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Effects of a pesticide and a parasite on neurological, endocrine, and behavioral responses of an estuarine fish

Violet Compton Renick[a,b,*], Kelly Weinersmith[b,c], Doris E. Vidal-Dorsch[d], Todd W. Anderson[a]

[a] San Diego State University, Department of Biology and Coastal and Marine Institute, 5500 Campanile Drive, San Diego, CA 92182, United States
[b] University of California Davis, Department of Environmental Science and Policy, One Shields Avenue, Davis, CA 95616, United States
[c] Rice University, BioSciences, 6100 Main Street, Houston, TX 77005, United States
[d] Southern California Coastal Water Research Project, 3535 Harbor Blvd. Suite 110, Costa Mesa, CA 92626-1437, United States

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ABSTRACT

In coastal waters, pesticides and parasites are widespread stressors that may separately and interactively affect the physiology, behavior, and survival of resident organisms. We investigated the effects of the organophosphate pesticide chlorpyrifos and the trematode parasite *Euhaplorchis californiensis* on three important traits of California killifish (*Fundulus parvipinnis*): neurotransmitter activity, release of the stress hormone cortisol, and behavior. Killifish were collected from a population without *E. californiensis*, and then half of the fish were experimentally infected. Following a 30 day period for parasite maturation, infected and uninfected groups were exposed to four concentrations of chlorpyrifos (solvent control, 1–3 ppb) prior to behavior trials to quantify activity, feeding behavior, and anti-predator responses. Water-borne cortisol release rates were measured non-invasively from each fish prior to infection, one-month post-infection, and following pesticide exposure. Killifish exposed to 3 ppb chlorpyrifos exhibited a $74.6 \pm 6.8\%$ and $60.5 \pm 8.3\%$ reduction in brain and muscle acetylcholinesterase (AChE) activity relative to controls. The rate of cortisol release was suppressed by each chlorpyrifos level relative to controls. Killifish exposed to the medium (2 ppb) and high (3 ppb) pesticide concentrations exhibited reduced activity and a decrease in mean swimming speed following a simulated predator attack. Muscle AChE was positively related to swimming activity while brain AChE was positively related to foraging behavior. No effects of the parasite were observed, possibly because of low metacercariae densities achieved through controlled infections. We found that sublethal pesticide exposure has the potential to modify several organismal endpoints with consequences for reduced fitness, including neurological, endocrine, and behavioral responses in an ecologically abundant fish.

1. Introduction

Coastal and marine environments consist of a myriad of chemical, biological, and physical stressors that challenge the survival of resident biota. Among marine and estuarine stressors, pollutants and parasites are ubiquitous and highly influential on the fitness and survival of resident organisms (Marcogliese and Pietrock, 2011). Pesticides and heavy metals are carried by run-off into coastal urban habitats where they can negatively affect physiological traits of resident organisms and have significant ecological consequences (McGourty et al., 2009; Dachs and Méjanelle, 2010), and parasites are common and significant biological stressors in marine systems (Lafferty, 2013). When considered separately, pollutants and parasites can exert considerable effects on the physiology, metabolism, reproduction, behavior, and survival of resident organisms. However, their potential interactive effects on organisms including fishes remain largely unknown (Sures, 2008a,b). As organisms are exposed to both stressors concurrently, it is crucial to understand the combined effect of pesticides and parasites on the host phenotype.

Pollutants can disrupt neurological and endocrine function as well as behavior. Among pollutants, organophosphate pesticides are one of the most globally pervasive and neurotoxic chemical groups (Saunders et al., 2012). Organophosphate pesticides can severely modify the neurological and endocrine responses

* Corresponding author at: Grossmont College, Department of Biology, 8800 Grossmont College Dr., San Diego, CA 92020, United States.
E-mail address: violetrenick@gmail.com (V.C. Renick).

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of non-target organisms even at relatively low concentrations (Grue et al., 1997). Organophosphate pesticides inhibit the acetylcholinesterase (AChE) enzyme which regulates acetylcholine, one of the most widely distributed neurotransmitters in vertebrates (Behra et al., 2002; Sandahl et al., 2005). Acetylcholine is key in transmitting neuronal messages to muscle cells in vertebrates (Tilton et al., 2011). AChE inhibition results in an accumulation of acetylcholine in the central and peripheral synapses, causing modified physiological and neuroendocrine processes (Behra et al., 2002; Sandahl et al., 2005; Tilton et al., 2011). Such physiological modifications can subsequently lead to changes in behaviors, including reduced swimming performance (Levin et al., 2004; Almeida et al., 2010; Yang et al., 2011), altered social behavior (Khalil et al., 2013), reduced foraging (Sandahl et al., 2005), and greater predation risk (Carlson et al., 1998).

The endocrine stress response is particularly sensitive to natural and anthropogenic stressors including pollutant exposure (Bisson and Hontela, 2002; Jobling and Tyler, 2003; Brar et al., 2010). In fish, this response is characterized primarily by the release of the corticosteroid cortisol by the hypothalamo–pituitary–interrenal (HPI) axis. However, links between sublethal organosphosphate exposure and the endocrine stress response have rarely been quantified in fishes (Oruç, 2010). These links may be important because alterations in cortisol release rates produce acute and chronic effects on an organism's biochemistry and physiology (including impacts on osmoregulation and energy metabolism; Øverli et al., 2002; Ezemonye and Ikpesu, 2013). Furthermore, changes to rates of cortisol release may have important implications for behavior, as cortisol is often strongly linked to activity and locomotion, sociality, reproduction, and foraging (Gregory and Wood, 1999; Øverli et al., 2002, 2005).

Parasites can also significantly impact the physiology, behavior, and survival of their hosts (Moore, 2002; Cézilly et al., 2013; Santos and Santos, 2013). Parasites that are transmitted trophically can be particularly effective at exerting changes to host phenotype, as they can manipulate the physiology and behavior of their hosts to increase predation by a definitive host species (Adamo, 2013; Lafferty and Shaw, 2013). For instance, the California killifish, *Fundulus parvipinnis*, infected with trematode parasite *Euhaplorchis californiensis* metacercariae (the larval stage which encysts on the surface of host brain tissue) display conspicuous behaviors including surfacing, flashing, and body contortions 3–4 times more frequently than do uninfected killifish. Parasitized killifish are 10 to 30 times more likely to be consumed by avian predators, the definitive hosts of *E. californiensis* (Lafferty and Morris, 1996). California killifish infected with *E. californiensis* have altered serotonergic and dopaminergic neurotransmitter activity (Shaw et al., 2009; Shaw and Øverli, 2012), and such changes in neurochemistry may be an explanatory factor behind the increased conspicuous behaviors of infected killifish. Serotonergic activity has downstream effects on cortisol synthesis (Winberg et al., 1997; Lim et al., 2013), and thus *E. californiensis* may also influence cortisol release. In addition to potential manipulation of cortisol release rates by the parasite, the stress of infection alone may increase the rate of cortisol release. Studies of the European eel, *Anguilla anguilla*, infected with the swim-bladder parasite *Anguillicola crassus* and simultaneously exposed to pollutants (cadmium and PCB-126) indicate that the stress of a parasite infection could exacerbate the cortisol response to other stressors such as pesticides (Sures et al., 2006). Interactions between *E. californiensis* and stressors such as pesticides have yet to be explored in California killifish.

When considered in combination, pesticides and parasites may act additively or synergistically to reduce the survival of exposed fishes (Sures, 2008a). However, few studies in ecotoxicology incorporate the effects of parasites, and there remain many gaps in knowledge surrounding their potential interactive effects (Lafferty, 1997; Sures, 2008b; Blanar et al., 2009). A greater understanding of the singular and interactive effects of pesticides and parasites is necessary because of the pervasiveness of both of these stressors in marine environments and their potential for dramatically reducing the fitness of fishes (Marcogliese and Pietrock, 2011). More specifically, the neurological and endocrine mechanisms and behavioral consequences underlying their interactive effects require further investigation.

In this study, we investigated the neurological, endocrine, and behavioral effects of the organophosphate pesticide chlorpyrifos and the trematode parasite *E. californiensis* in California killifish to provide a better understanding of their interactive effects on the behavior and survival of estuarine fishes. We based our study on this parasite-host system due to the relatively high numerical abundance and ecological importance of both species in Southern California estuaries, which is well documented in a rich source of literature detailing the ecological and physiological effects of parasitism by *E. californiensis* on California killifish (Shaw et al., 2010). Many other species of parasites can infect California killifish, but *E. californiensis* is typically the most abundant and prevalent parasite found in this host species (Lafferty, 2008). Moreover, the larval cercariae of *E. californiensis* are easily shed in the laboratory, making them a reliable and relevant source for experimental infection (Shaw et al., 2009). We measured the activity of AChE, release of the stress hormone cortisol, and behavioral modifications caused by both stressors. We asked the following questions: (1) Are there separate and interactive effects of pesticide exposure and parasitism on AChE activity and rates of cortisol hormone release in killifish? (2) What are the behavioral responses of killifish exposed to either or both stressors? (3) What are the relationships between the physiological responses (AChE activity and cortisol release rates) and altered behaviors of killifish?

2. Materials and methods

2.1. Fish collection and acclimation

California killifish were collected by beach seine from a small isolated lagoon at Camp Pendleton, California, USA (33°15′48.1″N, −117°26′20.4″W). This collection site was chosen because it historically lacked the trematode *E. californiensis* and the parasite's first intermediate host (the California black horn snail, *Cerithidea californica*). Prior to initiating the experiment, a subset of killifish were euthanized and dissected to confirm that experimental fish were not already infected with *E. californiensis*. Other parasitic trematodes were occasionally observed in dissected killifish, but their possible effects on behavior or physiology were controlled for through experimental infections in an environment free of infectious stages of other parasite species. Killifish were transported in aerated coolers to the San Diego State University (SDSU) Coastal and Marine Institute Laboratory (CMIL), in San Diego, CA. Fish were held in 37.5 L aquaria in filtered seawater (0.45 μm, 20 ± 1 °C, salinity: ~34 ppt) for four wk under a 14:10 h light:dark photoperiod. Fish were fed frozen *Artemia* brine shrimp (San Francisco Bay Brand, San Francisco, CA, USA) *ad libitum* once daily.

2.2. Cortisol collection and processing

Cortisol is released from the gills of fishes, where it can be extracted from the surrounding water (Scott et al., 2008). In various different species, cortisol release rates mirror concentrations of cortisol in the plasma, allowing for non-invasive and repeated measurements of cortisol and other steroid hormones (Ellis et al., 2013). The relationship between cortisol release rates and plasma cortisol is also observed in California killifish (Weinersmith, unpub-