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Compensatory response of fathead minnow larvae following a pulsed *in-situ* exposure to a seasonal agricultural runoff event

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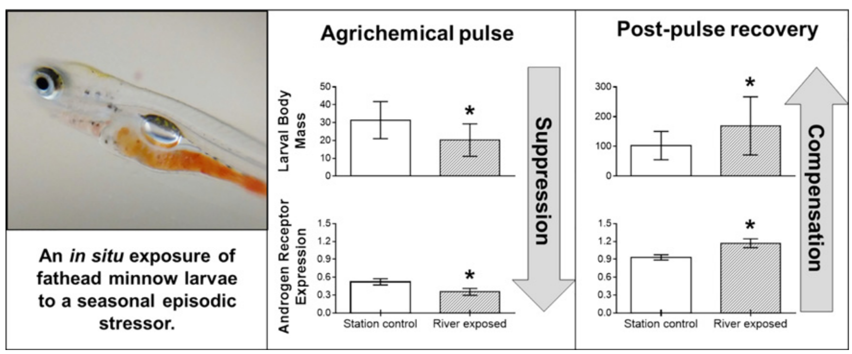
HIGHLIGHTS

- Fathead minnow larvae were maintained at the Elkhorn River Research Station for an *in-situ* exposure to a seasonally-occurring runoff.
- There was a 1.5– to 13-fold change in waterborne agrichemical contaminants including atrazine, acetochlor and metolachlor.
- Peaks in sediment contamination by agrichemicals was discordant with those of waterborne contaminants.
- Minnow larvae demonstrated compensation following reduction in size and androgenic gene expression by agrichemical exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Agriculturally-dominated waterways such as those found throughout the Midwestern United States often experience seasonal pulses of agrichemical contaminants which pose a potential hazard to aquatic organisms at varying life stages. The objective of this study was to characterize the developmental plasticity of fathead minnow larvae in a natural environment subject to a seasonal episodic perturbation in the form of a complex mixture of agricultural stressors. Fathead minnow larvae were maintained at the Elkhorn River Research Station for a 28-d *in situ* exposure to an agrichemical pulse event. Minnow larvae were sampled after 14 and 28 days to characterize developmental plasticity through growth measures and relative gene expression. Concentrations of agrichemical contaminants measured in water using polar organic chemical integrative samplers and composite sediment samples throughout the 28-d exposure were quantified using gas chromatography–mass spectrometry. Elevated concentrations of acetochlor, atrazine, and metolachlor were indicative of inputs from agricultural sources and were associated with reductions in body mass, condition factor, and androgenic gene expression in river exposed fathead minnow larvae. However, following a 14-d *in situ* depuration during the post-pulse period, river exposed larvae overcompensated in previously suppressed biological endpoints. These results indicate that fathead minnow larvae are capable of compensatory responses following episodic exposure to agrichemical stressors.

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1. Introduction

Successful development of any organism requires an accommodating environment. This is especially true for aquatic vertebrates such as fish that rely on a combination of environmental and genetic cues to regulate early life growth and ontogeny (Baroiller et al., 2009; Pittman et al. 2013). Diel and seasonal oscillations in abiotic factors such as temperature, dissolved oxygen and suspended solids can profoundly alter growth, metabolism and survival of fish larvae (Pérez-Domínguez et al. 2006; Pérez-Domínguez and Holt 2006; Shrimpton et al. 2007; Villamizar et al. 2012; Armstrong et al. 2013). Beyond growth and development, several abiotic factors are also recognized to influence sexual determination and differentiation in a wide variety of species (as reviewed by Devlin and Nagahama 2002). For example, exposure of West African cichlid larvae (*Pelvicachromis pulcher*) to slight acidic conditions (pH 5.5) during early development results in a female-biased sex ratio (Reddon and Hurd 2013). Although fish acclimate to natural variation in their native environments, the presence of anthropogenic stressors presents novel challenges for larval and juvenile fish.

Exposure to agricultural runoff is one such example of a widespread anthropogenic stressor that can influence larval fish. In many Midwestern streams, agrichemical concentration is seasonal with the highest concentrations occurring during the spring (Kolok et al. 2014). As a pollutant mixture, agricultural runoff contains fertilizers (Kaushal et al. 2011), pesticides (Schulz 2004; Vecchia et al. 2009; Lerch et al. 2011a), and veterinary pharmaceuticals (Kolok and Sellin 2008; Biswas et al. 2013; Jaimes-Correa et al. 2015) which move downstream as “pulses” that persist on an order of days to weeks depending on the hydrology of the affected watershed (Blann et al. 2009). Throughout the spring there will be a series of short-term pulsatile events which in composite will make up the overall spring pulse (Ali and Kolok 2015). The short-term peaks in agrichemicals tend to overlap with dramatic fluctuations in physicochemical parameters (*e.g.* temperature, dissolved oxygen, salinity, suspended solids) related to increased river discharge (Blann et al. 2009; Zhang et al. 2015). Early life stage fish may be particularly sensitive to the covariation among stressors, as larvae are balancing the metabolic demands of growth and organogenesis while simultaneously responding to their unpredictable surroundings.

In the Midwestern United States, agrichemical pulses follow precipitation events from May until July (Crawford 2001; Smiley et al. 2014; Zhang et al. 2015) making a predictable and natural setting for investigating their biological impacts. Indeed, field studies exposing fish to seasonally occurring agricultural runoff have documented endocrine disruption in the reproductive axis of otherwise intact adult females (Sellin et al. 2009; Sellin Jeffries et al. 2011a; Sellin Jeffries et al. 2011b; Knight et al. 2013; Ali and Kolok 2015; Zhang et al. 2015). Periods of elevated discharge and pesticide loads were associated with decreased expression of the steroid responsive genes vitellogenin (VTG), estrogen receptor subtype α (ERα) and androgen receptor (AR) (Knight et al. 2013; Ali and Kolok 2015; Zhang et al. 2015).

In larval fish, steroid receptors regulate gene expression, growth and organogenesis. Leet et al. (2012) found that fathead minnow larvae (*Pimphales promelas*) (0–45 days post fertilization), exposed to agricultural ditch water contaminated with pesticides and androgenic steroids under semi-natural conditions had increased body masses and a male biased sex ratio relative to lab water controls. More recently, Ali et al. (2016) reported that the brief *in situ* exposure of fathead minnow larvae, 5–12 days post hatch (dph), to an agrichemical pulse resulted in impaired growth and persistent suppression of the steroidogenic enzyme aromatase despite a 16 d recovery period in clean water.

Developmental plasticity presents a major challenge for understanding the response of larval fish towards episodic stressors. Plasticity allows the larvae to match its rate of development to oscillations within the environment (Pittman et al. 2013). Under adverse conditions development may be attenuated. When optimal conditions are restored, development may compensate, returning to a normal trajectory (Ali et al. 2003). Following a simulated cold front, red drum larvae (*Sciaenops ocellatus*) reared under diel thermocycles exhibited enhanced growth and feeding relative to larvae reared at constant temperatures (Pérez Dominguez et al. 2006). While thermal effects on developmental depression and compensation have been well studied, there is a paucity of literature that highlights the influence of weather-driven episodic exposure to physicochemical stressors from agricultural sources.

To date, there are very few studies that utilize larval fish for *in situ* exposures, and even fewer that investigate the impact of an episodic stressor like agricultural runoff. Furthermore, many of these studies only examine biological endpoints at a single time point after the exposure, an experimental design that fails to characterize how larval plasticity responds to intermittently polluted environments. The objective of this study is to characterize the developmental plasticity of fathead minnow larvae in a natural environment subject to a seasonal episodic perturbation in the form of a complex mixture of agricultural stressors. We hypothesized that 1) larval fish subjected to an agrichemical pulse under natural conditions would experience down regulations in endocrine function and growth immediately following exposure, and 2) the exposed larvae would show partial or complete compensation in endocrine function and growth following a recovery period in the field. To assess this, fathead minnow larvae were maintained at the Elkhorn River Research Station over the course of an agricultural runoff event. Larvae were assessed for changes in endocrine responsive gene markers and growth after 14 and 28 d following the start of the exposure and compared to controls maintained in clean water at the Elkhorn River Research Station.

2. Materials and methods

2.1. Animal production and maintenance

Fathead minnow larvae (*Pimphales promelas*) used for this experiment were obtained from the Animal Culture Unit at the University of Nebraska at Omaha, Omaha, NE. All procedures were conducted in compliance with protocols approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee (Protocol #98-075-110). All fish were maintained in dechlorinated tap water at 25 ± 1.0 °C. Beginning on April 15, 2015, breeding triads of adult fathead minnows were established consisting of one male and two females. Fish were housed in 30 L aquaria, divided into two compartments by a plastic, porous divider with two triads in each aquarium. Breeding triads were provided with a breeding tile (12-cm-long sections of polyvinyl chloride tubing 8 cm in diameter split in half lengthwise) on which eggs were laid.

After the triads began to breed, the tiles with eggs were removed daily from the aquaria and transferred to 1 L aerated beakers. Unfertilized and fungus infected eggs were removed daily, and surviving embryos all hatched by 5 days post fertilization. Upon hatching, the larvae were transferred to 1 L beakers at a density of 100 larvae per liter. A daily static renewal of the water within these beakers was conducted replacing approximately 80% of the total volume. All larvae were fed daily with a mixture of newly hatched (<24 h old) *Artemia nauplii* (INVE Aquaculture, Salt Lake City, UT).

2.2. Exposure at the Elkhorn River Research Station

Exposure of all fathead minnow larvae was conducted at the Elkhorn River Research Station (ERRS) where previous field studies have identified biological impacts and changes in endocrine responsive gene expression following *in situ* exposure to agricultural runoff (Knight et al. 2013; Ali and Kolok 2015; Zhang et al. 2015; Ali et al. 2016). The ERRS is an open-air facility located approximately 10 km upstream from the confluence of the Elkhorn and Platte Rivers, Nebraska, USA. The station