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Elevated temperatures increase the toxicity of pesticide mixtures to juvenile coho salmon

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ABSTRACT

Pesticide mixtures and elevated temperatures are parallel freshwater habitat stressors for Pacific salmon in the western United States. Certain combinations of organophosphate (OP) insecticides are known to synergistically increase neurotoxicity in juvenile salmon. The chemicals interact to potentiate the inhibition of brain acetylcholinesterase (AChE) and disrupt swimming behavior. The metabolic activation and detoxification of OPs involve temperature-sensitive enzymatic processes. Salmon are ectothermic, and thus the degree of synergism may vary with ambient temperature in streams, rivers, and lakes. Here we assess the influence of water temperature (12–21 °C) on the toxicity of ethoprop and malathion, alone and in combination, to juvenile coho salmon (*Oncorhynchus kisutch*). A mixture of ethoprop (0.9 μg/L) and malathion (0.75 μg/L) produced synergistic AChE inhibition at 12 °C, and the degree of neurotoxicity approximately doubled with a modest temperature increase to 18 °C. Slightly lower concentrations of ethoprop (0.5 μg/L) combined with malathion (0.4 μg/L) did not inhibit brain AChE activity but did produce a temperature-dependent reduction in liver carboxylesterase (CaE). The activity of CaE was very sensitive to the inhibitory effects of ethoprop alone and both ethoprop–malathion combinations across all temperatures. Our findings are an example of how non-chemical habitat attributes can increase the relative toxicity of OP mixtures. Surface temperatures currently exceed water quality criteria in many western river segments, and summer thermal extremes are expected to become more frequent in a changing climate. These trends reinforce the importance of pollution reduction strategies to enhance ongoing salmon conservation and recovery efforts.

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1. Introduction

Patterns of aquatic species decline and recovery are determined to a large extent by the availability and quality of habitat. Habitat attributes may be physical, biological, or chemical, and they can have interacting influences on fish and other taxa. In the western United States, myriad forms of habitat degradation since the late 1800s have led to decline of Pacific salmonids (*Oncorhynchus* spp.), to the extent that more than 20 population segments are now listed under the U.S. Endangered Species Act (ESA). Many of the drivers for salmon and steelhead losses are relatively well understood. These include habitat attributes that are physical (dams, impassable culverts, water diversions, sedimentation, etc.) and biological (riparian vegetation loss, non-native species, etc.). The contributions of chemical habitat degradation (e.g., the inputs of toxic chemical contaminants) to salmon declines are less well understood. Most ESA-listed salmonids are highly migratory during their freshwater phase, traversing habitats that receive chemical pollutants from agricultural, urban, municipal, and industrial sources (reviewed by Ross et al., 2013). Despite information gaps, there is growing concern that toxics are limiting salmon recovery and undermining costly physical habitat restoration activities (e.g., Naiman et al., 2012). Key questions at present are whether and to what extent chemicals interact in mixtures, and whether co-occurring (non-chemical) habitat perturbations render salmon more or less vulnerable to toxic chemical pollution.

Recent research on mixture toxicity has focused on agrochemicals, and in particular the organophosphate (OP) class of insecticides. Although several OPs were targeted for phaseout following the passage of the 1996 Food Quality Protection Act, these pesticides continue to be widely used on agricultural crops throughout the major western U.S. river basins that contain

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designated critical habitat for ESA-listed salmonids. In watersheds where OPs are applied, they are often transported to the surface waters of rivers and streams by spray drift, surface runoff, and agricultural return flows. They can occur in aquatic habitats together with other pesticides in complex mixtures (Gilliom, 2007) at low dissolved individual concentrations of a few parts per billion (μg/L) or less.

Common OP insecticides including diazinon, chlopyrifos, malathion, dichlorvos, fenitrothion, and ethoprop are known inhibitors of several enzymes in fish, insects, mammals, and other animals. A primary target is acetylcholinesterase (AChE), an enzyme that hydrolyzes the neurotransmitter acetylcholine at neuronal and neuromuscular synapses throughout the central and peripheral nervous system. Once taken up by fish, most sulfur-containing OP parent compounds are metabolized to oxon derivatives that bind AChE irreversibly. Enzyme inhibition leads to excess neurotransmitter accumulation in the clefts of synapses, thereby disrupting cholinergic signaling. For juvenile salmon, OP-induced neurotoxicity (AChE inhibition) corresponds closely with reductions in swimming and feeding behaviors (Sandahl et al., 2005). Many widely used OP insecticides also inhibit other esterases, notably carboxylesterase (CaE). Carboxylesterase is expressed primarily in the liver (Wheelock et al., 2005) and is known to play a substantial role in xenobiotic detoxification, including the hydrolysis of OP oxons (Casida & Quistad, 2004).

In mammals, certain combinations of OP insecticides have been known to cause greater-than-additive (synergistic) toxicity for many years (e.g., Frawley et al., 1957). These findings were recently extended to juvenile salmon, specifically for binary OP pairings of diazinon, chlorpyrifos, and malathion (Laetz et al., 2009). When present in a mixture, the OPs were much more neurotoxic than would be predicted from concentration–response curves for individual chemicals. A subsequent study showed that combinations of malathion-diazinon and malathion-ethoprop cause significant brain AChE inhibition in juvenile salmon at individual pesticide concentrations near or below a part per billion (Laetz et al., 2013). The enhanced toxicity of the mixtures is likely to involve CaE inhibition and, by extension, the prevention of OP detoxification. For example, in arthropods, mutations that increase the hydrolytic efficiency of CaE convey OP insecticide resistance (e.g., Jackson et al., 2013) while chemicals that inhibit CaE can greatly potentiate OP toxicity (Yuan and Chambers, 1996). The pharmacological inhibition of CaE similarly increases OP toxicity in fish (e.g., Shao-nan and De-Fang, 1996).

Although the precise mechanism has yet to be determined, OP mixture synergism in salmon is likely to involve brain and liver enzyme activity. In addition, toxicodynamic processes in fish and other aquatic species are often temperature-dependent. Changes in temperature influence rates of chemical uptake, metabolism, and depuration, and pesticide toxicities are likewise temperature dependent (Osterauer and Köhler, 2008; Harwood et al., 2009; Weston et al., 2009). Since juvenile salmon are ectothermic, the enzymatic processes that determine OP metabolism, detoxification, and synergism are likely to be influenced by ambient temperature fluctuations in freshwater habitats.

Surface water temperature is an important habitat attribute for threatened and endangered salmonid populations throughout California and the Pacific Northwest. As a consequence of temperature, many salmon-bearing waters in the western U.S. are currently classified as impaired under section 303(d) of the Clean Water Act (McCullough, 2010). Cold-water salmon and steelhead experience thermal stress at temperatures above approximately 18 °C, with thresholds varying across species and life stages (Richter and Kolmes, 2005). Juveniles seek thermal refugia in river networks, and ambient temperatures shape the distribution and abundance of salmonids over large spatial scales (Welsh et al., 2001). Moreover, future changes in stream flows and summer temperatures are expected to increase the frequency of thermal extremes (Arismendi et al., 2012) and alter the structure of coldwater fish populations (Mantua et al., 2010). Projected habitat loss due to increasing temperatures (Ruesch et al., 2012) is an increasingly important factor in salmon recovery planning (Battin et al., 2007).

In the present study we assess the relationship between surface water temperature and OP mixture toxicity to juvenile coho salmon (*O. kisutch*). We exposed salmon to ethoprop (*O*-ethyl *S*,*S*-dipropyl phosphorodithioate) and malathion (diethyl 2-[(dimethoxyphosphorothioyl)sulfanyl]butanedioate) individually and in mixtures at concentrations that are very low and yet known to synergistically increase neurotoxicity (Laetz et al., 2013). We measured brain AChE and liver CaE enzyme inhibition in coho held at ambient temperatures ranging from 12 °C to 21 °C. These temperatures are environmentally relevant and vary from non-stressful at 12 °C (a typical holding temperature for captive salmonids) to stressful but non-lethal at 21 °C.

2. Methods

2.1. Juvenile salmon

Juvenile coho salmon (*O. kisutch*) were obtained from the Northwest Fisheries Science Center's hatchery (NWFSC, Seattle, WA) and transferred to the Washington State University (WSU) Puyallup Research and Extension Center (Puyallup, WA) for the duration of the study. Fish were maintained in recirculating tanks of chilled, dechlorinated municipal water (hatchery water; temperature 11–13 °C, dissolved oxygen 95–100%, pH 7.0–8.0, total hardness as $CaCO_3$ 80–120 ppm, and alkalinity 80–180 ppm). Free ammonia was monitored colorimetrically and maintained at non-toxic levels (<0.05 ppm) by the periodic addition of a sodium bicarbonate solution. Fish were held on a 12:12 h light:dark schedule and fed commercial pellet food (Bio-Oregon, Warrenton, OR) daily. At the time of experiments, fish were 4–7 months old and averaged (±SD) 68 ± 7 mm in length and 3.5 ± 1.1 g in weight. Experiments were conducted in accordance with Washington State University's Institutional Animal Care and Use Committee (IACUC).

2.2. Pesticide exposures

Analytical grade ethoprop (CAS No. 13194-48-4; 98.4% pure) and malathion (CAS No. 121-75-5; 98.7% pure) were purchased from Chem Service (West Chester, PA). Pesticide stock solutions were constituted in methanol at the WSU Food and Environmental Quality Laboratory (Richland, WA) and shipped on ice to the Puyallup facility for use in experiments. Exposure concentrations (Table 1) were based on previous exposures (Laetz et al., 2009, 2013) with juvenile coho of a similar size and age. As shown previously (Laetz et al., 2013), ethoprop and malathion interact in a simple binary mixture to synergistically increase neurotoxicity. This toxicity occurs at concentrations that are a very small fraction of the EC_{50} (the concentration producing a 50% decrease in AChE activity) for either insecticide acting alone. Based on this earlier work, the two chemicals were combined in "high" and "low" mixtures, which correspond to a 0.02 and 0.01 cumulative EC_{50}, respectively. The high mixture contained ethoprop and malathion at 0.9 μg/L and 0.75 μg/L nominal concentrations, respectively, and the low mixture contained the same at 0.5 μg/L and 0.4 μg/L. At these aqueous concentrations, neither insecticide alone would be expected to produce measurable brain AChE inhibition based on EC_{50} values derived from earlier reported single-chemical concentration–response curves (91 μg/L and 74 μg/L for ethoprop and malathion, respectively; Laetz et al., 2013). Thus, absent