Review: The Interactions of Contaminants with Fish and Amphibian Behavior

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Abstract: Organisms in aquatic environments encounter a combination of contaminants, the potential interactive effects on interacting physiological systems controlling behavior need to be considered. The goal of this work is to explore the interactions of contaminants towards fish and amphibians while focusing on the four main ethological concepts. Information was gathered using scientific literature and the data base http://apps.isiknowledge.com. ISI web of knowledge provided a majority of the papers mentioned in this review. While reading various papers it was evident that the same contaminant could have various effects with different function at different life-stages. The understanding of these different life-stages assisted with organizing the main topics which were: reproduction, maturation, foraging, and activity. The scope of most studies allows investigation into certain effects due to time or financial constraints. Although the objective of this study may be fulfilled, a little further investigating could prove to go a long way.

1. Introduction

Endocrine disrupting chemicals (EDCs) have been detected worldwide including existence in the most remote locations such as the deep ocean and the Arctic (de Boer, Wester et al. 1998). However, there is a gap in the literature related to the effects on behavior of fish and amphibians due to both endocrine disruptors and byproducts of manufactured chemicals. Organisms in aquatic environments encounter a combination of contaminants, the potential interactive effects on interacting physiological systems controlling behavior need to be considered (Christen, Hickmann et al. 2010) . Persistent organic pollutants (POPs) are contaminants that are lipophilic and are resistant to biodegradation. Ironically the resistance to biodegradation is an important factor that makes these compounds so useful. Over 100 POPs are known to interfere with the endocrine system of wildlife.

DDT and polychlorinated biphenyls (PCBs) have declined since the 1970's, however chemical alterations have inserted more persistent and toxic congeners (Helander, Olsson et al. 2002). The endocrine-disrupting properties of these chemicals have been detected *in vitro* by binding to hormone receptors (White, Jobling et al. 1994). Most EDCs originated from agricultural, industrial, and municipal purposes (Clotfelter, Bell et al. 2004). Estrogenic chemicals are among the most widely spread EDCs in aquatic systems because waste waters have shown to be the primary source through contraceptive pills (Kolpin, Furlong et al. 2002). These contaminants are dangerous because they are readily absorbed into the body. Ethinyl estradiol has the ability to bioconcentrate in tissue, and is not only more effective in eliciting estrogenic responses but also more stable than the natural estrogen 17β-estradiol (Thorpe, Cummings et al. 2003). The high frequency of use and continual output of drugs from wastewater treatment plants and other sources stimulates continuous exposures that are not lethal. This low level exposure presents sub-lethal effects to the species of concern. One type of sub-lethal effect maybe primer effects which are long term effects such as unwanted growth in the gonads. The other sub-lethal effect is releaser effects this is a short term effect such as a delayed or no reaction to social cues. The information presented in this review will favor towards releaser effects. However the primer effects may have not been study within that species which further supports this paper's viewpoint regarding information gaps on behavior of fish and amphibians due to contaminants.

It is important to understand that contaminants in the body influence behavior. Behavior, in addition, influences how many contaminants are stored in the body. Ethology is the study of innate behavior known as instincts. Niko Tinbergen, described ethology as the biological study of behavior, and that science is characterized by an observable phenomenon, and by a type of approach, a method of study (the biological method) (Tinbergen

2005). The biological method is explored by four concepts commonly used in ethology to answer the questions about animal behavior they are: causation, development, evolution, and function.

Most review papers about contaminants focus on the aspect of causation and rarely discuss development, function, or evolution. An animal's behavior toward a stimulus and whether the stimulus modified the animal's behavior is causation. With causation there will always be an interaction of the internal state of the animal and an external stimulus. An example of causation would be the altered reproductive behavior of male African clawed frogs *(Xenopus laevis)* after exposure to the herbicide atrazine (ATZ). The stimulus or stressor in this case is the herbicide ATZ and the behavior affected is reproduction by the male African clawed frog.

As the animal ages the behavior towards that stimulus may change, due to changed sensitivity towards the contaminant, this is development. Continuing with the example of the African clawed frogs, development would be the age-related behavior of the frogs exposed to ATZ. In theory a juvenile African clawed frog behavioral response towards ATZ will be different from an adult African clawed frog. One reason that supports this is the idea that a juvenile African clawed frog has not fully developed physiologically for reproduction. Therefore exposure to ATZ may not trigger any behavioral response.

Exposure to the stimulus will make the animal display a behavior that will help or impair the animal's ability for survival and reproduction, this is function. When mentioning function it is worth noting behavioral systems with different functions are controlled by different physiological mechanisms. So in the case of the African clawed frogs the physiological response to the stressor ATZ may not only influence reproductive hormone causing feminization but also feeding functions. This review will focus on these functions in particular: reproduction, maturation, foraging, and activity.

Evolution examines the changes in behavior from the ancestral up to the derived form. Due to evolutionary events not all organisms respond the same to contaminants. For example other Xenopus species may not show adverse reproductive behavior when exposed to ATZ, but rather a reduction in escape ability from predators. This divergence of evolution makes one species more of a specialist as to where the other would be a generalist. A specialist is a species that has become adapted to a specific environment. This is ideal for the survival of that species, however the trade-off is, that unlike a generalist species it can only survive in that environment.

Behavioral changes are excellent biomarkers of stress and stressors in animals, given that behavior is the physical manifestation of the animal's internal neuronal, metabolic, and endocrine processes, and at the same time the integrated physiological response to its environment (Clotfelter, Bell et al. 2004). The present review explores the interactions of contaminants towards fish and amphibian behavior. All of the studies mentioned in this review will focus on fish and amphibians because their behaviors are observed and quantified in controlled settings. Separate reviews have been made for pharmaceuticals pesticides, and heavy metals but few have integrated the three contaminants and explore their interactions towards fish and amphibians. The goal of this work is to explore the interactions of contaminants towards fish and amphibians while focusing on the four main ethological concepts.

2. Methods

I identified the species that would be the focal point of the review; initially the review was going to consist of only fish and salamanders. It was understood from the beginning that a variety of fish species would be included, due to the fact that fish have such a wide range of diversity, that studying just one species or even one class would hinder the efficiency of this review. While gathering information, the inclusion of frogs into this review appeared to be a logical choice since there was a limited amount of information on just salamander toxicity. Information was gathered using scientific literature and the data base http://apps.isiknowledge.com. ISI web of knowledge provided a majority of the papers mentioned in this review. Insight from Dr. Miguel Mora provided expert knowledge which assisted with tailoring the searches to get optimal results. Even with the expert knowledge from Dr. Mora there was a period of becoming accustomed to ISI web of knowledge.

The keywords that were used: contaminants (also in the form of contam*), toxins (tox*), pollutants (pollu*). These words would be combined with fish, frogs, salamanders, and amphibians. Certain search combinations would not always display all the papers that were available even when it was attempted as a refine or related search. The best references came from the keyword "review" in the *Aquatic Toxicology* publication. This search provided specific publications rather than the other searches that where too general and would bring up a broad range of papers.

While reading various papers it was evident that the same contaminant could have various effects at different function at different life-stages. The understanding of these different life-stages assisted with organizing the main topics which were: reproduction, maturation, foraging, and activity. As the papers were compiled information received was sorted according to the four behavioral functions as well as classification as a primer effect or a releaser effect.

3. Results

3.1 Reproduction and Maturation

In fish, exposure to EDCs alters their reproductive physiology and morphology resulting in for example, the induction of female specific-proteins in male fish. Sexual behavior is a endpoint of endocrine disturbance (Toft, Edwards et al. 2003). In a study by Baatrup (2009) the mating behavior of male guppies (*Poecilia reticulata*) were examined. Male guppies were exposed to three antiandrogens: the fungicide Vinclozolin, the insecticide DDT metabolite p,p'-DDE and the pharmaceutical antiandrogen Flutamide which was used as a positive antiandrogenic control. Sexually mature male guppies perform courtship behavior almost continuously during photoperiods. The sexual instinct and courtship behavior was compromised after exposure to the lowest dose of Vinclozolin and p,p'-DDE. The releaser effect known as sigmoid display, in which the male assumes the shape of an "S" or "C" and vibrates while he swims sideways displaying his coloration, prevented the function of this reproductive behavior (Table 1).

Table 1
Summary of contaminant effects on fish behavior.

Contaminant	Species	Behavior	Reference
p,p'-DDE	male guppies (*Poecilia reticulate)*	Reduced courtship behavior	Baatrup (2009)
Ethinyl Estradiol	Zebra fish (*Danio rerio*)	Reduced aggression in males	Coe et al. (2008)
		Decreased competition for spawning substrate	Salierno and Kane (2009)
	Three spine stickleback (*Gasterosteus aculeatus*)	Hyper active feeding	Bell (2004)
		Increased activity which lead to being more susceptible to predation	Bell (2004)
	Fathead minnows (*Pimephales promelas*)	Increased risk of predation	McGee (2008)
	Sand Goby (*Pomatoschistus minutus*)	Failure in nest ad mate competition	(Saaristo, Craft et al. 2009)
Fluoxetine	Hybrid striped bass (morone saxatilis x morone chrysops)	Delayed feeding	Gaworecki (2008)
PBDE	Zebra fish (*Danio rerio*)	Delayed maturation	Lema (2007)

Bell (2001) examined the levels of ethinyl oestradiol found in the environment to see if it was suffient to alter the aggressive and courtship behavior of male three-spine stickleback (*Gasterosteus aculeatus*). Although it is unclear where naturally occurring oestrogens act along the hypothalamus-pituitary-gonadal axis in stickleback, if naturally occurring oestrogens play a role in regulating male reproductive behavior in three-spine stickleback, it is more likely to be inhibitory than stimulatory (Bell 2001). Males exposed to environmentally relevant levels of ethinyl oestradiol showed decreased aggressive response to male conspecific over time. 11-Ketotestosterone is one of the major androgens in male fish and is associated with dominate behavior (Coe, Hamilton et al. 2008). Bell was able to quantify the negative relationship between ethinyl oestradiol related to conspecific-related behaviors and 11-Ketotestosterone is negatively related to nest-related behaviors. Bell also noted that since oestrogens have important roles in female reproduction and physiology, oestrogenic endocrine disruptors may have more consequential effects on females than males (Table 1).

Some amphibian species have known to show behavioral patterns that can indirectly affect embryonic survival. Many amphibian species suffer increased mortality, especially during their aquatic stages, due to habitat acidification or water pollution (Gibbs, Whiteleather et al. 2005). Ortiz et al. (2007) examined the effects of ammonium nitrate exposure and water acidification on the oviposition behavior of female dwarf newts (Triturus pygmaeus). Female Triturus species lay eggs on aquatic plant leaves and wrap the leaf using it as an additional protective envelope. Ammonium nitrate is a commonly used chemical fertilizer. In a previous study Ortiz et al (2004) examined the effects of environmentally relevant concentrations of nitrate and ammonium and noted the deleterious effects, such as high mortality or delayed growth rates, in embryos and larvae of several amphibian species. Although, ammonium nitrate did not directly affect oviposition behavior, low pH decreased the percentage of wrapped eggs for females exposed to acid water compared to the controls. The primary mechanism acid toxicity altered was the process of osmoregulation and consequent inhibition of sodium intake. The alteration in sodium balance can force newts to increase energetic costs and alter respiratory function, which would induce females to gulp air with a greater frequency and prevent the oviposition process being completed. As a results eggs that are not wrapped get laid on the bottom of ponds and can be buried in the mud then die from suffocation (Table 2) .

Table 2
Summary of contaminants effects on amphibian behavior.

Contaminant	Species	Behavior	Reference
Atrazine	African clawed frogs (*Xenopus laevis*)	Antagonizes reproductive behavior	Hayes, Khoury et al. (2010)
	American Toads (*Bufo americanus*)	Antagonizes activity habitat selection	Mendez, Tillitt et al. (2009)
Carbaryl	Gray tree frogs (*Hyla versicolor*)	Antagonizes habitat selection	Vonesh and Kraus (2009)
DDT	Tiger Salamander (*Ambystoma tigrinum*)	Antagonizes maturation	Clark, Norris et al. (1998)
Estradiol	Tiger Salamander (*Ambystoma tigrinum*)	Antagonizes maturation	Norris, Carr et al. (1997)
	Western clawed frog (*Xenopus tropicalis*)	Antagonizes reproductive behavior in males	Gyllenhammar, Holm et al. (2009)
Fluoxetine	African clawed frogs (*Xenopus laevis*)	Antagonizes metamorphosis	Conners, Rogers et al. (2009)

Norris et al. (1997) studied the interactions of androgens and estradiol on sex accessory ducts of larval tiger salamanders (*Ambystoma tigrinum*). Tiger salamander larvae were treated with environmentally relevant concentrations of estradiol, testosterone, or dihydrotestosterone (DHT), or estradiol combined with either testosterone or DHT. Both of the androgens and estradiol alone at high dosages stimulated enlargement of connective tissue surrounding the duct, however combined androgen/estrogen treatment was more effective. Norris point outs that there is an obvious dose-response effect that may occur when using a single steroid, but there is a clear synergistic action of estrogen and androgen on Mullerian duct epithelium and surrounding connective tissue that is not related to the total amount of steroid administered. Wofflian ducts were stimulated only by androgen. The releaser effects of androgen and estrogen in this case suggest that endogenous estrogen is already present as the animal develops (Table 2).

3.2Activity and Foraging

Predator detection and response during early life-stages is vital to any species ability to mature to age of recruitment. Predation is one of the primary sources of mortality during the larval stage due to many predators search for prey at speeds that exceed larval capabilities. One avoidance technique is coordinated burst of swimming. McGee et al. (2008) studied predator avoidance performance of larval fathead minnows (*Pimphales promelas*) following short-term exposure to estrogen mixtures. The avoidance behavior that was examined in this study is known as C-start. C-start begins when the fish identifies the stimulus as a stressor and then larvae will bend its body into a C-shape and burst into locomotion away from the stressor stimulus. The primer effects were estrogen-specific for example E! had little effect during larval exposure. E2 concentration reduced total escape response in exposed larvae and EE2 had no effect. The inhibitory effect of E2 on total escape response carried over into the estrogen mixture exposure in larval fathead minnows. This behavioral assay and others like it provide a tool to test the effects of multiple chemical classes with suspected endocrine disrupting functionalities (Table 1).

Gaworecki et al. (2008) determined the effects of waterborne fluoxetine on the feeding behavior of hybrid striped bass (*morone saxatilis x morone chrysops*). Fluoextine is an antidepressant that acts as a selective serontonin reuptake inhibitor. Serotonin is as a neurotransmitter and hormone that regulates a wide range of behaviors like feeding activity. Fluoextine has been successful in improving mood levels, decreasing appetite, and aggression in humans. Fluoxetine exposure has also been shown to affect various aspects of behavior in aquatic organisms. Feeding behavior was chosen because previous studies demonstrated that changes in feeding behavior were related to neurotoxin exposure and neurotransmitter concentrations (Gaworecki, Roberts et al. 2009). The high frequency of use and continual output of drugs from wastewater treatment plants and other sources stimulates continuous exposures, which was the basis for the exposure levels in Gaworecki's study. Bass in the high treatment level took significantly longer to eat the first and second prey fish in comparison to the other groups. Feeding behavior is crucial for the development, function and evolution of an organism and is relevant to the assessment of environmental stressors such as sub-lethal contaminant exposure (Table 1).

Amphibians move across landscapes to reach suitable habitats for foraging and reproduction. These movements can occur at different life-stages whether as metamorphs leaving a body of water or adults coming and going from breeding sites. Amphibians in terrestrial environments obtain water through a highly vascularized pelvic patch of skin (Mendez, Tillitt et al. 2009). This can lead to chemicals being exchanged across this patch. Mendez et al. (2009) examined the behavioral response and kinectics of terrestrial atrazine exposure in American Toads (*Bufo americanus*). Toads usually hydrate on moist surfaces, such as soil, rather than in open water. Atrazine (ATZ) a widespread herbicide commonly applied to corn and sorghum remains as a concern among amphibian ecologist based on potential exposure and toxicity. The result of this study showed that toads absorbed ATZ across their pelvic patch. The study revealed peak concentrations of ATZ in liver, gall bladder, and intestine respectively. As with most other vertebrates this is a function for contaminants to be removed from the blood into the liver and then emptied into the gall bladder. American toads showed no significant behavior of avoiding ATZ-contaminated soils versus the non-contaminated control soil. This primer effect of lack of choice leads the researcher to suggest that toads cannot detect ATZ in the soil or their body (Mendez, Tillitt et al. 2009). The lack of choice is important when considering conservation and future studies of amphibians in terrestrial habitats (Table 2).

4. Discussion

Abnormal behavior is one of the most conspicuous endpoints produced by EDCs, but until recently it has been underused by ecotoxicologist (Clotfelter, Bell et al. 2004). Behavioral responses potentially integrate many inputs, including other sensory modalities over varying time periods (Fig 1). For example in the McGee study the effects of estrogen exposure on C-start performance may be the result of either direct disruption of the afferent estrogen-sensitive system, the efferent musculo-skeletal system, or the effects may be indirect by impairing the development of the hindbrain neurons (McGee, Julius et al. 2009).

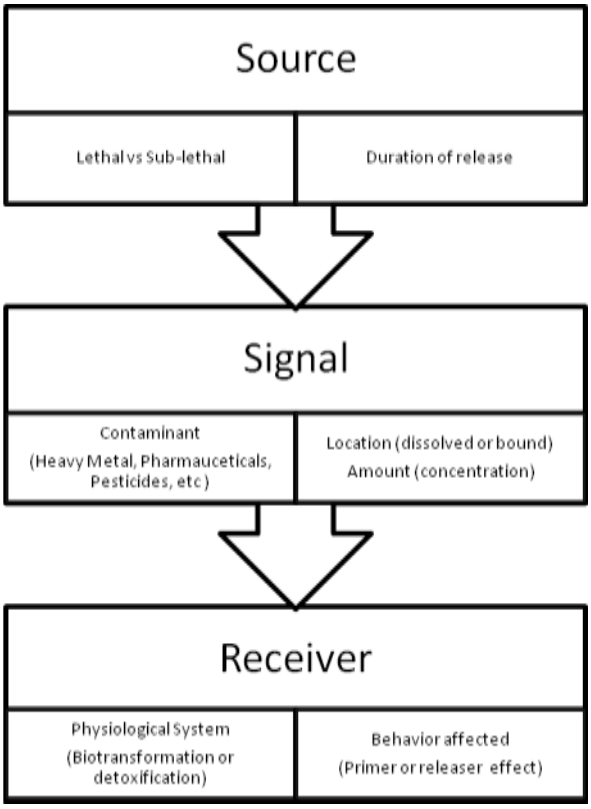

Fig 1. A general overview of transmission of sensory information. A source can discharge numerous substances but the signal depends on the concentration and the type of location that the receiver perceives with a response or lack thereof (Tierney, Baldwin et al. 2010).

Conducting laboratory research is an effective way to contribute to the knowledge base, however laboratories usually are not scenarios comparable to the exposure received in the wild. Identification of specific compounds responsible for endocrine disruption is difficult because of the large number of co-occurring EDCs, temporal variability in composition and concentrations, potential interactions among compounds, and diversity of biological endpoints (Barber, Lee et al. 2007). The scope of most studies allows investigation into certain affects due to time of financial constraints. Although the objective of the study may be fulfilled a little further investigating could prove to go a long way. The two classes of species mentioned in this review depend heavily on water to carry out daily activities; therefore contaminants have the ability to interfere with a host of behaviors that have not been adequately researched. It would be interesting to see long term exposures to determine if adaptation is possible to EDCs and/or other contaminants. Long term exposure experiments would help the understanding of the different affects contaminants have at various life stages. With more information available comparison of divergent and convergent species will be more efficient can become useful in the conservation realm. Similar to fish and hunting season the reduction in usage of certain chemicals during a time of year can prove to be vital towards specialist species conservation efforts.

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