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Do environmental factors affect male fathead minnow (*Pimephales promelas*) response to estrone? Part 2. Temperature and food availability

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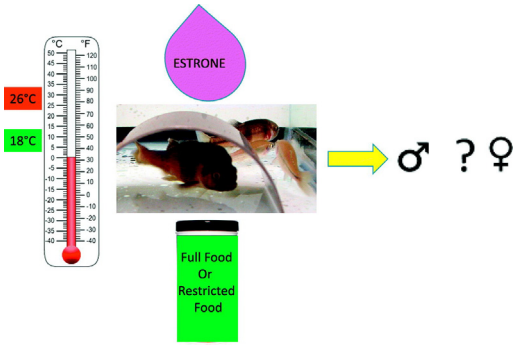
HIGHLIGHTS

- Fish exposure to estrogens occurs under variable temperature and nutrient conditions
- Fathead minnows were exposed to estrone (E1) at 18° and 26 °C, ± food restriction
- Temperature effect dominated, with lower testis weight and vitellogenin at 26 °C
- E1 interactions found for temperature/food for hematocrit, liver size and maturity
- 18 °C most relevant temperature to study endocrine disruption in fathead minnows

GRAPHICAL ABSTRACT



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ABSTRACT

Fish are subject to constantly changing environmental conditions and food availability, factors that may impact their response to endocrine disruptors (EDs). This may, in part, explain outcome discrepancies between field studies and laboratory exposures to EDs. This study assessed whether standard laboratory conditions for fish exposures adequately represent effects of ED exposure at two environmentally realistic temperatures. The impact of temperature and food availability on male fathead minnow response to estrone (E1) exposure was studied in two experiments (3 × 2 × 2 factorial design) with three E1 concentrations (range 0–135 ng/L); two temperatures (18 °C and 26 °C, the latter the prescribed laboratory temperature), and two feeding treatments (full fed vs. 25% of full fed) in a 21-day flow-through system. Morphometric endpoints [including body condition factor, somatic index of gonad (GSI) and liver (HSI), and secondary sex characteristics (SSC)], blood parameters [hematocrit (HCT), blood glucose, cortisol, and vitellogenin (VTG) concentrations], and histology of liver and testis were determined on day 22. High E1 consistently increased VTG, though interactions among E1, temperature and/or food on liver weight, HSI, and HCT were inconsistent between experiments. High temperature impacted the greatest number of parameters, independent of E1 treatment. Three sex-linked parameters were lower at high temperature (testis weight, GSI and VTG), and in Exp. 2SSC and gonad maturity rating were lower. At 26 °C, in Exp. 1 HSI and HCT decreased, and in Exp. 2 length, body and liver weight, and body condition factor were lower. Food

Abbreviations: (E1), estrone; (EtOH), ethanol; (ff), full fed; (GMR), gonad maturity rating; (GSI), gonadosomatic index; (HCT), hematocrit; (HSI), hepatosomatic index; (VTG), vitellogenin.

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restriction decreased GSI in Exp. 1, and blood glucose and liver weight in Exp. 2. At 26 °C several parameters were altered independent of E1 exposure, including three out of four measurements of sperm differentiation. Concordance between laboratory and field investigations of the biological effects of EDs may improve if environmentally-relevant exposure conditions, especially temperature, are employed.

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1. Introduction

In nature, fish are subject to constantly changing environmental conditions and limitations on food availability, potentially impacting their response to endocrine disruptors. Outcome discrepancies between field studies and laboratory exposures to endocrine disruptors may be a result of these conditions. In contrast, toxicity testing is often intentionally over-simplified, as consistent methodology allows for more legitimate comparison of results from different laboratories. For example, culture guidelines set forth by regulatory agencies have generally recommended that water quality parameters, including water temperature be kept constant (Table 1.2, Denny, 1987; U.S. Environmental Protection Agency, 1988). These studies have provided the framework for aquatic toxicologists to develop a vast ecotoxicological database of knowledge. There are, however, limitations to this reductionist approach. Fish populations are not genetically identical (Wang et al., 2016). Variability of individual organismal biology within treatments will inevitably lead to variation in dose responses. Other factors, including seasonal influences on fish reproduction (Denton and Yousef, 1975; Smith, 1978), further emphasize the limitations of standardized exposures. It is important to remember that the ultimate goal of data derived from toxicity testing is to create environmental standards that typically will be applied across habitats encompassing a broad geographical or climatic range. In the case of the fathead minnow (*Pimephales promelas*), a common laboratory model species in aquatic toxicology, spawning may begin as early as March at the edge of the southern-most habitat range, but may not reach peak reproductive season until June or July along its northern range in the Upper Midwest of the USA and Canada. Average temperatures for April and July are presented along with fathead minnow habitat in Fig. 1. The aim of the current study was, therefore, to assess whether standard laboratory conditions for fish exposures adequately represent effects of ED exposure at two environmentally realistic temperatures, 18 °C and 26 °C. Based on water temperature data throughout the range of the fathead minnow in the U.S. (Fig. 1) during peak spawning season (Gale and Bunyak, 1982; Smith, 1978), an environmentally relevant test temperature is approximately 18 °C.

By many accounts, climate change is accelerating, and estimations predict that this trajectory will persist (Murdoch et al., 2000; Adrian et al., 2009). It is expected that changes in gill ventilation and physiology (Evans, 1987; Blewett et al., 2012; Roberts, 2012), elevated metabolic rate (Evans and Claiborne, 2006), and increased estrogen receptor sensitivity (Blair et al., 2000), which occur at higher temperatures, may affect the outcome of E1 exposure in fish. In addition to variability of abiotic environmental conditions, food availability represents another point of departure between laboratory exposure conditions and realities in the environment. While ad libitum feeding is recommended for most toxicity testing, fish in aquatic ecosystems seldom have this luxury for extended periods of time and more likely face prolonged periods of limited food quality and quantity. Biological markers of estrogenic exposure might be enhanced in fish fed with an abundance of food when compared to those with limited food access. The synthesis of VTG in male fish, a well-established biomarker of estrogenic exposure (Sumpter and Jobling, 1995; Ankley et al., 2001; Matozzo et al., 2008; Bartell and Schoenfuss, 2012) may be limited by nutrient availability or may come at the expense of reduced gamete production under limited food availability. Such responses would not be observed in typical laboratory exposures, where food access is ad libitum. If so, detection of estrogenic endocrine disruption would be hindered in laboratory exposures using standard laboratory exposure conditions and the biomarker, VTG.

The overall objective of the current study was to examine the modulating quality of an abiotic (temperature) and biotic (food availability) factor on E1 exposure effects in male fathead minnows. Specifically, we tested the hypothesis that male fathead minnows exposed to E1 at higher temperatures and limited food will exhibit greater adverse effects of exposure than those at any other tested combination of temperature and food availability.

2. Materials and methods

2.1. Experimental design

Two successive 21-d flow-through exposure experiments were conducted at the Aquatic Toxicology Laboratory in St. Cloud, MN (Fig. S1). Previously published flow-through exposure protocols (Schoenfuss et al., 2008) were utilized and modified to reflect the three E1 concentrations, two temperature and two feeding regimes. Treatment groups consisted of two tanks with 10 to 12 mature (six-month-old) male fathead minnows per tank. Estrone concentrations (0, low, and high) were chosen to reflect environmental concentrations in surface waters as previously summarized from the literature (~20–120 ng/L, Fig. 1 of Dammann et al., 2011; Ankley et al., 2017). Based on the available literature, our low E1 treatment concentrations are found frequently in environments with anthropogenic inputs, while the high E1 treatment concentrations in this study reflect worst case environmental conditions, or may represent the totality of estrogenic activity measured in a body of water (often expressed as Estradiol Equivalency Quotient – EEQ; for example, Schultz et al., 2013). Each estrone treatment concentration (0, low and high) was delivered from a common mixing tank to assure that all aquaria in the same treatment received the same E1 concentration (Water flow diagram, SI Fig. 1). Estrone treatments were further replicated under two different temperatures (low ~18 °C and high ~26 °C) and feeding conditions (restricted to 0.75% body weight/d [25% ff] or full-fed at 3% body weight [ff]). On day 22 of the exposure, all fish were assessed for morphological characteristics (length, weight, body condition factor, secondary sex characteristics [SSC], hepatosomatic index [HSI] and gonadosomatic index [GSI]). Blood and tissue samples were collected for analysis of physiological biomarkers (hematocrit [HCT], blood glucose, plasma VTG and cortisol concentrations [0 and high E1 treatments only]) and histological endpoints.

2.2. Exposure chemicals

Estrone (Reference standard,Sigma-Aldrich, St. Louis, MO) exposure solutions were prepared daily and delivered as previously described (companion paper, Part 1.). Analysis of stock estrone solutions in ethanol diluted to ~100 pg/μL and analyzed by LCMSMS detected no estriol, 17α- or β-estradiol (detection limits 1 pg/ul on column). Water exchange rate was approximately seven exchanges/aquarium/d.

2.3. Water

2.3.1. Water quality

Water temperature was monitored continuously using a HOBO Data Logger (Onset Computer Corporation, Bourne, MA). Nominal temperatures were 18 °C and 26 °C, with measured mean low temperature for