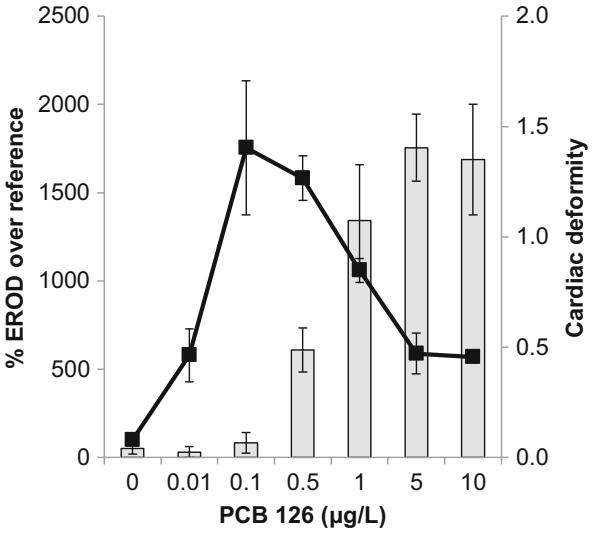

Fig. 2 Relationship between CYP1A activity levels and developmental cardiac deformities in response to a common AHR agonist, PCB 126. When F1 embryos from a reference *F. grandis* population are dosed with PCB 126, the CYP1A activity (*black line*) is induced as part of the AHR pathway response to the toxicant. At higher concentrations, CYP1A has lower activity levels, and embryos develop cardiac deformities (*gray bars*). The inverse relationship between the two is a well-documented occurrence, while the mechanistic connection is yet to be elucidated. A subset of these data was presented in Oziolor et al. (2014)

previously done in zebrafish (*Danio rerio*; Carney et al. 2004). The control and the mechanisms by which reduced responsiveness of the AHR pathway leads to protection from PAH toxicity in development remain unknown. Since the generation turnover time for *F. heteroclitus* is close to a year, there have likely been more than 50 generations, and perhaps as many as 85, since the onset of pollution at the AWI. The identification of an adaptation in a vertebrate species to novel toxicants is a rare and interesting event.

Results documenting PAH resistance in F_1-mummichog embryos from the AWI were quite interesting, but failed to clarify whether they represent evolutionary adaptation or a trans-generational epigenetic response to heavy contamination. In order to distinguish between the two options, later generations reared under common garden conditions (i.e., no PAH exposure) needed to be tested. However, studies conducted to date differ somewhat in their findings regarding the heritability of protection to F_2-progeny (Ownby et al. 2002; Meyer and Di Giulio 2002; Clark et al. 2013a). Ownby et al. (2002) found that the resistance was maintained completely in F_2-progeny, whereas Meyer and Di Giulio (2002) concluded that, while there was still resistance, F_2-progeny were less resistant than the F_1-generation. Nonetheless, high levels of mortality in AWI fish in the study seem to suggest that the more resistant fish might have been eliminated from contributing to the tested F_2-progeny because of fitness costs associated with the

resistance, and thus, the protective phenotype may have only appeared to not be heritable (Meyer and Di Giulio 2002). In addition, it is possible that epigenetic effects could be interacting with the adaptive change. The most thorough investigation of the heritability of PAH resistance in AWI mummichog was performed by Clark et al. (2013a). They found an interesting disconnect between the recalcitrance of the molecular pathway believed to be responsible for observed resistance, the AHR pathway, and the actual embryo resistance to cardiovascular defects. The F_2-progeny in their study regained partial AHR pathway responsiveness, yet fully retained their protection from embryotoxicity. They concluded that multiple pathways may be involved in PAH resistance, but that none of them seem to be fully genetically heritable (Clark et al. 2013a).

In addition to the physiological differences, which were shown to be at least partially heritable, *F. heteroclitus* from the AWI were genetically distant from geographically proximate populations (Mulvey et al. 2002). Combined, these results support the hypothesis that there has been a selective sweep caused by the high contamination at the location, causing these non-migratory fish to become resistant to PAH toxicity. As with most adaptations, fitness costs are to be expected, and a few have been found within the AWI population. Fluoranthene, a CYP1A-inhibiting PAH, which is also phototoxic, was found to affect AWI populations more severely in combination with UV light than observed for a reference population (Meyer and Di Giulio 2003). In addition, AWI larvae have been documented to have a reduced resistance to short-term hypoxia exposures (see chapter "Low-Oxygen Lifestyles"), as well as exhibiting decreased survival under common-garden laboratory conditions (see above), suggesting a general reduction in fitness (Meyer and Di Giulio 2003). While some fitness costs have been identified, several other attempts to identify further fitness costs have been unsuccessful. Glutathione expression is on average lower in polluted site fish, but sex and present exposure confound the significance of those results (Bacanskas et al. 2004). Some specific fitness costs were hypothesized as a result of the mechanistic basis for the PAH resistance; because of the lower CYP1A activity, *F. heteroclitus* from polluted sites were hypothesized to be more susceptible to pesticides deactivated by that enzyme (Clark and Di Giulio 2012). Contrary to this expectation, polluted site mummichogs were shown to be highly cross-resistant to the acute toxicity of two CYP1A-detoxified pesticides, a carbamate and a pyrethroid (Clark and Di Giulio 2012). In addition, even though the main contaminant load at the AWI stems from PAHs, *F. heteroclitus* from that site are also protected from the effects of PCBs (Clark et al. 2013b). Other research suggests that field-collected fish may have an impaired reproductive system and increased chromosomal damage compared to reference populations (Frederick et al. 2007; Jung et al. 2011). The issue with these studies is that they often have a difficulty differentiating between the effects of PAHs on fish in the field, versus the fitness costs driven by the adaptation of the fish. Thus, the potential adverse effects of PAHs in AWI mummichog may involve multiple physiological changes, some of which are caused by genetic alterations in the fish, while others could be caused simply by the exposure to a toxic mixture with very diverse toxicity pathways.

4.3 Polychlorinated Biphenyls

There are 209 known congeners of PCBs that can be produced to create mixtures of various properties, dependent on their chlorine content (Safe 1984). They were produced in the USA until the early 1970s with known uses in plastics, insulation, various dyes, carbonless paper, and transformers (ATSDR 2000). Because of their stability and resilience to chemical, thermal, and photo-degradation, PCBs are persistent in the environment (Buckman et al. 2004; ATSDR 2000). Nevertheless, they are still found in the production of certain dyes and paint (Hu and Hornbuckle 2010). Resembling 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), some co-planar PCB congeners can upregulate the aryl hydrocarbon receptor (AHR) pathway constitutively and cause cancer, apoptosis, and cardiac deformity in aquatic organisms and mammals (Zhang et al. 2012; Thackaberry et al. 2005; Gao et al. 2011; Jonsson et al. 2007; Antkiewicz et al. 2006; Carney et al. 2004). These properties of co-planar PCBs make them likely selective agents in heavily PCB-contaminated environments (U.S. Environmental Protection Agency 2004).

4.3.1 Atlantic Tomcod (*Microgadus tomcod*)

Heavy PCB contamination often occurs in urban estuarine regions. A well-known site with historic PCB contamination is the Hudson River (Fig. 1; Feng et al. 1998). Following 70 years of contamination, including the release of an estimated 1.3 million pounds of PCBs, a 200-mile stretch of the Hudson River was put on the National Priorities List as a Superfund site in 1984 (U.S. General Accounting Office 2000). Since then, the fate and distribution of these compounds have been modeled and studied extensively (Connolly et al. 2000). Accumulation and toxicity have been seen both in fish and migratory piscivorous birds in the area (Custer et al. 2012; Deshpande et al. 2013; Koenig et al. 2013; Fernandez et al. 2004). The historic contamination with highly toxic PCBs in the Hudson River resulted in multi-generational exposures and the subsequent evolutionary adaptation of Atlantic tomcod, providing resistance to many of the toxic effects associated with PCBs (Roy and Wirgin 1997). Despite the infamy of this site as a PCB-contaminated site, there have also been levels of TCDD found in juvenile fish collected from the Hudson River that may be among some of the highest levels of this contaminant found in natural populations (Fernandez et al. 2004). Thus, the contamination at this site is complicated by multiple highly toxic contaminants at very high concentrations, many of which have similar ecotoxicological properties.

Resistance to PCBs by Atlantic tomcod is one of the clearest and mechanistically best-elucidated investigations of human-induced evolution through contaminants (Wirgin et al. 2011). The extraordinary concentrations of PCBs in the Hudson River were predicted to be highly detrimental to aquatic life, including the resident Atlantic tomcod (Fernandez et al. 2004). Not unexpectedly, initial investigations identified increased levels of hepatic tumors and decreased life spans of populations

living in the Hudson River (Dey et al. 1993). Since a primary molecular mechanism linked to PCB toxicity is the activation of the AHR pathway, research quickly focused on potential alterations to this important pathway. Cytochrome P450 1A (CYP1A) was already discussed as a downstream regulated enzyme responsible for PAH degradation, but its activity is also highly induced in aquatic organisms exposed to co-planar PCBs and dioxins (Nacci et al. 1998; Courtenay et al. 1999). Populations of tomcod collected from the Hudson River did not increase the expression of CYP1A after exposure to PCBs, in contrast to fish from a proximate reference site (Roy and Wirgin 1997). Both populations had similarly increased levels of AHR expression, a normal response to PAHs, but their CYP1A expression was recalcitrant (Roy and Wirgin 1997). The downregulation of the pathway could not be attributed to the AHR repressor (AHRR), which was also found to be downregulated in all tissues, similar to CYP1A (Roy et al. 2006). Following these results, researchers sequenced AHR2, the more active form in fish, for tomcod from four reference populations and two populations in the polluted Hudson River. The sequence data elucidated the mechanistic basis of the phenotypic adaptation of these fish (Wirgin et al. 2011). A six base pair deletion in exon 10 was discovered in Hudson River tomcod, and kinetic tests confirmed that the mutated AHR2 protein had more than sevenfold reduced binding to a model dioxin (Wirgin et al. 2011). This mutation was close to fixation in polluted site populations, whereas it was extremely rare and was always heterozygous in reference site tomcod (Wirgin et al. 2011). Mitochondrial genome diversity revealed that the adaptation in polluted sites did not lead to a decrease in haplotype diversity, confirmed gene flow between resistant populations, and indicated the lack of significant gene flow to reference sites (Wirgin et al. 2011). However, Wirgin et al. (2011) concluded that the variant AHR2 allele, which is also present in some reference populations at low frequencies, likely predates anthropogenic pollution.

The identification of a molecular mechanism and the attribution of this change to anthropogenic pollution is the ultimate goal of evolutionary toxicology (Bickham 2011). Research on Atlantic tomcod populations in the Hudson River has quickly reached that target. In this case, the PCB pollution, likely with help from TCDD, in the Hudson River was intense enough to cause mortality in the resident Atlantic tomcod populations. In addition, PCBs are persistent enough to remain in the environment for enough time such that many generations of tomcod were exposed to the stress of pollution. Therefore, it seems that when a beneficial mutant allele is available in the populations originally exposed to the contaminants, as in the Atlantic tomcod, novel selective pressures can allow for the rapid fixation of this trait and for the success of populations that would normally collapse under these extreme anthropogenic selective pressures (Wirgin et al. 2011).

Estuaries polluted with elevated levels of PCBs are present throughout the world, and they have provided the opportunity to examine the possibility for additional phenotypic adaptations to anthropogenic contaminants (Nelson and Bergen 2012; Lakshmanan et al. 2010). Two species of killifish in the USA, both in the genus *Fundulus*, have also been found to have repeatedly evolved an

increased ability to survive in estuaries with high PCB contaminations (Oziolor et al. 2014; Nacci et al. 1999). Despite being genetically isolated, both species include multiple, disparate populations that have apparently evolved phenotypes resistant to PCB toxicity independently. Unfortunately, the exact molecular mechanism responsible for tolerance remains unknown in either species, but the rapid evolution that has occurred in both suggests that it results from selection on standing genetic variation present in ancestral populations, which—if it turns out to be similar in both species—could predate the *Fundulus heteroclitus–F. grandis* split (Whitehead et al. 2010; Oziolor et al. 2014). Research on *F. heteroclitus* and *F. grandis* reveals a diverse side of human-induced evolution, which has the potential to contribute greatly to our understanding of adaptational processes in response to anthropogenic pollution.

4.3.2 New Bedford Harbor Atlantic Killifish (*Fundulus heteroclitus*)

Another site on the Atlantic Coast of the United States, New Bedford Harbor (NBH), Massachusetts, was identified as a highly PCB-polluted estuary and placed on the National Priorities List in 1983 (Fig. 1; Nelson and Bergen 2012). Because of the high projected costs of remediating this site, it was placed on the EPA's Long Term Monitoring program, which allowed for frequent tracking of the contamination at the site (Nelson and Bergen 2012). The first dredging event removed 14,000 cubic yards of sediment with concentrations of over 4,000 μg/g of PCBs in 1995 (Bergen et al. 2005). Through 2009, approximately 200,000 cubic yards of sediment had been dredged from NBH, leading to a significant decrease in PCB concentrations (Nelson and Bergen 2012).

Following 2009, the projected 20-year remediation process led to multiple investigations of toxicity to aquatic organisms at NBH (Nelson and Bergen 2012). To investigate potential population-level impacts, *F. heteroclitus* was used as a model for effects on fish, since it is a resident non-migratory species in NBH (Munns et al. 1997). Laboratory studies suggested that *F. heteroclitus* reproduction might be impaired by exposure to high PCB concentrations, signifying that the toxicants could act as a selective pressure in the polluted estuary (Black et al. 1998). Interest in the effects of chronic PCB contamination on *F. heteroclitus* finally led to the discovery that NBH populations had adapted to resist the developmental toxicity of PCBs (Nacci et al. 1999).

Populations of *F. heteroclitus* collected from NBH had lower embryonic and larval mortality in comparison with reference site populations (Nacci et al. 1999). This resistance extended to at least the F_2-generation raised under common-garden conditions, confirming that the trait is genetic, rather than an example of physiological acclimation (Nacci et al. 2010). In addition, the resistant phenotype was also correlated with a reduced responsiveness of CYP1A activity, much like in the case of Atlantic tomcod from the Hudson River (Nacci et al. 1999). CYP1A has also been shown to be a sensitive biomarker of dioxin exposure and toxicity in *F. heteroclitus* (Toomey et al. 2001). In the process of studying the control of

CYP1A by the AHR pathway, initial tests identified AHR1 to be more strongly and ubiquitously expressed in tissues of NBH fish, while not being responsive to dioxins or PCBs (Karchner et al. 1999). Adult fish and hepatocyte cell cultures also revealed that NBH populations were much less sensitive in inducing CYP1A mRNA expression or enzyme activity when exposed to dioxins and a model PAH (Bello et al. 2001). The AHR pathway was capable of being activated to the same magnitude, but it only occurred at much higher doses of pollutants (Bello et al. 2001). Results in zebrafish suggest that dioxin-mediated cardiovascular defects are AHR2 dependent, and that the heart deformities are prevented when AHR2, and thus its downstream genes, was knocked down (Carney et al. 2004). On the other hand, knocking down only CYP1A had no effect (Carney et al. 2004). These results support the hypothesis that the resistance of NBH populations of *F. heteroclitus* to PCB-mediated deformities is based on a recalcitrant AHR pathway and, by extension, its main inducible downstream enzymes (Toomey et al. 2001; Bello et al. 2001).

One factor that makes this case particularly interesting is that there are very few identified fitness costs associated with adaptation to PCBs in *F. heteroclitus*. Studies have identified the lower capacity of NBH killifish to deal with oxidative stress (Harbeitner et al. 2013). Resistant populations were also found to have lower hepatic, but higher intestinal, expression of P-glycoprotein, an enzyme responsible for the excretion of moderately hydrophobic compounds (Bard et al. 2002). This difference between populations diminished after they were placed in common-garden laboratory conditions for more than 8 days (Bard et al. 2002). Other studies dealing with retinoid depletion by PCB exposures have been unable to identify a difference between reference and resistant populations of *F. heteroclitus* (Nacci et al. 2001). In addition, it was found that the PCB sensitivity of populations of *F. heteroclitus* correlated with the concentrations of PCBs at the sites of their collection (Nacci et al. 2002). Further studies into that trend determined that there were several exceptions to the correlation between contamination levels and resistance, which may result from the vast variability of contaminants present at different locations (Nacci et al. 2010).

The lack of mechanistic evidence to explain the differential sensitivity to dioxins in NBH populations led investigators to explore genetic and epigenetic markers to better understand the possible shifts in genes or their control. The efforts began with the sequencing of the CYP1A promoter region, but after CYP1A knockdown was identified to not be protective of PCB-induced teratogenesis, efforts were shifted to the AHR (Powell et al. 2004). The methylation patterns of AHR1 and AHR2 were not observed to have an effect on the PCB sensitivity of *F. heteroclitus*, but rather to affect the gene expression patterns in the fish (Aluru et al. 2011). With the development of new technology, population resequencing studies have become more affordable, and investigations have begun into the genotypic differences between the AHR pathway genes of these fish (Reitzel et al. 2014). These studies identified a significant population genetic differentiation between resistant and reference populations (Reitzel et al. 2014). The results suggest that selection is possible for the tested genes (AHR1, AHR2, and AHRR), but with the current

results, the study was unable to show strong significant differences that could identify the genetic basis of protection (Reitzel et al. 2014). Another exploration of the genetic variation between reference and resistant site fish through single-nucleotide polymorphism (SNP) analysis identified signs of positive selection in SNPs of AHR2 and CYP1A between these populations (Proestou et al. 2014). Such variation may suggest that the AHR pathway still represents a potential mechanism to explain part of the phenotypic differences in the responses of resistant killifish to contaminant-induced damage. Population genomics is a promising tool for the exploration of genetic effects on populations, and the *F. heteroclitus* populations at New Bedford Harbor are a promising start for this endeavor, but adaptation in killifish contains more complexity that still stands to be unraveled.

4.3.3 Newark Bay Atlantic Killifish (*F. heteroclitus*)

Shortly after the identification of the first adapted populations at NBH, another group was able to find a similar recalcitrant AHR phenotype in *F. heteroclitus* collected from Newark Bay (NB) (Fig. 1; Arzuaga and Elskus 2002). The NB estuary, part of the Passaic River in New Jersey, is a heavily contaminated area, which has been on the National Priorities List since 1984 as the "Diamond Alkali Superfund Site" (Crawford et al. 1995). In addition, the Passaic River runs parallel to the Hudson River discussed previously in terms of Atlantic tomcod adaptation. The PCB levels at the NB site are not as high as the ones at NBH, but the type of contamination is much more complex, involving high levels of polychlorinated dibenzo-dioxins and furans (PCDD/Fs), PCBs, and heavy metals (Crawford et al. 1995; Wenning et al. 1993; Armstrong et al. 2005). This has led to multiple fish consumption advisories for this area and to the identification of another population of contaminant-adapted *F. heteroclitus* (Pflugh et al. 2011; Arzuaga and Elskus 2002).

The initial studies identified a similar phenotype of resistance associated with protection from developmental toxicity and reduced CYP1A activity in *F. heteroclitus* populations collected from NB (Arzuaga et al. 2004; Arzuaga and Elskus 2002). In addition, de-methylating agents failed to revert either of those phenotypes, suggesting that epigenetics may not play a strong role in the observed resistance (Arzuaga et al. 2004). Similar to other adapted populations, NB fish were resistant not only to cardiovascular teratogenesis, but also to the induction of ROS by a PCB-simulating compound (Arzuaga and Elskus 2010; Arzuaga et al. 2006). These findings point to a similar phenotype of protection and shared fitness costs with *F. heteroclitus* populations from NBH. Additional studies on NB fish confirmed that complex contaminant exposures impose selection on populations by reducing the reproductive fitness of chronically exposed individuals (Bugel et al. 2010, 2011).

The discovery of three, geographically distant, populations of *F. heteroclitus* that have adapted to chronic contamination in a physiologically similar manner distinguishes this system from Atlantic tomcod. The tomcod population fixed a rare

mutation that allowed it to survive in conditions of high contamination. Conversely, *F. heteroclitus* seems to have been able to adapt at multiple locations, and gene flow among adapted populations is extremely unlikely. In addition, the genetic basis of this adaptation or the full extent of the adaptation has still not been fully determined.

One of the most fascinating findings about *F. heteroclitus* is that they seem to have adapted to heavy PCB pollution in a very similar manner to the way they have adapted to PAHs, including recalcitrance in AHR pathway enzymes and protection from cardiac deformities (Arzuaga and Elskus 2002; Van Veld and Westbrook 1995; Roy and Wirgin 1997). The vast geographic separation has been shown to prevent gene flow among these distant adapted populations, as would be expected for this species (Duvernell et al. 2008). In addition, it has been shown that selection at NBH and AWI has had an influence on the population genetic differentiation between adapted and non-adapted populations (Duvernell et al. 2008). Other populations of *F. heteroclitus* have also been shown to exhibit resistance, with levels of pollution similar to those previously described at NBH and NB (Nacci et al. 2010). The vast genetic and geographic distance between these locations and the similar mode of adaptation between sites with varying pollution led to the hypothesis that standing genetic variation in ancestral populations is responsible for the rapid evolution of protection in *F. heteroclitus* from contaminated habitats (Whitehead et al. 2012). Populations from various locations were compared in terms of their resistance and their transcriptomic profiles, revealing that distant resistant populations had profiles more similar to each other than to their most geographically proximate reference sites (Whitehead et al. 2012). This could allow for the rapid fixation of the trait and the possibility for multiple populations to acquire this resistance, despite low migration rates in *F. heteroclitus*.

4.3.4 Houston Ship Channel Gulf Killifish (*Fundulus grandis*)

While the *F. heteroclitus* case is fascinating at its current intricacy, there is another level of complexity—*F. grandis*. Gulf killifish are the sister species of *F. heteroclitus* and are found primarily along the US Gulf Coast (Fig. 1; Gonzalez et al. 2009). These two species have been shown to overlap and hybridize along a short portion of the northeastern Florida Atlantic coast (Gonzalez et al. 2009). *Fundulus grandis* is found ubiquitously along the Gulf of Mexico coast and has been studied as a relevant environmental model for hypoxia exposures, osmoregulation in euryhaline fishes, as well as physiological and toxicological responses to the Deepwater Horizon Oil Spill (Dubansky et al. 2013; Landry et al. 2003; Love and Rees 2002; Virani and Rees 2000). As an ecologically important coastal fish in the Gulf of Mexico, it was of particular interest that populations of *F. grandis* were identified to be resident, and in fact quite common, in the Houston Ship Channel (HSC) (Fig. 1; Oziolor et al. 2014).

The HSC is a heavily industrialized commercial waterway, heavily polluted with a mixture of PCBs, dioxins, and PAHs (Lakshmanan et al. 2010). These compounds

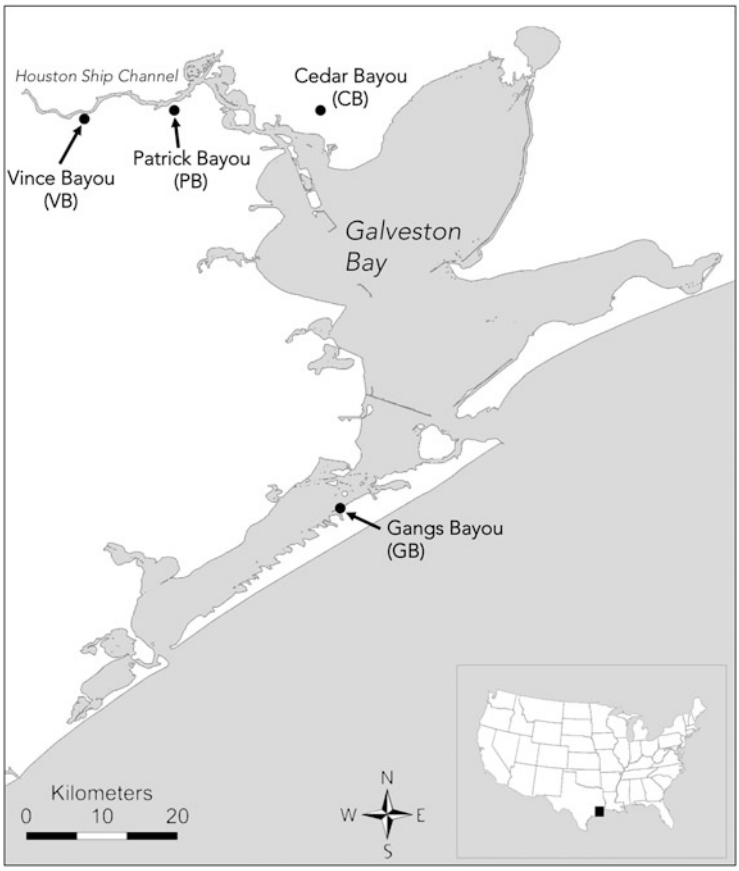

Fig. 3 Sampling sites from two Superfund sites within the industrialized portion of the Houston Ship Channel, Vince Bayou (VB), and Patrick Bayou (PB). A site with predicted intermediate contamination levels, Cedar Bayou (CB), was collected from a portion proximate to the HSC, while a reference population, Gangs Bayou (GB), was collected far from the high contamination regions

have been observed to accumulate in catfish (*Ictalurus punctatus*), reaching total PCB concentrations of 37 pg/g (Subedi and Usenko 2012). The spatial distributions of PCBs and PCDD/Fs have been studied intensely and were found to diminish as the channel continues through Galveston Bay (Howell et al. 2008, 2011). In addition, these levels of contamination have been observed historically over the last four decades allowing for chronic contamination of aquatic organisms over many generations (Yeager et al. 2007).

Recently, populations of *F. grandis*, collected from heavily contaminated areas in the HSC, were shown to exhibit a similar phenotypic resistance to PCB- and PAH-induced cardiovascular teratogenesis (Fig. 3; Oziolor et al. 2014). Cardiovascular teratogenesis occurs in embryos, if they come in contact with a toxicant at

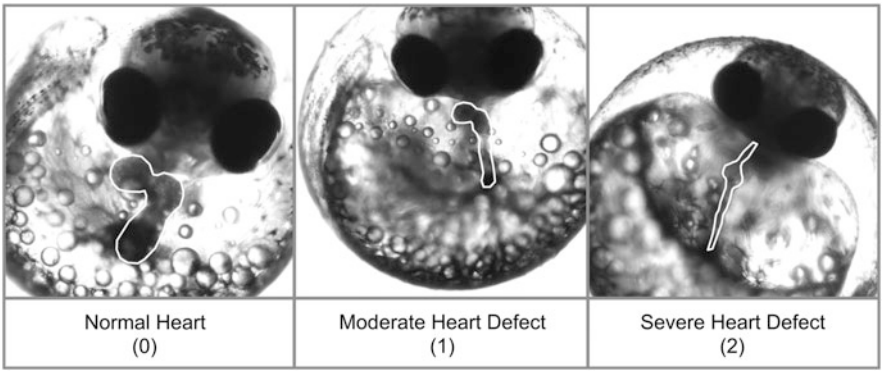

Fig. 4 Scoring scale for cardiovascular teratogenesis. Embryos were dosed at 24 h post-fertilization (hpf) with a toxicant and screened at 144 hpf. The scores were recorded blind to avoid bias for population or contamination levels from researcher performing the screening

sensitive developmental stages. Often, these deformities are scored qualitatively from a normal, two-chambered heart (0) to severely deformed string-heart (2) (Fig. 4). The protection from PCBs in HSC *F. grandis* was of much higher magnitude than that observed for PAHs, which correlated well with the toxicity equivalency of these classes of compounds in the HSC (Oziolor et al. 2014). The protection from cardiovascular teratogenesis in *F. grandis* population also correlated highly with a recalcitrant AHR pathway, as measured via CYP1A activity, suggesting a similar mode of action as seen in adapted *F. heteroclitus* populations (Oziolor et al. 2014). The different levels of resistance to PCBs and PAHs, even when evaluating a known AHR-mediated toxicity endpoint, suggest that there may be additional complexity to the observed adaptation, beyond a simple AHR recalcitrance. Biparental crosses of reference and resistant populations suggest that each parent contributes equally to the resistant phenotype, suggesting a genetic basis of this adaptation (Oziolor et al. 2014). More recently, a gradient of this adaptation has been found, where populations from the HSC with lower predicted contaminant exposure exhibit lower levels of protection and lower levels of AHR recalcitrance (Fig. 5).

The identification of a sister species that has undergone a phenotypically similar genetic adaptation to human-induced pollution suggests that the hypothesis of adaptation through preexisting genetic variation would now extend to their shared common ancestor. Thus, the alleles responsible for this adaptation may have been carried through populations and generations of *Fundulus* even before the split of the two species. Such an extended timeframe begets multiple questions, including the question of why these alleles persisted through time, the nature of the fitness costs associated with them, and the natural stressors that may have led to their evolution.

Expanding adaptation to human-induced novel selective pressures in *Fundulus* to multiple species has the potential to answer questions extending from toxicology to the basics of evolutionary biology. Through these adaptations, we can study not

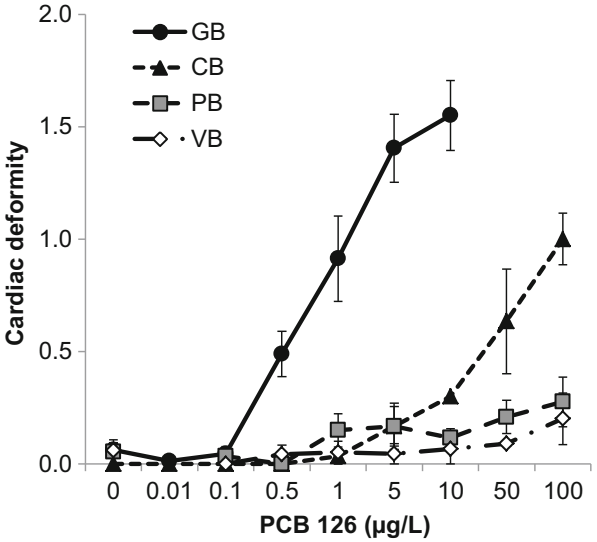

Fig. 5 Populations of *F. grandis* from chronically contaminated sites exhibit protection from cardiovascular deformities in a gradient-dependent manner. F1 embryos from the reference site, GB, show a normal dose–response curve for cardiovascular deformities in response to PCB 126, a known AHR agonist. The population from a site with predicted intermediate contamination, CB, develops deformities at higher concentrations of contaminant, showing significant levels of protection. On the other hand, sites within the industrialized portion of the HSC show no significant cardiac defects in response to PCB 126, revealing a >1,000× protection from contaminant induced deformities. A subset of these data was presented in Oziolor et al. (2014)

only the effect of current toxicants in the environment but also about the nature of evolutionary processes that have led to the continuous selection of alleles that would provide some protection from xenobiotics. This should lead to a better understanding of how natural biological systems may respond to current anthropogenic pollutants and how they cross-react with historical biological selective pressures. The study of these two species is at the forefront of evolutionary toxicology and the discovery of the molecular mechanisms and history of these adaptations will significantly improve our understanding of the evolutionary effects of anthropogenic contamination.

5 Conclusions and Outlook

While research has identified some long-term effects of legacy contaminants following chronic exposures, there are many chemicals that have not been investigated, and novel stressors being released every year. For most systems, we are far from having a comprehensive understanding of how organisms will respond to the changing selective landscape in their natural environments. A crucial

misconception with finding resistance to high concentrations of contaminants is that the public may interpret this to mean that contamination is acceptable or less of a problem, because organisms can adapt to it. Unfortunately, this is a misinterpretation of such results for two primary reasons. First, the fact that some species are able to adapt to pollution does not imply that it will happen in every organism, population, or pollution scenario. Second, the resistant population often has been changed in terms of its phenotypic response to more than just the compound it adapted to; in other words, adaptation to anthropogenic stressors almost certainly has fitness costs. Whether and how fish will respond to rising salinities in freshwater bodies and whether they will cope with higher radiation or temperature increases are questions stemming from already existing sources of human-induced alterations of natural selective regimes. Along with current levels of toxicity from pesticides or persistent pollutants that have already been introduced in the environment, a new direction of research will be to study the evolutionary consequences of constantly increasing levels of pharmaceuticals in aquatic environments. The toxicity of pharmaceuticals in the environment is being widely studied; but in terms of population adaptation, the effects of these compounds are still entirely unknown. There are multiple classes and types of anthropogenic effects on the environment, but our knowledge of how they shape fish populations is often limited to short exposure durations. In addition, as most of these compounds are found in very complex mixtures, it is of interest to understand how they interact with existing environmental stressors to change the selective landscape of resident organisms at varying locations. Future extremophile fish may evolve in response to already existing levels of alteration in the selective landscape within aquatic environments, and if the populations have sufficient standing genetic variation to adapt to these changes.

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