Review

Delayed Behavioral Effects of Early Life Toxicant Exposures in Aquatic Biota

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Abstract: Behavioral development occurs together with the development of the nervous system. Studies on mammals indicate that exposures to some chemicals during embryonic development at concentrations that do not produce anatomical malformations may nevertheless produce behavioral deficits later in life, an example of delayed effects. There have been reports of delayed effects in aquatic organisms. Delayed behavioral effects of mercury, chlorinated and other pesticides, polycyclic aromatic hydrocarbons (PAHs), and some synthetic hormones in the environment have been reported in fishes and invertebrates; in some cases behavioral effects are manifested years after the exposure. Another type of delayed behavioral effect results from exposure of mature females before fertilization (maternal exposure). Even when embryos and larvae are reared in clean water, offspring may manifest abnormal behaviors following maternal exposure. The reported behavioral changes are generally deleterious and compromise the fitness of the animal in its natural environment. Delayed effects and their impacts on fitness are not considered in standard short-term embryo bioassays, which will therefore underestimate neurotoxicity. The literature in the field is scattered and has not been reviewed. The objective of this paper is to review and synthesize what is known about delayed behavioral effects in aquatic biota.

Keywords: embryo; larva; behavior; delayed effect; fish; crustacean

1. Introduction

Behavioral development is a result of the development of the nervous system. Studies on mammals showed that exposures to some chemicals during embryonic development at concentrations lower than those produce anatomical malformations may nevertheless result in behavioral alterations later in life [1]. The study of behavioral abnormalities resulting from embryonic toxicant exposures has been called "behavioral teratology", and is an example of delayed effects. Behavior is a particularly sensitive response to environmental contaminants [2]. Exposures during certain stages of development may be particularly sensitive, though not many studies have examined this with respect to delayed effects. Observable differences in behavior may be found at concentrations of chemicals lower than those that affect biochemical biomarkers or physiology. Since behavior is a link between underlying physiological/biochemical processes and an animal's ecology [3], it is a particularly important type of response for animals in the field. As it is likely to occur in nature it can have effects at the population and community level [4]. Since exposure to some chemicals during early life stages can produce behavioral changes that are manifested later suggests that standard toxicity tests, which are terminated shortly after hatching and do not examine behavior, underestimate the toxicity of various toxicants.

Behaviors that have been examined include locomotor activity, as well as more complex behaviors such as predator/prey interactions, reproductive and social behaviors, which are particularly likely to have ecological repercussions that could affect populations. The transition from dependence on yolk to feeding is a critical stage for larval stages of many animals [5]. The ability to capture food effectively and to avoid being eaten are literally matters of life and death for organisms in nature.

There have also been reports of transgenerational effects in which adults are exposed to toxicants and responses seen in their offspring. For example, in one (nonbehavioral) study, offspring of killifish or mummichogs, *Fundulus heteroclitus* fed mercury-contaminated food were less able to successfully reproduce and had altered sex ratios [6]. These delayed and transgenerational behavioral effects are the subject of this review, which focuses primarily on fishes. In recent years the zebrafish, *Danio rerio*, has become a favored model organism for early life toxicity studies because of knowledge of its genome. However, other species such as killifish (*Fundulus heteroclitus*), fathead minnows (*Pimephales promelas*), medakas (*Oryzias latipes*), and sticklebacks (*Gasterosteus aculeatus*) have been studied over a longer time period and it is important to understand effects on a variety of species with different life histories, behaviors, and ecology. It is likely that the majority of *D. rerio* studies have been motivated by considering the fish as a "model" organism (comparable to the laboratory rat), while many of the studies on other species have been motivated by interest in the aquatic environment and its residents.

In this paper, delayed behavioral effects of environmental chemicals on aquatic organisms are reviewed in Part One, and then potential underlying mechanisms are discussed in greater depth in Part Two. The majority of papers cited focus on fishes with relatively few covering invertebrates because of a paucity of such studies on invertebrate taxa.

2. Environmental Chemicals

2.1. Mercury

Metals released from mining and industrial processes are among the major contaminants in aquatic environments. Mercury, the most toxic, is also released into the atmosphere from burning coal, where it can be transported long distances before being deposited far from its source. Mercury and especially its organic form methylmercury (meHg) is particularly neurotoxic, so effects on behavior have been intensively studied. Mammalian studies indicate mercury is a behavioral teratogen [7]. In addition to numerous experimental studies in laboratory animals, there is epidemiological evidence. The town of Minamata Japan, where in the 1950s and '60s a factory discharged Hg into the bay from which people ate fish that had accumulated the contaminant, was a site of Hg poisoning of the human population. Prenatally-exposed individuals exhibited brain damage, mental retardation and/or psychomotor retardation [8]. There was a high frequency of cerebral palsy and other neurological problems in children, including those whose mothers did not exhibit symptoms. Some of their mother's body burden of methylmercury (meHg) had been transferred through the placenta to the fetus, and symptoms appeared after birth. Minamata was a "wake-up call" that made people aware of the risks of eating fish with high mercury levels.

Studies have shown that mercury exposure of fish embryos can produce behavioral changes in larvae after hatching, even though the larvae are maintained in clean water. In some cases, the effects are temporary and disappear over time. Weis and Weis [9] examined behavior of mummichog or killifish (*Fundulus heteroclitus*) larvae after embryonic exposure to 5 and 10 µg/L meHg, concentrations lower than those that produced anatomical malformations in this species. Larvae were maintained in clean water. Prey capture ability of early larvae was impaired when they first began to feed, but after about one week after hatching the prey capture ability was comparable to controls, showing that this effect was temporary. Authors speculated that the exposure may have caused a retardation of neurological development that was subsequently compensated for, and therefore no long lasting effects were produced. Larvae that had been exposed as embryos were also more susceptible to predation by grass shrimp (*Palaemonetes pugio*) or adult mummichogs [10]. Larvae that had been exposed to meHg as embryos generally had increased activity levels, which could serve to attract a predator resulting in increased susceptibility to capture [11]. Exposure of larvae themselves to meHg was relatively ineffective in producing behavioral changes, but after exposure of both embryos and larvae, effects on prey capture were greater than embryonic exposure alone [12]. Larvae from parents taken from a Hg-contaminated site were more resistant to the effects of meHg exposure [13]. Social behavior, in terms of incipient schooling, was also impaired in larvae that had been exposed to meHg as embryos [14]. When maintained in groups, these larvae had a greater frequency of collisions (bumped into each other more often) than controls. This response also disappeared by four weeks after hatching, indicating that the effect was reversible.

In contrast, Samson *et al.* [15] exposed zebrafish (*Danio rerio*) embryos to 0, 5, 10 or 15 µg meHg/L for varying amounts of time. Larvae were kept in clean water. Continuous embryonic exposure to 15 µg/L resulted in delayed mortality: by day 3 post-hatch, activity was reduced; by day 5, post-hatch larvae were moribund with faint heartbeat, edema and vertebral flexures. Most embryos were dead by Day 6

post hatch. Shorter embryonic exposures (24-hours) to 15 μg/L caused reduced activity and impaired prey capture; by day 4 ph, larvae showed signs of delayed mortality. Continuous exposure or exposure during only the last 24 h of development to 10 μg/L reduced activity, which did not improve. Prey capture was impaired after continuous embryo exposure to 10 μg/L, even after four days in clean water. Single-day embryonic exposures to 10 μg/L did not affect activity or prey capture of larvae, however, showing that shorter exposure periods caused less severe effects. Thus, in contrast to the mummichog experiments, the delayed effects seen here did not appear to be reversible. This was supported by reports that adult zebrafish that had been exposed to as little as 0.01 μMmeHg as embryos showed decreased learning ability [16,17]. Learning was studied in adults (4 months after developmental exposure) using food delivery on alternating sides of the aquarium. Low concentrations of meHg (<0.1 μM) delayed learning, while fish exposed to higher concentrations were unable to learn the task.

Alvarez *et al.* [18] fed adult female Atlantic croaker meHg-contaminated food at three levels for one month. Fish were then induced to spawn and meHg levels in the eggs were measured. Behavioral performance of maternally-exposed and control larvae was measured at different developmental stages: end of yolk absorption, end of oil absorption, and 4 and 11 days after oil absorption. Behaviors analyzed included survival skills related to foraging and predator evasion: routine behavior (rate of travel, active swimming speed, activity) and startle response to a visual and a vibratory stimulus (responsiveness, reactive distance, response distance, response duration, average response speed, and maximum response speed). Maternally-transferred meHg impaired these behaviors, which were considered survival skills, since they relate directly to feeding and to predator avoidance.

Fjeld *et al.* [19] found long-delayed effects of embryonic meHg exposure. Embryos of grayling (*Thymallus thymallus*) were exposed to meHg during the first 10 days of development. Three years later impaired feeding efficiencies and reduced competitive abilities were found in fish from groups which had accumulated Hg > 0.27 μg·g^{-1}. Exposed fish were 15%–24% less efficient than controls. In competition experiments, controls captured 2–6 times as many prey as fish exposed as embryos three years prior to the analysis. Therefore, the authors concluded that embryonic exposures caused some irreparable alterations in the brains of the fish, since effects were seen long after exposure. The concentration of 0.27 μg·g^{-1} Hg, which appeared to be a threshold for this effect, can be found in eggs of piscivorous fishes in lakes with substantial atmospheric deposition of Hg.

2.2. Other Metals

While lead (Pb) is a well known neurotoxicant, there are relatively few studies on delayed behavioral effects of this metal on aquatic organisms. Rice *et al.* [20] exposed newly fertilized zebrafish embryos to lead (as $PbCl_2$) at 0, 10, or 30 nM for 24 h. They then tested larvae at 168 h post fertilization for response to a mechanosensory stimulus (taps on the tank) and recorded the startle response. Larvae responded in a concentration-dependent pattern for latency (time lag) to react, maximum turn velocity, time to reach V_{max} and escape time. At higher exposure concentrations, more larvae failed to respond or stopped responding before control larvae. When fish exposed as embryos became adults, they were tested for visual responses to a rotating black bar. Visual responses were significantly reduced after embryonic exposure to 30 nM.

Decreased pH in freshwater was a concern in previous decades due to acid rain, and numerous studies of effects of this environmental problem were performed [21]. Acidity mobilizes aluminum from soils, where it is a natural constituent and not a pollutant. The combination of low pH and accompanying aluminum in freshwater can impair the development of gill Na^+, K^+-ATP-ase, (the enzyme primarily responsible for osmoregulation) and thus salinity tolerance, which is essential for salmon to develop before migrating to saline waters [22]. Exposures as short as 12 h to relatively mild acidity (pH 5.2, 31 mg·L^{-1} aluminum) can compromise salinity tolerance [22]. Atlantic salmon (*S. salar*) smolts (the stage that begins to migrate downriver) released into an acidic river in Norway often had no adult returns and had only one-tenth the returns of smolts transported to and released at the mouth of the river or in a nearby limed river [23]. These return rates were strongly correlated with the effect of acidity on gill Na^+, K^+-ATPase activity and osmotic balance of fish held in cages at the different sites. Therefore, the smolt stage appears to be particularly sensitive to acid rain and other pollutants. By affecting the development of salinity tolerance, migration to the sea can be impaired, although whether this impaired migration is a "behavioral effect" might be questionable.

Tributyltin, formerly commonly used in anti-fouling paints on ships, is a well-known and potent androgenic endocrine disruptor, with mollusks being the most sensitive taxonomic group. Fish are also sensitive. In addition to its androgenic effects, TBT has been shown to have delayed (transgenerational) behavioral effects. Japanese medaka (*Oryzias latipes*) were fed daily [24] on diets containing tributyltin (TBT), polychlorinated biphenyls (PCBs), a combination of TBT and PCBs, or a control diet with nothing added. Contaminant doses were 1 μg/g body weight per day for 3 weeks. In addition to adverse effects on reproductive success of these adults, TBT significantly reduced embryo and larval survival in eggs from females from the TBT diet, indicating transgenerational toxicity. The percentages of larval swim-up behavior in TBT-treated groups were significantly lower than those of controls, and hatched larvae exhibited erratic swimming or lack of activity. PCB produced similar but less severe effects compared to TBT. With both TBT and PCB exposures, additive effects were found on spawning success but effects on success of eggs and swim-up behavior of larvae were antagonistic.

Powers *et al.* [25] exposed zebrafish from 0 to 5 days post-fertilization (embryos plus early larvae) to different concentrations of silver to assess effects on embryonic development. They then tested whether lower concentrations altered larval behavior and subsequent survival. Concentrations ≥ 3 μM reduced embryonic survival, while 1 μM delayed hatching but did not affect survival. Concentrations as low as 0.1 μM delayed inflation of the swim bladder and impaired swimming activity, which persisted past the time when swim bladders inflated.

Steele [26] found that 72-hour embryonic exposures of sea catfish (*Arias felis*) and sheepshead (*Archosargus probatocephalus*) to copper initially caused hyperactivity and altered the frequency and degree of turning. However, after one week in clean water exposed fish of both species showed extreme hypoactivity, which was the opposite of the behavior shortly after exposure and different from that of control fish. Delayed effects of copper exposure on orientation exacerbated the abnormal turning behavior seen immediately following exposure.

2.3. Pesticides

Dichlorodiphenyltrichloroethane (DDT) is a chlorinated organic pesticide. For years it was the most popular pesticide in the world because it could kill hundreds of different kinds of insects and was relatively stable so that a single application could continue to kill insects for a long time. Its environmental persistence and its toxic effects on non-target organisms eventually led to its banning in most countries in the 1970s. Because of its persistence, it remains in aquatic sediments and continues to cycle through ecosystems decades later. It is toxic to aquatic organisms, including fish that were frequently killed by agricultural spraying. At lower concentrations it can cause behavioral effects, including developmental neurotoxic effects. Parental exposure to DDT (2.0 or 10.0 μg per 100 g fish per day in the diet for 1 month) affected behavior of larvae of Atlantic croaker, *Micropogonias undulatus* produced by these adults [27]. Responses of larvae to separate visual and vibratory stimuli, as well as routine swimming activity, were quantified using motion analysis equipment. The proportion of larvae responding to a vibratory stimulus, burst and routine swimming speeds, duration of activity, and pause duration were affected by parental exposure. Burst speeds in response to the visual stimulus were lower than in controls. These changes may decrease survival by increasing predation rates on the larvae and/or decreasing their feeding rates.

There have been some studies on newer types of pesticides that are more specifically targeted to insect biology. Diflubenzuron (Dimilin®) is a chitin-synthesis inhibitor and as such, is likely to affect crustaceans as well as insects, since both have chitin in their cuticles and both molt their cuticles. But effects of this chemical on crustaceans are not limited to effects on the molt cycle. Photobehavior of grass shrimp (*Palaemonetes pugio*) larvae was altered after they had been exposed as embryos to 0.3 μg L^{-1} of [28]. Exposed shrimp had strongly negative responses and avoided high and low light intensity, while controls had positive phototaxis at high light intensity. The depression of positive phototaxis and increase in negative phototaxis was greatest at 0.5 μg·L^{-1}. Reversals in phototactic responses were observed when embryos were exposed at different stages of development. Altered phototaxis could alter the maintenance of proper depth in the water column, which is important for larval retention in an estuary. The swimming pattern and velocity of larval grass shrimp were also altered by embryonic diflubenzuron exposure [29]. Low concentrations (0.3 μg L^{-1}) increased the swimming speed of the larvae, while higher concentrations decreased it; this may be a hormetic response. Hormesis is a phenomenon in which lower concentrations of a chemical have "positive" effects (e.g., greater speed), but higher concentrations have "negative" effects [30]. Larvae with higher exposure tended to be found lower in the water column than controls.

2.4. Polychlorinated Biphenyls (PCBs) and Dioxins

Chemically related to DDT, PCBs and dioxins are also chlorinated hydrocarbons. Unlike DDT, they were not sprayed in the environment, but due to their stability, non-flammability, and insulating properties, PCBs were used in hundreds of industrial processes, some of which are electrical, heat transfer, and hydraulic equipment. They entered the environment accidentally during their manufacture and use, and because of their persistence, still remain in aquatic sediments decades after their use has ceased. McCarthy *et al.* [31] examined effects of PCBs (Aroclor 1254) on Atlantic croaker

(*Micropogonias undulatus*) larvae. Adult fish were given a dietary administration of 0 (control) or 0.4 (dosed) mg Aroclor 1254 kg^{-1} fish d^{-1} for 2 weeks during the final stages of gonadal recrudescence. Fertilized eggs collected from control and dosed adults immediately after spawning contained 0 and 0.66 μg Aroclor 1254 g^{-1} egg, respectively. Growth of exposed larvae was significantly lower than that of controls, with dosed larvae showing a 4 days delay in attaining the same size as control larvae. Behaviors examined were routine swimming speed and activity and the response to a startle (transient vibratory) stimulus. Routine swimming speed and activity were similar for both groups, but there was a difference in response to the stimulus. The percentage of control larvae responding to the stimulus and their burst speeds increased with age, but no such increase was found in dosed larvae. Therefore, authors concluded that environmentally realistic body burdens of Aroclor 1254 transfer to the eggs, reduced larval growth and impaired their startle responses (a "survival skill"), which could make them more susceptible to predation. This has major implications for survival in the wild.

Dioxin (2,3,7,8, TCDD) is a persistent contaminant that is very toxic at very low concentrations. It is considered one of the most toxic halogenated hydrocarbons. It was never manufactured on purpose, but is a byproduct of manufacture of some herbicides, and is produced by burning plastics and in paper mills. Fish embryos are particularly sensitive and develop anatomical deformities including craniofacial defects. Zebrafish embryos were exposed to 25, 50, or 100 pg/mL (note units are pg rather than the usual *u*g or ng) for only one hour at 4 h post-fertilization then raised in clean water for 21 or 90 days [32]. In 21-day larvae that had been exposed to 50 or 100 pg/mL, the lower jaw was reduced in size by 5% and prey capture was reduced by 10%. Despite effects being minor, they occurred after only one hour of exposure during early embryonic development. This experimental design of brief exposures could have led to an investigation into sensitive periods, but since they did not do 1-hour exposures during other stages of development, they could not gain insight into sensitive periods. The decreased feeding could have been due to the reduced size of the jaw rather than neurobehavioral mechanisms. However, feeding ability was also reduced in larvae without malformations of the jaw. Similarly, *Fundulus heteroclitus* exposed to TCDD or to PCB-126 at concentrations that did not cause anatomical malformations captured 20%–40% fewer prey, a substantial reduction [33,34].

Marit and Weber [35] exposed zebrafish larvae between 48 and 96 h post fertilization to 1, 0.1, or 0.01 ng/L TCDD (or DMSO solvent control 0.005%), then raised them in clean water for 90 days. Exposed fish had equal survivability, no significant increase in gross deformities and no change in cytochrome P450 1A (CYP1A) activity. However, critical swimming speed and dorsal aorta diameter were significantly decreased in TCDD-exposed fish at 90 days. Furthermore, whole body triglycerides were significantly elevated in TCDD-exposed fish both before and after swim testing. Sublethal exposure during development caused a persistent decrease in swimming endurance. The cause of the persistent decrease in swimming endurance was not known, but authors felt it may be related to behavioral adaptations limiting swimming capabilities, failure to mobilize triglyceride stores, reduced blood flow to the periphery, or a combination of these factors.

2.5. Contaminants of Emerging Concern: Flame Retardants

Polybrominated diphenyl ethers (PBDE) are commonly used as flame retardants in furniture and other household products. They are of environmental concern because in aquatic environments they

leach out and are persistent and lipophilic and likely to accumulate in sediments and to biomagnify up food webs. High levels have been detected in wildlife. These chemicals are known to be endocrine disruptors, especially of the thyroid gland, which is closely associated with behavior and development of behavior [36]. Juvenile *F. heteroclitus* that had been exposed as embryos to the PBDE mixture DE-71 performed poorly in a maze, indicating impaired cognitive ability [37]. Garcia-Reyero *et al.* [38] exposed zebrafish embryos (*Danio rerio*) 4 h–8 days post-fertilization to sediments spiked with brominated diphenylether (BDE)-209, another flame retardant. The larvae accumulated the chemical. Although larvae did not have visible changes in motor neuron or neuromast development or on thyroid function, they did exhibit abnormal behavior, indicating (again) that behavior is a more sensitive response. Using video tracking software, they found that fish exposed to 13.7 mg/kg sediment exhibited increased swimming velocity and distance compared to controls, while those exposed to 20 mg/kg showed decreased velocity and distance compared to controls. This appears to be another example of hormesis, a fairly common phenomenon in toxicology [30]. Altered locomotion has also been observed in offspring of adult zebrafish exposed to BDE-209, indicating intergenerational transfer to progeny of exposed fish [39]. Parental BDE treatment led to delayed hatch and delayed motor neuron development, which was associated with slower locomotion under normal light conditions but hyperactivity after changing light/dark photoperiod stimulation.

2.6. Contaminants of Emerging Concern: Pharmaceuticals and Personal Care Products

Pharmaceuticals get into the aquatic environment because they are not removed by current technology in sewage treatment plants [40]. While only low concentrations (low ng/L) are found in the environment, pharmaceuticals are designed to produce effects at very low concentrations, and it is not surprising that they affect aquatic biota such as fishes as well as humans, since they have similar biology. Effects in fish in the laboratory have often been shown to be similar to effects in mammals. Some pharmaceuticals found in the environment are synthetic hormones, such as birth control pills. Those pharmaceuticals related to reproduction may have delayed effects on reproductive behavior in resident species [41]. Ethinyl estradiol (EE_2) exposure of juveniles can have delayed effects. Maunder *et al.* [42] exposed juvenile sticklebacks (*Gasterosteus aculeatus*) to 1.75 ng·L^{-1} and 27.7 ng·L^{-1} EE_2 for 4 weeks post-hatch and reared them in clean water until they matured. Exposure to the higher concentration caused the occurrence of ovotestis in males, which had less intense nuptial coloration (the bright colors that males develop in the breeding season), and built fewer nests, in which fewer eggs were deposited. The group exposed to 1.75 ng·L^{-1} also built significantly fewer nests than controls but did not develop an ovotestis, indicating that the behavioral response of nest building was more sensitive than anatomical changes.

Parental exposure to some pharmaceuticals can produce behavioral changes in the offspring. Adult zebrafish were exposed for 6 weeks to 10 μg·L^{-1} of carbamazepine (CBZ, an anticonvulsant and mood stabilizer) or gemfibrozil (GEM, which lowers lipid levels), and embryos were reared in clean water until sexual maturity and then assessed for reproductive output and behavior [43]. Male F_1 had greater reproductive effects than females. When a control F_1 male was crossed with a F_1 female whose parents were CBZ or GEM exposed, there were no differences in embryo production compared to controls. However, when a control F_1 female was crossed with either a CBZ or GEM F_1 male, 50% fewer

embryos were produced. Both CBZ and GEM F_1 males had reduced courtship behavior [43], but the specific deficits in courtship displays were drug specific.

Antidepressants are commonly detected in wastewater and in the aquatic environment. Since these are designed to modulate neurotransmitters (serotonin, dopamine and norepinephrine), aquatic animals that take up the chemicals into their nervous systems and have receptors sensitive to activation by these chemicals are likely to be affected [44]. The most widely prescribed antidepressants are selective serotonin uptake inhibitors (SSRIs) including Prozac (fluoxetine), zoloft (sertraline), and venlafaxine. Both mammals and teleost fish exhibit a close relationship between the brain neurotransmitter systems and the hypothalamic-pituitary-interrenal axis (the teleost equivalent of the hypothalamic-pituitary-adrenal axis). Fathead minnows (*Pimephales promelas*) exposed as embryos to fluoxetine or venlafaxine and maintained in clean water for 12 days posthatch, had impaired C-start escape, as analyzed by high-speed videos [45]. The C-start is a reflex that involves a extensive bending of the body into a C-shape, followed by an explosive burst of high-speed locomotion away from the threat stimulus. On the other hand, larvae exposed for 12 d posthatch did not exhibit altered escape responses when exposed to fluoxetine, but were affected by venlafaxine and bupropion exposure. Mixtures of these four antidepressant pharmaceuticals slowed predator avoidance behaviors in larvae regardless of the exposure window. The impacts of reduced C-start performance are likely to be increased mortality by predation, with obvious ecological relevance.

Fong and Ford [46] reviewed recent studies on the effects of antidepressants on mollusks and crustaceans and showed that molluscan reproductive and locomotory systems are affected by antidepressants at environmentally relevant concentrations. Antidepressants affect spawning and larval release in bivalves and disrupt locomotion and reduce fecundity in snails. In crustaceans, antidepressants affect amphipod activity patterns, photo- and geotactic behavior, crayfish aggression, and daphnid reproduction and development. They did not specifically cover delayed effects, but noted that many dose response curves showed effects at low concentrations but not at higher concentrations, and suggested that future studies test a broader range of concentrations.

Serotonin and dopamine regulate locomotion in mollusks. Newborn cuttlefish (*Sepia officianalis*) were perinatally exposed to fluoxetine (1 and 100 ng/L) and behaviors were subsequently assessed. Efficiency at striking at prey was reduced at 1 ng/L. Perinatal exposure at both concentrations led to significant changes in memory processing. To evaluate learning and memory, they used a "prawn-in-the-tube" procedure in which a shrimp (prey) is placed inside a glass tube to determine whether cuttlefish learned to inhibit their predatory behavior because of pain when their tentacles hit the glass tube. Exposed animals at both concentrations had impaired learning abilities and those exposed to the higher concentration had reduced memory retention [47]. Hatchlings exposed to 1 ng/L showed a decrease in camouflage and an increase of sand digging behavior, which might make them more visible to predators in nature. When tested again two weeks later, however, they seemed to have recovered and behavioral alterations were no longer observed—showing a transitory effect of the antidepressant [47].

2.7. Oil and Hydrocarbons

Petroleum hydrocarbons (including a great number of both linear alkanes and polycyclic aromatic hydrocarbons, PAHs) have been a long-standing problem in the marine environment due to oil spills and the resultant mortality of large numbers of birds and marine mammals. Following the Exxon Valdez oil spill in Prince William Sound, Alaska, 260,000–750,000 barrels of crude oil were released into the environment. The oil spread over more than one thousand miles of coastline, and pockets of oil remained sequestered in places where degradation was inhibited, such as subsurface sediments or under mussel beds. When exposed to the air, oil undergoes a process called "weathering", in which some volatile components evaporate and some components are degraded by sunlight or microbes. The chemical composition and physical characteristics of the oil change due to these processes. The sequestered oil does not weather, and weathered oil can still be toxic.

The spill occurred in March, coinciding with the breeding season for a number of species. Many studies were performed on effects of the oil on fish embryos, as many species were undergoing embryonic development at the time of the spill. Heintz *et al.* [48] incubated pink salmon *Oncorhynchus gorbuscha* embryos under three conditions: direct contact with oil-coated gravel, effluent from oil-coated gravel, and direct contact with gravel coated with very weathered (many months) oil. Mortalities and PAH accumulation in both direct-contact and effluent exposure experiments were not significantly different, indicating that PAH accumulation was mediated by aqueous transport. Delayed effects were noted [49]. Pink salmon that were exposed to 5.4 $\mu g \cdot L^{-1}$ PAHs as embryos had decreased marine survival compared to unexposed salmon. A delayed effect on growth was found in juveniles that survived embryonic exposure to doses as low as 18 $\mu g \cdot L^{-1}$ PAH. Reduced growth could account in part for the reduced marine survival of the released fish, since smaller fish are more vulnerable to predation than larger ones. Years later, the same team investigated CYP1A induction, survival and other responses that continued long after exposure to determine delayed impacts [50]. Survival was assessed at eyeing and emergence, and periodically for the next 5 mo. Emergent fry were examined for gross and cellular abnormalities, then grown for 5 mo in clean water to measure delayed impacts on growth. Growth was considered an integrator of intermediate and long-term delayed responses because failure to grow rapidly reduces marine survival. Results from a series of experiments demonstrated that CYP1A induction is related to a variety of lethal and sublethal effects including poorer marine survival, reduced growth and abnormalities. The lowest observed effective aqueous concentration of total PAH that caused physiological responses, including reduced size 6 mo after exposure ended (<0.94 $\mu g \cdot L^{-1}$), was less than the lowest concentration that caused significant CYP1A induction (<3.7 $\mu g \cdot L^{-1}$). Although this was not a study of behavioral effects per se, it is a comprehensive and multi-faceted study of long-term delayed effects that may be associated with behavior (reduced growth can be a result of reduced feeding), so is included here.

To examine toxicological effects of heavy oil (HO, oil with high viscosity and density) Kawaguchi *et al.* [51] performed embryonic exposure experiments and morphological and behavioral analyses in pufferfish (*Takifugu rubripes*) larvae. Fertilized eggs were exposed to 50 $mg \cdot L^{-1}$ of HO for 8 days then transferred to clean seawater before hatching. The hatched larvae were observed for their swimming behavior and morphology. Exposed larvae appeared normal but exhibited an abnormal

swimming pattern and structurally disorganized midbrain, which controls movement. These results suggest that the abnormal midbrain structure may alter normal swimming behavior in HO-exposed fish.

Effects of individual PAHs can also be studied. Coho salmon (*Oncorhynchus kisutch*) that were exposed to benzo[*a*] pyrene at either 24 h post-fertilization or 1 week prior to hatching had reduced ability to swim upstream after hatching [52].

3. Discussion of Possible Mechanisms

Effects of contaminant exposures on complex behaviors are due to underlying effects on the nervous and endocrine systems. Effects of pollutants on behavior are likely caused by interference with these systems, including neurological development, neurotransmitters, and sensory receptors. Chemoreception, especially olfaction, as well as vision and hearing are important senses that can be impaired by contaminants [53]. In addition, many behaviors are affected by the endocrine system, which is very sensitive to environmental contaminants. Many anatomical, physiological, and pathological effects (e.g., cancer) of exposure to endocrine disruptors at early life stages also do not manifest themselves until much later in life [54]. These possible mechanisms are expounded on below.

3.1. Neurotransmitters and Brain Development

Brain neurotransmitter levels and enzyme functions are related to behaviors, so it is likely that altered neurotransmitters induced by toxicants will result in altered behaviors. One of the most common indicators of altered neural function is altered acetylcholinesterase (AchE) in the brain. AchE breaks down the transmitter acetylcholine after it diffuses across the synapse in cholinergic neurons. Organophosphate pesticides inhibit fish AchE, as this is their "mode of action", as do many carbamate pesticides [55]. Embryonic exposure to organophosphate pesticides (diazinon, chlorpyrifos, and parathion) produced changes in mobility of larvae of zebrafish, associated with changes in AChE [56].

Alterations in dopamine and/or serotonin have been seen in contaminant-exposed fish, but levels are not consistently increased or decreased. Killifish (*F. heteroclitus*) after mercury exposure or collected from polluted sites, which had reduced activity and prey capture, also had reduced serotonin in their brains [57,58]. In contrast, Gulf killifish (*F. grandis*) showed decreased dopamine and norepinephrine after exposure to PCBs (Aroclor 242), along with greatly increased activity levels, but no change in serotonin [59]. Yu *et al.* [60] found that 10, 100 and 1000 ng·L^{-1} TBT reduced prey capture by *Sebastiscus marmoratus* and altered neurotransmitters. Dopamine levels in the fish brains increased in a dose-dependent manner and 5-hydroxytryptamine (serotonin) and norepinephrine levels decreased in TBT exposed fish compared to controls. Embryonic exposure of zebrafish to the pesticide chlorpyrifos, which decreases dopamine and serotonin in *D. rerio* larvae [61] caused a reduction in subsequent spatial discrimination and learning ability [62].

Contaminant-induced changes in brain development can cause behavioral alterations. Changes in sensory motor brain anatomy were associated with reduced activity and delayed escape responses in *D. rerio* after exposure to phenyl-cyclopropylamine (PCPA) [63], lead [64], and trimethyltin [65]. Changes in brain development can be linked to decreased learning ability. Embryonic exposure of *D. rerio* to meHg caused a reduction in cell body density in the telencephalon, which reduced learning ability at four months of age [16]. Using a quantitative stereological technique, Hill *et al.* [66] showed

that TCDD substantially reduces the capacity for embryonic brain development in zebrafish, causing a 30% reduction in total neuronal number in the brain of 168-h old larvae. Using transgenic GFP-expressing zebrafish lines, they linked this to decreased expression of key developmentally regulated genes, namely *neurogenin* and *sonic hedgehog*. This disruption of neuronal development was considered to provide the basis for understanding the neurotoxic and behavioral effects of these compounds. In these studies, effects were seen specifically in the anatomy of the developing nervous system; these would be expected to produce permanent behavioral deficits.

In contrast, a common general response of fish embryos to low concentrations of contaminants is a general retardation of development [67]. If larvae that were exposed as embryos are not as mature as controls, it is likely that their behaviors such as feeding and predator avoidance would be poorer than controls. But one might also predict that they could catch up with controls and that the behavioral deficits would not be permanent. Such responses were seen with methylmercury in killifish [9,14].

3.2. Sense Organs

The olfactory system of fishes is open to the environment and particularly sensitive to metals including Hg [68], although inorganic Hg and meHg localize in different parts of the olfactory system. Many metals directly enter the olfactory system where they can disrupt normal function [69]. By accumulating in and damaging cells of the olfactory system, toxicants can disrupt transmission of information from olfactory lobes to higher levels of the brain. Olfactory receptor neurons can be a transport route of contaminants to the olfactory bulbs and the brain, with resulting effects on the functioning of the nervous system. Cadmium appears to move along olfactory neurons by axonal transport [40]. Some studies have shown a connection between altered behavior and altered olfactory system. Rehnberg and Schreck [70] showed reduced avoidance of the amino acid L-serine (a potent odor to fish) by coho salmon (*Oncorhynchus kisutch*) exposed to copper and mercury. Hg but not Cu inhibited serine binding to the olfactory epithelium. Cu produced morphological lesions in olfactory, taste, and lateral line receptor systems of juvenile coho salmon (*Oncorhynchus kisutch*), and 30 min exposure to 20 $\mu g \cdot L^{-1}$ Cu reduced the olfactory response to a natural odorant (10^{-5} M L-serine) by 82% [71]. Embryonic copper exposure caused olfactory disruption in fathead minnows (*P. promelas*) which subsequently impaired discrimination between control and alarm substance in a maze [72]. Exposure to Cd caused cell death in the olfactory epithelium and changes in sensory cells, which were linked to altered aversion behavior in zebrafish larvae [73]. It is possible that when fish are maintained in clean water that olfactory neurons will regenerate [74] and normal behaviors would eventually be restored, but this was not investigated in these studies.

The olfactory system is very important for social communication in some fishes—for detection of predators and prey, and social recognition. Ward *et al.* [75] found that environmentally relevant concentrations of 4-nonylphenol, can impair social recognition. A 1 h exposure to 0.5 $\mu g \cdot L^{-1}$ altered the response of juvenile banded killifish, *Fundulus diaphanus*, to conspecific chemical cues, while 1–2 $\mu g \cdot L^{-1}$ caused fish to orient away from exposed conspecifics.

The lateral line, a series of sense organs found in fishes used to detect movement and vibration in the surrounding water, is another sense organ that can be altered by toxicants to produce behavioral changes. For example, Johnson *et al.* [76] found that copper exposure reduced the number of

functional neuromasts in *D. rerio* larvae, which was associated with decreased ability to maintain equilibrium. The developing visual system can also be damaged by toxicants including PAHs, tributyltin (TBT), and estrogens, with subsequent behavioral effects. Both EE_2 and TBT increased the size of the pupil in the pipefish *Syngnathus abaster*, and increased their activity (number of movement bursts) [77]. However, exposure to the two chemicals in combination reduced the pupil size and caused larvae to become more lethargic. Developmental exposure to TCDD reduced the number of retinal ganglion cells in rainbow trout (*Oncorhynchus mykiss*), which was associated with decreased ability to capture prey [78].

3.3. Endocrine System

Many studies have shown linkages between hormones and behavior. Many pollutants can act as agonists or antagonists to hormones, and the hormones themselves are also present in the environment as pharmaceuticals. McGee *et al.* [79] found that embryonic exposure to estrogens altered the C-start response in larvae of *P. promelas*, but estrogens did not have this effect when larvae themselves were exposed for 12 days. Early life exposures to endocrine disruptors may affect much later stages in terms of reproductive ability. Male *P. promelas* larvae that were exposed to a nonylphenoloxylate-octylphenolethoxylate (estrogen mimic) mixture had reduced ability to compete for spawning sites when they became adults [80].

Thyroid hormones influence many processes in fish, including neural development, metabolism, maturation (smoltification in salmonids and metamorphosis in flatfish), and behavior. Many chemicals, including chlorinated hydrocarbons, PAHs, chlorinated paraffins, organophosphate pesticides, carbamate pesticides, cyanide compounds, methyl bromide, phenols, ammonia, sex steroids, and pharmaceuticals, affect the fish thyroid, which has implications for behavior. About 40 fish species have been shown to have thyroid alterations in response to contaminants (reviewed by Brown *et al.* [81].Turbot larvae (*Scophthalmus maximus*) larvae after exposure to the water soluble fraction of crude oil had increased levels of thyroxine, elevated cortisol, and reduced swimming activity [82]. Perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) affected endocrine signaling in Atlantic salmon (*Salmo salar*) embryos and larvae after exposure to 100 $\mu g \cdot L^{-1}$ from egg for 52 days, followed by 1 week recovery [83]. Exposure altered expression of thyroid receptor α and β, thyroid-stimulating hormone, and T_4 outer-ring deiodinase. Killifish, *Fundulus heteroclitus*, from Piles Creek, New Jersey (contaminated with metals, PCBs and more) are sluggish with poor prey capture and predator avoidance. They have extremely enlarged thyroid glands, with elevated follicular cell heights, and contain elevated plasma thyroxine (T4), but not plasma or tissue T3 [84]. Reference site fish held in conditions simulating Piles Creek also developed elevated T4 and impaired predatory behavior.

3.4. Mechanisms of Transgenerational Effects

The majority of toxicants including endocrine disruptors do not promote genetic mutations or alterations in DNA sequence. However, a variety of environmental contaminants including metals, halogenated organics, and solvents have been linked to epigenetic effects, some of which may be transgenerational [85,86]. Epigenetics is the science of how the environment can produce heritable

changes in an individual during its development as well as the development of its descendants, without changing the DNA sequence. This is a revolutionary concept that has only recently come to the fore and is somewhat controversial. Most of the work thus far has been in mammals. Epigenetic changes can link the environment to altered gene expression that could lead to altered phenotypes including behavior. An important aspect of epigenetics for ecotoxicology is that exposures at one time or in one generation can result in effects at a much later time and in subsequent generations. Exposure to chemicals early in development can result in abnormalities later in life, and chemically induced epigenetic effects in one generation may be passed down to future generations in the absence of the stressor. A chemical can cause long-lasting effects that alter cell function, even when the chemical is no longer present [87]. Many reported effects of such studies are on physiology, fertility, and other reproductive functions [88–90]. However, some are behavioral effects. For example, female mice were fed a diet with or without the endocrine disruptor bisphenol-a (BPA) before mating and throughout gestation, which caused offspring (exposed *in utero*) to have altered social behavior. In addition, the F2 and F4 generations, which had not been exposed at all, also exhibited altered social behavior [91].

Elevated levels of a chemical detected in a population today may cause epigenetic effects that are not manifested for years. Furthermore, benefits resulting from contaminant removal may not become apparent for generations [92]. Since epigenetic changes in gene expression can be passed to subsequent generations in the absence of the stressor chemical, then those changes may persist when the stressor has been removed from the environment.

Mechanisms of epigenetic inheritance appear to be quite well conserved across vertebrates. One proposed mechanism for explaining transgenerational neuroendocrine effects is alterations in DNA methylation. There is an extensive literature in mammals relating endocrine disruptors and DNA methylation [93,94]. There is also a body of literature (also in mammals) linking DNA methylation to brain function and behavior. For example, evidence indicates that DNA methylation may serve as a contributing mechanism in memory formation and storage and in synaptic function in forebrain neurons [95,96]. DNA methylation has also been linked to behaviors. Elliott *et al.* [97] found that methylation regulated the expression of a particular gene (*Crf*) and that chronic social stress induced long-term demethylation of this gene. Demethylation was observed in defeated mice that displayed social avoidance, and knockdown of *Crf* attenuated the stress-induced social avoidance behavior. It is likely that future studies on aquatic species will be able to link environmental contaminants to DNA methylation to transgenic effects on neural development and behavior.

4. Discussion, Conclusions, and Recommendations for Future Research

This review has reported numerous instances of delayed behavioral effects when embryonic fishes or crustaceans are exposed to a variety of different contaminants, or when adults are exposed and their offspring demonstrate altered behaviors. It was surprising and disappointing to note how few studies have been done on invertebrates, many of which are commonly used in many kinds of laboratory studies and some of which are frequently used in toxicity testing, including embryo-larval tests (e.g., bivalve mollusks, sea urchins, mysids). Extending studies of delayed behavioral effects to the wide variety of invertebrate taxa will no doubt lead to a plethora of interesting and important discoveries.

In most embryonic exposure experiments, scientists exposed embryos throughout their development. It was noteworthy that there were very few papers that attempted to pinpoint sensitive periods in development by exposing embryos for shorter periods at different times in development, although in traditional teratology, such approaches are fairly common. In one cited study [15] of meHg and zebrafish, embryos were exposed throughout the three days of development but also during single-day periods. While effects were less severe than in those exposed for the full three days, there were no notable differences among the groups that were exposed on day one, day two, or day three, so no sensitive period could be determined. In the study of dioxin and zebrafish embryos [32] embryos were exposed only for one hour at 4 h post-fertilization, which resulted in somewhat abnormal jaw structure and reduced prey capture. Embryos at four to five hours post-fertilization are in the early part of gastrulation, at the very beginning of cell reorganization and before differentiation [98]. This stage is before any rudiments of the nervous system (or skeletal system) have begun to form, so it is difficult to understand how feeding behavior could be affected after a short exposure so early in development. It seems more probable that dioxin, which is highly lipophilic, accumulates in the yolk at that time, and produces its toxic effects at later stages when the nervous system is actively developing. This would be an interesting avenue for future research to pursue.

It was also striking and disappointing how few investigators attempted to ascertain whether the alterations they observed in behavior were temporary or permanent, which is very important. The vast majority of studies did the behavioral testing at a single point in time and did not repeat them to see if affected animals recovered or improved over time. Some effects of the antidepressant, fluoxetine on juvenile cuttlefish were found to be transient [47]. In two studies on fish ([9] and [14]) effects of meHg on prey capture by killifish larvae were found to be transient. However, in another meHg study on grayling, Fjeld *et al.* [19] saw deleterious effects on prey capture when fish were three years old. It is safe to conclude, as the authors did, that those were permanent effects. Lead exposure of killifish larvae for four weeks produced decreases in activity, prey capture ability, and predator avoidance, but the effects were temporary—four weeks after larvae were returned to clean water all these behaviors were comparable to controls [99].

Whether effects (delayed or otherwise) are temporary or permanent is particularly important in terms of overall impacts on fitness and survival in the wild. Larval mortality in fishes and invertebrates is normally very high, but further increases in mortality rates can result in major decreases in populations [100]. Decreased feeding will result in smaller larvae, which are more vulnerable to starvation and predation. Therefore, if important behaviors (prey capture, predator avoidance, *etc.*) of exposed animals return to normal relatively quickly, they will have a much greater chance of survival in nature. Whether effects are temporary or permanent can differ depending on the species of animal. Adult killifish (*F. heteroclitus*) with lifetime exposure to Hg and other chemicals in a contaminated estuary in northern New Jersey had impaired prey capture. Their behavior did not improve after six weeks in clean water, during which they did not depurate mercury from their brains [101]. In contrast, adult blue crabs (*Callinectes sapidus*) from a similar environment with similar impaired prey capture did increase their prey capture ability after six weeks in clean water and did depurate mercury [102].

Progress has been made recently in elucidating the neuro- and endocrine cellular and biochemical mechanisms underlying behavioral changes, but much more work needs to be performed for more

thorough understanding. Understanding the mechanism(s) underlying a particular behavioral effect has major importance for whether the effect is transitory or permanent, as discussed above. Teratological or pathological alterations in brain structure are likely to be permanent, but differences in maturity of the nervous system or levels of neurotransmitters or hormones could be modified over time. However, while hormone levels can be modified over time, most effects of early life exposure to endocrine disrupting chemicals appear to be long-lasting. The new field of transgenerational effects promises to revolutionize many aspects of biology, including ecotoxicology. At present, most of the research has been on mammals, so the field is wide open for exploration in aquatic organisms. Of particular interest and concern in this regard is whether or not such deleterious effects would be reversible in the future generations that were not exposed to the chemical that initially triggered the response.

In addition to future work elucidating mechanisms, it is also important to increase the number of studies that connect behavioral effects to changes in ecology of the affected organisms. Foraging and predator avoidance are critical to larval survival, and survival to reproductive age is closely related to efficient feeding during larval stages. Appropriate social behavior (e.g., schooling) and reproductive behavior are also important for population success. Therefore there is a clear connection, in theory, between behaviors of early life stages and the population, but the connections have not been elucidated in many cases. Since behaviors are closely associated with fitness in the environment, more work is needed in unraveling and demonstrating the ecological consequences of altered behaviors. Since many of the studies used concentrations of toxicants greater than those seen in the environment, priority for future research (and regulation) should be given to chemicals that produce effects at concentrations close to those found in the environment.

Acknowledgments

Conflicts of Interest

The author declares no conflict of interest.

References

1. Kimmel, C.A. Behavioral teratology: Overview. *Environ. Health Persp*. **1976**, *18*, 73.
2. Weiss, B.; Levine, T.E. Studies on the behavioral toxicology of environmental contaminants. *Environ. Health Persp*. **1976**, *13*, 31–35
3. Amiard-Triquet, C. Behavioural disturbances: The missing link between sub-organismal and supra-organismal responses to stress? Prospects based on aquatic research. *Hum. Ecol. Risk Assess.* **2009**, *15*, 87–110.
4. Weis, J.S.; Smith, G.; Zhou, T.; Santiago-Bass, C.; Weis, P. Effects of contaminants on behavior: Biochemical mechanisms and ecological consequences. *BioScience* **2001**, *51*, 209–217.
5. Li, S.; Mathias, J. The critical period of high mortality of larval fish—A discussion based on current research. *Chin. J. Oceanog. Limnol.* **1987**, *5*, 80–96.

6. Matta, M.; Linse, J.; Cairncross, C.; Francendese, L.; Kocan, R.M. Reproductive and transgenerational effects of methylmercury or Aroclor 1268 on *Fundulus. heteroclitus*. *Environ. Toxicol. Chem.* **2001**, *20*, 327–335.

7. Choi, B.H. Effects of Prenatal Methylmercury Poisoning upon Growth and Development of Fetal Nervous System. In *Reproductive and Developmental Toxicity of Metals*; Clarkson, T.W., Nordberg, G.F., Sager, P.R., Eds.; Plenum Press: New York, NY, USA, 1983; pp. 473–495.

8. Clarkson, T.W. Mercury: Major issues in environmental health. *Environ. Health Persp.* **1992**, *100*, 31–38.

9. Weis, J.S.; Weis, P. Effects of embryonic exposure to methylmercury on larval prey capture ability in the mummichog, *Fundulus. heteroclitus*. *Environ. Toxicol. Chem.* **1995**, *14*, 153–156.

10. Weis, J.S.; Weis, P. Effects of embryonic and larval exposure to methylmercury on larval swimming performance and predator avoidance in the mummichog, *Fundulus. heteroclitus*. *Can. J. Fish. Aquat. Sci.* **1995**, *52*, 2168–2173.

11. Zhou, T.; Weis, J.S. Swimming behavior and predator avoidance in three populations of *Fundulus. heteroclitus* larvae after embryonic and/or larval exposure to methylmercury. *Aquat. Toxicol.* **1998**, *43*, 131–148.

12. Zhou, T.; Scali, R.; Weis, J.S. Effects of methylmercury on ontogeny of prey capture ability and growth in three populations of larval *Fundulus. heteroclitus*. *Arch. Environ. Contam. Toxicol.* **2001**, *41*, 47–54.

13. Zhou, T.; Scali, R.; Weis, P.; Weis, J.S. Behavioral effects in mummichog larvae (*Fundulus. heteroclitus*) following embryonic exposure to methylmercury. *Mar. Environ. Res.* **1996**, *42*, 45–49.

14. Ososkov, I.; Weis, J.S. Development of social behavior in larval mummichogs after embryonic exposure to methylmercury. *Trans. Am. Fish. Soc.* **1996**, 125, 983–987.

15. Samson, J.C.; Goodridge, R.; Olubatuyi, F.; Weis, J.S. Delayed effects of embryonic exposure of zebrafish (*Danio. rerio*) to methylmercury (MeHg). *Aquat.Toxicol.* **2001**, *51*, 369–376.

16. Smith, L.E.; Carvan, M.J.; Dellinger, J.A.; Ghorai, J.K.; White, D.B.; Williams, F.E.; Weber, D.N. Developmental selenomethionine and methylmercury exposures affect zebrafish learning. *Neurotox. Teratol.* **2010**, *32*, 246–255.

17. Xu, X.; Lamb, C.; Smith, M.; Schaefer, L.; Carvan, M.J., III.; Weber, D.N. Developmental methylmercury exposure affects avoidance learning outcomes in adult zebrafish. *J. Toxicol. Environ. Health Sci.* **2012**, *4*, 85–91.

18. Alvarez, M.C.; Murphy, C.A.; Rose, K.A.; McCarthy, I.D.; Fuiman, L.A. Maternal body burden of methylmercury impairs survival skills of offspring in Atlantic croaker (*Micropogonias. undulatus*). *Aquat. Toxicol.* **2006**, *80*, 329–337.

19. Fjeld, E.; Haugen, T.O.; Vollestad, L.A. Permanent impairment in the feeding behavior of grayling (*Thymallus. thymallus*) exposed to methylmercury during embryogenesis. *Sci. Total Environ.* **1998**, *213*, 247–254.

20. Rice, C.; Ghorai, J.K.; Zalewski, K.; Weber, D.N. Developmental lead exposure causes startle response deficits in zebrafish. *Aquat. Toxicol.* **2011**, *105*, 600–608.

21. Bricker, O.P.; Rice, K.C. Acid Rain. *Ann. Rev. Earth Planet. Sci.* **1993**, *21*, 151–174.

22. Staurnes, M.; Kroglund, F.; Rosseland, B.O. Water quality requirement of Atlantic salmon (*Salmo. salar*) in water undergoing acidification or liming in Norway. *Water Air Soil Pollut.* **1996**, *85*, 347–352.

23. Staurnes, M.; Hansen, L.P.; Fugelli, K.; Haraldstad, O. Short-term exposure to acid water impairs osmoregulation, seawater tolerance, and subsequent marine survival of smolt of Atlantic salmon (*Salmo. salar* L.). *Can. J. Fish. Aquat. Sci.* **1996**, *53*, 1695–1704.

24. Nirmala, K.; Oshima, Y.; Lee, R.; Imada, N.; Honjo, T.; Kobayashi, K. Transgenerational toxicity of tributyltin and its combined effects with polychlorinated biphenyls on reproductive processes in Japanese medaka (*Oryzias. latipes*). *Environ. Toxicol. Chem.* **1999**, *18*, 717–721.

25. Powers, C.M.; Yen, J.; Linney, E.A.; Seidler, F.; Slotkin, T. Silver exposure in developing zebrafish (*Danio. rerio*): Persistent effects on larval behavior and survival. *Neurotoxicol. Teratol.* **2010**, *32*, 391–397.

26. Steele, C.W. Latent behavioural toxicity of copper to sea catfish, *Arius felis* and sheepshead, *Archosargus. probatocephalus*. *J. Fish. Biol.* **1985**, 27, 643–654.

27. Faulk, C.K.; Fuiman, L.; Thomas, P. Parental exposure to *ortho, para*-dichlorodiphenyltrichloroethane impairs survival skills of Atlantic croaker (*Micropogonias. undulatus*) larvae. *Envir. Toxicol. Chem.* **1999**, *18*, 254–262

28. Wilson, J.E.; Forward, R.B.; Costlow, J.D. Effects of Embryonic Exposure to Sublethal Concentrations of Dimilin® on the Photobehavior of Grass Shrimp Larvae. In *Marine Pollution and Physiology: Recent Advances*; Vernberg, F.J., Thurberg, F.P., Calabrese, A., Vernberg, W.B., Eds.; University of South Carolina Press: Columbia, SC, USA, 1985; pp. 377–396.

29. Wilson, J.E.; Forward, R.B.; Costlow, J.D. Delayed Effects of Diflubenzuron on the Swimming and Vertical Distribution of *Palaemonetes. pugio* Larvae. In *Pollution Physiology of Estuarine Organisms*; Vernberg, W.B., Calabrese, A., Thurberg, F.P., Vernberg, F.J., Eds.; University of South Carolina Press: Columbia, SC, USA, 1987; pp. 351–371.

30. Calabrese, E.; Baldwin, L.A. Defining hormesis. *Hum. Exp. Toxicol.* **2002**, *21*, 91–97.

31. McCarthy, I.; Fuiman, L.A.; Alvarez, M.C. Aroclor 1254 affects growth and survival skills of Atlantic croaker *Micropogonias. undulatus* larvae. *Mar. Ecol. Prog. Ser.* **2003**, *252*, 295–301.

32. Chollett, D.; Perez, K.E.; King-Heiden, T.C. Embryonic exposure to 2,3,7,8, TCDD impairs prey capture by zebrafish larvae. *Environ. Toxicol. Chem.* **2014**, *33*, 784–790.

33. Couillard, C.M.; Légaré, B.; Bernier, A.; Dionne, Z. Embryonic exposure to environmentally relevant concentrations of PCB126 affect prey capture ability of *Fundulus. heteroclitus* larvae. *Mar. Environ. Res.* **2011**, *71*, 257–265.

34. Rigaud, C.; Couillard, C.M.; Pellerin, J.; Légaré, B.; Gonzalez, P.; Hodson, P.V. Relative potency of PCB 126 to TCDD for sublethal embryotoxicity in the mummichog (*Fundulus. heteroclitus*). *Aquat.Toxicol.* **2013**, *129*, 203–214.

35. Marit, J.S.; Weber, L.P. Persistent effects on adult swim performance and energetics in zebrafish developmentally exposed to 2,3,7,8 tetrachlorodibenzo-p-dioxin. *Aquat. Toxicol.* **2012**, *106–107*, 131–139.

36. Zhou, T.; Ross, D.G.; de Vito, M.J.; Crofton, K. Effect of short-term *in vivo* exposure to polybrominated diphenylethers on thyroid hormones and hepatic enzyme activities in weanling rats. *Toxicol. Sci.* **2001**, *61*, 76–82.

37. Timme-Laragy, A.R.; Levin, E.D.; Di Giulio, R.T. Developmental and behavioral effects of embryonic exposure to the polybrominated diphenyl mixture DE-71 in the killifish (*Fundulus. heteroclitus*). *Chemosphere* **2006**, *62*, 1097–1104.

38. Garcia-Reyero, N. Effects of BDE-209 contaminated sediments on zebrafish development and potential implications for human health. *Environ. Int*. **2014**, *63*, 216–223.

39. He, J.; Yang, D.; Wang, C.; Liu, W.; Liao, J.; Xu, T.; Bai, C.; Chen, J.; Lin, K.; Huang, C.; Dong, Q. Chronic zebrafish low dose decabrominateddiphenyl ether (BDE-209) exposure affected parental gonad development and locomotion in F1 offspring. *Ecotoxicology* **2011**, *20*, 1813–1822.

40. Corcoran, J.; Winter, M.J.; Tyler, C.R. Pharmaceuticals in the aquatic environment: A critical review of the evidence for health effects in fish. *Crit. Revs. Toxicol*. **2010**, *40*, 287–304.

41. Soffker, M.; Tyler, C.R. Endocrine disrupting chemicals and sexual behaviours in fish—A critical review on effects and possible consequences. *Crit. Revs. Toxicol*. **2012**, *42*, 653–668.

42. Maunder, R.J.; Matthiessen, P.; Sumpter, J.P.; Pottinger, T.G. Impaired reproduction in three-spined sticklebacks exposed to ethinyletradiol as juveniles. *Biol. Repro*. **2007**, *77*, 999–1006

43. Galus, M.; Rangarajan, S.; Lai, A.; Shaya, L.; Balshine, S.; Wilson, J.Y. Effects of chronic, parental pharmaceutical exposure on zebrafish (*Danio. rerio*) offspring. *Aquat. Toxicol*. **2014**, *151*, 124–134.

44. Schultz, M.M.; Furlong, E.T.; Kolpin, D.W.; Werner, S.L.; Schoenfuss, H.L.; Barber, L.B.; Blazer, V.S.; Norris, D.O.; Vajda, A.M. Antidepressant pharmaceuticals in two U.S. effluent-impacted streams: Occurrence and fate in water and sediment, and selective uptake in fish neural tissue. *Environ. Sci. Technol*. **2010**, *44*, 1918–1925.

45. Painter, M.M.; Buerkley, M.A.; Julius, M.L.; Vajda, A.M.; Norris, D.O.; Barber, L.B.; Furlong, E.T.; Schultz, M.M.; Schoenfuss, H.L. Antidepressants at environmentally relevant concentrations affect predator avoidance behavior of larval fathead minnows (*Pimephales. promelas*). *Environ. Toxicol. Chem.* **2010**, *28*, 2677–2684

46. Fong, P.P.; Ford, A.T. The biological effects of antidepressants on the Molluscs and Crustaceans: A review. *Aquat. Toxicol*. **2014**, *151*, 4–13.

47. Di Poi, C.; Darmaillacq, A.-S.; Dickel, L.; Boulouard, M.; Bellanger, C. Effects of perinatal exposure to waterborne fluoxetine on memory processing in the cuttlefish *Sepia officianalis*. *Aquat. Toxicol*. **2013**, *132–133*, 84–91.

48. Heintz, R.; Short, J.W.; Rice, S.D. 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. *Environ. Toxicol. Chem*. **1999**, *18*, 494–503

49. Heintz, R.A.; Rice, S.D.; Wertheimer, A.C.; Bradshaw, R.F.; Thrower, F.P.; Joyce, J.E.; Short, J.W. Delayed effects on growth and marine survival of pink salmon *Oncorhynchus. gorbuscha* after exposure to crude oil during embryonic development. *Mar. Ecol. Prog. Ser.* **2000**, *208*, 205–216.

50. Carls, M.G.; Heintz, R.A.; Marty, G.D.; Rice, S.D. Cytochrome P4501A induction in oil exposed pink salmon *Oncorhynchus gorbuscha* embryos predicts reduced survival potential. *Mar. Ecol. Prog. Ser.* **2005**, *301*, 253–265.

51. Kawaguchi, M.; Sugahara, Y.; Watanabe, T.; Irie, K.; Ishida, M.; Kurokawa, D.; Kitamura, S.; Takata, H.; Handoh, I.C.; Nakayama, K.; *et al.* Nervous system disruption and concomitant

behavioral abnormality in early hatched pufferfish larvae exposed to heavy oil. *Environ. Sci. Pollut. Res*. **2012**, 19, 2488–2497.

52. Ostrander, G.K.; Landolt, M.; Kocan, R. The ontogeny of coho salmon (*Oncorhynchus. kisutch*) behavior following embryonic exposure to benzo[a]pyrene. *Aquat. Toxicol*. **1988**, *13*, 325–346.

53. Lemby, A.; Smith, R.J. A behavioral assay for assessing effects of pollutants on fish chemoreception. *Ecotoxicol. Environ. Saf.* **1986**, *11*, 210–218.

54. Colborn, T.; Vom Saal, F.; Soto, A. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ. Health Persp*. **1993**, *101*, 378–384

55. Scott, G.R.; Sloman, K.A. The effects of environmental pollutants on complex fish behaviour: Integrating behavioural and physiological indicators of toxicity. *Aquat. Toxicol*. **2004**, *68*, 369–392.

56. Yen, J.; Donerly, S.; Levin, E.D.; Linney, E.A. Differential acetylcholinesterase inhibition of chlorpyrifos, diazinon and parathion in larval zebrafish. *Neurotox. Teratol*. **2011**, *33*, 735–741.

57. Smith, G.M.; Khan, A.T.; Weis, J.S.; Weis, P. Behavior and brain correlates in mummichogs (*Fundulus. heteroclitus*) from polluted and unpolluted environments. *Mar. Environ. Res*. **1995**, *39*, 329–333.

58. Zhou, T.; Rademacher, D.; Steinpreis, R.E.; Weis, J.S. Neurotransmitter levels in two populations of larval *Fundulus. heteroclitus* after methylmercury exposure. *Comp. Biochem. Physiol. Part C* **1999**, *124*, 287–294.

59. Fingerman, S.W.; Russell, L.C. Effects of the polychlorinated biphenyl Aroclor 1242 on locomotor activity and the neurotransmitters dopamine and norepinephrine in the brain of Gulf killifish, *Fundulus. grandis*. *Bull. Environ. Contam. Toxicol*. **1980**, *25*, 682–687.

60. Yu, A.; Wang, X.; Zuo, Z.; Cai, J.; Wang, C. Tributyltin exposure influences predatory behavior, neurotransmitter content and receptor expression in *Sebastiscus. marmoratus. Aquat. Toxicol*. **2013**, *128–129*,158–162.

61. Cerutti, D.; Williams, P.; Limney, E.; Levin, E.D. Zebrafish provide a sensitive model of persisting neurobehavioral effects of developmental chlorpyrifos exposure: Comparison with nicotine and pilocarpine effects and relationship to dopamine deficits. *Neurotox. Teratol*. **2010**, *32*, 99–108.

62. Levin, E.D.; Chrysanthis, E.; Yacisin, E.; Linney, E. Chlorpyrifos exposure of developing zebrafish: effects on survival and long-term effects on response latency and spatial discrimination. *Neurotox. Teratol*. **2003**, *25*, 51–57.

63. Jie, Z.; Li, T.; Jia-Yun, H.; Qiu, J.; Ping-Yao, Z.; Houyan, S. Trans-2-phenyl-cyclopropylamine induces nerve cells apoptosis in zebrafish mediated by depression of LSD1 activity. *Brain Res. Bull*. **2009**, *80*, 79–84.

64. Dou, C.; Zhang, J. Effects of lead on neurogenesis during zebrafish embryonic brain development. *J. Hazard. Mater*. **2011**, *194*, 277–282.

65. Chen, J.; Huang, C.; Zheng, L.; Simonich, M.; Bai, C.; Tanguay, R.; Dong, Q. Trimethyltin chloride (TMT) neurobehavioral toxicity in embryonic zebrafish. *Neurotox. Teratol*. **2011**, *33*, 721–726.

66. Hill, A.; Howard, C.V.; Strahle, U.; Cossins, A. Neurodevelopmental defects in zebrafish (*Danio. rerio*) at environmentally relevant dioxin (TCDD) concentrations. *Toxicol. Sci*. **2003**, *76*, 392–399.

67. Weis, J.S.; Weis, P. Effects of environmental pollutants on early fish development. *Revs. Aquat. Sci.* **1989**, *1*, 45–74.

68. Baatrup, E.; Doving, K.B.; Winberg, S. Differential effects of mercurial compounds on the electro-olfactogram (EOG) of salmon (*Salmo. salar* L.). *Ecotox. Environ. Saf.* **1990**, *20*, 269–276.

69. Olsen, K.H. Effects of Pollutants on Olfactory Related Behaviors in Fish and Crustaceans. In *Chemical Communications in Crustaceans*; Breithaupt, T., Thiel, M., Eds.; Springer: New York, NY, USA, 2011; pp. 507–529.

70. Rehnberg, B.C.; Schreck, C.B. Acute metal toxicology of olfaction in coho salmon: Behavior, receptors, and odor-metal complexation. *Bull. Environ. Contam. Toxicol.* **1986**, *36*, 579–586.

71. McIntyre, J.; Baldwin, D.H.; Beauchamp, D.A.; Scholz, N.L. Low-level copper exposures increase visibility and vulnerability of juvenile coho salmon to cutthroat trout predators. *Ecol. Appl.* **2012**, *22*, 1460–1471.

72. Carreau, N.D.; Pyle, G.G. Effect of copper exposure during embryonic development on chemosensory function of juvenile fathead minnows (*Pimephales. promelas*). *Ecotoxicol. Environ. Saf.* **2005**, *61*, 1–6.

73. Matz, C.J.; Krone, P.H. Cell death, stress-responsive transgene activation, and deficits in the olfactory system of larval zebrafish following cadmium exposure. *Environ. Sci. Tech.* **2007**, *41*, 5143–5148.

74. Cancalon, P. Degeneration and regeneration of olfactory cells induced by $ZnSO_4$ and other chemicals. *Tissue Cell* **1982**, *14*, 717–733.

75. Ward, A.J.; Duff, A.J.; Horsfall, J.S.; Currie, S. Scents and scents-ability: Pollution disrupts social recognition and shoaling in fish. *Proc. Biol. Soc.* **2008**, *275*, 101–105

76. Johnson, A.; Carew, E.; Sloman, K.A. The effects of copper on the morphological and functional development of zebrafish embryos. *Aquat. Toxicol.* **2007**, *84*, 431–438.

77. Sarria, M.; Santos, M.; Reis-Henriques, M.A.; Viera, N.; Monteiro, N.M. The unpredictable effects of mixtures of androgenic and estrogenic chemicals on fish early life. *Environ. Int.* **2011**, *37*, 416–424.

78. Carvalho, P.S.; Tillitt, D.E. 2,3,7,8-TCDD effects on visual structure and function in swim-up rainbow trout. *Environ. Sci. Tech.* **2004**, *38*, 6300–6306.

79. McGee, M.R.; Julius, M.L.; Vajda, A.M.; Norris, D.O.; Barber, L.B.; Schenfuss, H.L. Predator avoidance performance of larval fathead minnow (*Pimephales. promelas*) following short-term exposure to estrogen mixtures. *Aquat. Toxicol.* **2009**, *91*, 355–361.

80. Bistodeau, T.J.; Barber, L.B.; Bartell, S.E.; Cediel, R.A.; Grove, K.J.; Klaustermeier, J.; Woodard, J.C.; Lee, K.E.; Schoenfuss, H.L. Larval exposure to environmentally relevant mixtures of alkylphenolethoxylates reduces reproductive competence in male fathead minnows. *Aquat. Toxicol.* **2006**, *79*, 268–277.

81. Brown, S.B.; Adams, A.; Cyr, D.G.; Eales, G. Contaminant effects on the teleost fish thyroid. *Environ. Toxicol. Chem.* **2004**, *23*, 1680–1701.

82. Stephens, S.M.; Alkindi, A.Y.; Waring, C.; Brown, J.A. Corticolsteroid and thyroid responses of larval and juvenile turbot exposed to the water-soluble fraction of crude oil. *J. Fish. Biol.* **1997**, *50*, 953–964.

83. Spachmo, B.; Arukwe, A. Endocrine and developmental effects in Atlantic salmon (*Salmo. salar*) exposed to perfluorooctane sulfonic or perfluorooctane carboxylic acids. *Aquat.Toxicol*. **2012**, *108*, 112–124.

84. Zhou, T.; John-Alder, H.B.; Weis, P.; Weis, J.S. Thyroidal status of mummichogs (*Fundulus. heteroclitus*) from a polluted *versus* a reference habitat. *Environ. Toxicol. Chem.* **1999**, *18*, 2817–2823.

85. Grossniklaus, U.; Kelly, W.G.; Ferguson-Smith, A.C.; Pembrey, M.; Lindquist, S. Transgenerational epigenetic inheritance: How important is it? *Nat. Rev. Genet.* **2013**, *14*, 228–235.

86. Morgan, D.; Whitelaw, E. The case for transgenerational epigenetic inheritance in humans. *Mamm. Genome* **2008**, *19*, 394–397.

87. Crews, D.; Gore, A. Epigenetic synthesis: A need for a new paradigm for evolution in a contaminated world. *F 1000 Biol. Rep*. **2012**, *4*, doi: 10.3410/B4–18.

88. Pöikki, M.; Kangassalo, K.; Rantala, M. Transgenerational effects of heavy metal pollution on immune defense of the blowfly, *Protophormia. terraenovaei. PLoS One* **2012** 7, e38832.

89. Crews, D.; Gore, A.C.; Hsu, T.S.; Dangleben, N.L.; Spinetta, M.; Schallert, T.; Anway, M.D.; Skinner, M.K. Transgenerational epigenetic imprints on mate preference. *Proc. Nat. Acad. Sci. USA* **2007**, *104*, 5942–5946.

90. Anway, M.D.; Cupp, A.S.; Uzumcu, M.; Skinner, M.K. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* **2005**, *308*, 1466–1469.

91. Wolstenholme, J.T. Edwards, M.; Shetty, S.R.; Gatewood, J.D.; Taylor, J.A.; Rissman, E.F.; Connelly, J.J. Gestational exposure to bisphenol a produces transgenerational changes in behaviors and gene expression. *Endocrinology* **2012**, *153*, 3828–3838.

92. Head, J.A.; Dolinoy, D.; Basu, N. Epigenetics for ecotoxicologists. *Environ. Toxicol. Chem*. **2012**, *31*, 221–227.

93. Gore, A. Developmental programming and endocrine disruptor effects on reproductive neuroendocrine systems. *Front. Neuroendocrin.* **2008**, *29*, 358–374

94. Walker, D.; Gore, A. Transgenerational neuroendocrine disruption of reproduction. *Nat. Rev. Endocrinol.* **2011**, *7*,197–207

95. Day, J.J.; Sweatt, J.D. DNA methylation and memory formation. *Nat. Neurosci.* **2010**, *13*, 1319–1323.

96. Feng, J.; Zhou, Y.; Campbell, S.L.; Le, T.; Li, E.; Sweatt, J.D.; Silva, A.J.; Fan, G. Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. *Nat. Neurosci*. **2010**, *13*, 423–430.

97. Elliott, E.; Ezra-Nevo, G.; Regev, L.; Neufeld-Cohen, A.; Chen, A. Resilience to social stress coincides with functional DNA methylation of the *Crf.* gene in adult mice. *Nat. Neurosci.* **2010**, *13*, 1351–1353

98. Kimmel, C.B.; Ballard, W.W.; Kimmel, S.R.; Ullmann, B.; Schilling, T.F. Stages of embryonic development of the zebrafish. *Devel. Dynam*. **1995**, *203*, 253–310.

99. Weis, J.; Weis, P. Effects of exposure to lead on behavior of mummichog larvae. *J. Exp. Mar. Biol. Ecol.* **1998**, *222*, 1–10.

100. Fuiman, L.A.; Cowan, J.; Smith, M.E.; O'Neal, J.P. Behavior and recruitment success in fish larvae: Variation with growth rate and the batch effect. *Can. J. Fish. Aquat. Sci.* **2005**, *62*, 1337–1349.

101. Smith, G.M.; Weis, J.S. Predator/prey interactions of the mummichog, *Fundulus. heteroclitus*: Effects of living in a polluted environment. *J. Exp. Mar. Biol. Ecol.* **1997**, *209*, 75–87.

102. Reichmuth, J.M.; Roudez, R.; Glover, T.; Weis, J.S. Differences in prey capture behavior in populations of blue crab (*Callinectes. sapidus* Rathbun) from contaminated and clean estuaries in New Jersey. *Estuaries Coasts* **2009**, *32*, 298–308.