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EFFECTS ON FISH OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) AND NAPHTHENIC ACID EXPOSURES

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1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic pollutants that contain two or more fused aromatic rings composed of hydrogen and carbon. PAHs can also have alkyl groups, such as methyl and ethyl groups, substituted for one or more of their hydrogen atoms, and are generally considered to include heterocyclic aromatic compounds, where sulfur, oxygen, or nitrogen can commonly be substituted for one or more of the carbon atoms in their aromatic rings. PAHs make up a substantial portion of many fossil fuels, including crude oil, oil shales, and tar sands. The petroleum-derived, or petrogenic, PAHs usually have two to three fused rings, are highly alkylated, and are also known as low molecular weight PAHs. When petroleum products and other organic materials such as wood are subject to incomplete combustion, higher molecular weight PAHs (generally thought of as having four to seven fused rings) can be formed as a result of condensation processes. These compounds are generally unsubstituted and are commonly referred to as pyrogenic PAHs. The simplest PAH is naphthalene, consisting of two aromatic benzene rings. Phenanthrene and anthracene are three-ringed compounds, and as the number of rings increases, the number of possible structures multiplies (Figure 4.1). Thus, as a class, PAHs are composed of literally thousands of structures, making their classification, nomenclature, and analysis, as well as studies of their environmental fate and effects, extremely complex.

PAHs are derived from both natural and anthropogenic sources and are released from a wide range of industries and everyday activities. Petrogenic PAHs are associated with petroleum exploration, extraction, transport, and refining, as well as with oil seeps, and can also be formed biogenically. Petrogenic PAHs are made up of 85% or more alkyl congeners, based on analyses of a range of fresh and weathered crude oils and refined petroleum products (Wang et al., 2003). Pyrogenic PAHs are released into the atmosphere and surface waters as a result of fossil fuel and wood combustion, as well as natural forest and grassland fires, and from volcanoes. Unlike many other organic chemical contaminants that are

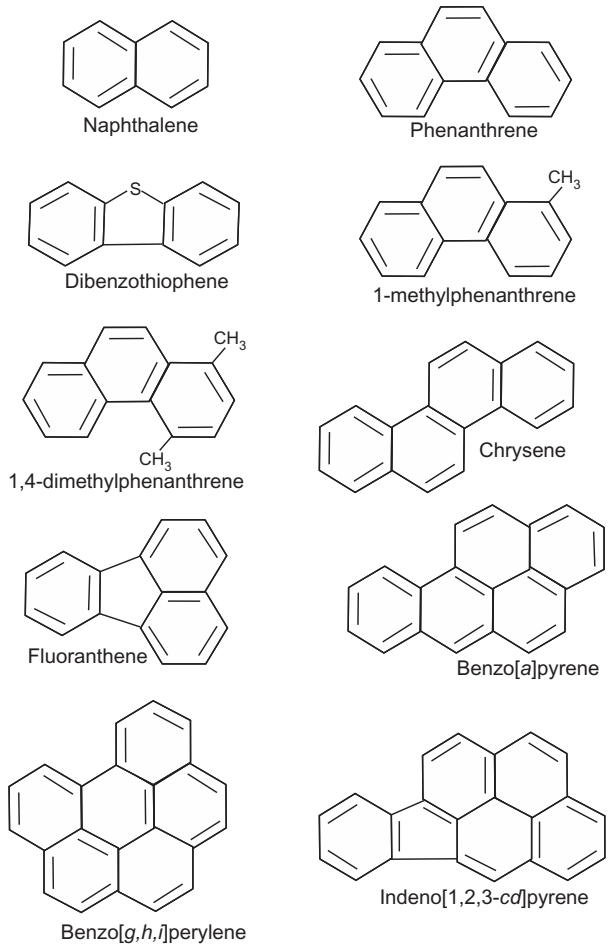

Figure 4.1. Chemical structures of polycyclic aromatic hydrocarbons commonly detected in environmental samples.

manufactured and regulated, PAHs continue to be released on a global scale. Given the multitude of sources, and especially our continued global reliance on petroleum as an energy source, PAHs will continue to be chemical contaminants of concern for decades to come.

In this chapter, we briefly review transformation of PAHs in the aquatic environment, highlighting their efficient metabolism in fish. We also review the evidence that links PAH exposure to a wide range of biological dysfunctions in fish, including neoplasia, reduced reproductive success and other types of endocrine disruption, immunotoxicity, postlarval growth and somatic condition, transgenerational impacts, and finally, recent findings showing that embryonic development of fish is severely affected by

extremely low concentrations of PAH exposure. We also include a brief review of the effects of naphthenic acids on fish because these compounds are increasingly recognized as major factors in the toxicity of process waters from a variety of petroleum sources, most notably the immense oil sands deposits found in Alberta, Canada.

2. TRANSFORMATIONS OF PAHs IN THE AQUATIC ENVIRONMENT

2.1. Physical and Chemical Degradation

PAHs are persistent chemical contaminants that are distributed in environmental matrices, including sediments, soils, water, and air. Lower molecular weight PAHs (those containing two to three fused benzene rings) can be found in all of these matrices, whereas higher molecular weight compounds (those containing four to seven fused benzene rings) are more tightly associated (physically and chemically) with sediments/soils and particles than the other abiotic sample types. PAHs in air can be altered via chemical oxidation and photochemical processes (Lima et al., 2005), whereas in sediments/soils and the uppermost portion of the water column, degradation of PAHs, particularly lower molecular weight PAHs, occurs via photooxidation (McElroy et al., 1989; Bertilsson and Widenfalk, 2002). In addition to parent PAHs, oxygenated PAH metabolites formed during these degradation processes can remain associated with sediments up to six months after initial addition to the water column and thus can remain in the environment for extended periods of time (Hinga and Pilson, 1987).

In sediment and water samples, certain microorganisms (e.g., bacteria, fungi) have been shown to mineralize PAHs under aerobic conditions, particularly those compounds that contain two- and three-fused rings (e.g., naphthalene, fluorene), to their basic elements or to biodegrade these compounds to more polar degradation products (Meador et al., 1995; Lima et al., 2005). Information on PAH microbial degradation pathways and identification of degradation products is beyond the scope of this chapter but details on these processes and products can be found in Cerniglia and Heitkamp (1989), Juhasz and Naidu (2000), and Bamforth and Singleton (2005). Some research studies have demonstrated that pyrene, a four-ring PAH, can be mineralized by certain strains of bacteria (e.g., *Mycobacterium*) under optimum growing conditions in the laboratory, but it is unclear if this occurs in the natural environment (Heitkamp et al., 1988; Heitkamp and Cerniglia, 1989). In contrast, other higher molecular weight PAHs (e.g., five- and six-ring compounds) are not readily degraded by microbes and thus are

more likely to accumulate in these environmental media (particularly in fine-grained sediments with high organic carbon content) (Meador et al., 1995; Juhasz and Naidu, 2000). Under anoxic conditions, PAHs persist in sediments, particularly in organic sediments (Heitkamp and Cerniglia, 1989).

2.2. Biotransformation in the Aquatic Food Web

In aquatic organisms, exposure to PAHs can occur through dermal exposure, respiration, or consumption of contaminated prey (e.g., annelids, mollusks) or sediment (Varanasi et al., 1989). Biotransformation of PAHs in aquatic organisms occurs to varying degrees depending on a number of factors, including the rate of uptake, metabolic capability, physical condition, feeding strategy, and age (James, 1989; Varanasi et al., 1989). Invertebrates are capable of PAH uptake from their environment and have been shown to have varying levels of PAH-metabolizing capability (James, 1989). Mollusks generally have lower PAH-metabolizing capability compared to certain species of polychaetes, crustaceans, and fish (Meador et al., 1995). In contrast to persistent organic pollutants, PAHs are readily metabolized by teleost fish, with the initial oxidation occurring through the action of cytochrome P4501A, creating epoxides (see Chapter 1 for a complete review of biotransformation). PAH epoxides are short-lived reactive compounds that can hydrolyze nonenzymatically to form phenols or be enzymatically converted by epoxide hydrolase to form dihydrodiols. PAH epoxides, through the action of glutathione-S-transferases, can also be conjugated to glutathione and eventually converted to mercapturic acids. The more stable diols and phenols are also further metabolized in fish through the actions of UDP-glucuronosyl transferases or sulfotransferases, creating glucuronide and sulfate conjugates, respectively. These now highly polar conjugated metabolites are then excreted into urine or secreted into the bile for rapid elimination through the gastrointestinal tract (Roubal et al., 1977; Krahn et al., 1984; Varanasi et al., 1989; Collier and Varanasi, 1991). As a result of this rapid metabolism and elimination in fish, concentrations of parent PAHs are negligible in muscle and other tissues. Thus, bile and urine are good matrices for determining recent exposure to PAHs in teleost fish, with bile being preferred because of its ease in collection. Interestingly, differences in the metabolism of the four-ring PAH, benzo[*a*]pyrene (BaP), including differences in the types and proportions of metabolites formed, have been demonstrated between two species of freshwater fish (Sikka et al., 1991; Steward et al., 1991). These differences could contribute to variations in susceptibility to these carcinogenic compounds among fish species. Differences in glutathione-S-transferases

may also help explain differential susceptibility to chemically induced carcinogenesis among fish species (Collier et al., 1992).

A number of analytical methods have been developed to measure PAH metabolites in fish bile and are reviewed in Beyer et al. (2010). Oil spills, including the *Deepwater Horizon* (*DWH*) spill in the Gulf of Mexico in 2010, have led to a need for additional methods (including rapid analytical methods that measure PAHs and PAH metabolites) to determine PAH exposure in seafood or protected species (e.g., marine mammals, sea turtles) where animals cannot be lethally sampled. For example, a rapid, sensitive HPLC-fluorescence method that measures 14 carcinogenic and noncarcinogenic PAHs in seafood tissues was developed by the U.S. Food and Drug Administration (Gratz et al., 2010) during the *DWH* spill and was used by federal and Gulf state analytical laboratories as part of the seafood safety response (Ylitalo et al., 2012). Development of new analytical methods such as this can provide important information on PAH exposure in aquatic organisms.

3. HEPATIC NEOPLASIA AND RELATED LESIONS IN WILD FISH

A number of case studies in wild fish have strongly linked the occurrence of hepatic neoplasms and neoplasia-related toxicopathic liver lesions to PAH exposure, as measured by ΣPAHs in sediments, PAH metabolites or fluorescent aromatic compounds (FACs) in fish bile, PAH-DNA adducts in liver, or components of the natural diet of these species. The only convincing case studies linking PAH exposure to neoplasia in wild fish are those involving liver neoplasms and a spectrum of hepatic lesions involved in the histogenesis of liver neoplasia. The following sections provide summaries of these interesting and illuminating case studies.

3.1. Mummichog/Killifish, *Fundulus heteroclitus* (Elizabeth River, Virginia, Chesapeake Bay; Delaware Estuary)

Studies in this region on the highly territorial, nonmigratory estuarine mummichog (also referred to as killifish) began with the discovery of an epizootic of a suite of toxicopathic hepatic lesions, including hepatocellular, cholangiocellular, and pancreatic neoplasms, preneoplastic focal lesions (commonly referred to as foci of cellular alteration or altered hepatocellular foci (FCA/AHF)), and lesions manifesting cytotoxicity in fish inhabiting a site in the Elizabeth River, Virginia, that was grossly PAH-contaminated by a nearby facility that pressure-treated wood with creosote (Vogelbein et al., 1990; reviewed in Vogelbein and Unger, 2006). The prevalence of some of these lesions at this site exceeded 70%, and less severely PAH-contaminated

sites in the Elizabeth River and James River estuary showed intermediate prevalences of these toxicopathic lesions; they were not detected in fish from relatively uncontaminated areas. Overall, field studies incorporating samplings at 12 sites in the region have shown a strong association between occurrence of these lesions and PAH exposure, with prevalences tracking well along a PAH exposure gradient. Recent studies using stepwise logistic regression have confirmed these trends statistically, whereby both total sediment PAH concentrations and TOC-normalized PAH concentrations exhibited a significantly positive relationship with the risk of occurrence of toxicopathic liver lesions, especially for neoplasms and preneoplastic focal lesions (Vogelbein et al., 2008). Moreover, as was also shown in English sole, immunohistochemical studies on cytochrome P4501A (CYP1A) and glutathione-S-transferase localization in the preneoplastic focal lesions and hepatic neoplasms (Van Veld et al., 1991, 1992) indicate that the hepatocytes that make up these lesions have undergone an adaptive response consistent with the selective resistance to cytotoxicity or resistant hepatocyte model of hepatocarcinogenesis (Solt et al., 1977).

An abundant literature surrounds the issue of toxicity resistance in this population of mummichog/killifish from the grossly creosote-contaminated Atlantic Wood site in the Elizabeth River (reviewed in Van Veld and Nacci, 2008; see that review for more specific references). This collective body of research includes the demonstration of resistance or enhanced tolerance to acute toxicity (i.e., skin and fin erosion, and death) associated with exposure to Atlantic Wood sediment in adults from the Elizabeth River as compared to those from reference sites (the York River), dramatically reduced hepatic CYP1A inducibility in adult Atlantic Wood mummichog as compared to mummichog from uncontaminated reference sites either by interparenteral (i.p.) injections of 3-methylcholanthrene or by exposure to two dilutions of sediment (10%, 30%) from the Atlantic Wood site (Van Veld and Westbrook, 1995), and resistance to teratogenicity in the form of cardiac abnormalities in the offspring of Elizabeth River fish (Ownby et al., 2002). This resistance in the Elizabeth River mummichog is at least partially heritable in laboratory-reared offspring from reproductively mature adults from the Elizabeth River, with resistance to toxicity declining from the F1 to subsequent generations, suggesting that both physiological acclimation and genetic adaptation are components of this resistance (Meyer and Di Giulio, 2002). The mechanism for this resistance to PAH-associated toxicity in both adults and offspring of Elizabeth River mummichog likely involves alterations in the aryl hydrocarbon receptor (AhR)-mediated pathways, specifically those related to CYP1A induction. CYP1A expression and inducibility is depressed in Elizabeth River mummichog, and this inducibility persisted in F1 embryos and larvae reared in the laboratory. However, CYP1A inducibility rose to

normal levels in the mature adults developing from these Elizabeth River F1 embryos and in subsequent generations.

Recent studies relate more specifically to differential susceptibility and potential resistance to hepatic carcinogenesis in offspring of this population of Atlantic Wood/Elizabeth River mummichog that are tolerant to PAH toxicity, as compared to those from a reference site, Kings Creek (Wills et al., 2010a). Larvae from Elizabeth River and Kings Creek parents were exposed to BaP for two 24-hr periods, seven days apart, at doses of 50, 100, 200, and 400 μg/L. Fish were sampled for histological examination at three and nine months after the second exposure. Briefly, results showed no toxicopathic, neoplasia-related liver lesions at any dose in either Elizabeth River or Kings Creek juveniles at three months post-exposure, while Kings Creek fish at nine months post-exposure, in the 200 μg/L dose, showed an incidence of 8% (2/25) eosinophilic FCA, compared to 0% in Elizabeth River fish. At the highest dose in the Kings Creek juveniles at nine months, the incidence of eosinophilic FCA was 30%, with two of these fish showing hepatic adenomas or carcinomas (6.7%); in Elizabeth River fish, 2/30 (6.7%) were affected with eosinophilic FCA, and none developed hepatic neoplasms. Even though the sample sizes in these experiments were relatively small, there was a significantly higher ($p = 0.018$) incidence of FCA in Kings Creek juveniles at the highest dose and at nine months (30%), as compared to Elizabeth River juveniles (6.7%) at the same dose and sampling time. Although these results are not definitive, this lower incidence may be related to the reduced inducibility of hepatic CYP1 enzymes in Atlantic Wood/Elizabeth River mummichog.

However, the most convincing evidence supporting a causal link between exposure to sediment-associated PAHs dominated by high molecular weight species and these neoplasms and neoplasia-related lesions was the experimental induction, in a four-year sediment and dietary exposure regimen in mummichog, of high prevalences (up to 40%) of altered hepatocellular foci and moderate prevalences (8.7%) of hepatic neoplasms. This experimental evidence is perhaps the strongest in existence for a direct cause-and-effect relationship between chronic PAH exposure and hepatic neoplasia in fish (Vogelbein and Unger, 2006).

Separate field studies targeting mummichog from five sites in the Delaware Estuary watershed (Pinkney and Harshbarger, 2006) also detected FCA/AHF and hepatic neoplasms at dramatically higher prevalences from sites with elevated concentrations of ΣPAHs in sediments compared to fish from sites with low ΣPAHs. Although this smaller study did not demonstrate a direct statistical correlation between lesion prevalences and PAH exposure, the overall findings support the evidence from the above studies and in a different geographical location.

3.2. Winter Flounder, *Pleuronectes americanus* (Northeast Atlantic Coast)

Multiyear, multisite studies investigating toxicopathic hepatic lesions, including neoplasms, in winter flounder from urban marine and estuarine sites in the northeastern United States have shown significant associations with PAH exposure, in addition to DDT, its metabolites, and chlordanes, but not PCBs (Johnson et al., 1993). Specifically, stepwise logistic regression techniques indicated that significant risk factors for the occurrence of neoplasms were low-molecular-weight PAHs (ΣLAHs) and chlordanes in stomach contents, with relatively low proportions of the variation in risk of lesion occurrence attributed to PAHs (7%) compared to chlordanes (22%). However, substantial proportions (14–29%) of the intersite variation in prevalence of the unique lesion termed hydropic vacuolation of biliary epithelial cells and hepatocytes (HydVac) (Moore et al., 1997) were accounted for by exposure to both ΣLAHs and high- (ΣHAHs) molecular-weight PAHs. HydVac is considered a lesion manifesting cytotoxicity but is clearly also involved in the early stages of the histogenesis of liver neoplasms. However, significant associations between risk of HydVac occurrence and exposure to DDT, its metabolites, and chlordanes, were also shown, and in the case of chlordane explained an even higher proportion (16–43%) of the variation in lesion risk (Johnson et al., 1993). Statistical analysis (by factor and canonical correlation analyses) of an earlier, more limited dataset in winter flounder from similar sites in the northeastern U.S. Atlantic Coast essentially confirmed these associations (Chang et al., 1998). Prevalences of HydVac and hepatic neoplasms dropped considerably and significantly between 1987 and 1999 at the Deer Island flats sewage outfall site in Boston Harbor, presumably resulting from reductions in the chemical content of sludge, source reduction programs, and transfer of the sewage effluent release to an offshore site (Moore et al., 1996; personal communication to Mark Myers). These findings strongly support a causal role for PAHs and certain pesticides in the etiology of these lesions. The current view is that PAHs probably play a role in initiating hepatic neoplasia in winter flounder, but exposure to chlorinated pesticides may play a stronger promotional, cytotoxic, and overall role in the genesis of the toxicopathic lesions and hepatic neoplasms in winter flounder (Moore and Stegeman, 1994; Moore et al., 1997).

3.3. Brown Bullhead Catfish, *Ameiurus nebulosus* (Tributaries of the Southern Great Lakes Region, Chesapeake Bay)

Studies on this benthic species, especially those investigating the Black and Cuyahoga rivers in Ohio, both tributaries entering Lake Erie, have also

implicated high-molecular-weight PAHs in sediments as the probable etiologic agents for the hepatic tumors observed (reviewed in Baumann et al., 1991, 1996). Bullhead from areas with high neoplasm prevalences also had significantly elevated hepatic aryl hydrocarbon hydroxylase activity and hepatic DNA adduct concentrations (Balch et al., 1995). Geographically broader studies using multiple histopathological and biochemical biomarkers (Arcand-Hoy and Metcalfe, 1999) found significant prevalences of hepatic neoplasms in the PAH-polluted sites of the Detroit River in Michigan (15%), Black River in Ohio (9.5%), and Hamilton Harbor, Ontario, Canada (6%), with no tumors found in fish from relatively clean reference sites. The liver lesion data were consistent with results of biochemical biomarkers such as bile FACs and hepatic ethoxyresorufin-O-deethylase activity. Other field and caging studies in the Detroit River in Michigan (Maccubbin and Ersing, 1991; Leadley et al., 1998, 1999) found higher hepatic neoplasm prevalences at sites with elevated concentrations of sediment PAHs and FACS in bile (Leadley et al., 1999). Although evidence in the brown bullhead does not reflect the same degree of statistical rigor as the case studies in other species, it collectively supports a strong association between toxicopathic hepatic lesions, especially neoplasms and FCA/AHF, and environmental PAH exposure. Additional supportive field evidence is seen in the clear pattern of initial reduction in age-specific liver neoplasm prevalence after the closing of a coking plant on the Black River with subsequent reduction in PAH exposure (Baumann and Harshbarger, 1995), followed by a brief increase in tumor prevalence three years after dredging activities and resuspension of PAHs in 1990, and then a decrease in age-specific tumor prevalence in 1994 and 1998 (Baumann and Harshbarger, 1998). In this case, reduction in the hypothesized causal variable (PAH exposure) preceded the reduction in the effect, satisfying the required temporal order for cause and effect in epidemiological studies (Susser, 1986).

Separate and more recent field studies with this species in tributaries of the Chesapeake Bay (Pinkney et al., 2009) have shown high levels of PAH exposure, as measured by sediment ΣPAHs, bile FACs (PAH-like metabolites), and hepatic cytochrome P450 activity and PAH-DNA adducts. In addition, higher prevalences of toxicopathic hepatic lesions (including FCA/AHF and neoplasms) have been evidenced in fish from urban sites in the Anacostia River near Washington, DC, as compared to reference sites in the Tuckahoe River. In a truncated dataset including only sites from the Anacostia River and the Tuckahoe River reference site where bile FACs data were available (reviewed in Pinkney et al., 2009), PAH-like metabolites in bile were a significant predictor variable for the occurrence of liver neoplasms in brown bullhead. However, the overall statistical relationship between PAH exposure and the occurrence of hepatic

neoplasms and FCA/AHF in the full dataset of sampling sites for this species in this geographic region is not particularly strong or consistent.

3.4. European Flounder, *Platichthys flesus* (North Sea)

Field studies with this species in the Dutch (Vethaak and Wester, 1996) and German (Koehler, 2004) coastal waters of the North Sea, and complementary mesocosm studies in coastal Dutch waters (Vethaak et al., 1996), have documented toxicopathic hepatic lesions involved in the histogenesis of hepatic neoplasia, including FCA/AHF, neoplasms, and HydVac that have been generally associated (with a lesser degree of statistical rigor and certainty) with exposure to environmental contaminants such as PAHs and PCBs. However, a specific statistical association with PAH exposure has not been demonstrated, and the spatial pattern of liver neoplasm prevalence has not suggested a straightforward relationship to PAH exposure. Moreover, there has been a long-term, significant decline in liver neoplasms and related lesions in this species in the North Sea (verified in a linear logistic model) since the early 1990s (Vethaak et al., 2009); these changes have been attributed to improved water and sediment quality over the last 15 to 20 years in Dutch coastal waters (Vethaak et al., 2009). The current view held by European research groups on this issue is that exposures of wild European flounder to tumor promoters such as PCBs and other polyhalogenated hydrocarbons (PHHs) are probably more important risk factors in hepatocarcinogenesis in this species than exposures to genotoxic PAHs (Koehler, 2004; Vethaak et al., 2009).

3.5. English Sole, *Parophrys vetulus* (Puget Sound, Washington, and U.S. Pacific Coast)

Over 35 years of research exists on the occurrence of hepatocellular and biliary neoplasms and a spectrum of other toxicopathic lesions involved in the stepwise histogenesis of liver neoplasia in English sole from Puget Sound (Myers et al., 1987) and other Pacific Coast estuaries (Myers et al., 1994). This histogenesis parallels the experimental histogenesis of liver neoplasia in rats (Farber and Sarma, 1987) and fish (Bailey et al., 1996; Boorman et al., 1997). Within the context of the sequential, stepwise progression of hepatic neoplasia, these hepatic lesions represent the phases of cytotoxicity, compensatory regeneration and proliferation, preneoplastic foci of cellular alteration (FCA) or altered hepatocellular foci (AHF), and hepatocellular and biliary neoplasms. Their geographical patterns of occurrence, combined with chemical contaminant exposure data in English sole at their sites of capture, provide especially strong evidence for a causal relationship with

exposure to environmental PAHs and to a lesser degree, certain chlorinated hydrocarbons (CHs) such as PCBs, DDT and its metabolites, and chlordanes (see reviews/articles by Myers and Fournie, 2002; Myers et al., 2003; Johnson et al., 2008, 2013). These studies are based on the initial hypothesis and subsequently derived evidence that these liver lesions in English sole are the result of exposure to PAHs (as hepatoxicants and initiators of carcinogenesis) and, of secondary importance, exposure to chlorinated compounds such as PCBs, DDT and its metabolites, and chlordanes (as hepatotoxicants and nongenotoxic promoters of carcinogenesis) (see specific references in articles by Myers et al., 1987, 2003). To date, evidence from 12 field studies and somewhat fewer laboratory studies that have validated the field findings fulfill all of the classic criteria for causation in epidemiological studies of cancer and other diseases (Colton and Greenberg, 1983; Mausner and Bahn, 1974) as reviewed in Myers et al. (2003).

These criteria, with a brief description of the evidence within them (see Myers et al., 2003 and Collier, 2003 for specific references and more detail) for English sole include the following.

1. **Strength of the association:** Toxicopathic liver lesions were initially correlated ($p<0.001$) with sediment ΣPAHs in a 32-site Puget Sound study, followed by an 11-site Puget Sound study showing correlations with concentrations of fluorescent aromatic compounds (FACs) in bile ($0.001<p<0.01$, depending on lesion type). Subsequent multisite, multiyear field studies in Puget Sound attributed varying proportions of the toxicopathic liver lesion risk to PAHs (12–54%) and far less to PCB (1–8.5%) exposure (see pp. 69–75 of Myers et al., 2003).
2. **Consistency of the association:** The association of the occurrence of these lesions with PAH exposure (as assessed by PAHs in sediments, stomach contents, bile FACs, and as hepatic PAH-DNA adducts) by multivariate analytical techniques such as stepwise logistic regression has held true for over 12 separate multisite, multiyear field studies in Puget Sound, the Pacific Coast from Southern California to Alaska conducted by the Northwest Fisheries Science Center of NOAA Fisheries, or in conjunction with Washington Department of Fish and Wildlife, and Environment Canada, in both adult and subadult English sole (see pp. 75–78 of Myers et al., 2003).
3. **Toxicological and biological plausibility:** The PAHs sole are exposed to in these studies include known higher molecular weight hepatocarcinogens (e.g., BaP) in mammals (see review by Myers et al., 1987 for multiple references) and fish (Metcalfe et al., 1988; Hawkins et al., 1995; Hendricks et al., 1985; Reddy et al., 1999).The spectrum of toxicopathic liver lesions and their complex patterns of co-occurrence closely parallel

those involved in the histogenesis of liver neoplasia in fish (Hendricks et al., 1984; pp. 79–82 in review of Myers et al., 2003) and mammals (Farber and Sarma, 1987). Moreover, laboratory studies in English sole on the uptake and disposition of PAHs such as BaP show that such PAHs can be metabolized/activated by CYP1A to proximate carcinogens that covalently bind to and, in a dose-dependent fashion, form persistent hydrophobic adducts of PAH metabolites with hepatic DNA (reviewed in French et al., 1996). These PAH-DNA adducts have the potential to represent the molecular lesions involved in initiation of hepatocarcinogenesis, and their concentrations have been shown to be significant risk factors for the occurrence of lesions early in the sequential histogenesis of hepatic neoplasia in sole (reviewed in Reichert et al., 1998). Further findings in English sole from PAH-contaminated environments (Myers et al., 1998), showing significantly reduced CYPIA immunostaining in hepatic neoplasms and FCA/AHF, and reduced PAH-DNA adduct concentrations in hepatic neoplasms, as compared to the surrounding normal liver, indicate that hepatic neoplasia in this species follows the selective resistance to cytotoxicity paradigm of hepatocarcinogenesis (Solt et al., 1977). Due to their reduced capacity for CYP1A-mediated activation of PAHs to their cytotoxic and mutagenic intermediates, with a consequent reduction in formation of covalent PAH-DNA adducts from these reactive intermediates, preneoplastic and neoplastic hepatocytes and neoplastic biliary epithelial cells in English sole possess an adaptive phenotype that is resistant to the cytotoxicity of agents such as PAHs that require metabolic activation to exert their toxicity (see Myers et al., 1987, 2003; Solt et al., 1977; Farber and Sarma, 1987; Roomi et al., 1985).

In summary, in the resistant hepatocyte model of chemically induced carcinogenesis, upon exposure to chemical carcinogens such as PAHs, uncommon hepatocytes are produced that have become altered through carcinogen-induced mutations (e.g., formation of PAH-DNA adducts), followed by cell proliferation to "fix" the mutation(s), resulting in fully initiated hepatocytes (Solt and Farber, 1976; Columbano et al., 1981; Farber, 1990). These initiated hepatocytes possess adaptive and resistant phenotype possessing mechanisms (e.g., reduced CYP1A expression and decreased activation of toxicants to cytotoxic and mutagenic intermediates) that allow for their survival and proliferation in an environment of toxicant exposure that inhibits the proliferation or survival of surrounding normal cells. These initiated, resistant hepatocytes are protected from the deleterious effects of acute exposure to cytotoxicants, possess a growth advantage in the liver, and therefore form foci of altered hepatocytes (FCA/AHF) and eventually neoplasms.

4. **Temporal sequence (i.e., exposure precedes disease):** Lesions manifesting cytotoxicity and occurring earlier in the histogenetic sequence of neoplasia are first observed and are the most common lesion type in younger fish, whereas the preneoplastic and neoplastic lesions first occur in fish >4 years of age, and their prevalence is strongly associated with increasing fish age (see pp. 82–83 in Myers et al., 2003). Moreover, a unique case study involving site remediation measures and English sole monitoring undertaken in previously creosote-contaminated Eagle Harbor, Washington (Myers et al., 2008), clearly shows a significantly decreasing trend from 1993 to 2004 in PAH exposure (measured by bile FACs and hepatic DNA adduct concentrations) that is paralleled by a significant and decreasing trend in risk of toxicopathic hepatic lesion occurrence. The prevalence of toxicopathic liver lesions in sole from Eagle Harbor has remained stable and at near-reference relative risks from 1997 to 2011. Other studies (1989–2009) targeting English sole from the Seattle Waterfront in Elliott Bay of Puget Sound, Washington, as part of the Puget Sound Assessment and Monitoring Program (PSAMP; now referred to as the Puget Sound Ecosystem Monitoring Program), conducted in conjunction with Washington Department of Fish and Wildlife, have further corroborated this parallel reduction in toxicopathic liver lesion prevalence, with reduced PAH exposure in English sole from a different geographic area in Puget Sound. Results from the PSAMP provide convincing evidence that the incidence of toxicopathic liver disease has declined sharply at the Seattle Waterfront site from a peak of nearly 40% in 1996 to 5% in 2009. This trend has been accompanied by reductions in PAH exposure as measured by FACs in the bile of the same English sole (Puget Sound Partnership, 2010; Collier et al., 2013) (Figure 4.2, Table 4.1). This improvement in a biomarker of fish health likely reflects the efforts to achieve source control and clean up PAH-contaminated sediments in this area.
5. **Dose–response/biological gradient:** Previously discussed correlations and associations in multiple field studies of lesion prevalence with PAH exposure parameters (sediment ΣPAHs, bile FACs, hepatic PAH-DNA adducts) fulfill this criterion. Also, data from three separate field studies show that risk of occurrence of the early hepatic lesions rises significantly with hepatic DNA adduct concentrations, as a dosimeter of chronic PAH exposure. Finally, segmented "hockey-stick" regression analyses of hepatic lesion prevalence in multiple studies have shown relatively consistent thresholds of sediment PAH exposure at which certain hepatic lesion types are first observed (Table 4.1) (see pp. 83–86 in review by Myers et al., 2003; also review by Johnson et al., 2008).

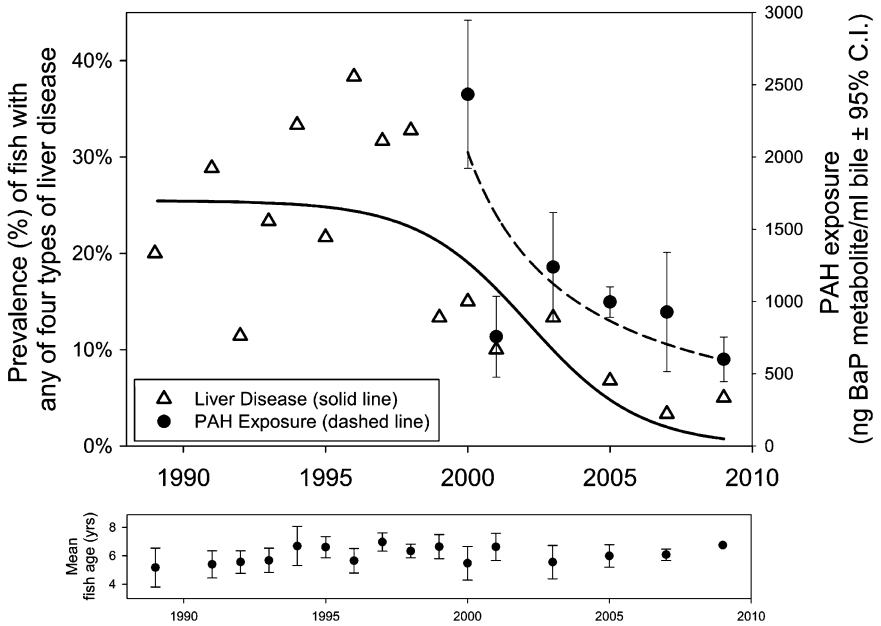

Figure 4.2. Twenty-year trend (1989–2009) in liver disease, and partially overlapping 10-year trend (2000–2009) in PAH exposure in English sole from Elliott Bay, Puget Sound, Washington State. The prevalence of liver disease was calculated using the number of 60 fish per year exhibiting any of four toxicopathic lesion types; preneoplastic foci of cellular alteration, neoplasms, hepatocellular nuclear pleomorphism/megalocytosis, or non-neoplastic proliferative lesions (hepatocellular/biliary regeneration). PAH exposure was estimated as the concentration of benzo[*a*]pyrene (BaP) equivalents in fish bile. Mean age of fish is shown because increasing age is positively associated with increasing prevalence of liver disease. Adapted from Puget Sound Partnership, 2010, and personal communication from J. E. West and S. M. O'Neill (Washington Department of Fish and Wildlife).

6. **Specificity of the association:** Because of the significant covariance of sediment concentrations of PAHs, PCBs, and certain pesticides in our Pacific Coast-wide English sole studies, it is not possible to attribute the toxicopathic hepatic lesions strictly to PAH exposure (see pp. 86–89 in Myers et al., 2003). However, over multiple studies, the PAHs remain the strongest (explaining the highest proportion of variation in lesion prevalence among all of the chemical exposure variables) and most consistent risk factor for occurrence of these lesions. The PCBs and pesticides contribute less to the overall risk, but are significant and plausible risk factors, as nongenotoxic promoters of liver neoplasia. Additional supportive data for the specificity of the PAH-hepatic lesion association come from results of field studies on sole conducted in Kitimat, British Columbia, in the vicinity of an

Table 4.1
Estimated effects levels associated with increasing sediment PAH concentration for selected liver lesions, indicators of reproductive dysfunction, and DNA damage in English sole. Adapted from Johnson et al. (2002).

PAHs (ppb dry wt)	Any Toxicopathic Liver Lesion (%)	Liver Neoplasms (%)	Inhibited Gonadal Development (%)	Inhibition of Spawning (%)	Infertile Eggs, % of Eggs Spawned	DNA Damage (nmol adducts/ mol bases)
50	0	0	15	12	38	5
100	0	0	15	12	38	5
1000	9	0	15	17	42	25
2000	18	0	15	25	48	36
3000	24	1	15	30	51	43
5000	31	3	18	35	55	51
10,000	40	6	27	43	61	63
100,000	71	16	58	69	80	100

aluminum smelter (Johnson et al., 2009). In this area, prevalences of the same liver lesions were associated with exposure to sediment PAHs, in the relative absence of other confounding chemicals such as PCBs. However, results also showed lower lesion prevalences than might be expected (based on ΣPAHs in sediments) at sites closest to the smelter, in addition to evidence of reduced actual uptake of PAHs in lower-than-expected bile FACs and hepatic PAH-DNA adduct concentrations. This finding suggests the limited bioavailability to English sole of smelter-derived, soot-based PAHs. At sites more distant from the smelter, hepatic lesion prevalences were comparable to those at sites with sediment PAHs from more typical, urban, industrial sources.

7. **Supportive experimental evidence:** The combined data on uptake, metabolism/activation, and binding of PAHs like BaP to DNA with dose-dependent adduct formation, with the potential to represent molecular lesions involved in initiating hepatocarcinogenesis (discussed in (3) above) also fit well into this criterion (see pp. 89–90 in the review by Myers et al., 2003). However, the most important experimental evidence in support of a causal relationship is the laboratory induction of early toxicopathic hepatic lesions (hepatocellular nuclear pleomorphism/ hepatic megalocytosis, hepatocellular regeneration, and preneoplastic focal lesions) in sole by 12 monthly injections into the dorsal sinus of either BaP or a PAH-rich extract from Eagle Harbor, followed by a six-month grow-out (Schiewe et al., 1991).

In addition to the above studies, case studies in the following species and locales were less extensively documented and showed less specific and less proven relationships between similar neoplasia-related lesions and PAH or combined chlorinated hydrocarbon (e.g., PCBs) exposure: white croaker (*Genyonemus lineatus*) and starry flounder (*Platichthys stellatus*), U.S. Pacific Coast (Myers et al., 1994; Stehr et al., 1998; McCain et al., 1992); dab (*Limanda limanda*) from the North Sea and British coastal waters (Kranz and Dethlefsen, 1990; Kohler et al., 1992; Bucke and Feist, 1993; Feist et al., 2004; Stentiford et al., 2003, 2009, 2010); and Atlantic tomcod (*Microgadus tomcod*) from the Hudson River estuary (Smith et al., 1979; Dey et al., 1993).

4. EFFECTS ON REPRODUCTION

There is substantial evidence that PAHs can cause reproductive impairment in a variety of organisms, including fish, through their ability to disrupt endocrine function, and their cytotoxic and mutagenic effects on germ cells (Tuvikene, 1995; Nicolas, 1999; Logan, 2007; Meador, 2008).

4.1. Fecundity of Females

Studies conducted over the past 30 years have shown that PAH exposure is associated with decreased plasma estradiol concentrations, reduced ovarian estradiol production, suppression of vitellogenesis, reduced ovarian growth, ovarian atresia, and reduced fecundity in female fish (e.g., Johnson et al., 1998, 2008; Anderson et al., 1996; Ridgway et al., 1999). More recent studies have confirmed a relationship between PAH exposure and reproductive impairment in female fish. A number of studies in both the field and the laboratory have documented the fact that exposure to PAHs, in mixtures or as single compounds (e.g., naphthalene and BaP), can depress plasma 17-β estradiol in female fish (Monteiro et al., 2000a; Sol et al., 2000; Tintos et al., 2006, 2007; Pollino et al., 2009), as well as suppress the *in vitro* synthesis of reproductive steroids by ovarian tissue (Monteiro et al., 2000b). Such alterations in reproductive hormones are often associated with suppressed vitellogenin production and poor ovarian growth, as reviewed by Nicolas (1999) and Logan (2007). Recent examples of these effects in fish include a study by Pait and Nelson (2009), who found significant negative correlations between sediment PAH concentrations and both gonado-somatic index (GSI) and plasma vitellogenin concentrations in killifish from the Chesapeake Bay in the United States. Similarly, Bugel et al. (2010) found that female killifish from Newark Bay, with elevated concentrations of PAH

metabolites in bile, had decreased gonad weight, inhibited gonadal development, and decreased hepatic vitellogenin production. In a laboratory study, Khan (2013) reported reduced GSI and inhibited oocyte development in female cod exposed to a water-soluble fraction of crude oil.

Reduced egg production and declines in GSI have also been documented in zebrafish (*Danio rerio*) exposed to BaP in the water column at concentrations as low as 3 μg/L (Hoffmann and Oris 2006). These authors also found that transcription of several genes related to reproduction, including genes for the steroid metabolizing enzymes, 20 β-hydroxysteroid dehydrogenase and aromatase CYP19A2, and vitellogenin, were affected at BaP exposure concentrations in the 1.5–3.0 μg/L range.

4.2. Fertility of Males

While the reproductive toxicity of PAHs in female fish is well established, less information is available on the effects of PAHs on reproductive function in male fish. In laboratory rodents and humans, exposure to PAHs has been linked to reduced testosterone production, poor sperm quality, and infertility (Ramesh and Archibong, 2011). There is some evidence that these effects occur in fish as well, but results are contradictory. In several studies conducted prior to 2000, PAH exposure was associated with declines in plasma androgen concentrations and/or reduced testicular development in male fish (Burton et al., 1985; McMaster et al., 1996; Truscott et al., 1983; Kiceniuk and Khan, 1987). Sundt and Björkblom (2011) observed inhibited testicular development and a reduction in the quantity of mature sperm in prespawning male Atlantic cod (*Gadus morhua*) exposed to PAHs in produced water for 12 weeks, at concentrations comparable to those found in the environment. Testicular development was altered, showing a rise in the number of spermatogonia and primary spermatocytes and a reduction in the quantity of mature sperm in the produced water-exposed fish compared to control. Khan (2013) reported reduced GSI and disrupted testicular development and sperm production in Atlantic cod exposed to the water-soluble fraction of crude oil. Holth et al. (2008) observed changes in the expression of genes associated with the reproductive system in zebrafish exposed to produced water containing PAHs, at concentrations of 0.54 to 5.4 ppb for 1 to 13 weeks, but found no effects on reproduction or recruitment.

Researchers report mixed effects of PAHs on reproductive steroid concentrations and metabolism in male fish. Martin-Skilton et al. (2006, 2008) found that exposure of juvenile Atlantic cod and turbot (*Scophthalmus maximus*) to North Sea crude oil in water or Prestige fuel oil in the diet increased the metabolism of testosterone (precursor to the biologically

active androgen, 11-ketotestosterone, in male fish) and/or reduced circulating concentrations of testosterone in the plasma. However, increased plasma testosterone concentrations and increased *in vitro* gonadotropic hormone (GTH)-stimulated testosterone production have been reported in several fish species (rainbow trout, *Oncorhynchus mykiss*; goldfish, *Carassius auratus*; rainbowfish, *Melanotaenia fluvatilis*; and grouper, *Epinephelus areolatus*) exposed to a variety of PAHs, including naphthalene, β-naphthoflavone, BaP, and retene (Evanson and Van Der Kraak, 2001; Wu et al., 2003; Pollino et al., 2009). A study by Sun et al. (2011) may shed some light on these conflicting findings. These researchers examined the effects of waterborne phenanthrene at doses of 0.06, 0.6, and 6 μg/L on testicular development in a species of rockfish, *Sebastiscus marmoratus*. After 50 days' exposure, the gonadosomatic indices and percentage of sperm produced, concentrations of salmon-type gonadotropin-releasing hormone, follicle-stimulating hormone, luteinizing hormone mRNA, 17β-estradiol, and γ-glutamyl transpeptidase activity were all depressed at the 0.06 and 0.6 μg/L doses, but were comparable to the control animals at the 6 μg/L dose. The researchers also found that these effects were related to phenanthrene accumulation in the brain, which increased with exposure to the two moderate doses of phenenathrene, but declined again at the highest exposure concentration. The decline in brain phenanthrene at the highest dose was related to changes in glutathione-S-transferase activity. Sun et al. (2011) hypothesized that the U-shaped dose–response curve for the effects of phenanthrene on testicular function was mediated via alteration of biotransformation enzyme activity in the brain. Although there is little additional information on the effects of phenanthrene on brain GST activity in fish, other studies have documented upregulation of hepatic GST activity in fish exposed to phenanthrene (Shallaja and D'Silva, 2003; Correia et al., 2007; Oliveira et al., 2008; Pathiratne and Hemachandra, 2010). However, results are somewhat inconsistent and dose dependent; for example, some researchers report GST induction at low doses of phenanthrene but little effect or suppression at higher doses (Oliveira et al., 2008; Pathiratne and Hemachandra, 2010).

Results of field studies of male reproductive function at PAH-contaminated sites are mixed. Bugel et al. (2010) evaluated the reproductive health of male Atlantic killifish inhabiting the heavily industrialized Newark Bay, which is contaminated with PAHs as well as other contaminants. The Newark Bay males had decreased gonad weight, altered testis development, and decreased gonadal aromatase mRNA expression. Similarly, Pait and Nelson (2005) observed a significant negative correlation between GSI of male killifish and PAH concentrations in sediments in Chesapeake Bay. However, in both of these studies, killifish were exposed to other contaminants in combination with PAHs, which may also have contributed

to the observed effects. On the other hand, Sol et al. (2008) found little correlation between exposure to chemical contaminants, including PAHs, and either 11-ketotestosterone concentrations or gonad weight in male English sole from Puget Sound. Similarly, male California halibut (*Paralichthys californicus*) exposed to sediments from natural oil seeps did not show a dose-responsive decrease in testosterone concentrations or GSI (Seruto et al., 2005). Differences in the timing, duration, and level of exposure, the specific PAHs to which fish were exposed, and other contaminants present at these study sites, could all contribute to the conflicting responses observed in these studies.

PAHs are genotoxic compounds known to reduce sperm quantity and quality in humans and laboratory rodents (Xia et al., 2009; Ramesh and Archibong, 2011) and have strong cytotoxic effects on the testis in marine mammals (Godard et al., 2006). However, less information is available on their effects on sperm quality in fish. Nagler and Cyr (1997) observed reduced sperm quality in male plaice exposed to sediments with high concentrations of PAHs. White et al. (1999) found reduced egg hatchability and larval survival in offspring of fathead minnow (*Pimephales promelas*) exposed to BaP, but whether this finding was associated with genotoxic effects on sperm was not determined. Bohne-Kjersem et al. (2009) observed changes in some proteins and enzymes (Pzp-(pregnancy zone protein)-resembling protein and alpha-2-antiplasmin) involved in mammalian spermatogenesis, sperm capacitation, and fertilization in juvenile Atlantic cod exposed to crude oil at a concentration of 0.25 mg/L. These studies suggest an effect of PAHs on sperm quality in male fish.

4.3. Mechanisms of Endocrine Disruption

PAHs exert their endocrine disrupting effects through various mechanisms. Like dioxin, they can influence steroid production and metabolism through their interaction with the aryl hydrocarbon receptor and other dioxin-responsive elements (Williams et al., 1998; Navas and Segner, 2001; Villeneuve et al., 2002; Logan, 2007) (see Chapter 1), and they have also been shown to interact with other receptors and responsive elements that play a role in regulation of endocrine processes, including cAMP responsive elements, estrogen-responsive elements (EREs), and elements related to other nuclear receptors, including peroxisome proliferator-activated receptor, retinoid X receptor, and retinoic acid receptor (Cheshenko et al., 2008; Bilbao et al., 2010).

Several studies have demonstrated that PAHs can alter the expression and activity of the P450 enzyme aromatase, which is involved in the conversion of androgens into estrogens, generally suppressing activity and

reducing estrogen synthesis (see reviews by Cheshenko et al., 2008; Le Page et al., 2011). In fish, there are two isoforms of aromatase—a predominantly ovarian form, CYP19A1, and a brain form, CYP19A2. The effects of BaP and other PAHs on aromatase activity have been investigated in multiple fish species including mummichog, gray mullet (*Chelon labrosus*), Atlantic cod, turbot (*Schophthalmus maximus*), and zebrafish (Kazeto et al., 2004; Hoffmann and Oris, 2006; Patel et al., 2006; Martin-Skilton, 2006, 2008; Dong et al., 2008; Bilbao et al., 2010). The degree to which the expression of these enzymes is altered appears to vary with dose, length of exposure, and life stage, although of the two forms, CYP19A2 appears to be the most consistently sensitive to PAH exposure.

PAHs may also affect endocrine processes through their influence on brain monoaminergic neurotransmitters, such as dopamine, serotonin, and noradrenaline. In a series of studies, Gesto and colleagues (Gesto et al., 2006, 2008, 2009) established that exposure of immature rainbow trout to the naphthalene altered levels of these three neurotransmitters in several brain regions, including the hypothalamus, preoptic region, pituitary, and brain stem. This could be significant, as serotonin is known to play a role in the modulation of diverse physiological processes, including reproduction, in fish (Rahman et al., 2011).

Exposure to PAHs may also alter estrogen and testosterone metabolism, although the effects are not necessarily consistent among studies. For example, while earlier studies suggested that PAH exposure might inhibit estrogen metabolism in fish (Snowberger and Stegeman, 1987; Stein et al., 1988), Butala et al. (2004) reported that exposure to BaP increased formation of the estradiol metabolites, 2- and 4-hydoxyestradiol, in liver, gill, and gonad microsomes of channel catfish (*Ictalurus punctatus*). Martin-Skilman et al. (2006, 2008) observed increased UDP-glucuronosyltransferase activity in juvenile turbot exposed to fuel oil, and increased glucuronidation and clearance of testosterone in turbot exposed to crude oil.

5. EFFECTS ON EMBRYONIC AND LARVAL DEVELOPMENT

Of the many aspects of fish physiology potentially affected by PAHs, a wealth of laboratory and field studies indicate that embryonic development is the most sensitive process identified to date. In particular, developing fish embryos are especially sensitive to low concentrations of waterborne PAHs derived from spilled crude oil or its refined products. Largely because of the 1989 *Exxon Valdez* oil spill in Prince William Sound, AK, the impacts of crude oil exposure on fish development have been intensively studied for

over two decades. This line of research has led to the identification of two major toxic pathways for injury to fish early life history stages. The first is the recognition of cardiac-specific toxicity associated primarily with the tricyclic families of PAHs that are most abundant in petroleum products, that is, fluorenes, dibenzothiophenes, and phenanthrenes. This form of toxicity is specific to the organogenesis phase of development during which the initial embryonic heart tube begins to function and undergoes looping and morphogenesis of atrial and ventricular chambers to form the larval heart. A second major pathway is light-induced toxicity (phototoxicity). Phototoxicity is not necessarily specific to a particular stage of development, but occurs in fish species (and other organisms) that have translucent early life history stages with little pigment. Some PAHs produce phototoxic reactions in cells and tissues, through activation by ultraviolet (UV) radiation and generation of reactive oxygen species, which leads to membrane damage and cell death (Arfsten et al., 1996; Yu, 2002). In laboratory studies with diverse aquatic animals, this UV-enhanced phototoxicity of PAHs occurs at much lower tissue concentrations that produce acute toxicity in the absence of UV light (Diamond, 2003). Although the precise compounds responsible for phototoxicity of petroleum products are currently unidentified, they are presumed to be polyaromatic compounds due to the necessity of this chemical structure for activity (Veith et al., 1995).

The elucidation of tricyclic PAH cardiotoxicity followed directly from field studies that assessed the impacts of the *Exxon Valdez* spill. Initial efforts focused on the two most important commercial fish species in Prince William Sound at the time of the spill: pink salmon (*Oncorhynchus gorbuscha*) and Pacific herring (*Clupea pallasi*). Due to the spawning habits of both species, they in essence put their embryos in harm's way by depositing fertilized eggs adjacent to (herring) or in stream deltas that crossed (salmon) oiled shorelines. Samples collected from pink salmon redds showed elevated rates of embryonic lethality up to four years following the spill (Rice et al., 2001), while herring larvae sampled in plankton tows along oiled shorelines showed high rates of morphological abnormalities, the most frequent of which was fluid accumulation (edema) around the heart (Marty et al., 1997a). Laboratory studies showed that exposure to Alaska North Slope crude oil (ANSCO), representative of oil from the hold of the *Exxon Valdez*, caused the same types of abnormalities in salmon and herring embryos observed in the field in a dose-dependent manner (Carls et al., 1999; Heintz et al., 1999; Marty et al., 1997a, 1997b). Since that time, multiple studies have demonstrated a common syndrome of embryonic oil exposure marked by fluid accumulation (edema or ascites) and variable degrees of craniofacial and body axis malformation (Figure 4.3). These effects have been documented for geologically distinct crude oils (ANSCO,

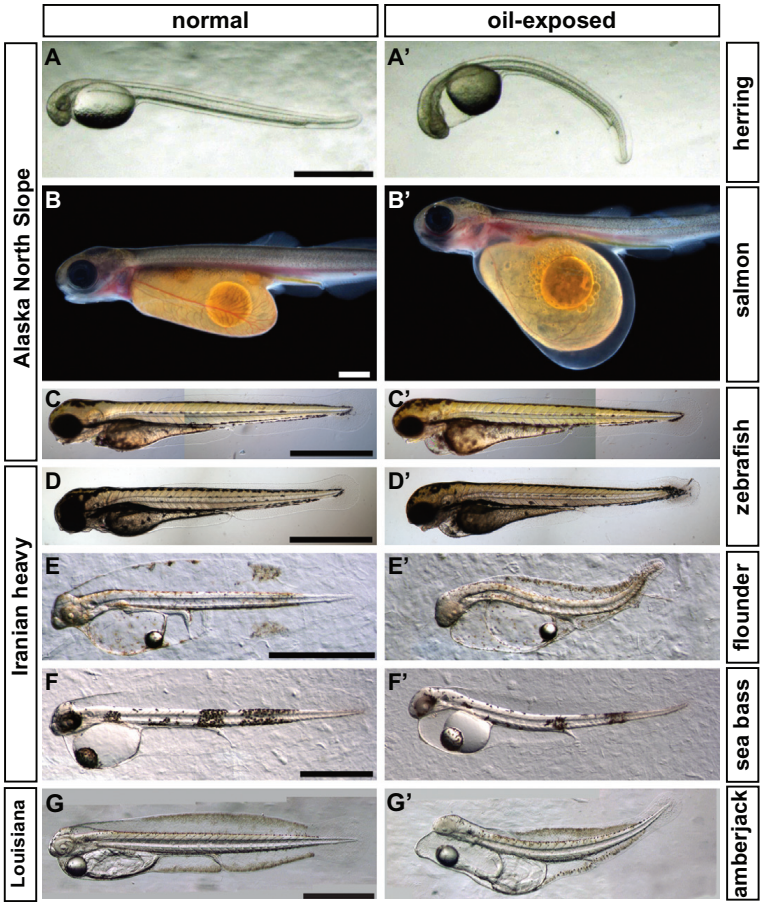

Figure 4.3. Crude oil exposure during embryogenesis causes a common fluid accumulation syndrome across multiple species of fish, geological sources of oil, and exposure methods. Left panels show control embryos with the egg shell dissected or newly hatched larvae; right panels are corresponding oil-exposed clutch mates. All exposures occurred from shortly after fertilization to the hatching stage. (A) Pacific herring and (B) pink salmon exposed to ANSCO using oiled gravel columns; (C) zebrafish exposed to a mechanically dispersed water-accommodated fraction of ANSCO; (D) zebrafish exposed to Iranian heavy crude oil using an oiled gravel column; (E) olive flounder (*Paralichthys olivaceus*) and (F) Japanese sea bass (*Lateolabrax japonicas*) exposed to Iranian heavy crude oil using an oiled gravel column; (G) Yellowtail amberjack (*Seriola lalandi*) exposed to a mechanically dispersed water-accommodated fraction of a Louisiana crude oil. (A) Incardona et al., 2009; (B) Carls and Incardona, unpublished; (C) and (D) Jung et al., 2013; (E) and (F) Jung and Incardona, unpublished; (G) Incardona et al., unpublished.

Iranian heavy, Bass Strait, and Medium South American (Couillard, 2002; Incardona et al., 2005; Jung et al., 2013; McIntosh et al., 2010; Pollino and Holdway, 2002) and in a diversity of fish species beyond pink salmon and Pacific herring, including mummichog (Couillard, 2002), zebrafish (Carls

et al., 2008; Incardona et al., 2005), rainbowfish (Pollino and Holdway, 2002), and Atlantic herring (*Clupea harengus*) (McIntosh et al., 2010). Importantly, the syndrome associated with crude oil exposure is independent of the method used to expose embryos and is identical whether aqueous PAHs are derived by passage of water though oiled gravel generator columns, physical dispersion of oil droplets, or chemical dispersion with detergents (Carls et al., 2008; Incardona et al., 2005; Jung et al., 2013) (Figures 4.3 and 4.4).

A series of studies using the zebrafish model determined that heart failure and the corresponding loss of circulatory function underlie the majority of the morphological defects associated with "canonical" crude oil toxicity described above (Incardona et al., 2011a). The characteristic accumulation of fluid in edematous embryos is a secondary or downstream consequence of severe cardiac malfunction, including heart malformation. Comparison between the effects of single PAHs and petrogenic PAH mixtures from crude oil in zebrafish embryos established that individual PAHs produce different forms of toxicity via distinct mechanistic pathways (Incardona et al., 2004, 2005, 2006, 2011b; Scott et al., 2011). A major finding was that the tricyclic compounds fluorene, dibenzothiophene, and phenanthrene drive most of the toxicity in petrogenic PAH mixtures (Figure 4.4). Importantly, as spilled oiled is weathered, the fractions of these tricyclic PAH classes within the total dissolved PAHs measured in water increases relative to the smaller two-ring compounds over time. The alkylated derivatives in particular become the dominant compounds during prolonged periods of weathering (Carls et al., 1999; Heintz et al., 1999; Short and Heintz, 1997). These three-ring compounds cause cardiac dysfunction relatively early in the organogenesis phase of development, when the heart is both taking shape and starting to circulate blood to other still-forming organs. The developing heart is unable to effectively pump blood, which leads to gross injury in the form of heart malformation, edema, craniofacial defects, and body axis defects (Incardona et al., 2004, 2005).

The primary effects of the tricyclic PAHs on cardiac function are most likely a consequence of ion channel blockade (Incardona et al., 2004, 2005, 2009). The observed abnormalities in heart rhythm (slowed or irregular heart rate) and contractility (pumping ability) are consistent with tricyclics acting as pharmacological agents to block the sodium, potassium, or calcium channels that together regulate the electrical activity of the heart. Consistent with this, the physiological effects of crude oil on the fish heart are indistinguishable from the side effects of some human drugs such as the antihistamine terfenadine, which were removed from the market after they were found to induce fatal arrhythmias (De Bruin et al., 2005; Mitcheson et al., 2000). In zebrafish, PAHs (Incardona et al., 2004, 2005) and drugs

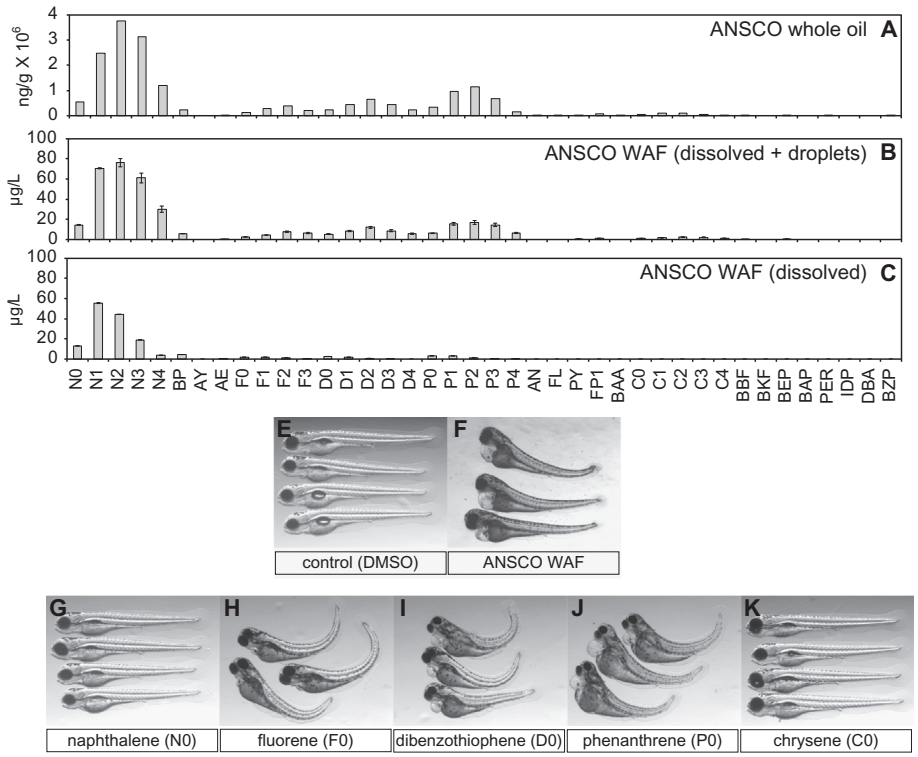

Figure 4.4. Individual tricyclic compounds in crude oil are sufficient to produce cardiotoxicity in zebrafish embryos similar to the entire petrogenic PAH mixture. (A) PAHs measured in ANSCO are shown for the whole oil after artificially weathering to remove monoaromatic compounds; (B) a mechanically dispersed water-accommodated fraction containing both aqueous (dissolved) PAHs and whole oil droplets, and (C) the same water-accommodated fraction after filtration to remove droplets, leaving behind only the aqueous fraction. Although the mixtures are dominated by naphthalenes, the next most abundant classes are the tricyclic compounds. (E–K) Hatching stage zebrafish larvae exposed to (E) solvent control (dimethylsulfoxide); (F) mechanically dispersed water-accommodated fraction of ANSCO containing 1.5 mg/L ΣPAHs; (G) 10 mg/L naphthalene; (H) 10 mg/L fluorene; (I) 10 mg/L dibenzothiophene; (J) 10 mg/L phenanthrene; and (K) 2 mg/L chrysene. Embryos are representative of studies described in Incardona et al. (2004 and 2005). Exposures were performed in small-volume (2.5 mL) multiwall polystyrene plates with high nominal concentrations of PAHs above the solubility limit of each compound. Actual aqueous concentrations were not measured but had to be at or below the solubility limit for each compound (e.g., about 7 µM for phenanthrene).

that have ion channel blocking side effects (Langheinrich et al., 2003; Milan et al., 2003) cause the same arrhythmias. These insights from the zebrafish model were extended to Pacific herring in controlled laboratory exposures using ANSCO (Incardona et al., 2009). Moreover, reduced heart rate and pericardial edema were documented in both caged and naturally

spawned herring embryos collected from shorelines in San Francisco Bay up to two years after they were oiled in the aftermath of the 2007 *Cosco Busan* bunker oil spill (Incardona et al., 2012a).

The *Cosco Busan* field studies completed a full circle in which a gross syndrome was identified in field samples collected after the *Exxon Valdez* spill; the specific target organ and toxic mechanisms leading to that syndrome were identified in laboratory studies with a model fish species (zebrafish); and then the same specific defects observed in laboratory studies were confirmed in embryos collected from the impacted zone of another oil spill 18 years later. At the same time, this ground-truthing of petrogenic PAH cardiotoxicity in the field was overshadowed by a more dramatic toxicity observed in herring spawn at oiled shorelines that was subsequently determined to be a result of phototoxicity (Incardona et al., 2012a, 2012b). During the initial assessment three months following the *Cosco Busan* spill, differential toxicity was observed in herring embryos that incubated either in the shallow subtidal zone, beneath at least a meter of turbid San Francisco Bay water, or in the potentially sun-lit intertidal zone (also called the photic zone). While sublethal cardiac impacts were observed in subtidal embryos, embryos deposited in the intertidal zone of oiled sites developed to the hatching stage (e.g., fully pigmented eyes) before succumbing to lethality that involved extensive tissue deterioration (Incardona et al., 2012a).

A previous study in Pacific herring demonstrated how canonical crude oil sublethal toxicity could be converted to a more potent and acutely lethal toxicity by an interaction with UV radiation (Barron et al., 2003). As was the case for canonical petrogenic PAH toxicity, the zebrafish model provided a useful system for assessing the phototoxic potential of bunker oils typically used to power large ships like the *Cosco Busan*. A comparison of two different bunker oils with ANSCO in a simple zebrafish embryo phototoxicity assay demonstrated that the bunker oils contained an even more potent phototoxic activity than the unrefined crude oil (Hatlen et al., 2010). Embryos exposed to bunker oil in a dark incubator showed all the signs of canonical petrogenic PAH cardiotoxicity. When transferred to clean water and exposed briefly to sunlight, however, they responded with a rapid cytolytic response in transparent tissues, leading to massive tissue disruption and mortality (Hatlen et al., 2010). A broadscan GC/MS analysis of bunker oil compounds in water-accommodated fractions showed that a wide range of heterocyclic and potentially polar aromatic compounds are present at much higher concentrations in the bunker oils relative to unrefined crude oil (Hatlen et al., 2010). Assays in zebrafish embryos exposed to single PAH compounds showed that the PAHs most abundant in both crude and bunker oils did not produce strong phototoxic reactions, nor did the most abundant

three-ringed heterocycles such as carbazole (Incardona et al., 2012b). The precise compounds causing bunker (or crude) oil phototoxicity remain unclear.

Regardless of the identity of the phototoxic compounds, assays in Pacific herring embryos did show that bunker oil remained potently phototoxic after at least two months of weathering (Incardona et al., 2012b). Moreover, exposure to effluent from gravel columns coated with *Cosco Busan* bunker oil containing ΣPAH concentrations less than 2 μg/L resulted in a tissue disruption pattern that was essentially identical both to that observed in zebrafish assays using water-accommodated fractions (WAFs) from mechanically dispersed oil (Hatlen et al., 2010) and to dead embryos collected from intertidal zones at oiled sites three months after the *Cosco Busan* spill (Incardona et al., 2012a, 2012b) (Figure 4.5).

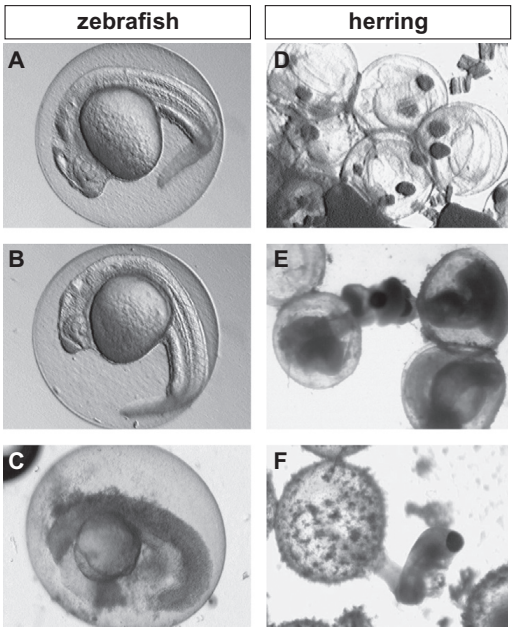

Figure 4.5. Phototoxicity of bunker oil in zebrafish and herring embryos. Left column, zebrafish embryos at 24 hours of development. (A) No-oil control following a 2-h exposure to ambient sunlight. (B) Embryo exposed to bunker oil for 24 hours followed by a 2-h outdoor exposure protected from light with aluminum foil. (C) Embryo exposed to bunker oil for 24 hours followed by a 2-h exposure to ambient sunlight outdoors. Right column, herring embryos. (D) Healthy hatching stage herring embryos. (E) Dead hatching stage embryos collected from the intertidal zone of the *Cosco Busan* spill site. (F) Dead hatching stage embryo following laboratory combined exposure to *Cosco Busan* bunker oil (expo 1.8 μg/L) and sunlight. (A–C) Hatlen et al., 2010; (D–F) Incardona et al., 2012b.

These recent findings of both major pathways of early life stage toxicity for PAHs (or other aromatics from petroleum) have important implications for the future of research on this topic. The recognition of cardiotoxicity as the driver for embryonic malformation syndromes has identified important hypotheses for the nature of delayed mortality that can occur in fish that survive very low-concentration PAH exposure during embryogenesis. Mark and recapture studies in pink salmon repeatedly demonstrated a reduction in marine survival for externally normal juvenile fish that were exposed to ANSCO as embryos (Heintz, 2007; Heintz et al., 2000). A parallel study using zebrafish showed that embryos exposed to ANSCO (ΣPAH 20–30 μg/L) grew into adults with no external abnormalities, but with abnormal heart shape that was associated with reduced aerobic performance as measure by critical swimming speed (Hicken et al., 2011). It is therefore possible that the observed reduced marine survival in the pink salmon studies was due to cardiovascular impairment resulting from mild heart defects downstream of cardiotoxicity during organogenesis. Future studies are likely to focus on cardiac anatomy and cardiovascular performance in oil-exposed salmon and herring.

The *Cosco Busan* studies highlight how poorly characterized crude oil and its products like bunker oil remain, with respect to both chemical complexity and biological effects. Identification of the primary phototoxic compounds will require a major effort using novel approaches in analytical chemistry and a focus on the "unresolved complex mixture," which is typically a large fraction in bunker oils. These chemical approaches will also need to be integrated into a program assessing biological effects.

6. TRANSGENERATIONAL EFFECTS

The transgenerational effects of chemical contaminants, mediated through epigenetic mechanisms, are an emerging area of research (Skinner et al., 2011). Epigenetics is defined as the study of mitotically or meiotically heritable changes in gene function that occur without a change in the DNA sequence (Vandegehuchte and Janssen, 2011). Epigenetic regulation of gene expression typically occurs through DNA methylation or histone modification (Head et al., 2012). Environmental influences, including toxic chemicals, can alter DNA methylation and epigenetic programming in both germ and somatic cells (Skinner et al., 2011). When an epigenetic mutation occurs in a somatic cell, it may lead to disease in the affected individual, but it will not be transmitted to the next generation. However, if the germ line is modified, it can affect not only the offspring of the

individual with the mutation, but also subsequent generations (Skinner et al., 2011). Research on humans and rodents shows that exposure to PAHs can cause epigenetic modifications to somatic- and germ-cell lines, potentially leading to transgenerational effects. Exposure to PAHs in cigarette smoke can cause changes in DNA methylation in lung tissue that are correlated with the development of lesions associated with tumorigenesis (Phillips and Goodman, 2009; Liu et al., 2009, Tommasi et al., 2010). There is also some evidence that maternal smoking may be associated with altered DNA methylation patterns in the fetus that can influence nervous system and brain development (Stein, 2012), or even increase the risk of adult-onset diseases such as asthma, reduced fecundity, and obesity (Suter et al., 2010). In another recent study, Manikkam et al. (2012) found that exposure to jet fuel promoted early-onset female puberty and reduced fertility in both male and female rats not only in the exposed generation and its offspring, but in the unexposed F3 generation. These conditions appeared to be mediated through changes in DNA methylation observable in the sperm of all exposure lineage males.

Exposure to PAHs may also lead to changes in DNA methylation in fish (Fang et al., 2010), including changes associated with carcinogenesis (Fang et al., 2010; Mirbahai et al., 2011a, 2011b). Changes in DNA methylation in liver neoplasms have been observed in both laboratory-reared zebrafish, in which tumors were induced with a model carcinogen (Mirbahai et al., 2011a), and wild dab that developed neoplasms as a result of environmental exposure to PAHs (Mirbahai et al., 2011b). These studies indicate that epigenetic mechanisms occur in fish, that they are affected by exposure to PAHs, and that they may play a role in disease occurrence. There are also reports of multigenerational effects of PAH exposure. For example, when White et al. (1999) exposed fathead minnow to BaP, hatch frequency of eggs produced by F1 females was reduced and the survival of the F2 larvae was poor. Several studies also indicate that fish populations from contaminated areas have developed resistance to the effects of PAHs that may be heritable (Wirgin and Waldman, 2004). For example, Elizabeth River and other U.S. Atlantic urban coastal populations of killifish have adapted to high concentrations of PAHs by having reduced responsiveness to these compounds in the aryl-hydrocarbon receptor (AHR) pathway (Ownby et al., 2002; Meyer et al., 2002; Wills et al., 2010b). This alteration is at least partially heritable through the F2 generation and is thought to play a role in mediating their resistance to teratogenicity and lethality caused by PAHs (Meyer and Di Giulio, 2002, 2003; Meyer et al., 2002). However, the mechanisms underlying the development of PAH resistance in killifish are not completely understood. Although the genetic basis for resistance has not been fully elucidated (Mulvey et al., 2002, 2003; Hahn et al., 2004;

Meyer et al., 2005; Oleksiak et al., 2011), there is little evidence for epigenetic changes associated with modified AHR pathway functioning. Resistant and sensitive populations display no consistent differences in either AHR or AHR promoter DNA methylation patterns (Arzuaga et al., 2004; Timme-Laragy et al., 2005; Aluru et al., 2011), and there is no evidence that the resistant phenotype is passed on to future generations through epigenetically modified germ cells. Thus, as yet, there appear to be no reports in fish of true transgenerational effects of PAHs occurring as a result of epigenetic modifications to fish germ lines.

7. OTHER EFFECTS WITH CONSEQUENCES FOR SURVIVAL OF POSTLARVAL STAGES

7.1. Effects on Growth and Condition

Normal growth and adequate energy reserves are critical to the survival of juvenile fish and have a strong influence on the reproductive potential of adults. Several studies conducted prior to 2000 indicated that exposure to PAHs in crude oil or from urban and industrial sources could lead to reduced growth rates and reduced condition indices in fish (Al-Yakoob et al., 1996; Carls et al., 1996; Kubin, 1997; Moles and Norcross, 1998; Ridgway et al., 1999). In recent years these findings have been confirmed and expanded to include the effects of PAHs on lipid profiles and energy metabolism.

Reduced growth and/or condition factors have been documented in several studies conducted since 2000 in which fish were exposed to PAH mixtures comparable to those present in urban sediments. Rice et al. (2000) found significantly reduced weight in juvenile English sole fed polychaete worms reared on sediments containing 3000–4000 μg/kg dry wt of PAHs, after an exposure period of only 12 days. The growth rate was markedly lower (0.05–0.1% per day) in exposed fish than in control fish (1.1–1.2% per day). Meador et al. (2006) reported reduced weight in juvenile Chinook (*Oncorhynchus tshawytscha*) salmon fed a diet containing PAHs at a dose of 18 μg/g fish/day, as well as increased variation in weight distribution in Chinook salmon at a dietary dose of 0.7 μg/PAHs/ g fish/day Saborido-Rey et al. (2007) also showed reductions in the growth and condition factor in turbot exposed to dietary PAHs at concentrations from 25 to 260 μg/g dry wt.

Several other studies document reduced growth and condition indices in fish exposed to PAHs in the form of crude oil and related petroleum products. Heintz et al. (2000) report reduced growth of juvenile pink salmon

exposed as embryos to water contaminated with PAHs from gravel coated with weathered crude oil at doses as low as 18 μg/L PAH. Kerambrun et al. (2012) exposed juvenile sea bass (*Dicentrarchus labrax*) to PAHs in the form of Arabian light crude oil for 48 and 96 hours, at concentrations ranging from 11 to 49 μg/L, and fish were then held for 26–28 days in clean seawater. They found that the 48-hour exposure led to a significant decrease in specific growth rate (length) and in RNA:DNA ratios, which are an indicator of growth rate. Significant decreases in the specific growth rates for both length and weight, recent growth rate estimated from otolith analysis, RNA:DNA ratio, and the Fulton's K condition index were observed in sea bass 26 d after the 96-h exposure. Holth et al. (2008) reported reduced condition factor in zebrafish exposed to PAHs in produced water at concentrations as low as 0.54 μg/L. Claireaux et al. (2004) reported reduced condition factor in common sole (*Solea solea*) exposed to fuel oil for 24 h and then transferred to clean water and monitored for two months. Similar reductions in condition factor were found in sole caught from sites exposed to the Erika oil spill (Gilliers et al., 2006). Amara et al. (2007) found that juvenile common sole from contaminated estuaries along the French coast of the Southern Bight of the North Sea and the Eastern English Channel had lower growth rates (estimated from otolith microstructure) and lower condition index than sole from less contaminated estuaries in the same region, but other contaminants as well as PAHs were present at the impacted sites.

Several investigators report changes in energy metabolism, particularly lipid metabolism, in fish exposed to PAHs. Meador et al. (2006) observed reductions in total body lipid and triacylglycerol (TAG) in juvenile Chinook salmon fed a PAH-contaminated diet at a dose of 18 μg/g fish/day and a reduction in plasma triglyceride and lipase concentrations at a dose of 2.3 μg/g fish/day. Bohne-Kjersem et al. (2009) found evidence from a proteonomic study of an effect of crude oil exposure on lipid metabolism. In juvenile cod, both prepro-apolipoprotein and apolipoprotein B, which are important regulators of triglyceride and cholesterol metabolism, were differentially expressed after 24 days of exposure to crude oil at concentrations from 0.06 to 1 mg/L. Holth et al. (2008) also observed effects on genes associated with lipid metabolism in zebrafish exposed to PAHs in produced water at concentrations ranging from 0.54 to 5.4 μg/L for 1, 7, and 13 weeks. In a mesocosm study, Claireaux et al. (2004) showed that the ratio of TAG to structural lipids (TAG:ST ratio) declined by 75% relative to controls in juvenile sole exposed for 24 hr to PAHs in petroleum fuel (0.1 and 0.5% fuel in water), then held for three months in clean seawater. Kerambrun et al. (2012) found a similar trend in TAG:ST ratios in juvenile Atlantic cod exposed to light crude oil (total PAH concentrations of 10–50 μg/L), but these differences were not statistically significant, perhaps

because feeding with commercial pellets minimized these nutritional deficits. Amara et al. (2007) report lower TAG:ST ratios in juvenile common sole from PAH-contaminated estuaries along the French coast of the Southern Bight of the North Sea and the Eastern English Channel compared to sole from less affected estuaries in the region; however, other contaminants in addition to PAHs were also present at the affected sites and may have contributed to their results.

Other researchers report changes in plasma glucose concentrations and other measures associated with energy metabolism in fish exposed to PAHs. Tintos et al. (2007) found that exposure of immature female rainbow trout to naphthalene (intraperitoneally injected or in slow-release coconut oil implants at doses of 10 and 50 mg/kg body wt) led to increased glycogenolysis in the liver, use of exogenous glucose, and glycolysis and decreased gluconeogenesis. They hypothesized that the increased energy production in liver might be related to increased detoxification activity in liver after PAH exposure. Gesto et al. (2008) observed depressed plasma glucose concentrations in rainbow trout exposed to naphthalene, BaP, or β-naphthoflavone and subjected to prolonged stress (high stock density for 72 hr). Meador et al. (2006) showed decreased plasma glucose concentrations in juvenile Chinook salmon fed a diet containing 18 μg/g fish/day of PAHs. Several additional studies report short-term increases in plasma glucose concentrations in fish exposed to PAHs, often associated with increased concentrations of plasma cortisol and so likely a response to the stress of toxicant exposure (Tintos et al., 2008; Oliveira et al., 2007). In a field study, Oliveira et al. (2011) observed high glucose and lactate concentrations in plasma of the gray golden mullet (*Liza aurata*), from a PAH-contaminated site in comparison to fish from an uncontaminated site.

Contaminants, including PAHs, may also affect growth, condition, and energy metabolism through their effects on thyroid function. Brown et al. (2004) reviewed the effect of chemical contaminants on fish thyroid function and identified several studies in which exposure to model PAHs or water-soluble fractions of crude oil led to changes in plasma concentrations of the thyroid hormones triiodothyronine (T3) and thyroxine (T4). However, the specific effects found in these studies were not consistent, with PAH exposure sometimes leading to increases and in other cases to decreases in T3 and T4 concentrations. More recent studies are similar in their outcome. For example, Wu et al. (2003) found that exposure to dietary BaP at concentrations of 0.25 and 12.5 μg/g body wt led to increases in free T3 concentrations in plasma of juvenile grouper (*Epinephelus areolatus*). However, in a field study, Oliveira et al. (2011) found low plasma T3 concentrations in golden gray mullet from a PAH-contaminated site in Portugal. Similarly, He et al. (2012) found that exposure of embryonic

rockfish (*Sebasticus marmoratus*) to pyrene reduced concentrations of T3 in whole embryos and downregulated thyroid receptor gene expression in rockfish embryos, but no effect was found on T4 concentrations. In adult eel (*Anguilla anguilla*), exposure to β-naphthoflavone significantly decreased plasma T4 concentrations, whereas thyroid-stimulating hormone and T3 remained constant (Teles et al., 2005). Other studies suggest that separate classes of contaminants, such as PCBs, are more strongly correlated than PAHs with alterations in thyroid function in fish (Brar et al., 2010).

7.2. Immunotoxicity

PAHs have been found to modulate both the innate and adaptive immune response in fish (reviewed in Rice and Arkoosh, 2002; Burnett, 2005; Reynaud and Deschaux, 2006). The immune system protects against internal and external insults. A properly functioning immune system is an important fitness trait that is critical for survival of the individual and the population (Segner et al., 2012a). An altered immune system due to contaminant exposure, either in conjunction with other stressors (Jacobson et al., 2003) or alone (Arkoosh et al., 2000, 2001, 2010), can result in increased disease susceptibility. This increase in disease susceptibility can lead to potential population-level effects (Arkoosh et al., 1998; Loge et al., 2005; Spromberg and Meador, 2005). Extrapolating changes due to contaminants from the individual or molecular level to alterations in the population or community is a key concept in ecotoxicology (Newman and Clements, 2008).

The mechanisms of PAH toxicity on the immune system in mammals have been well studied, but few studies exist in fish. However, the mechanisms of PAH toxicity are thought to be conserved from fish to mammals (Reynaud and Deschaux, 2006). Two potential mechanisms are: (1) metabolic activation (P450-mediated biotransformation) of PAH is required for immunotoxicity (Reynaud et al., 2001; Carlson et al., 2004a, b); and (2) PAHs can modulate intracellular calcium levels, suggesting that inhibition of the immune response is calcium dependent (Reynaud et al., 2001, 2003).

Innate or nonspecific immunity is the first line of defense against pathogen invasion (Magnadóttir, 2006; Whyte, 2007). Innate immunity prevents pathogens from attacking, invading, and multiplying on or in the host. Fish have integumental, humoral, and cellular innate defenses against pathogens (Bols et al., 2001; Ellis, 2001). Most studies examining the effects of PAHs on innate immunity have focused on humoral and cellular components, and not on the integumental component.

Studies examining the effect of PAHs on the humoral component of the innate immune system have investigated lysozyme and complement activity (Hutchinson et al., 2003; Bado-Nilles et al., 2009). *In vitro* exposure of European sea bass (*Dicentrarchus labrax* Linné) to a number of individual PAHs produced both an enhancement and a suppression of alternative complement activity and lysozyme concentration, depending on the concentration and the PAH examined (Bado-Nilles et al., 2009). However, dab exposed to sediments spiked with PAHs did not have an altered lysozyme concentration (Hutchinson et al., 2003). Therefore, it is apparent that the effects of PAH exposure on the humoral component of the immune system depend not only on the concentration of the PAH but also on the method of exposure.

Studies examining the effect of PAHs on the cellular component of the innate immune system have focused mainly on phagocytes (Reynaud et al., 2001, 2004; Carlson et al., 2002). Kidney phagocytes from Japanese medaka (*Oryzias latipes*) injected with BaP had a reduced amount of reactive oxygen species (ROS) activity (Carlson et al., 2002). Also, dab exposed to sediments contaminated with PAHs had a reduced amount of ROS activity in the kidney (Hutchinson et al., 2003). However, the kidney phagocytes of common carp (*Cyprinus carpio* L.) exposed to 3-methylcholanthrene had an enhanced production of ROS (Reynaud et al., 2001). Likewise, ROS production increased in kidney phagocytes of English sole exposed to a mixture of PAHs (Clemons et al., 1999). Phagocytes are critical for the optimal functioning of the innate immune system, as well as the adaptive immune system. Humoral and cellular pattern recognition receptors (PRRs) recognize pattern recognition proteins (PRPs) on microbes and malignant or "nonself" host tissue. The PRP on macrophage cells triggers phagocytosis and eventually leads to activation of adaptive or specific immunity (Janeway et al., 2005; Magnadóttir, 2010).

Adaptive or specific immunity takes longer to develop than innate immunity and consists of both cellular and humoral components (reviewed in Burnett, 2005). Cellular components of adaptive immunity in fish have been examined with mitogen assays. The mitogen assay examines the ability of critical immune cells, for example, B and T cells, to proliferate in response to selective plant lectins and pharmacologic agents. Studies examining the immunotoxicological effects of PAHs on lymphocyte proliferation have exposed fish either through their diets (Connelly and Means, 2010), in cell culture (Reynaud et al., 2003), or by injection (Reynaud and Deschaux, 2005).

Depending on the PAH used and the route of exposure, both immunosuppression and immunostimulatory effects of the mitogen response have been observed. For example, bluegills (*Lepomis macrochirus*) exposed individually to

either two- (2-methylnaphthalene) or three-ringed (2-aminoanthracene and 9,10-dimethylanthracene) PAHs in their diets exhibit either increased or suppressed mitogenesis, depending on the PAH. However, a mixture of these same PAHs suppressed the response (Connelly and Means, 2010). Suppression to B- and T-cell mitogens was also observed in common carp exposed to 3-methylcholanthrene either intraperitoneally or in culture after the cells were isolated (Reynaud et al., 2003; Reynaud and Deschaux, 2005). Japanese medaka injected with BaP also had a suppressed mitogen response to both T- and B-cell mitogens (Carlson et al., 2002).

The humoral or soluble components of adaptive immunity include the production of immunoglobulins (antibodies) and cytokines (Segner et al., 2012b). Few studies have examined the effect of PAHs on the humoral components of adaptive immunity. Rainbow trout intraperitoneally injected with retene, an alkyl PAH, had an increased antibody titer to *Aeromonas salmonicida* relative to fish that were not exposed to the PAH (Hogan et al., 2010). The plaque-forming cell (PFC) assay determines the numbers of B-cells producing antibodies to a hapten. Juvenile Chinook salmon exposed to 7,12,-dimethylbenz[a]anthracene (DMBA) had suppressed primary and secondary PFC responses (Arkoosh et al., 1994). Japanese medaka were also found to have a suppressed primary PFC response after intraperitoneal injection with BaP.

Cellular assays examining various components of the innate or adaptive immune system are important in examining the immunotoxicity of PAHs. However, they are not measures of whole fish toxicity resulting from PAH exposure (Segner et al., 2012a). A more integrated and holistic approach for assessing the immunotoxicity of a chemical examines the host's susceptibility to a pathogen (Arkoosh and Collier, 2002; Arkoosh et al., 2005). With the disease challenge assay, immunotoxicity is examined with an activated rather than a resting immune system (Köllner et al., 2002). Few studies report the toxicity of environmentally relevant PAH concentrations on disease susceptibility in fish. Juvenile rainbow trout fed a diet containing a mixture of 10 high-molecular-weight PAHs were found to be more susceptible to the pathogen *Aeromonas salmonicida* (Bravo et al., 2011). Japanese medaka injected with BaP and juvenile Chinook salmon injected with a mixture of PAHs or DMBA were also more susceptible to the pathogens *Yersinia ruckeri* (Carlson et al., 2002) and *Listonella anguillarum* (Arkoosh et al., 2001), respectively. Interestingly, zebrafish exposed simultaneously to phenanthrene and the bacteria *Mycobacterium marinum* demonstrated a greater survival than those exposed to phenanthrene alone (Prosser et al., 2011). The authors hypothesized that the bacteria-induced inflammatory response interfered with the metabolism of phenanthrene to more toxic compounds, suggesting an antagonistic interaction between the

stressors. This hypothesis is supported by previous studies demonstrating that proinflammatory cytokines can inhibit CYP1A biotransformation of the parent PAH compound to more potentially toxic metabolites (reviewed in Morgan, 2001). However, the exact mechanisms that result in the suppression of the CYPlA biotransformation pathway are not known (Prosser et al., 2011).

Studies with fish also exist that examine the immunotoxic effects of petroleum products such as heavy oil (Nakayama et al., 2008; Song et al., 2012a, 2012b), oil-produced water (Perez-Casanova et al., 2010, 2012), the water-soluble fraction of oil (Kennedy and Farrell, 2008; Danion et al., 2011a, 2011b), and creosote (Karrow et al., 1999, 2001). The immunotoxic effects of these products are thought to be correlated to the PAH component of the petroleum products (Reynaud and Deschaux, 2006). Functional immunological end-points and gene expression have been found to be sensitive to these products, and the effects are species and product specific (Barron, 2012).

In summary, the observed response of the teleost immune system to PAH exposure has been variable. The majority of studies investigating the effects on teleost immune function have focused mainly on individual PAHs, for example, BaP, 3-methylcholanthrene, and the synthetic DMBA (Hogan et al., 2010). A number of variables influence a host's susceptibility to these PAHs and how the contaminant ultimately influences the function of the immune system. These variables include the host, the PAH or PAH mixture used, the PAH concentration, and the route of PAH exposure (e.g., *in vivo*, *in vitro*, and *in situ*) (Tryphonas et al., 2005). A uniform approach to PAH exposure assessment on immune function would facilitate comparisons of the effects of individual and mixed PAHs on a single species as well as across species.

8. NAPHTHENIC ACIDS AS A NEW CONCERN

8.1. Background

Naphthenic acids are complex mixtures of organic compounds that are natural (and corrosive) constituents of petroleum. Naphthenic acids are present in produced waters from certain petroleum deposits, most notably the immense oil sands deposits in northern Alberta, Canada. Naphthenic acids are also found in waters discharged from oil production platforms in the North Sea (Røe Utvik, 1999). Because they can make up as much as 3–4% by weight of petroleum and are generally more water soluble than PAHs, the environmental toxicology of naphthenic acids is receiving increasing attention. This is especially true in areas such as Alberta, where

the rate of extraction of bitumen is increasing, with concomitant production of millions of gallons of produced water contaminated with both naphthenic acids and PAHs. It has been estimated that 100 tonnes of naphthenic acids are being released from the daily processing of 500,000 tonnes of oil sands in Alberta (Clemente and Fedorak, 2005); large retention ponds have been built to handle the discharges of produced waters.

In terms of their chemistry, naphthenic acids are alkyl-substituted saturated cyclic and noncyclic carboxylic acids with the general chemical formula $C_nH_{2n+z}O_2$, where n indicates the number of carbon atoms and z is zero or a negative even integer that specifies the hydrogen deficiency arising from ring structures. When z is divided by −2, the result is the number of rings in the compound (see Figure 4.6). The acyclic portions of naphthenic acids can be highly branched, contributing to the complexity of these mixtures.

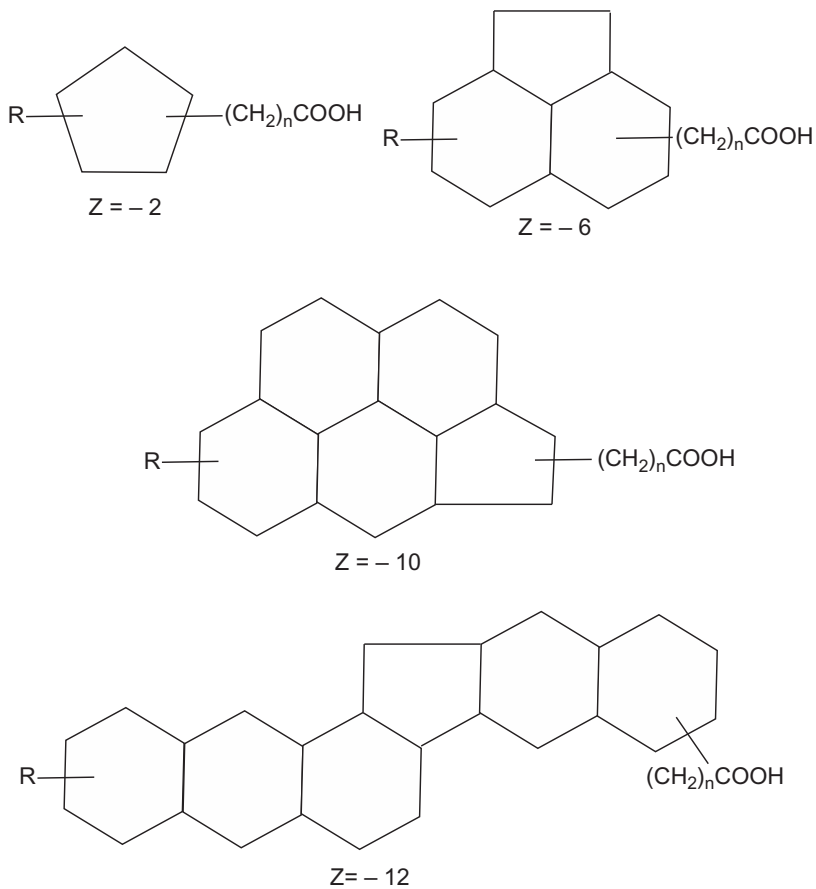

Figure 4.6. Chemical structure of four types of naphthenic acids.

8.2. Analytical Methods and Tissue Residues

The standard methods for measuring naphthenic acids in water involve extracting the samples and analyzing for the carboxylic acid moiety using Fourier transform infrared spectroscopy (Jivraj et al., 1995). When this method is used on biological samples, however, it will also detect naturally occurring fatty acids (which do fit the naphthenic acid formula given above, when $z = 0$). In order to ascertain the uptake of naphthenic acids in fish, Young et al. (2007) developed a method to qualitatively determine the presence of petroleum-derived naphthenic acids in fish tissues, involving anion exchange chromatography, followed by derivatization and GC/MS. Their results demonstrated that naphthenic acids were taken up by rainbow trout exposed via feeding or via waterborne routes, and that fish exposed to produced waters from oil sands extraction also accumulated naphthenic acids in their bodies. The method was subsequently modified to use GC/MS in single ion-monitoring mode, allowing for estimation of concentrations down to 0.1 mg/kg wet weight tissue (Young et al., 2008). Application of this method to fish captured near oil sands deposits or downstream of oil sands operations showed that several individuals of three species of fish (walleye (*Stizostedion vitreum*), northern pike (*Esox lucius*), and lake whitefish (*Coregonus clupeaformis*)) had concentrations of free naphthenic acids as high as 2.8 mg/kg wet weight. However, a subsequent sampling of several fish from the same area did not show any fish having detectable concentrations (>0.1 mg/kg) of free naphthenic acids in their muscle tissue. However, values were reported for apparent naphthenic acids in liver tissue, which the authors contend were not due to naphthenic acids because the chromatogram did not show an unresolved hump characteristic of naphthenic acids (Young et al., 2011). This same paper reported analysis of several tissues from rainbow trout that had been exposed to waterborne naphthenic acids for 10 days at a target concentration of 3 mg/L. Data from analyses of heart, liver, gills, kidney, and muscle tissue of four individual fish showed that liver had the highest concentrations (88 ± 54 mg/kg wet wt), followed by kidney and gill, with heart and muscle tissue lowest. Naphthenic acids are thought to rapidly depurate from fish after exposure (Young et al., 2008). A comparatively small number of studies have investigated either the toxicity of commercially available naphthenic acid mixtures to fish or the toxicity of environmentally relevant naphthenic acids, extracted from oil sands process waters or oil production platform process waters. Studies of process waters themselves are not reviewed here because of the confounding effects of other contaminants, notably the PAHs covered in the rest of this chapter. Determining the methods for bioremediation of naphthenic acids in oil sands process waters is an area of active research, reviewed in Quagraine et al. (2005).

8.3. Toxicology of Naphthenic Acids

Naphthenic acids are acutely toxic in bioassays using *Daphnia magna* as test organisms, and LC_{50}s were in the low (< 1 to 10) millimolar range. Toxicity generally increased with increases in molecular weight (Frank et al., 2009), though the addition of a second carboxylic acid moiety resulted in reduced acute toxicity. Juvenile (2-month-old) salmonids exposed to naphthenic acids for 10 days showed 50% mortality at a water concentration of 25 mg/L (Dokholyan and Magomedov, 1984). Exposure of fish (wild-caught yellow perch *Perca flavescens* and laboratory-exposed Japanese medaka) to commercially available naphthenic acid mixtures has demonstrated that these compounds cause increased embryo deformities and reduced length at hatch (Peters et al., 2007). Exposure of these same species to oil sands process waters containing naphthenic acids resulted in less toxicity than would have been predicted based on the concentrations of naphthenic acids alone. The authors speculated that either differences in naphthenic acid compositions or the presence of highly toxic impurities in the commercial mixture may have accounted for this difference.

Petrogenic naphthenic acids (extracted from North Sea oil platform process waters) have recently been shown to act as estrogen receptor agonists and androgen receptor antagonists *in vitro*, using yeast cell assays (Thomas et al., 2009). Evidence arguing against naphthenic acids being reproductive toxins in fish comes from the work of Lister et al. (2008). In that study, when goldfish were exposed to oil sands process waters, gonads of both males and females showed reduced steroidogenesis, but when naphthenic acids from those same process waters were extracted and administered to goldfish for 7 days, the reductions in steroidogenesis were not observed, suggesting that compounds other than naphthenic acids were responsible for the effects of the whole process water. Conversely, a recent study in fathead minnows showed that naphthenic acids extracted from oil sands process waters did cause reproductive dysfunction, reducing female fecundity, as well as concentrations of reproductive hormones and secondary sexual characteristics in males (Kavanagh et al., 2012). These authors also showed that $HCO3^{-}$ ions, but not Na^{+}, Cl^{-}, or $SO4^{-}$ ions, could ameliorate the toxicity of naphthenic acids extracted from oil sands process waters. This is important because process waters often contain substantial amounts of salts.

To see if exposure to naphthenic acids caused histopathological changes in fish, one study using yellow perch as the test organism compared the effects of commercially available naphthenic acids to naphthenic acids extracted from oil sands process water (Nero et al., 2006). The authors reported 100% mortality at low ppm (i.e., between 1 and 10) concentrations

of both mixtures, within 96 hours, and in fish exposed to lower concentrations of both mixtures for three weeks, there were increases in cellular proliferation in the gills (epithelial, mucous, and chloride cells). No histological changes were observed in the liver of exposed fish compared to control fish.

One recent study examined the immunotoxic effects of naphthenic acids in fish, using naphthenic acids that were extracted from oil sands process waters and then injected intraperitoneally into rainbow trout (MacDonald et al., 2013). The authors demonstrated that there were no appreciable concentrations of PAHs present in the extracted material; they did so by testing for induction of CYP1A and fluorescent aromatic compounds in the bile, described earlier in this chapter as indicators of PAH exposure in vertebrate species. While there were indications of immunotoxicity in the naphthenic acid-exposed fish, other fish in the same study, injected with BaP, manifested different and more severe symptoms. The authors suggested that the extracted naphthenic acids "act via a generally toxic mechanism rather than by specific toxic effects on immune cells." (p. 95) In another recent study, Hagen et al. (2012) investigated the effects of waterborne mixtures of commercial naphthenic acids on immune responses in goldfish and concluded that acute exposure (1 week) of these compounds caused the upregulation of several pro-inflammatory cytokines and increased resistance to parasitic infection, whereas longer term exposures (8 weeks) resulted in opposite effects on both end-points.

As this discussion makes clear, there is a paucity of information on the toxicology of naphthenic acids in fish. Considerably more information is needed about the occurrence and biological exposures of naphthenic acids in aquatic systems, and about the ecotoxicological relevance of those exposures. Recent advances in techniques for extraction and analysis of naphthenic acids should lead to more investigations utilizing environmentally realistic exposures, and greater clarity concerning the environmental effects of these petroleum-derived substances.

9. FUTURE DIRECTIONS

While substantial progress has been made in understanding the effects of PAHs and related compounds on fish, a number of questions remain to be addressed. The findings highlighted in this chapter showing the exquisite sensitivity of fish early life stages to petrogenic compounds both through cardiotoxicity and phototoxicity, as well as the carcinogenicity of PAHs and their impacts on the metabolic, immune, and reproductive systems, together

with continued reliance on fossil fuels as a global energy source, are compelling reasons to increase research on the toxicology of PAHs and other petrochemicals. Future research needs to include work on analytical chemistry, the mechanisms of PAH toxicity, the effects of mixtures and multiple stressors, the monitoring of remediation, and improved risk assessment.

9.1. Better Analytical Chemistry

Better analytical chemistry will help determine what specific compounds are responsible for the variably toxic effects of PAHs. Some specific needs include better characterization of the PAH content of petroleum, particularly the components of fresh and weathered oil that have not previously been well studied (e.g., Aeppli et al., 2012) and the relationship of different classes of PAHs to toxicity.

9.2. Understanding the Mechanisms of Toxicant Action

The mechanisms underlying many toxic effects of PAHs, including certain embryo deformities, immunotoxicity, endocrine disruption, carcinogenicity, and phototoxicity, are still not completely understood, particularly for the wide array of alkyl PAH and alkyl monoaromatics typical of oil. There is also a need for better assessment of the additive, antagonistic, and synergistic effects of mixtures of PAHs, as well as PAHs in combination with other contaminants. The potential for PAHs to cause epigenetic transgenerational effects in fish is also as yet uncertain. Increased application of genomic techniques should increase our understanding of the underlying mechanisms responsible for the phenotypic effects demonstrated in earlier studies of impaired immunocompetence, reproductive impairment, metabolic disturbance, liver disease, and other impacts. Proteomics in concert with genomics can help determine whether the effects of PAH exposure are at the level of transcription or translation (Pruett et al., 2007).

9.3. Continued Use of Field Study Approaches

Research approaches in future field studies that integrate several sublethal end-points strongly associated with PAH exposure, such as toxicopathic lesions involved in hepatic neoplasia, reproductive effects (e.g., inhibited gonadal development, inhibition of spawning, percentage infertile eggs), with PAH exposure indicators such as sediment ΣPAH concentrations, FACs in bile, and hepatic PAH-DNA adducts in resident fish (e.g.,

English sole; see Table 4.1) are strongly encouraged. Such epizootiological studies have been instrumental in establishing PAHs as a risk factor for neoplasia and related lesions, and could serve a similar purpose for other toxic effects. We also recommend increased use of these above end-points in field studies that evaluate the recovery of fish health at sites that have undergone significant remediation, in order to show the effectiveness of management actions. Examples demonstrating that remediation can lead to improved ecological conditions and improved fish health include English sole from Eagle Harbor (Myers et al., 2008) and Elliott Bay, Washington (State of the Sound, 2009; Figure 4.2), brown bullhead in the Black River, Ohio (Baumann and Harshbarger, 1998), and winter flounder in Boston Harbor, Massachusetts (Moore et al., 1996).

9.4. Cumulative Effects with Other Stressors

We have little information on the cumulative and interactive effects of PAHs and nonchemical stressors, particularly as they relate to the delayed effects of PAHs exposure. Development of methods to test the capacity of PAH-exposed larvae, fry, and other sensitive life stages to handle natural environmental stressors (e.g., temperature flux, salinity stress, predator–prey interactions) would be very useful for estimating risks to recruitment and population abundance.

9.5. Modeling of Population Impacts

Future studies should use modeling approaches to link end-points, such as disease susceptibility, growth and metabolic alterations, endocrine disruption, neoplasia-related processes in liver, and early life history impacts, to demographic traits (e.g., survival and reproduction) that can lead to population-level effects (Spromberg and Meador, 2005, 2006).

9.6. Improved Framework for Regulation of PAHs to Protect Fish

Finally, there is a need for improved regulation of PAHs for the protection of fish. Currently, sediment quality standards set by various regulatory agencies are usually based on toxicity to benthic invertebrates (Long et al., 1998), which may avoid impacts on the fish prey base but is not necessarily protective of direct toxicity to fish. At best, standards are based on critical body residues in fish in combination with biota-sediment bioaccumulation factors (Meador et al., 2002a, 2002b). However, in the case of PAHs, which are readily metabolized in fish, this approach is not applicable. We advocate regulations based on studies targeting resident fish

in their natural habitat and incorporating a suite of integrated, sublethal effects of PAHs, and PAH exposure thresholds for these effects in these fish (Johnson et al., 2002). Use of injury thresholds based on dietary PAH exposure or levels of PAH metabolites in fish bile are two additional approaches for regulating PAH exposure in fish (e.g., see Meador et al., 2008).

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REFERENCES

Aeppli, C., Carmichael, C. A., Nelson, R. K., Lemkau, K. L., Graham, W. M., Redmond, M. C., Valentine, D. L. and Reddy, C. M. (2012). Oil weathering after the *Deepwater Horizon* disaster led to the formation of oxygenated residues. *Environmental Science and Technology* **46**, 8799–8807.

Al-Yakoob, S. N., Gundersen, D. and Curtis, L. (1996). Effects of the water-soluble fraction of partially combusted crude oil from Kuwait's oil fires (from Desert Storm) on survival and growth of the marine fish *Menidia beryllina*. *Ecotoxicology and Environmental Safety* **35**, 142–149.

Aluru, N., Karchner, S. I. and Hahn, M. E. (2011). Role of DNA methylation of AHR1 and AHR2 promoters in differential sensitivity to PCBs in Atlantic killifish, *Fundulus heteroclitus*. *Aquatic Toxicology* **101**, 288–294.

Amara, R., Meziane, T., Gilliers, C., Hermel, G. and Laffargue, P. (2007). Growth and condition indices in juvenile sole *Solea solea* measured to assess the quality of essential fish habitat. *Marine Ecology Progress Series* **351**, 201–208.

Anderson, M. J., Miller, M. R. and Hinton, D. E. (1996). In vitro modulation of 17-β-estradiol-induced vitellogenin synthesis: effects of cytochrome P4501A1 inducing compounds on rainbow trout (*Oncorhynchus mykiss*) liver cells. *Aquatic Toxicology* **34**, 327–350.

Arcand-Hoy, L. D. and Metcalfe, C. D. (1999). Biomarkers of exposure of brown bullheads (*Ameiurus nebulosus*) to contaminants in the lower Great Lakes, North America. *Environmental Toxicology and Chemistry* **18**, 740–749.

Arfsten, D. P., Schaeffer, D. J. and Mulveny, D. C. (1996). The effects of near ultraviolet radiation on the toxic effects of polycyclic aromatic hydrocarbons in animals and plants: a review. *Ecotoxicology and Environmental Safety* **33**, 1–24.

Arkoosh, M. R., Clemons, E., Myers, M. and Casillas, E. (1994). Suppression of B-cell mediated immunity in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) after exposure to either a polycyclic aromatic hydrocarbon or to polychlorinated biphenyls. *Immunopharmacology and Immunotoxicology* **16**, 293–314.

Arkoosh, M. R., Casillas, E., Clemons, E., Kagley, A. N., Olson, R., Reno, P. and Stein, J. E. (1998). Effect of pollution on fish diseases: potential impacts on salmonid populations. *Journal of Aquatic Animal Health* **10**, 182–190.

Arkoosh, M. R., Casillas, E., Clemons, E., Huffman, P., Kagley, A., Collier, T. and Stein, J. E. (2000). Increased susceptibility of juvenile Chinook salmon to infectious disease after exposure to chlorinated and aromatic compounds found in contaminated urban estuaries. *Marine Environmental Research* **50**, 470–471.

Arkoosh, M. R., Clemons, E., Huffman, P., Kagley, A. N., Casillas, E., Adams, N., Sanborn, H. R., Collier, T. K. and Stein, J. E. (2001). Increased susceptibility of juvenile Chinook salmon to vibriosis after exposure to chlorinated and aromatic compounds found in contaminated urban estuaries. *Journal of Aquatic Animal Health* **13**, 257–268.

Arkoosh, M. R. and Collier, T. K. (2002). Ecological risk assessment paradigm for salmon: analyzing immune function to evaluate risk. *Human and Ecological Risk Assessment* **8**, 265–276.

Arkoosh, M. R., Boylen, D., Stafford, C. L., Johnson, L. L. and Collier, T. K. (2005). Use of disease challenge assay to assess immunotoxicity of xenobiotics. In *Techniques in Aquatic Toxicology* (ed. G. K. Ostrander), pp. 19–38. New York: Taylor and Francis Group.

Arkoosh, M. R., Boylen, D., Dietrich, J. P., Anulacion, B. F., Ylitalo, G. M., Bravo, C. F., Johnson, L. L., Loge, F. J. and Collier, T. K. (2010). Disease susceptibility of salmon exposed to polybrominated diphenyl ethers (PBDEs). *Aquatic Toxicology* **98**, 51–59.

Arzuaga, X., Calcaño, W. and Elskus, A. (2004). The DNA de-methylating agent 5-azacytidine does not restore CYP1A induction in PCB resistant Newark Bay killifish (*Fundulus heteroclitus*). *Marine Environmental Research* **58**, 517–520.

Bado-Nilles, A., Quentel, C., Thomas-Guyon, H. and Le Floch, S. (2009). Effects of two oils and 16 pure polycyclic aromatic hydrocarbons on plasmatic immune parameters in the European sea bass, *Dicentrarchus labrax* (Linne). *Toxicology in Vitro* **23**, 235–241.

Bailey, G., Williams, D. and Hendricks, J. (1996). Fish models for environmental carcinogenesis: the rainbow trout. *Environmental Health Perspectives* **104**, 5–21.

Balch, G. C., Metcalfe, C. D., Reichert, W. L. and Stein, J. E. (1995). Biomarkers of exposure of brown bullheads to contaminants in Hamilton Harbor, Ontario. In *Biomonitors and Biomarkers as Indicators of Environmental Change: A Handbook* (eds. F. M. Butterworth, L. D. Corkum and J. Guzman-Rincon), pp. 249–273. New York: Plenum Press.

Bamforth, S. M. and Singleton, I. (2005). Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *Journal of Chemical Technology and Biotechnology* **80**, 723–736.

Barron, M. G., Carls, M. G., Short, J. W. and Rice, S. D. (2003). Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope crude oil to Pacific herring eggs and larvae. *Environmental Toxicology and Chemistry* **22**, 650–660.

Barron, M. G. (2012). Ecological impacts of the *Deepwater Horizon* oil spill: implications for immunotoxicity. *Toxicologic Pathology* **40**, 315–320.

Baumann, P. C., Mac, M. J., Smith, S. B. and Harshbarger, J. C. (1991). Tumor frequencies in walleye (*Stizostedion vitreum*) and brown bullhead (*Ictalurus nebulosus*) and sediment contaminants in tributaries of the Laurentian Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 1804–1810.

Baumann, P. C. and Harshbarger, J. C. (1995). Decline in liver neoplasms in wild brown bullhead catfish aftercoking plant closes and environmental PAHs plummet. *Environmental Health Perspectives* **103**, 168–170.

Baumann, P. C., Smith, I. R. and Metcalfe, C. D. (1996). Linkages between chemical contaminants and tumors in benthic Great Lakes fish. *Journal of Great Lakes Research* **22**, 131–152.

Baumann, P. C. and Harshbarger, J. C. (1998). Long term trends in liver neoplasm epizootics of brown bullhead in the Black River, Ohio. *Environmental Monitoring and Assessment* **53**, 213–223.

Bertilsson, S. and Widenfalk, A. (2002). Photochemical degradation of PAHs in freshwaters and their impact on bacterial growth—influence of water chemistry. *Hydrobiologia* **469**, 23–32.

Beyer, J., Jonsson, G., Porte, C., Krahn, M. M. and Ariese, F. (2010). Analytical methods for determining metabolites of polycyclic aromatic hydrocarbon (PAH) pollutants in fish bile: a review. *Environmental Toxicology and Pharmacology* **30**, 224–244.

Bilbao, E., Raingeard, D., de Cerio, O. D., Ortiz-Zarragoitia, M., Ruiz, P., Izagirre, U., Orbea, A., Marigómez, I., Cajaraville, M. P. and Cancio, I. (2010). Effects of exposure to Prestige-like heavy fuel oil and to perfluorooctane sulfonate on conventional biomarkers and target gene transcription in the thicklip grey mullet *Chelon labrosus*. *Aquatic Toxicology* **98**, 282–296.

Bohne-Kjersem, A., Skadsheim, A., Goksøyr, A. and Grøsvik, B. E. (2009). Candidate biomarker discovery in plasma of juvenile cod (*Gadus morhua*) exposed to crude North Sea oil, alkyl phenols and polycyclic aromatic hydrocarbons (PAHs). *Marine Environmental Research* **68**, 268–277.

Bols, N. C., Brubacher, J. L., Ganassin, R. C. and Lee, L. E. J. (2001). Ecotoxicology and innate immunity in fish. *Developmental and Comparative Immunology* **25**, 853–873.

Boorman, G. A., Botts, S., Bunton, T. E., Fournie, J. W., Harshbarger, J. C., Hawkins, W. E., Hinton, D. E., Jokinen, M. P., Okihiro, M. S. and Wolfe, M. J. (1997). Diagnostic criteria for degenerative, inflammatory, proliferative nonneoplastic and neoplastic liver lesions in medaka (*Oryzias latipes*): consensus of a National Toxicology Program Pathology Working Group. *Toxicologic Pathology* **25**, 202–210.

Brar, N. K., Waggoner, C., Reyes, J. A., Fairey, R. and Kelley, K. M. (2010). Evidence for thyroid endocrine disruption in wild fish in San Francisco Bay, California, USA. Relationships to contaminant exposures. *Aquatic Toxicology* **96**, 203–215.

Bravo, C. F., Curtis, L. R., Myers, M., Meador, J. P., Johnson, L. L., Buzitis, J., Collier, T. K., Morrow, J. D., Laetz, C. A., Loge, F. J. and Arkoosh, M. R. (2011). Biomarker response and disease susceptibility in juvenile rainbow trout *Oncorhynchus mykiss* fed a high molecular weight PAH mixture. *Environmental Toxicology and Chemistry* **30**, 1–11.

Brown, S. B., Adams, B. A., Cyr, D. G. and Eales, J. G. (2004). Contaminant effects on the teleost fish thyroid. *Environmental Toxicology and Chemistry* **23**, 1680–1701.

Bucke, D. and Feist, S. W. (1993). Histopathological changes in the livers of dab, *Limanda limanda (L.)*. *Journal of Fish Diseases* **16**, 281–296.

Bugel, S. M., White, L. A. and Cooper, K. R. (2010). Impaired reproductive health of killifish (*Fundulus heteroclitus*) inhabiting Newark Bay, NJ, a chronically contaminated estuary. *Aquatic Toxicology* **96**, 182–193.

Burnett, K. G. (2005). Impact of enviromental toxicants and natural variables on the immune system of fishes. In *Biochemistry and Molecular Biology of Fishes. Vol. VI, Environmental Toxicology* (eds. T. P. Mommsen and T. W. Moon), pp. 231–253. Amsterdam: Elsevier.

Burton, D., Burton, M. P., Truscott, B. and Idler, D. R. (1985). Epidermal cellular proliferation and differentiation in sexually mature male *Salmo salar* with androgen levels depressed by oil. *Proceedings of the Royal Society of London Series B Biological Sciences* **225**, 121–128.

Butala, H., Metzger, C., Rimoldi, J. and Willett, K. L. (2004). Microsomal estrogen metabolism in channel catfish. *Marine Environmental Research* **58**, 489–494.

Carls, M. G., Holland, L., Larsen, M., Lum, J. L., Mortensen, D. G., Wang, S. Y. and Wertheimer, A. C. (1996). Growth, feeding, and survival of pink salmon fry exposed to food contaminated with crude oil. *American Fisheries Society Symposium* **18**, 608–618.

Carls, M. G., Rice, S. D. and Hose, J. E. (1999). Sensitivity of fish embryos to weathered crude oil: part I. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval Pacific herring (*Clupea pallasi*). *Environmental Toxicology and Chemistry* **18**, 481–493.

Carls, M. G., Holland, L., Larsen, M., Collier, T. K., Scholz, N. L. and Incardona, J. P. (2008). Fish embryos are damaged by dissolved PAHs, not oil particles. *Aquatic Toxicology* **88**, 121–127.

Carlson, E., Li, Y. and Zelikoff, J. (2002). Exposure of Japanese medaka (*Oryzias latipes*) to benzo[a]pyrene suppresses immune function and host resistance against bacterial challenge. *Aquatic Toxicology* **56**, 289–301.

Carlson, E. A., Li, Y. and Zelikoff, J. T. (2004a). Suppressive effects of benzo[a]pyrene upon fish immune function: evolutionarily conserved cellular mechanisms of immunotoxicity. *Marine Environmental Research* **58**, 731–734.

Carlson, E. A., Li, Y. and Zelikoff, J. T. (2004b). Benzo[a]pyrene-induced immunotoxicity in Japanese medaka (*Oryzias latipes*): relationship between lymphoid CYP1A activity and humoral immune suppression. *Toxicology and Applied Pharmacology* **201**, 40–52.

Cerniglia, C. E. and Heitkamp, M. A. (1989). Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment. In *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment* (ed. U. Varanasi), pp. 41–68. Boca Raton, FL: CRC Press.

Chang, S., Zdanowicz, V. S. and Murchelano, R. A. (1998). Associations between liver lesions in winter flounder (*Pleuronectes americanus*) and sediment chemical contaminants from north-east United States estuaries. *ICES Journal of Marine Science: Journal du Conseil* **55**, 954–969.

Cheshenko, K., Pakdel, F., Segner, H., Kah, O. and Eggen, R. I. L. (2008). Interference of endocrine disrupting chemicals with aromatase CYP19 expression or activity, and consequences for reproduction of teleost fish. *General and Comparative Endocrinology* **155**, 31–62.

Claireaux, G., Désaunay, Y., Akcha, F., Aupérin, B., Bocquené, G., Budzinski, H., Cravedi, J.-P., Davoodi, F., Galois, R., Gilliers, C., Goanvec, C., Guérault, D., Imbert, N., Mazéas, O., Nonnotte, G., Nonnotte, L., Prunet, P., Sébert, P. and Vettier, A. (2004). Influence of oil exposure on the physiology and ecology of the common sole *Solea solea*: experimental and field approaches. *Aquatic Living Resources* **17**, 335–351.

Clemente, J. S. and Fedorak, P. M. (2005). A review of the occurrence, analyses, toxicity and biodegradation of napthenic acids. *Chemosphere* **60**, 585–600.

Clemons, E., Arkoosh, M. R. and Casillas, E. (1999). Enhanced superoxide anion production in activated peritoneal macrophages from English sole (*Pleuronectes vetulus*) exposed to polycyclic aromatic compounds. *Marine Environmental Research* **47**, 71–87.

Collier, T. and Varanasi, U. (1991). Hepatic activities of xenobiotic metabolizing enzymes and biliary levels of xenobiotics in English sole (*Parophrys vetulus*) exposed to environmental contaminants. *Archives of Environmental Contamination and Toxicology* **20**, 462–473.

Collier, T. K., Singh, S. V., Awasthi, Y. C. and Varanasi, U. (1992). Hepatic xenobiotic metabolizing enzymes in two species of benthic fish showing different prevalences of contaminant-associated liver neoplasms. *Toxicology and Applied Pharmacology* **113**, 319–324.

Collier, T. K. (2003). Forensic ecotoxicology: establishing causality between contaminants and biological effects in field studies. *Human and Ecological Risk Assessment* **9**, 259–266.

Collier, T. K., Chiang, M. W. L., Au, D. W. T. and Rainbow, P. S. (2013). Biomarkers currently used in environmental monitoring. In *Ecological Biomarkers: Indicators of Ecotoxicological Effects* (eds. C. Amiard-Triquet, J.-C. Amiard and P. S. Rainbow), pp. 385–409. Boca Raton, FL: CRC Press Taylor and Francis Group.

Colton, T. and Greenberg, E. R. (1983). Cancer epidemiology. In *Statistics in Medical Research: Methods and Issues, with Applications in Cancer Research* (eds. V. Mike and K. E. Stanley), pp. 23–70. Wiley Subscription Services, Inc., A Wiley Company.

Columbano, A., Rajalakshmi, S. and Sarma, D. S. (1981). Requirement for cell proliferation for the initiation of liver carcinogenesis as assayed by three different procedures. *Cancer Research* **41**, 2079–2083.

Connelly, H. and Means, J. C. (2010). Immunomodulatory effects of dietary exposure to selected polycyclic aromatic hydrocarbons in the bluegill (*Lepomis macrochirus*). *International Journal of Toxicology* **29**, 532–545.

Correia, A. D., Goncalves, R., Scholze, M., Ferreira, M. and Reis-Henriques, M. A. (2007). Biochemical and behavioral responses in gilthead seabream (*Sparus aurata*) to phenanthrene. *Journal of Experimental Marine Biology and Ecology* **347**, 109–122.

Couillard, C. M. (2002). A microscale test to measure petroleum oil toxicity to mummichog embryos. *Environmental Toxicology* **17**, 195–202.

Danion, M., Le Floch, S., Lamour, F., Guyomarch, J. and Quentel, C. (2011a). Bioconcentration and immunotoxicity of an experimental oil spill in European sea bass (*Dicentrarchus labrax* L.). *Ecotoxicology and Environmental Safety* **74**, 2167–2174.

Danion, M., Le Floch, S., Kanan, R., Lamour, F. and Quentel, C. (2011b). Effects of in vivo chronic hydrocarbons pollution on sanitary status and immune system in sea bass (*Dicentrarchus labrax* L.). *Aquatic Toxicology* **105**, 300–311.

De Bruin, M. L., Pettersson, M., Meyboom, R. H. B., Hoes, A. W. and Leufkens, H. G. M. (2005). Anti-HERG activity and the risk of drug-induced arrhythmias and sudden death. *European Heart Journal* **26**, 590–597.

Dey, W. P., Peck, T. H., Smith, C. E. and Kreamer, G.-L. (1993). Epizoology of hepatic neoplasia in Atlantic tomcod (*Microgadus tomcod*) from the Hudson River estuary. *Canadian Journal of Fisheries and Aquatic Sciences* **50**, 1897–1907.

Diamond, S. A. (2003). Photoactivated toxicity in aquatic environments. In *UV Effects in Aquatic Organisms and Ecosystems* (eds. D. Hader and G. Joir), pp. 219–250. New York: John Wiley & Sons.

Dokholyan, B. K. and Magomedov, A. K. (1984). Effect of sodium naphthenate on survival and some physiological–biochemical parameters of some fishes. *Journal of Icthyology* **23**, 125–132.

Dong, W., Wang, L., Thornton, C., Scheffler, B. E. and Willett, K. L. (2008). Benzo(a)pyrene decreases brain and ovarian aromatase mRNA expression in *Fundulus heteroclitus*. *Aquatic Toxicology* **88**, 289–300.

Ellis, A. E. (2001). Innate host defense mechanisms of fish against viruses and bacteria. *Developmental and Comparative Immunology* **25**, 827–839.

Evanson, M. and Van Der Kraak, G. J. (2001). Stimulatory effects of selected PAHs on testosterone production in goldfish and rainbow trout and possible mechanisms of action. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* **130**, 249–258.

Fang, X., Dong, W., Thornton, C., Scheffler, B. and Willett, K. L. (2010). Benzo(a)pyrene induced glycine N-methyltransferase messenger RNA expression in *Fundulus heteroclitus* embryos. *Marine Environmental Research* **69** (Suppl. 1), S74–S76.

Farber, E. and Sarma, D. S. R. (1987). Biology of disease—hepatocarcinogenesis: a dynamic cellular perspective. *Laboratory Investigations* **56**, 4–22.

Farber, E. (1990). Clonal adaptation during carcinogenesis. *Biochemical Pharmacology* **39**, 1837–1846.

Feist, S., Lang, T., Stentiford, G. and Koehler, A. (2004). Use of liver pathology of the European flatfish dab (*Limanda limanda L.*) and flounder (*Platichthys flesus L.*) for monitoring. *ICES Techniques in Marine Environmental Sciences*, 38. Copenhagen: ICES.

Frank, R. A., Fischer, K., Burnison, B. K., Arsenault, G., Headley, J. V., Peru, K. M., Van Der Kraak, G. and Solomon, K. R. (2009). Effect of carboxylic acid content on the acute toxicity of oil dands naphthenic acids. *Environmental Science and Technology* **43**, 266–271.

French, B. L., Reichert, W. L., Hom, T., Nishimoto, M., Sanborn, H. R. and Stein, J. E. (1996). Accumulation and dose-response of hepatic DNA adducts in English sole (Pleuronectes vetulus) exposed to a gradient of contaminated sediments. *Aquatic Toxicology* **36**, 1–16.

Gesto, M., Tintos, A., Soengas, J. L. and Míguez, J. M. (2006). Effects of acute and prolonged naphthalene exposure on brain monoaminergic neurotransmitters in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* **144**, 173–183.

Gesto, M., Soengas, J. L. and Míguez, J. M. (2008). Acute and prolonged stress responses of brain monoaminergic activity and plasma cortisol levels in rainbow trout are modified by PAHs (naphthalene, β-naphthoflavone and benzo(a)pyrene) treatment. *Aquatic Toxicology* **86**, 341–351.

Gesto, M., Tintos, A., Soengas, J. L. and Míguez, J. M. (2009). β-Naphthoflavone and benzo(a) pyrene alter dopaminergic, noradrenergic, and serotonergic systems in brain and pituitary of rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicology and Environmental Safety* **72**, 191–198.

Gilliers, C., Le Pape, O., Désaunay, Y., Bergeron, J. P., Schreiber, N., Guerault, D. and Amara, R. (2006). Growth and condition of juvenile sole (*Solea solea L.*) as indicators of habitat quality in coastal and estuarine nurseries in the Bay of Biscay with a focus on sites exposed to the Erika oil spill. *Scienta Marina* **70S1**, 183–192.

Godard, C. A. J., Wise, S. S., Kelly, R. S., Goodale, B., Kraus, S., Romano, T., O'Hara, T. and Wise, J. P., Sr (2006). Benzo[a]pyrene cytotoxicity in right whale (*Eubalaena glacialis*) skin, testis and lung cell lines. *Marine Environmental Research* **62** (Suppl. 1), S20–S24.

Gratz, S., Mohrhaus, A., Gamble, B., Gracie, J., Jackson, D., Roetting, J., et al. (2010). *Screen for the presence of polycyclic aromatic hydrocarbons in select seafoods using LC-Fluorescence*. *Laboratory Information Bulletin No. 4475*. Silver Spring, MD: U.S. Food and Drug Administration.

Hagen, M. O., Garcia-Garcia, E., Oladiran, A., Karpman, M., Mitchell, S., El-Din, M. G., Martin, J. W. and Belosevic, M. (2012). The acute and sub-chronic exposures of goldfish to naphthenic acids induce different host defense responses. *Aquatic Toxicology* **109**, 143–149.

Hahn, M. E., Karchner, S. I., Franks, D. G. and Merson, R. R. (2004). Aryl hydrocarbon receptor polymorphisms and dioxin resistance in Atlantic killifish (*Fundulus heteroclitus*). *Pharmacogenetics and Genomics* **14**, 131–143.

Hatlen, K., Sloan, C. A., Burrows, D. G., Collier, T. K., Scholz, N. L. and Incardona, J. P. (2010). Natural sunlight and residual fuel oils are an acutely lethal combination for fish embryos. *Aquatic Toxicology* **99**, 56–64.

Hawkins, W. E., Walker, W. W. and Overstreet, R. M. (1995). Carcinogenicity tests using aquarium fish. *Toxicology Mechanisms and Methods* **5**, 225–263.

He, C., Zuo, Z., Shi, X., Sun, L. and Wang, C. (2012). Pyrene exposure influences the thyroid development of *Sebastiscus marmoratus* embryos. *Aquatic Toxicology* **124–125**, 28–33.

Head, J. A., Dolinoy, D. C. and Basu, N. (2012). Epigenetics for ecotoxicologists. *Environmental Toxicology and Chemistry* **31**, 221–227.

Heintz, R. A., Short, J. W. and Rice, S. D. (1999). Sensitivity of fish embryos to weathered crude oil: part II. Increased mortality of pink salmon (*Oncorhynchus gorbuscha*) embryos incubating downstream from weathered *Exxon Valdez* crude oil. *Environmental Toxicology and Chemistry* **18**, 494–503.

Heintz, R. A., Rice, S. D., Wertheimer, A. C., Bradshaw, R. F., Thrower, F. P., Joyce, J. E. and Short, J. W. (2000). Delayed effects on growth and marine survival of pink salmon *Oncorhynchus gorbuscha* after exposure to crude oil during embryonic development. *Marine Ecology Progress Series* **208**, 205–216.

Heintz, R. A. (2007). Chronic exposure to polynuclear aromatic hydrocarbons in natal habitats leads to decreased equilibrium size, growth, and stability of pink salmon populations. *Integrated Environmental Assessment and Management* **3**, 351–363.

Heitkamp, M. A., Franklin, W. and Cerniglia, C. E. (1988). Microbial metabolism of polycyclic aromatic hydrocarbons: isolation and characterization of a pyrene-degrading bacterium. *Applied and Environmental Microbiology* **54**, 2549–2555.

Heitkamp, M. A. and Cerniglia, C. E. (1989). Polycyclic aromatic hydrocarbon degradation by a *Mycobacterium sp*. in microcosms containing sediment and water from a pristine ecosystem. *Applied and Environmental Microbiology* **55**, 1968–1973.

Hendricks, J., Meyers, T. and Shelton, D. (1984). Histological progression of hepatic neoplasia in rainbow trout (*Salmo gairdneri*). *National Cancer Institute Monograph Series* **65**, 321–336.

Hendricks, J. D., Meyers, T. R., Shelton, D. W., Casteel, J. L. and Bailey, G. S. (1985). Hepatocarcinogenicity of benzo[a]pyrene to rainbow trout by dietary exposure and intraperitoneal injection. *Journal of the National Cancer Institute* **74**, 839–851.

Hicken, C. E., Linbo, T. L., Baldwin, D. H., Myers, M. S., Holland, L., Larsen, M., Scholz, N. L., Collier, T. K., Rice, G. S., Stekoll, M. S. and Incardona, J. P. (2011). Sub-lethal exposure to crude oil during embryonic development alters cardiac morphology and reduces aerobic capacity in adult fish. *Proceedings of the National Academy of Sciences U S A* **108**, 7086–7090.

Hinga, K. R. and Pilson, M. E. Q. (1987). Persistence of benz[a]anthracene degradation products in an enclosed marine ecosystem. *Environmental Science and Technology* **21**, 648–653.

Hoffmann, J. L. and Oris, J. T. (2006). Altered gene expression: a mechanism for reproductive toxicity in zebrafish exposed to benzo[a]pyrene. *Aquatic Toxicology* **78**, 332–340.

Hogan, N. S., Lee, K. S., Kollner, B. and van den Heuvel, M. R. (2010). The effects of the alkyl polycyclic aromatic hydrocarbon retene on rainbow trout (*Oncorhynchus mykiss*) immune response. *Aquatic Toxicology* **100**, 246–254.

Holth, T. F., Nourizadeh-Lillabadi, R., Blaesbjerg, M., Grung, M., Holbech, H., Petersen, G. I., Alestrom, P. and Hylland, K. (2008). Differential gene expression and biomarkers in zebrafish (*Danio rerio*) following exposure to produced water components. *Aquatic Toxicology* **90**, 277–291.

Hutchinson, T. H., Field, M. D. R. and Manning, M. J. (2003). Evaluation of non-specific immune functions in dab, *Limanda limanda* L., following short-term exposure to sediments contaminated with polyaromatic hydrocarbons and/or polychlorinated biphenyls. *Marine Environmental Research* **55**, 193–202.

Incardona, J. P., Collier, T. K. and Scholz, N. L. (2004). Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. *Toxicology and Applied Pharmacology* **196**, 191–205.

Incardona, J. P., Carls, M. G., Teraoka, H., Sloan, C. A., Collier, T. K. and Scholz, N. L. (2005). Aryl hydrocarbon receptor-independent toxicity of weathered crude oil during fish development. *Environmental Health Perspectives* **113**, 1755–1762.

Incardona, J. P., Day, H. L., Collier, T. K. and Scholz, N. L. (2006). Developmental toxicity of 4-ring polycyclic aromatic hydrocarbons in zebrafish is differentially dependent on AH receptor isoforms and hepatic cytochrome P450 1A metabolism. *Toxicology and Applied Pharmacology* **217**, 308–321.

Incardona, J. P., Carls, M. G., Day, H. L., Sloan, C. A., Bolton, J. L., Collier, T. K. and Scholz, N. L. (2009). Cardiac arrhythmia is the primary response of embryonic Pacific herring (*Clupea pallasi*) exposed to crude oil during weathering. *Environmental Science and Technology* **43**, 201–207.

Incardona, J. P., Collier, T. K. and Scholz, N. L. (2011a). Oil spills and fish health: exposing the heart of the matter. *Journal of Exposure Science and Environmental Epidemiology* **21**, 3–4.

Incardona, J. P., Linbo, T. L. and Scholz, N. L. (2011b). Cardiac toxicity of 5-ring polycyclic aromatic hydrocarbons is differentially dependent on the aryl hydrocarbon receptor 2 isoform during zebrafish development. *Toxicology and Applied Pharmacology* **257**, 242–249.

Incardona, J. P., Vines, C. A., Anulacion, B. F., Baldwin, D. H., Day, H. L., French, B. L., Labenia, J. S., Linbo, T. L., Myers, M. S., Olson, O. P., Sloan, C. A., Sol, S., Griffin, F. J., Menard, K., Morgan, S. G., West, J. E., Collier, T. K., Ylitalo, G. M., Cherr, G. N. and Scholz, N. L. (2012a). Unexpectedly high mortality in Pacific herring embryos exposed to the 2007 *Cosco Busan* oil spill in San Francisco Bay. *Proceedings of the National Academy of Sciences U S A* **109**, E51–58.

Incardona, J. P., Vines, C. A., Linbo, T. L., Myers, M. S., Sloan, C. A., Anulacion, B. F., Boyd, D., Collier, T. K., Morgan, S., Cherr, G. N. and Scholz, N. L. (2012b). Potent phototoxicity of marine bunker oil to translucent herring embryos after prolonged weathering. *PLoS One* **7**, e30116.

Jacobson, K. C., Arkoosh, M. R., Kagley, A. N., Clemons, E. R., Collier, T. K. and Casillas, E. (2003). Cumulative effects of natural and anthropogenic stress on immune function and disease resistance in juvenile Chinook salmon. *Journal of Aquatic Animal Health* **15**, 1–12.

James, M. O. (1989). Biotransformation and deposition of PAH in aquatic invertebrates. In *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment* (ed. U. Varanasi), pp. 69–91. Boca Raton, FL: CRC Press.

Janeway, C., Travers, P., Walport, M. and Shlomchik, M. (2005). Innate immunity. In *Immunobiology* (eds. C. Janeway, P. Travers, M. Walport and M. Shlomchik), 6th ed., pp. 37–100. New York: Garland Science Publishing.

Jivraj, M. N., MacKinnon, M. and Fung, B. (1995). *Naphthenic acid extraction and quantitative analysis with FT-IR spectroscopy*. *Syncrude Analytical Manuals,* 4th ed. Edmonton, AB: Syncrude Canada Ltd.

Johnson, L. L., Stehr, C. M., Olson, O. P., Myers, M. S., Pierce, S. M., Wigren, C. A., McCain, B. B. and Varanasi, U. (1993). Chemical contaminants and hepatic lesions in winter flounder (*Pleuronectes americanus*) from the northeast coast of the United States. *Environmental Science and Technology* **27**, 2759–2771.

Johnson, L. L., Landahl, J. T., Kubin, L. A., Horness, B. H., Myers, M. S., Collier, T. K. and Stein, J. E. (1998). Assessing the effects of anthropogenic stressors on Puget Sound flatfish populations. *Journal of Sea Research* **39**, 125–137.

Johnson, L. L., Collier, T. K. and Stein, J. E. (2002). An analysis in support of sediment quality thresholds for polycyclic aromatic hydrocarbons (PAHs) to protect estuarine fish. *Aquatic Conservation: Marine and Freshwater Ecosystems* **12**, 517–538.

Johnson, L. L., Arkoosh, M. R., Bravo, C. F., Collier, T. K., Krahn, M. M., Meador, J. P., Myers, M. S., Reichert, W. L. and Stein, J. E. (2008). The effects of polycyclic aromatic hydrocarbons in fish from Puget Sound Washington. In *The Toxicology of Fishes* (eds. R. T. DiGiulio and D. E. Hinton), pp. 874–919. Boca Raton, FL: CRC Press.

Johnson, L. L., Ylitalo, G. M., Myers, M. S., Anulacion, B. F., Buzitis, J., Reichert, W. L. and Collier, T. K. (2009). Polycyclic Aromatic Hydrocarbons and Fish Health Indicators in the Marine Ecosystem in Kitimat, British Columbia. U.S. Department of Commerce, NOAA Tech. Memo., NMFS-NWFSC-98. p. 123.

Johnson, L. L., Anulacion, B. F., Arkoosh, M. R., Burrows, D. G., da Silva, D. A. M., Dietrich, J. D., Myers, M. S., Spromberg, J. A. and Ylitalo, G. M. (2013). Effects of legacy persistent organic pollutants (POPs) in fish—current and future challenges. In *Fish Physiology in Review* (ed. K. B. Tierney), Elsevier.

Juhasz, A. L. and Naidu, R. (2000). Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. *International Biodeterioration and Biodegradation* **45**, 57–88.

Jung, J.-H., Hicken, C. E., Boyd, D., Anulacion, B. F., Carls, M. G., Shim, W. J. and Incardona, J. P. (2013). Geologically distinct crude oils cause a common cardiotoxicity syndrome in developing zebrafish. *Chemosphere* **91**, 1146–1155.

Karrow, N. A., Boermans, H. J., Dixon, D. G., Hontella, A., Solomon, K. R., Whyte, J. J. and Bols, N. C. (1999). Characterizing the immunotoxicity of creosote to rainbow trout (Oncorhynchus mykiss): a microcosm study. *Aquatic Toxicology* **45**, 223–239.

Karrow, N. A., Bols, N. C., Wyte, J. J., Solomon, K. R., Dixon, D. G. and Boermans, H. J. (2001). Effects of creosote exposure on rainbow trout pronephros phagocyte activity and the percentage of lymphoid B cells. *Journal of Toxicology and Environmental Health Part A* **63**, 363–381.

Kavanagh, R. J., Frank, R. A., Burnison, B. K., Young, R. F., Fedorak, P. M., Solomon, K. R. and Van Der Kraak, G. (2012). Fathead minnow (*Pimephales promelas*) reproduction is impaired when exposed to a naphthenic acid extract. *Aquatic Toxicology* **116-117**, 34–42.

Kazeto, Y., Place, A. R. and Trant, J. M. (2004). Effects of endocrine disrupting chemicals on the expression of CYP19 genes in zebrafish (*Danio rerio*) juveniles. *Aquatic Toxicology* **69**, 25–34.

Kennedy, C. J. and Farrell, A. P. (2008). Immunological alternations in juvenile Pacific herring, *Clupea pallasi,* exposed to aqueous hydrocarbons derived from crude oil. *Environmental Pollution* **153**, 638–648.

Kerambrun, E., Le Floch, S., Sanchez, W., Thomas Guyon, H., Meziane, T., Henry, F. and Amara, R. (2012). Responses of juvenile sea bass, *Dicentrarchus labrax*, exposed to acute concentrations of crude oil, as assessed by molecular and physiological biomarkers. *Chemosphere* **87**, 692–702.

Khan, R. A. (2013). Effects of polycyclic aromatic hydrocarbons on sexual maturity of Atlantic cod, *Gadus morhua*, following chronic exposure. *Environment and Pollution* **2**, 1–10.

Kiceniuk, J. W. and Khan, R. A. (1987). Effect of petroleum hydrocarbons on Atlantic cod, *Gadus morhua*, following chronic exposure. *Canadian Journal of Zoology* **65**, 490–494.

Koehler, A. (2004). The gender-specific risk to liver toxicity and cancer of flounder (*Platichthys flesus (L.)*) at the German Wadden Sea coast. *Aquatic Toxicology* **70**, 257–276.

Kohler, A., Deisemann, H. and Lauritzen, B. (1992). Histological and cytochemical indices of toxic injury in the liver of dab *Limanda limanda*. *Marine Ecology Progress Series* **91**, 141–153.

Köllner, B., Kotterba, G. and Fisher, U. (2002). Evaluation of immune functions of rainbow trout—how can environmental influences be detected? *Toxicology Letters* **131**, 83–95.

Krahn, M. M., Myers, M. S., Burrows, D. G. and Malins, D. C. (1984). Determination of metabolites of xenobiotics in the bile of fish from polluted waterways. *Xenobiotica* **14**, 633–646.

Kranz, H. and Dethlefsen, V. (1990). Liver anomalies in dab *Limanda limanda* from the southern North Sea with special consideration given to neoplastic lesions. *Diseases of Aquatic Organisms* **9**, 171–185.

Kubin, L. A. (1997). A study of growth in juvenile English sole exposed to sediments amended with aromatic compounds [Master's Thesis]. Bellingham, WA: Western Washington University.

Langheinrich, U., Vacun, G. and Wagner, T. (2003). Zebrafish embryos express an orthologue of HERG and are sensitive toward a range of QT-prolonging drugs inducing severe arrhythmia. *Toxicology and Applied Pharmacology* **193**, 370–382.

Le Page, Y., Vosges, M., Servili, A., Brion, F. and Kah, O. (2011). Neuroendocrine effects of endocrine disruptors in teleost fish. *Journal of Toxicology and Environmental Health, Part B* **14**, 370–386.

Leadley, T. A., Balch, G., Metcalfe, C. D., Lazar, R., Mazak, E., Habowsky, J. and Haffner, G. D. (1998). Chemical accumulation and toxicological stress in three brown bullhead (*Ameiurus nebulosus*) populations of the Detroit River, Michigan, USA. *Environmental Toxicology and Chemistry* **17**, 1756–1766.

Leadley, T. A., Arcand-Hoy, L. D., Haffner, G. D. and Metcalfe, C. D. (1999). Fluorescent aromatic hydrocarbons in bile as a biomarker of exposure of brown bullheads (*Ameiurus nebulosus*) to contaminated sediments. *Environmental Toxicology and Chemistry* **18**, 750–755.

Lima, A. L. C., Farrington, J. W. and Reddy, C. M. (2005). Combustion-derived polycyclic aromatic hydrocarbons in the environment—a review. *Environmental Forensics* **6**, 109–131.

Lister, A., Nero, V., Farwell, A., Dixon, D. G. and Van Der Kraak, G. (2008). Reproductive and stress hormone levels in goldfish (*Carassius auratus*) exposed to oil sands process-affected water. *Aquatic Toxicology* **87**, 170–177.

Liu, W.-b., Liu, J.-y., Ao, L., Zhou, Z.-y., Zhou, Y.-h., Cui, Z.-h., Yang, H. and Cao, J. (2009). Dynamic changes in DNA methylation during multistep rat lung carcinogenesis induced by 3-methylcholanthrene and diethylnitrosamine. *Toxicology Letters* **189**, 5–13.

Logan, D. T. (2007). Perspective on ecotoxicology of PAHs to fish. *Human and Ecological Risk Assessment: An International Journal* **13**, 302–316.

Loge, F., Arkoosh, M. R., Ginn, T. R., Johnson, L. L. and Collier, T. K. (2005). Impact of environmental stressors on the dynamics of disease transmission. *Environmental Science and Technology* **39**, 7329–7336.

Long, E. R., Field, L. J. and MacDonald, D. D. (1998). Predicting toxicity in marine sediments with numerical sediment quality guidelines. *Environmental Toxicology and Chemistry* **17**, 714–727.

Maccubbin, A. and Ersing, N. (1991). Tumors in fish from the Detroit River. *Hydrobiologia* **219**, 301–306.

MacDonald, G. Z., Hogan, N. S., Kollner, B., Thorpe, K. L., Phalen, L. J., Wagner, B. D. and van den Heuvel, M. R. (2013). Immunotoxic effects of oil sands-derived naphthenic acids to rainbow trout. *Aquatic Toxicology* **126**, 95–103.

Magnadóttir, B. (2006). Innate immunity of fish (overview). *Fish and Shellfish Immunology* **20**, 137–151.

Magnadóttir, B. (2010). Immunological control of fish diseases. *Marine Biotechnology* **12**, 361–379.

Manikkam, M., Guerrero-Bosagna, C., Tracey, R., Haque, M. M. and Skinner, M. K. (2012). Transgenerational actions of environmental compounds on reproductive disease and identification of epigenetic biomarkers of ancestral exposures. *PLoS One* **7**, e31901.

Martin-Skilton, R., Thibaut, R. and Porte, C. (2006). Endocrine alteration in juvenile cod and turbot exposed to dispersed crude oil and alkylphenols. *Aquatic Toxicology* **78** (Suppl.), S57–S64.

Martin-Skilton, R., Saborido-Rey, F. and Porte, C. (2008). Endocrine alteration and other biochemical responses in juvenile turbot exposed to the *Prestige* fuel oil. *Science of The Total Environment* **404**, 68–76.

Marty, G. D., Hose, J. E., McGurk, M. D., Brown, E. D. and Hinton, D. E. (1997a). Histopathology and cytogenetic evaluation of Pacific herring larvae exposed to petroleum hydrocarbons in the laboratory or in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1846–1857.

Marty, G. D., Short, J. W., Dambach, D. M., Willits, N. H., Heintz, R. A., Rice, S. D., Stegeman, J. J. and Hinton, D. E. (1997b). Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome P4501A induction in pink salmon larvae

continuously exposed to oil-contaminated gravel during development. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **75**, 989–1007.

Mausner, J. S. and Bahn, A. K. (1974). *Epidemiology: An Introductory Text*. Philadelphia: Saunders.

McCain, B. B., Chan, S. L., Krahn, M. M., Brown, D. W., Myers, M. S., Landahl, J. T., Pierce, S., Clark, R. C. and Varanasi, U. (1992). Chemical contamination and associated fish diseases in San Diego Bay. *Environmental Science and Technology* **26**, 725–733.

McElroy, A. E., Farrington, J. W. and Teal, J. M. (1989). Bioavailability of polycyclic aromatic hydrocrabons in aquatic environment. In *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment* (ed. U. Varanasi), pp. 1–39. Boca Raton, FL: CRC Press.

McIntosh, S., King, T., Wu, D. and Hodson, P. V. (2010). Toxicity of dispersed weathered crude oil to early life stages of Atlantic herring (*Clupea harengus*). *Environmental Toxicology and Chemistry* **29**, 1160–1167.

McMaster, M. E., Van Der Kraak, G. J. and Munkittrick, K. R. (1996). An epidemiological evaluation of the biochemical basis for xteroid hormonal cepressions in fish exposed to industrial wastes. *Journal of Great Lakes Research* **22**, 153–171.

Meador, J. P., Stein, J. E., Reichert, W. L. and Varanasi, U. (1995). Bioaccumulation of polycyclic Aromatic hydrocarbons by marine organisms. In *Reviews of Environmental Contamination and Toxicology* (ed. G. Ware), pp. 79–165. New York: Springer.

Meador, J. P., Collier, T. K. and Stein, J. E. (2002a). *Use of Tissue and Sediment-based Threshold Concentrations of Polychlorinated Biphenyls (PCBs) to Protect Juvenile Salmonids Listed under the U.S. Endangered Species Act. Aquatic Conservation: Marine and Freshwater Ecosystems*. Hoboken, NJ: John Wiley & Sons, pp. 493–516.

Meador, J. P., Collier, T. K. and Stein, J. E. (2002b). Determination of a tissue and sediment threshold for tributyltin to protect prey species of juvenile salmonids listed under the U.S. Endangered Species Act. *Aquatic Conservation: Marine and Freshwater Ecosystems* **12**, 539–551.

Meador, J. P., Sommers, F. C., Ylitalo, G. M. and Sloan, C. A. (2006). Altered growth and related physiological responses in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from dietary exposure to polycyclic aromatic hydrocarbons (PAHs). *Canadian Journal of Fisheries and Aquatic Sciences* **63**, 2364–2376.

Meador, J. P. (2008). Polycyclic aromatic hydrocarbons. In *Encyclopedia of Ecology* (eds. S. E. Jorgensen and B. Fath), pp. 2881–2891. Oxford: Academic Press.

Metcalfe, C. D., Cairns, V. W. and Fitzsimons, J. D. (1988). Experimental induction of liver tumours in rainbow trout (*Salmo gairdneri*) by contaminated sediment from Hamilton Harbour, Ontario. *Canadian Journal of Fisheries and Aquatic Sciences* **45**, 2161–2167.

Meyer, J. and Di Giulio, R. T. (2002). Patterns of heritability of decreased EROD activity and resistance to PCB 126-induced teratogenesis in laboratory-reared offspring of killifish (*Fundulus heteroclitus*) from a creosote-contaminated site in the Elizabeth River, VA, USA. *Marine Environmental Research* **54**, 621–626.

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

Meyer, J. N. and Di Giulio, R. T. (2003). Heritable adaptation and fitness costs in killifish (*Fundulus beteroclitus*) inhabiting a polluted estuary. *Ecological Applications* **13**, 490–503.

Meyer, J. N., Volz, D. C., Freedman, J. H. and Di Giulio, R. T. (2005). Differential display of hepatic mRNA from killifish (*Fundulus heteroclitus*) inhabiting a Superfund estuary. *Aquatic Toxicology* **73**, 327–341.

Milan, D. J., Peterson, T. A., Ruskin, J. N., Peterson, R. T. and MacRae, C. A. (2003). Drugs that induce repolarization abnormalities cause bradycardia in zebrafish. *Circulation* **107**, 1355–1358.

Mirbahai, L., Williams, T. D., Zhan, H., Gong, Z. and Chipman, J. K. (2011a). Comprehensive profiling of zebrafish hepatic proximal promoter CpG island methylation and its modification during chemical carcinogenesis. *BMC Genomics* **12**, 3.

Mirbahai, L., Yin, G., Bignell, J. P., Li, N., Williams, T. D. and Chipman, J. K. (2011b). DNA methylation in liver tumorigenesis in fish from the environment. *Epigenetics* **6**, 1319–1333.

Mitcheson, J. S., Chen, J., Lin, M., Culberson, C. and Sanguinetti, M. C. (2000). A structural basis for drug-induced long QT syndrome. *Proceedings of the National Academy of Sciences U S A* **97**, 12329–12333.

Moles, A. and Norcross, B. L. (1998). Effects of oil-laden sediments on growth and health of juvenile flatfishes. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 605–610.

Monteiro, P. R. R., Reis-Henriques, M. A. and Coimbra, J. (2000a). Plasma steroid levels in female flounder (*Platichthys flesus*) after chronic dietary exposure to single polycyclic aromatic hydrocarbons. *Marine Environmental Research* **49**, 453–467.

Monteiro, P. R. R., Reis-Henriques, M. A. and Coimbra, J. (2000b). Polycyclic aromatic hydrocarbons inhibit in vitro ovarian steroidogenesis in the flounder (*Platichthys flesus L.*). *Aquatic Toxicology* **48**, 549–559.

Moore, M. J. and Stegeman, J. J. (1994). Hepatic neoplasms in winter flounder *Pleuronectes americanus* from Boston Harbor, Massachusetts, USA. *Diseases of Aquatic Organisms* **20**, 33–48.

Moore, M. J., Shea, D., Hillman, R. E. and Stegeman, J. J. (1996). Trends in hepatic tumours and hydropic vacuolation, fin erosion, organic chemicals and stable isotope ratios in winter flounder from Massachusetts, USA. *Marine Pollution Bulletin* **32**, 458–470.

Moore, M. J., Smolowitz, R. M. and Stegeman, J. J. (1997). Stages of hydropic vacuolation in the liver of winter flounder *Pleuronectes americanu*s from a chemically contaminated site. *Diseases of Aquatic Organisms* **31**, 19–28.

Morgan, E. (2001). Regulation of cytrochrome P450 by inflammation mediators: why and how? *Drug Metabolism and Disposition* **29**, 207–212.

Mulvey, M., Newman, M. C., Vogelbein, W. and Unger, M. A. (2002). Genetic structure of *Fundulus heteroclitus* from PAH-contaminated and neighboring sites in the Elizabeth and York rivers. *Aquatic Toxicology* **61**, 195–209.

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

Myers, M. S., Rhodes, L. D. and McCain, B. B. (1987). Pathologic anatomy and patterns of occurrence of hepatic neoplasms, putative preneoplastic lesions, and other idiopathic hepatic conditions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. *Journal of the National Cancer Institute* **78**, 333–363.

Myers, M. S., Stehr, C. M., Olson, O. P., Johnson, L. L., McCain, B. B., Chan, S.-L. and Varanasi, U. (1994). Relationships between toxicopathic hepatic lesions and exposure to chemical contaminants in English sole (*Pleuronectes vetulus*), starry flounder (*Platichthys stellatus*), and white croaker (*Genyonemus lineatus*) from selected marine sites on the Pacific Coast,USA. *Environmental Health Perspectives* **102**, 200–215.

Myers, M. S., French, B. L., Reichert, W. L., Willis, M. L., Anulacion, B. F., Collier, T. K. and Stein, J. E. (1998). Reductions in CYPIA expression and hydrophobic DNA adducts in liver neoplasms of English sole (*Pleuronectes vetulus*): further support for the "resistant hepatocyte" model of hepatocarcinogenesis. *Marine Environmental Research* **46**, 197–202.

Myers, M. S. and Fournie, J. W. (2002). Histopathological biomarkers as integrators of anthropogenic and environmental stressors. In *Biological Indicators of Aquatic Ecosystem Stress* (ed. S. M. Adams), pp. 221–287. Bethesda, MD: American Fisheries Society.

Myers, M. S., Johnson, L. L. and Collier, T. K. (2003). Establishing the causal relationship between polycyclic aromatic hydrocarbon (PAH) exposure and hepatic neoplasms and neoplasia-related liver lesions in English sole (*Pleuronectes vetulus*). *Human and Ecological Risk Assessment: An International Journal* **9**, 67–94.

Myers, M. S., Anulacion, B. F., French, B. L., Reichert, W. L., Laetz, C. A., Buzitis, J., Olson, O. P., Sol, S. and Collier, T. K. (2008). Improved flatfish health following remediation of a PAH-contaminated site in Eagle Harbor, Washington. *Aquatic Toxicology* **88**, 277–288.

Nagler, J. J. and Cyr, D. G. (1997). Exposure of male american plaice (*Hippoglossoides platessoides*) to contaminated marine sediments decreases the hatching success of their progeny. *Environmental Toxicology and Chemistry* **16**, 1733–1738.

Nakayama, K., Kitamura, S. I., Murakami, Y., Song, J. Y., Jung, S. J., Oh, M. J., Iwata, H. and Tanabe, S. (2008). Toxicogenomic analysis of immune system-related genes in Japanese flounder (*Paralichthys olivaceus*) exposed to heavy oil. *Marine Pollution Bulletin* **57**, 445–452.

Navas, J. M. and Segner, H. (2001). Estrogen-mediated suppression of cytochrome P4501A (CYP1A) expression in rainbow trout hepatocytes: role of estrogen receptor. *Chemico-Biological Interactions* **138**, 285–298.

Nero, V., Farwell, A., Lee, L. E., Van Meer, T., MacKinnon, M. D. and Dixon, D. G. (2006). The effects of salinity on naphthenic acid toxicity to yellow perch: gill and liver histopathology. *Ecotoxicology and Environmental Safety* **65**, 252–264.

Newman, M. C. and Clements, W. H. (2008). *Ecotoxicology: A Comprehensive Treatment*. Boca Raton, FL: CRC Press, Taylor and Francis Group.

Nicolas, J.-M. (1999). Vitellogenesis in fish and the effects of polycyclic aromatic hydrocarbon contaminants. *Aquatic Toxicology* **45**, 77–90.

Oleksiak, M. F., Karchner, S. I., Jenny, M. J., Mark Welch, D. B. and Hahn, M. E. (2011). Transcriptomic assessment of resistance to effects of an aryl hydrocarbon receptor (AHR) agonist in embryos of Atlantic killifish (*Fundulus heteroclitus*) from a marine Superfund site. *BMC Genomics* **12**, 263–280.

Oliveira, M., Pacheco, M. and Santos, M. A. (2007). Cytochrome P4501A, genotoxic and stress responses in golden grey mullet (*Liza aurata*) following short-term exposure to phenanthrene. *Chemosphere* **66**, 1284–1291.

Oliveira, M., Pacheco, M. and Santos, M. A. (2008). Organ specific antioxidant responses in golden grey mullet (*Liza aurata*) following a short-term exposure to phenanthrene. *Science of the Total Environment* **396**, 70–78.

Oliveira, M., Pacheco, M. and Santos, M. A. (2011). Fish thyroidal and stress responses in contamination monitoring—An integrated biomarker approach. *Ecotoxicology and Environmental Safety* **74**, 1265–1270.

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

Pait, A. S. and Nelson, J. O. (2009). A survey of indicators for reproductive endocrine disruption in *Fundulus heteroclitus* (killifish) at selected sites in the Chesapeake Bay. *Marine Environmental Research* **68**, 170–177.

Patel, M. R., Scheffler, B. E., Wang, L. and Willett, K. L. (2006). Effects of benzo(a)pyrene exposure on killifish (*Fundulus heteroclitus*) aromatase activities and mRNA. *Aquatic Toxicology* **77**, 267–278.

Pathiratne, A. and Hemachandra, C. K. (2010). Modulation of ethoxyresorufin O-deethylase and glutathione S-transferase activities in Nile tilapia (*Oreochromis niloticus*) by polycyclic aromatic hydrocarbons containing two to four rings: implications in biomonitoring aquatic pollution. *Ecotoxicology* **19**, 1012–1018.

Perez-Casanova, J. C., Hamoutene, D., Samuelson, S., Burt, K., King, T. L. and Lee, K. (2010). The immune response of juvenile Atlantic cod (*Gadus morhua* L.) to chronic exposure to produced water. *Marine Environmental Research* **70**, 26–34.

Perez-Casanova, J. C., Hamoutene, D., Hobbs, K. and Lee, K. (2012). Effects of chronic exposure to the aqueous fraction of produced water on growth, detoxification, and immune factors of Atlantic cod. *Ecotoxicology and Environmental Safety* **86**, 239–249.

Peters, L. E., MacKinnon, M., Van Meer, T., van den Heuvel, M. R. and Dixon, D. G. (2007). Effects of oil sands process-affected waters and naphthenic acids on yellow perch (*Perca flavescens*) and Japanese medaka (*Oryzias latipes*) embryonic development. *Chemosphere* **67**, 2177–2183.

Phillips, J. M. and Goodman, J. I. (2009). Inhalation of cigarette smoke induces regions of altered DNA methylation (RAMs) in SENCAR mouse lung. *Toxicology* **260**, 7–15.

Pinkney, A. E. and Harshbarger, J. C. (2006). Tumor prevalence in mummichogs from the Delaware Estuary watershed. *Journal of Aquatic Animal Health* **18**, 244–251.

Pinkney, A. E., Harshbarger, J. C. and Rutter, M. A. (2009). Tumors in brown bullheads in the Chesapeake Bay watershed: analysis of survey data from 1992 through 2006. *Journal of Aquatic Animal Health* **21**, 71–81.

Pollino, C. A. and Holdway, D. A. (2002). Toxicity testing of crude oil and related compounds using early life stages of the crimson-spotted rainbowfish (*Melanotaenia fluviatilis*). *Ecotoxicology and Environmental Safety* **52**, 180–189.

Pollino, C. A., Georgiades, E. and Holdway, D. A. (2009). Physiological changes in reproductively active rainbowfish (*Melanotaenia fluviatilis*) following exposure to naphthalene. *Ecotoxicology and Environmental Safety* **72**, 1265–1270.

Prosser, M. P., Unger, M. A. and Vogelbein, W. K. (2011). Multistressor interactions in the zebrafish (*Danio rerio*): concurrent phenanthrene exposure and *Mycobacterium marinum* infection. *Aquatic Toxicology* **102**, 177–185.

Pruett, S. B., Holladay, S. D., Prater, M. R., Yucesoy, B. and Luster, M. I. (2007). The promise of genomics and proteomics in immunotoxicology and immunopharmacology. In Target Organ Toxicology Series, *Immunotoxicology and Immunopharmacology* (eds. R. Luebke, R. House and I. Kimber), pp. 79–95. Boca Raton, FL: CRC Press, Taylor and Francis Group.

Puget Sound Partnership (2010). 2009 State of the Sound. Puget Sound Partnership, Olympia, WA. Publication No. PSP09-08.

Quagraine, E., Peterson, H. and Headley, J. (2005). *In situ* bioremediation of naphthenic acids contaminated tailing pond waters in the Athabasca Oil Sands Region—demonstrated field studies and plausible options: a review. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering* **40**, 685–722.

Rahman, M. S., Khan, I. A. and Thomas, P. (2011). Tryptophan hydroxylase: a target for neuroendocrine disruption. *Journal of Toxicology and Environmental Health, Part B* **14**, 473–494.

Ramesh, A. and Archibong, A. E. (2011). Reproductive toxicity of polycyclic aromatic hydrocarbons: occupational relevance. In *Reproductive and Developmental Toxicology* (ed. R. C. Gupta), pp. 577–591. San Diego, CA: Academic Press.

Reddy, A. P., Spitsbergen, J. M., Mathews, C., Hendricks, J. D. and Bailey, G. (1999). Experimental hepatic tumorigenicity by environmental hydrocarbon dibenzo(a)pyrene. *Journal of Environmental Pathology, Toxicology and Oncology* **18**, 261–269.

Reichert, W. L., Myers, M. S., Peck-Miller, K., French, B., Anulacion, B. F., Collier, T. K., Stein, J. E. and Varanasi, U. (1998). Molecular epizootiology of genotoxic events in marine fish: linking contaminant exposure, DNA damage, and tissue-level alterations. *Mutation Research/Reviews in Mutation Research* **411**, 215–225.

Reynaud, S., Duchiron, C. and Deschaux, P. (2001). 3-Methylcholanthrene increases phorbol 12-myristate 13-acetate-induced respiratory burst activity and intracellular calcium levels

in common carp (*Cyprinus carpio* L) macrophages. *Toxicology and Applied Pharmacology* **175**, 1–9.

Reynaud, S., Duchiron, C. and Deschaux, P. (2003). 3-Methylcholanthrene inhibits lymphocyte proliferation and increases intracellular calcium levels in common carp (*Cyprinus carpio* L). *Aquatic Toxicology* **63**, 319–331.

Reynaud, S., Duchiron, C. and Deschaux, P. (2004). 3-Methylcholanthrene induces lymphocyte and phagocyte apoptosis in common carp (*Cyprinus carpio* L) in vitro. *Aquatic Toxicology* **66**, 307–318.

Reynaud, S. and Deschaux, P. (2005). The effects of 3-methylcholanthrene on lymphocyte proliferation in the common carp (*Cyprinus carpio* L.). *Toxicology* **211**, 156–164.

Reynaud, S. and Deschaux, P. (2006). The effects of polycyclic aromatic hydrocarbons on the immune system of fish: a review. *Aquatic Toxicology* **77**, 229–238.

Rice, C. A., Myers, M. S., Willis, M. L., French, B. L. and Casillas, E. (2000). From sediment bioassay to fish biomarker—connecting the dots using simple trophic relationships. *Marine Environmental Research* **50**, 527–533.

Rice, C. D. and Arkoosh, M. R. (2002). Immunological indicators of environmental stress and disease susceptibility in fishes. In *Biological Indicators of Stress in Aquatic Ecosystem Stress* (ed. S. M. Adams), pp. 187–220. Bethesda, MD: American Fisheries Society Publication.

Rice, S. D., Thomas, R. E., Carls, M. G., Heintz, R. A., Wertheimer, A. C., Murphy, M. L., Short, J. W. and Moles, A. (2001). Impacts to pink salmon following the *Exxon Valdez* oil spill: persistence, toxicity, sensitivity, and controversy. *Reviews of Fisheries Science* **9**, 165–211.

Ridgway, L. L., Chapleau, F., Comba, M. E. and Backus, S. M. (1999). Population characteristics and contaminant burdens of the white sucker (*Catostomus commersoni*) from the St. Lawrence River near Cornwall, Ontario and Massena, New York. *Journal of Great Lakes Research* **25**, 567–582.

Røe Utvik, T. I. (1999). Chemical characterisation of produced water from four offshore oil production platforms in the North Sea. *Chemosphere* **39**, 2593–2606.

Roomi, M. W., Ho, R. K., Sarma, D. S. R. and Farber, E. (1985). A common biochemical pattern in preneoplastic hepatocyte nodules generated in four different models in the rat. *Cancer Research* **45**, 564–571.

Roubal, W. T., Collier, T. K. and Malins, D. C. (1977). Accumulation and metabolism of carbon-14 labeled benzene, naphthalene, and anthracene by young coho salmon (*Oncorhynchus kisutch*). *Archives of Environmental Contamination and Toxicology* **5**, 513–529.

Saborido-Rey, F., Domínguez-Petit, R., Tomás, J., Morales-Nin, B. and Alonso-Fernandez, A. (2007). Growth of juvenile turbot in response to food pellets contaminated by fuel oil from the tanker "Prestige". *Marine Ecology Progress Series* **345**, 271–279.

Schiewe, M. H., Weber, D. D., Myers, M. S., Jacques, F. J., Reichert, W. L., Krone, C. A., Malins, D. C., McCain, B. B., Chan, S.-L. and Varanasi, U. (1991). Induction of foci of cellular alteration and other hepatic lesions in English sole (*Parophrys vetulus*) exposed to an extract of an urban marine sediment. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 1750–1760.

Scott, J. A., Incardona, J. P., Pelkki, K., Shepardson, S. and Hodson, P. V. (2011). AhR2-mediated; CYP1A-independent cardiovascular toxicity in zebrafish (*Danio rerio*) embryos exposed to retene. *Aquatic Toxicology* **101**, 165–174.

Segner, H., Moller, A. M., Wenger, M. and Casanova-Nakayama, A. (2012a). Fish immunotoxicology: research at the crossroads of immunology, ecology and toxicology. In *Interdisciplinary Studies on Environmental Chemistry—Environmental Pollution and Ecotoxicology* (eds. M. Kawaguchi, K. Misaki, H. Sato, T. Yokokawa, T. Itai, et al.), pp. 1–12. Tokyo: TERRAPUB.

Segner, H., Wenger, M., Moller, A. M., Kollner, B. and Casanova-Nakayama, A. (2012b). Immunotoxic effects of environmental toxicants in fish—how to assess them? *Environmental Science and Pollution Research* **19**, 2465–2476.

Seruto, C., Sapozhnikova, Y. and Schlenk, D. (2005). Evaluation of the relationships between biochemical endpoints of PAH exposure and physiological endpoints of reproduction in male California Halibut (*Paralichthys californicus*) exposed to sediments from a natural oil seep. *Marine Environmental Research* **60**, 454–465.

Shallaja, M. S. and D'Silva, C. (2003). Evaluation of impact of PAH on a tropical fish, Oreochromis mossambicus using multiple biomarkers. *Chemosphere* **53**, 835–841.

Sikka, H. C, Steward, A. R., Kandaswami, C., Rutkowski, J. P., Zaleski, J., Kumar, S., Earley, K. and Gupta, R. C. (1991). Metabolism of benzo(a)pyrene and persistence of DNA adducts in the brown bullhead (*Ictalurus nebulosus*). *Comparative Biochemical Physiology* **100C** (1-2), 25–28.

Short, J. W. and Heintz, R. A. (1997). Identification of *Exxon Valdez* oil in sediments and tissues from Prince William Sound and the Northwestern Gulf of Alaska based on a PAH weathering model. *Environmental Science and Technology* **31**, 2375–2384.

Skinner, M. K., Manikkam, M. and Guerrero-Bosagna, C. (2011). Epigenetic transgenerational actions of endocrine disruptors. *Reproductive Toxicology* **31**, 337–343.

Smith, C. E., Peck, T. H., Klauda, R. J. and McLaren, J. B. (1979). Hepatomas in Atlantic tomcod *Microgadus tomcod* (Walbaum) collected in the Hudson River estuary in New York. *Journal of Fish Diseases* **2**, 313–319.

Snowberger, E. A. and Stegeman, J. J. (1987). Patterns and regulation of estradiol metabolism by hepatic microsomes from two species of marine teleosts. *General and Comparative Endocrinology* **66**, 256–265.

Sol, S. Y., Johnson, L. L., Horness, B. H. and Collier, T. K. (2000). Relationship between oil exposure and reproductive parameters in fish collected following the *Exxon Valdez* oil spill. *Marine Pollution Bulletin* **40**, 1139–1147.

Sol, S., Johnson, L., Boyd, D., Olson, O. P., Lomax, D. and Collier, T. (2008). Relationships between anthropogenic chemical contaminant exposure and associated changes in reproductive parameters in male English sole (*Parophrys vetulus*) collected from Hylebos Waterway, Puget Sound, Washington. *Archives of Environmental Contamination and Toxicology* **55**, 627–638.

Solt, D. B. and Farber, E. (1976). New principle for analysis of chemical carcinogenesis. *Nature* **263**, 701–703.

Solt, D. B., Medline, A. and Farber, E. (1977). Rapid emergence of carcinogen-induced hyperplastic lesions in a new model for the sequential analysis of liver carcinogenesis. *American Journal of Pathology* **88**, 595–618.

Song, J. Y., Nakayama, K., Kokushi, E., Ito, K., Uno, S., Koyama, J., Rahman, M. H., Murakami, Y. and Kitamura, S. I. (2012a). Effect of heavy oil exposure on antibacterial activity and expression of immune-related genes in Japanese flounder *Paralichthys olivaceus*. *Environmental Toxicology and Chemistry* **31**, 828–835.

Song, J. Y., Ohta, S., Nakayama, K., Murakami, Y. and Kitamura, S. I. (2012b). A time-course study of immune response in Japanese flounder *Paralichthys olivaceus* exposed to heavy oil. *Environmental Science and Pollution Research* **19**, 2300–2304.

Spromberg, J. A. and Meador, J. P. (2005). Population-level effects on Chinook salmon from chronic toxicity test measurement endpoints. *Integrated Environmental Assessment and Management* **1**, 9–21.

Spromberg, J. A. and Meador, J. P. (2006). Relating chronic toxicity responses to population-level effects: a comparison of population-level parameters for three salmon species as a function of low-level toxicity. *Ecological Modelling* **199**, 240–252.

Stehr, C. M., Johnson, L. L. and Myers, M. S. (1998). Hydropic vacuolation in the liver of three species of fish from the U.S. West Coast: lesion description and risk assessment associated with contaminant exposure. *Diseases of Aquatic Organisms* **32**, 119–135.

Stein, J. E., Hom, T., Sanborn, H. and Varanasi, U. (1988). The metabolism and disposition of 17β-estradiol in English sole exposed to a contaminated sediment extract. *Marine Environmental Research* **24**, 252–253.

Stein, R. A. (2012). Epigenetics and environmental exposures. *Journal of Epidemiology and Community Health* **66**, 8–13.

Stentiford, G. D., Longshaw, M., Lyons, B. P., Jones, G., Green, M. and Feist, S. W. (2003). Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. *Marine Environmental Research* **55**, 137–159.

Stentiford, G. D., Bignell, J. P., Lyons, B. P. and Feist, S. W. (2009). Site-specific disease profiles in fish and their use in environmental monitoring. *Marine Ecology Progress Series* **381**, 1–15.

Stentiford, G. D., Bignell, J. P., Lyons, B. P., Thain, J. E. and Feist, S. W. (2010). Effect of age on liver pathology and other diseases in flatfish: implications for assessment of marine ecological health status. *Marine Ecology Progress Series* **411**, 215–230.

Steward, A. R, Kandaswami, C., Chidambaram, S., Ziper, C., Rutkowski, J. P. and Sikka, H. C. (1991). Disposition and metabolic fate of benzo-a-pyrene in the common carp. *Aquatic Toxicology* **20** (4), 205–218.

Sun, L., Zuo, Z., Luo, H., Chen, M., Zhong, Y., Chen, Y. and Wang, C. (2011). Chronic exposure to phenanthrene influences the spermatogenesis of male *Sebastiscus marmoratus*: U-shaped effects and the reason for them. *Environmental Science and Technology* **45**, 10212–10218.

Sundt, R. C. and Björkblom, C. (2011). Effects of produced water on reproductive parameters in prespawning Atlantic cod (*Gadus morhua*). *Journal of Toxicology and Environmental Health, Part A* **74**, 543–554.

Susser, M. (1986). Rules of inference in epidemiology. *Regulatory Toxicology and Pharmacology* **6**, 116–128.

Suter, M., Abramovici, A., Showalter, L., Hu, M., Shope, C. D., Varner, M. and Aagaard-Tillery, K. (2010). In utero tobacco exposure epigenetically modifies placental CYP1A1 expression. *Metabolism* **59**, 1481–1490.

Teles, M., Oliveira, M., Pacheco, M. and Santos, M. A. (2005). Endocrine and metabolic changes in *Anguilla anguilla L.* following exposure to beta-naphthoflavone—a microsomal enzyme inducer. *Environment International* **31**, 99–104.

Thomas, K. V., Langford, K., Petersen, K., Smith, A. J. and Tollefsen, K. E. (2009). Effect-directed identification of naphthenic acids as important in vitro xeno-estrogens and anti-androgens in North Sea offshore produced water discharges. *Environmental Science and Technology* **43**, 8066–8071.

Timme-Laragy, A. R., Meyer, J. A., Waterland, R. A. and Di Giulio, R. T. (2005). Analysis of CpG methylation in the killifish CYP1A promoter. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* **141**, 406–411.

Tintos, A., Gesto, M., Alvarez, R., Míguez, J. M. and Soengas, J. L. (2006). Interactive effects of naphthalene treatment and the onset of vitellogenesis on energy metabolism in liver and gonad, and plasma steroid hormones of rainbow trout *Oncorhynchus mykiss*. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* **144**, 155–165.

Tintos, A., Gesto, M., Míguez, J. M. and Soengas, J. L. (2007). Naphthalene treatment alters liver intermediary metabolism and levels of steroid hormones in plasma of rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicology and Environmental Safety* **66**, 139–147.

Tintos, A., Gesto, M., Míguez, J. M. and Soengas, J. L. (2008). β-Naphthoflavone and benzo(a) pyrene treatment affect liver intermediary metabolism and plasma cortisol levels in rainbow trout *Oncorhynchus mykiss*. *Ecotoxicology and Environmental Safety* **69**, 180–186.

Tommasi, S., Kim, S. I., Zhong, X., Wu, X., Pfeifer, G. P. and Besaratinia, A. (2010). Investigating the epigenetic effects of a prototype smoke-derived carcinogen in human cells. *PLoS One* **5**, e10594.

Truscott, B., Walsh, J. M., Burton, M. P., Payne, J. F. and Idler, D. R. (1983). Effect of acute exposure to crude petroleum on some reproductive hormones in salmon and flounder. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology* **75**, 121–130.

Tryphonas, H., Fournier, M., Bakley, E. R., Smits, J. and Brousseau, P. (2005). *Investigative Immunotoxicology*. Boca Raton, FL: CRC Press.

Tuvikene, A. (1995). Responses of fish to aromatic hydrocarbons. *Annales Zoologici Fennici* **32**, 295–309.

Van Veld, P., Ko, U., Vogelbein, W. K. and Westbrook, D. J. (1991). Glutathione S-transferase in intestine, liver and hepatic lesions of mummichog (*Fundulus heteroclitus*) from a creosote-contaminated environment. *Fish Physiology and Biochemistry* **9**, 369–376.

Van Veld, P. A., Vogelbein, W. K., Smolowitz, R., Woodin, B. R. and Stegeman, J. J. (1992). Cytochrome P450IA1 in hepatic lesions of a teleost fish (*Fundulus heteroclitus*) collected from a polycyclic aromatic hydrocarbon-contaminated site. *Carcinogenesis* **13**, 505–507.

Van Veld, P. A. and Westbrook, D. J. (1995). Evidence for depression of cytochrome P4501A in a population of chemically resistant mummichog (*Fundulus heteroclitus*). *Environmental Science* **3**, 221–234.

Van Veld, P. A. and Nacci, D. E. (2008). Toxicity resistance. In *The Toxicology of Fishes* (eds. R. T. Di Guilio and D. E. Hinton), pp. 597–641. Boca Raton, FL: CRC Press.

Vandegehuchte, M. and Janssen, C. (2011). Epigenetics and its implications for ecotoxicology. *Ecotoxicology* **20**, 607–624.

Varanasi, U., Stein, J. E. and Nishimoto, M. (1989). Biotransformation and disposition of polycyclic aromatic hydrocarbons in fish. In *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment* (ed. U. Varanasi), pp. 93–149. Boca Raton, FL: CRC Press.

Veith, G. D., Mekenyan, O. G., Ankley, G. T. and Call, D. J. (1995). A QSAR analysis of substituent effects on the photoinduced acute toxicity of PAHs. *Chemosphere* **30**, 2129–2142.

Vethaak, A., Jol, J., Meijboom, A., Eggens, M., Rheinallt, T., Wester, P., van de Zande, T., Bergman, A., Dankers, N., Ariese, F., Baan, R., Everts, J., Opperhuizen, A. and Marquenie, J. (1996). Skin and liver diseases induced in flounder (*Platichthys flesus*) after longterm exposure to contaminated sediments in large-scale mesocosms. *Environmental Health Perspectives* **104**, 1218–1229.

Vethaak, A. and Wester, P. (1996). Diseases of flounder *Platichthys flesus* in Dutch coastal and estuarine waters, with particular reference to environmental stress factors. II. Liver histopathology. *Diseases of Aquatic Organisms* **26**, 99–116.

Vethaak, A. D., Jol, J. G. and Pieters, J. P. F. (2009). Long-term trends in the prevalence of cancer and other major diseases among flatfish in the southeastern North Sea as indicators of changing ecosystem health. *Environmental Science and Technology* **43**, 2151–2158.

Villeneuve, D. L., Khim, J. S., Kannan, K. and Giesy, J. P. (2002). Relative potencies of individual polycyclic aromatic hydrocarbons to induce dioxinlike and estrogenic responses in three cell lines. *Environmental Toxicology* **17**, 128–137.

Vogelbein, W. K., Fournie, J. W., Van Veld, P. A. and Huggett, R. J. (1990). Hepatic neoplasms in the mummichog *Fundulus heteroclitus* from a creosote-contaminated site. *Cancer Research* **50**, 5978–5986.

Vogelbein, W. K. and Unger, M. A. (2006). Liver carcinogenesis in a non-migratory fish: The association with polycyclic aromatic hydrocarbon exposure. *Bulletin of European Association of Fish Pathologists* **26**, 11–20.

Vogelbein, W.K., Unger, M.A., Gauthier, D.T., 2008. The Elizabeth River Monitoring Program 2006–2007: Association between mummichog liver histopathology and sediment chemical contamination. A Final Report. Virginia Dept. of Environmental Quality. p. 34.

Wang, Z., Hollebone, B. P., Fingas, M., Fieldhouse, B., Sigouin, L., Landriault, M., Smith, P., Noonan, J. and Thouin, G. (2003). Characteristics of Spilled Oils, Fuels, and Petroleum Products: 1. Composition and Properties of Selected Oils. EPA/600/R-03/072. Research Triangle Park, NC: U.S. Environmental Protection Agency, 280 pp.

White, P. A., Robitaille, S. and Rasmussen, J. B. (1999). Heritable reproductive effects of benzo [a]pyrene on the fathead minnow (*Pimephales promelas*). *Environmental Toxicology and Chemistry* **18**, 1843–1847.

Whyte, K. S. (2007). The innate immune response of finfish—A review of current knowledge. *Fish and Shellfish Immunology* **23**, 1127–1151.

Williams, D. E., Lech, J. J. and Buhler, D. R. (1998). Xenobiotics and xenoestrogens in fish: modulation of cytochrome P450 and carcinogenesis. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **399**, 179–192.

Wills, L. P., Jung, D., Koehrn, K., Zhu, S., Willett, K. L., Hinton, D. E. and Di Giulio, R. T. (2010a). Comparative chronic liver toxicity of benzo[a]pyrene in two populations of the Atlantic killfish with different exposure histories. *Environmental Health Perspectives* **118**, 1376–1381.

Wills, L. P., Matson, C. W., Landon, C. D. and Di Giulio, R. T. (2010b). Characterization of the recalcitrant CYP1 phenotype found in Atlantic killifish (*Fundulus heteroclitus*) inhabiting a Superfund site on the Elizabeth River, VA. *Aquatic Toxicology* **99**, 33–41.

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

Wu, R. S. S., Pollino, C. A., Au, D. W. T., Zheng, G. J., Yuen, B. B. H. and Lam, P. K. S. (2003). Evaluation of biomarkers of exposure and effect in juvenile areolated grouper (*Epinephelus areolatus*) on foodborne exposure to benzo[a]pyrene. *Environmental Toxicology and Chemistry* **22**, 1568–1573.

Xia, Y., Zhu, P., Han, Y., Lu, C., Wang, S., Gu, A., Fu, G., Zhao, R., Song, L. and Wang, X. (2009). Urinary metabolites of polycyclic aromatic hydrocarbons in relation to idiopathic male infertility. *Human Reproduction* **24**, 1067–1074.

Ylitalo, G. M., Krahn, M. M., Dickhoff, W. W., Stein, J. E., Walker, C. C., Lassitter, C. L., Garrett, E. S., Desfosse, L. L., Mitchell, K. M., Noble, B. T., Wilson, S., Beck, N. B., Benner, R. A., Koufopoulos, P. N. and Dickey, R. W. (2012). Federal seafood safety response to the *Deepwater Horizon* oil spill. *Proceedings of the National Academy of Sciences* **109**, 20274–20279.

Young, R. F., Orr, E. A., Goss, G. G. and Fedorak, P. M. (2007). Detection of naphthenic acids in fish exposed to commercial naphthenic acids and oil sands process-affected water. *Chemosphere* **68**, 518–527.

Young, R. F., Wismer, W. V. and Fedorak, P. M. (2008). Estimating naphthenic acids concentrations in laboratory-exposed fish and in fish from the wild. *Chemosphere* **73**, 498–505.

Young, R. F., Michel, L. M. and Fedorak, P. M. (2011). Distribution of naphthenic acids in tissues of laboratory-exposed fish and in wild fishes from near the Athabasca oil sands in Alberta, Canada. *Ecotoxicology and Environmental Safety* **74**, 889–896.

Yu, H. (2002). Environmental carcinogenic polycyclic aromatic hydrocarbons: photochemistry and phototoxicity. *Journal of Environmental Science and Health (C) Environmental Carcinogenesis and Ecotoxicological Review* **20**, 149–183.

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