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Thermal modulation of anthropogenic estrogen exposure on a freshwater fish at two life stages

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ABSTRACT

Human-mediated environmental change can induce changes in the expression of complex behaviors within individuals and alter the outcomes of interactions between individuals. Although the independent effects of numerous stressors on aquatic biota are well documented (e.g., exposure to environmental contaminants), fewer studies have examined how natural variation in the ambient environment modulates these effects. In this study, we exposed reproductively mature and larval fathead minnows (*Pimephales promelas*) to three environmentally relevant concentrations (14, 22, and 65 ng/L) of a common environmental estrogen, estrone (E1), at four water temperatures (15, 18, 21, and 24 °C) reflecting natural spring and summer variation. We then conducted a series of behavioral experiments to assess the independent and interactive effects of temperature and estrogen exposure on intra- and interspecific interactions in three contexts with important fitness consequences; reproduction, foraging, and predator evasion. Our data demonstrated significant independent effects of temperature and/or estrogen exposure on the physiology, survival, and behavior of larval and adult fish. We also found evidence suggesting that thermal regime can modulate the effects of exposure on larval survival and predator-prey interactions, even within a relatively narrow range of seasonally fluctuating temperatures. These findings improve our understanding of the outcomes of interactions between anthropogenic stressors and natural abiotic environmental factors, and suggest that such interactions can have ecological and evolutionary implications for freshwater populations and communities.

1. Introduction

Human-mediated environmental changes to aquatic ecosystems are occurring at an unprecedented rate, with potentially severe repercussions for resident wildlife. Habitat alteration or loss (e.g., nutrient loading, increased sedimentation, or physical changes due to land-use), invasive species introductions, over-harvesting, and influxes of aquatic contaminants have globally recognized, clear, and adverse effects on the health and viability of aquatic biota (Global Biodiversity Outlook II, 2006; Keister et al., 2010). Such stressors are typically studied in isolation; however, interactions among multiple anthropogenic stressors, or between stressors and natural abiotic environmental factors such as dissolved oxygen, pH, salinity, UV radiation or temperature (Crain et al., 2008; Häder and Gao, 2015; Heugens et al., 2001; Holmstrup et al., 2010; Laskowski et al., 2010) have the potential to modulate or exacerbate the impacts of human-mediated environmental change at both individual and population levels. For example, interactions between inputs of inorganic nutrients and organic matter have been shown to alter the dynamics of food webs in marine intertidal ecosystems (O'Gorman et al., 2012). Changes in the toxicities of aquatic contaminants in response to variation in UV-B exposure or salinity are also well documented (Hall and Anderson, 1995; Pelletier et al., 2006). Although the outcomes of these multi-factor interactions are often cumulative or synergistic, they can also be unpredictable (Christensen et al., 2006; Muthukrishnan and Fong, 2014; O'Gorman et al., 2012; Shears and Ross, 2010; see also Crain et al., 2008; Darling and Cote, 2008), or vary across space or time (Molinos and Donohue, 2010; Newman and Clements, 2008), including life stage (Przeslawski et al., 2015; Salice et al., 2011). Thus, concerted efforts to understand the impacts of anthropogenic change under more complex, real-world scenarios are of key importance for predicting and mitigating adverse effects on aquatic ecosystems.

Freshwater fish populations are often geographically restricted, and are likely to be especially vulnerable to declines in abundance or extirpation due to anthropogenic stress (Dudgeon et al., 2006; Heino et al., 2009). Among the most pressing threats to freshwater fish is chemical pollution; urban, industrial and agricultural runoffs, and wastewater treatment plants, continually discharge contaminants into rivers and streams (Kolpin et al., 2002), many of which bind to organismal hormone receptors and disrupt the normal endocrine functioning of

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exposed individuals (Kuiper et al., 1998). Because rates of introduction typically exceed chemical half-lives (Daughton, 2002), endocrine disrupting chemicals (EDCs) are common in the environment during critical life stages, such as during early development or at reproductive maturity. Exposure to EDCs has been shown to induce a variety of adverse molecular, behavioral, and physiological effects in both juvenile and adult fish (Bhandari et al., 2015; McGee et al., 2009; Niemuth and Klaper, 2015; Saaristo et al., 2010; van Aerle et al., 2002; Ward and Blum, 2012). Furthermore, empirical work and population modeling have convincingly demonstrated that these individual-level effects can dramatically impair the viability and sustainability of aquatic populations (Brown et al., 2015; Kidd et al., 2007; Palace et al., 2009).

Efforts to assess the impacts of EDCs on natural populations, however, are complicated by the fact that rates of chemical degradation in the environment (Starner et al., 1999), and uptake and elimination by organisms (Gordon, 2003), are dependent on the ambient temperature of the environment (Cairns et al., 1975; Heugens et al., 2001). In fish and other ectothermic aquatic species, temperature governs a wide array of fundamental physiological processes, including sexual determination, rates of early development, cellular signaling, biochemical reactions, and basal metabolic activity (Crockett and Londraville, 2006; Ospina-Alvarez and Piferrer, 2008), with potential to modulate the responses of organisms to toxicants in various ways (Brown et al., 2015; Hallare et al., 2005; Heugens et al., 2001, 2003; Khan et al., 2006). For example, increases in temperature have been shown to exacerbate EDC-induced production of vitellogenin (*vtg*; an egg yolk protein precursor normally only found in females) in juvenile salmonids (Körner et al., 2008; Korsgaard et al., 1986; Mackay and Lazier, 1993), and to influence EDC-induced skewed sex ratios in zebrafish (Brown et al., 2015). At higher temperatures, EDC exposure also synergistically increases mortality and impairs embryogenesis (Osterauer and Kohler, 2008). Cumulatively, the data collected to date suggest that chemical toxicants can interact with the thermal conditions to influence mortality and physiological impairment (Gordon, 2003; Heugens et al., 2001).

By comparison, little is known regarding the interactive effects of temperature and EDC exposure on the behavior of fish and other aquatic organisms (Manciocco et al., 2014). This deficit is significant, because an individual's behavior represents integrated physiological and developmental responses to the environment (Clotfelter et al., 2004), and altered inter- and intraspecific trait-mediated behavioral interactions that impact individual fitness, such as predator-prey relationships, competition for resources, or reproduction, have potential to reduce population abundances and alter the structure and function of aquatic communities (Clotfelter et al., 2004; Kidd et al., 2014). In this study, we conducted a factorial experiment in the laboratory to determine the extent to which temperature modulates the survival, development, reproductive physiology and interspecific (foraging ability, predator evasion) and intraspecific (male-male competition) behavioral interactions of a freshwater fish, the fathead minnow (*Pimephales promelas*), exposed to a common environmental estrogen, estrone (E1), during larval development and at sexual maturity. Our aims were threefold; first, we tested the general hypothesis that temperature modulates the dose-dependent effects of estrogen exposure at both larval and adult life stages. Second, we assessed the extent to which independent and interactive effects of E1 exposure and temperature differ across fitness contexts, specifically predator evasion, foraging efficiency, and territorial defense. Third, we compared the general susceptibility of fish to behavioral impairment during early development and at sexual maturity. To date, most single studies have focused on the effects of exposure at a single life stage (but see Oliveira et al., 2009; Parrott and Blunt, 2005; Schultz et al., 2012 for examples to the contrary); but growth and survival during the early stages of life, and successful reproduction at maturity, all directly impact individual fitness. Thus, knowledge regarding the effects of contaminant exposure at multiple life stages is a prerequisite to accurately assessing and predicting impacts under complex, real-world scenarios.

2. Material and methods

2.1. Experimental design

To test the hypothesis that the biological effects of estrogen exposure are modulated by ambient temperature, we exposed breeding groups of fathead minnows (two mature females, one male) to a low, medium or high concentration of E1 (i.e., $E1_{LOW}$, $E1_{MED}$, or $E1_{HIGH}$) dissolved in EtOH, or to EtOH alone (Control), at one of four temperatures (15, 18, 21, 24 °C) for 30 days (16 total treatments; 10–14 breeding groups per treatment). These temperatures reflect natural spring and summer seasonal variation in northern temperate streams, rivers and lakes and are well within the thermal tolerance limits for *P. promelas* (Pyron and Beitinger, 1993). Throughout the exposure period we monitored the fecundity and fertility of females and males. Beginning on day 10 and lasting through day 17, we collected one clutch of eggs from each breeding pair and placed it in a breeding basket in the parental aquarium. On days 29 and 30, we tested the parental subjects in two behavioral assays designed to assess the independent and interacting effects of temperature and estrogen on the foraging ability of males and females, and the territorial aggression of resident male fish towards a conspecific male intruder. We conducted two additional assays to assess the predator escape performance and foraging ability of exposed and control 21-day-old larval fish reared at different temperatures. All subjects were sacrificed immediately following the completion of testing via a lethal concentration of $NaCO_2$-buffered MS-222 (Western Chemical, WA, USA). The subjects were dissected (adults) or stored in RNA*later*® (Thermo-Fisher Scientific, MA, USA) (larvae) for use in a separate study. All procedures, and care and maintenance protocols, were approved by the Institutional Animal Care and Use Committee (protocol number 8-73) at St. Cloud State University.

2.2. Subjects, housing and maintenance

Six-month-old, reproductively mature *P. promelas* were purchased from a laboratory culturing facility (Environmental Consulting and Testing, WI, USA) and shipped to St. Cloud State University at bi-monthly intervals between March and July 2015. We chose *P. promelas* to test the hypothesis that the biological effects of estrogen exposure are modulated by ambient temperature because this species is widespread in North America, and considered to be a model species for ecotoxicology research (Ankley and Villeneuve, 2006). Upon arrival (day 0), the fish were introduced directly into the exposure apparatus and permitted to acclimate to their surroundings for 24 h before the experiment was started (day 1). During this time holding temperatures were increased or decreased as necessary to reach the experiment-specific ambient water temperature. The fish were maintained under a 16 h light: 8 h dark photoperiod, and fed an ad libitum diet of frozen brine shrimp (*Artemia franciscana*, San Francisco Bay Brand Inc., CA, USA) and bloodworms (*Glycera* spp.) twice daily for the duration of the experiment. F1 generation larvae were fed newly hatched brine shrimp (Brine Shrimp Direct, UT, USA) twice daily, beginning two days after hatching. The aquaria were cleaned of debris and monitored daily for mortality.

2.3. Exposure chemicals

Estrone is a common natural estrogen discharged in wastewater effluent, and is representative of a broad class of steroidal hormones and other chemicals with estrogenic activity. In a U.S. national survey, Kolpin et al. (2002) reported concentrations of E1 in rivers ranging from <5 ng/L to 112 ng/L.

Powdered estrone (≥99% purity) was obtained from Sigma-Aldrich (St. Louis, MO) and dissolved in 100% ethanol (1687.5 μg/mL). In accordance with EPA guidelines for short-term exposure studies, this solution was then serially diluted with EtOH to produce low, medium (5×) and high (25×) treatment stock solutions with nominal concentrations of