2017

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

Jonathan M. Ali
*University of Nebraska Medical Center*

John Sangster
*University of Nebraska - Lincoln*, john.sangster@unl.edu

Daniel D. Snow
*University of Nebraska-Lincoln*, dsnow1@unl.edu

Shannon L. Bartelt-Hunt
*University of Nebraska-Lincoln*, sbartelt2@unl.edu

Alan S. Kolok
*University of Nebraska at Omaha*, akolok@unomaha.edu

Science of the Total Environment 603–604 (2017) 817–826

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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

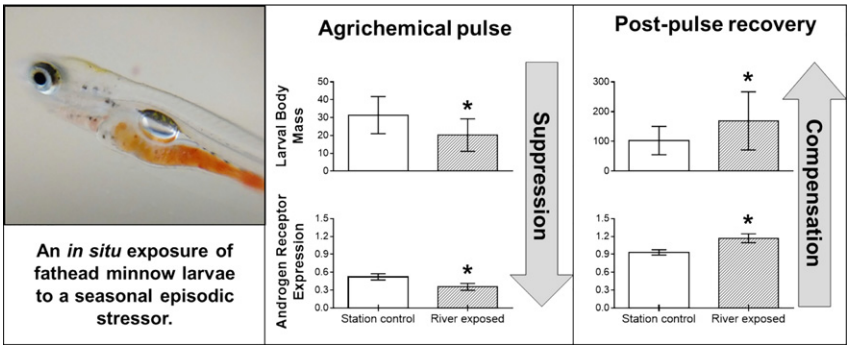
Jonathan M. Ali [a], Jodi L. Sangster [b,c], Daniel D. Snow [d], Shannon L. Bartelt-Hunt [b], Alan S. Kolok [a,e,*]

[a] Department of Environmental, Agricultural and Occupational Health, University of Nebraska - Medical Center, Omaha, NE 68198-6805, United States
[b] Department of Civil Engineering, University of Nebraska-Lincoln, Peter Kiewit Institute, Omaha, NE 68182-0178, United States
[c] USDA-ARS, U.S. Salinity Laboratory, Riverside, CA 92507-4617, United States
[d] Nebraska Water Center and School of Natural Resources, University of Nebraska-Lincoln, Lincoln, NE 68583-0844, United States
[e] Department of Biology, University of Nebraska at Omaha, Omaha, NE 68182-0040, United States

## 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




## ARTICLE INFO

*Article history:*
Received 23 January 2017
Received in revised form 9 March 2017
Accepted 9 March 2017
Available online 3 April 2017

Editor: Henner Hollert

*Keywords:*
Agricultural runoff
Fathead minnow larvae
Gene expression
*In situ* exposure
Pesticides
Compensation

## 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.



* Corresponding author at: Department of Biology, University of Nebraska at Omaha, 6001 Dodge St, Omaha, NE 68182-0040, United States.
*E-mail address:* akolok@unomaha.edu (A.S. Kolok).

http://dx.doi.org/10.1016/j.scitotenv.2017.03.093
0048-9697/

## 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

is equipped to pump water continuously from the Elkhorn River into stainless steel mesocosms capable of supporting aquatic organisms (Ali and Kolok 2015; Zhang et al. 2015; Ali et al. 2016).

The sampling regime for water, sediment and biological samples is outlined in Fig. 1A. Biological sampling consisted of a subset of fish taken 14-d after the start of the exposure (Exposure 1) to evaluate the immediate impact of the pulse, while the remaining fish were sampled 14 d after the pulse had subsided to evaluate their post-pulse recovery (Exposure 2). Passive samplers (POCIS) were deployed over three distinct 14-d windows (*i.e.* pre-pulse, pulse and post-pulse) (see Section 2.3 Water and sediment sampling) to evaluate changes in aqueous agrichemical contamination. Finally, weekly sediment samples (S) were collected across the larval fish exposure to quantify the overall contribution of sediment-associated agrichemicals to the *in-situ* exposure.

The start of the agrichemical pulse was determined using atrazine test strips (Abraxis, Warminster, PA) as described in previous studies within the Elkhorn River watershed (Knight et al. 2013; Ali and Kolok 2015; Zhang et al. 2015). Briefly, beginning April 15, 2015 atrazine test strips were used to test whether concentrations of atrazine in surface water in the Elkhorn River were above or below 3 $\mu g \cdot L^{-1}$. These test strips have been demonstrated to accurately detect the presence of atrazine and other triazine herbicides at 3 $\mu g \cdot L^{-1}$ in surface water (US EPA 2004), with further confirmation of there accuracy by previously collected field data (Knight et al. 2013; Ali and Kolok 2015). Tests were conducted every three to four days where the detection of three consecutive positive atrazine strips determined the start of the spring agrichemical pulse on May 8, 2015 (Fig. 1B). After the deployment of fathead minnow larvae atrazine strip tests were conducted weekly and following rainfall events.

Fathead minnow larvae, 5 days post-hatch (dph) were deployed from May 8 until June 5, 2015 and sampled at the end of Exposure 1 (19 dph) and Exposure 2 (33 dph) (Fig. 1A). During the exposure, larvae treatment groups were maintained at the ERRS in 3 L, half-crescent

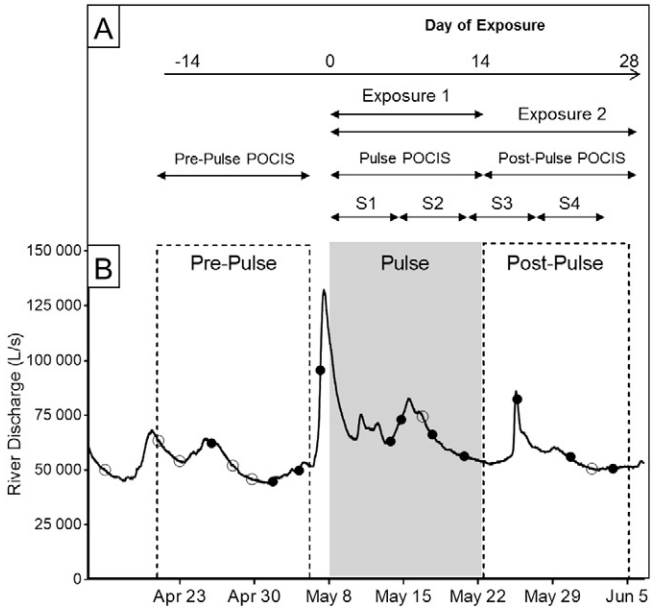



**Fig. 1.** Experimental design (A) for exposure of fathead minnow larvae at the Elkhorn River Research Station above corresponding temporal changes in Elkhorn River discharge (B). Exposure periods for fathead minnow larvae (Exposures 1 and 2), water sampling periods by polar organic chemical integrative samplers (POCIS), and sediment collection periods indicated by labelled lines. The temporal changes in discharge based on data collected from the USGS Elkhorn River (06800500) gaging station at Waterloo, Nebraska, USA. Detection of the agrichemical pulse conducted using atrazine test strips (see Materials and methods). Positive atrazine test strip results are shown as solid circles, and negative test results are represented as open circles. Pre-pulse, pulse and post-pulse periods indicated by boxes.

shaped, stainless steel chambers designed to fit inside the 10 L mesocosms (Ali et al. 2016). A portion of the 3 L chamber was made of 86 μm stainless steel mesh, allowing for river water to flow freely between the mesocosm and the larval chamber, without the loss of larvae. The 10 L mesocosms are maintained within a larger insulation tank (16.5 L) that circulates river water to maintain ambient water temperature of the Elkhorn River.

Two treatment groups, station control and river exposed, were maintained in separate mesocosms at the ERRS for a 28-day exposure (5–33 dph). One hundred larvae were transferred into each mesocosm at the start of the exposure. Station control larvae were transferred to a mesocosm at the ERRS that was supplied with dechlorinated tap water replacing the total volume by 80% daily. River exposed larvae were placed in a mesocosm at the ERRS that received continuous water flow directly from the Elkhorn River. Both treatment groups were fed, *ad libitum*, newly hatched *A. nauplii* once daily. Both station controls and river exposed mesocosms were placed within insulating tubs that circulated Elkhorn River water outside of the exposure chamber to maintain similar water temperatures. Additionally, both mesocosms were supplied with air-stones to control for differences in dissolved oxygen between treatment groups.

At the end of Exposure 1 and Exposure 2, larvae were transferred to aerated coolers containing water from their respective mesocosms and transported to the laboratory. Larvae from each treatment group at the end of Exposure 1 ($n = 35$–36) and Exposure 2 ($n = 20$) were euthanized using a lethal dose of MS-222. Body mass and length were collected from both age groups for morphometric analysis. Whole larvae were flash frozen in liquid nitrogen then stored at −80 °C for analysis of gene expression by RT-qPCR.

### 2.3. Water and sediment sampling

Water quality measurements including temperature (°C), pH, conductivity ($\mu S \cdot cm^{-1}$), dissolved oxygen ($mg \cdot L^{-1}$) and suspended solids ($mg \cdot L^{-1}$) in both station control and river exposed mesocosms were taken daily. Sampling for aqueous pesticides was conducted using polar organic chemical integrative samplers (POCIS) (Environmental Sampling Technologies, St. Joseph, MO, USA). Prior to deployment, all POCIS were soaked in 2 L Nanopure water (19.7 MΩ) for 24 h. A single POCIS was deployed within the receiving 10-L mesocosm for each of the three sampling periods; a Pre-pulse (April 21–May 5), Pulse (May 8–22), and Post-pulse (May 22–June 5). A laboratory blank was maintained in dechlorinated laboratory tap water for 14 d to evaluate the laboratory water used for the station control mesocosm. At the end of deployment POCIS were stored separately at −20 °C until further analysis.

Suspended solids carried in the Elkhorn River accumulated as shoals of sediment in the bottom of the larval mesocosms over the course of the 28-d exposure. Sediment was collected weekly from these shoals for physical and chemical analysis. A composite sample was collected weekly using glass jars and frozen at −20 °C for pesticide analysis by the Water Sciences Laboratory at University of Nebraska Lincoln (described below). The remaining bulk sample was stored at room temperature for approximately 48 h allowing suspended solids to settle before removing excess water. The remaining sediment was dried at 100 °C until a constant mass was reached (12–24 h). Dry sediment samples were analyzed using standard methods to determine texture (Gee and Or 2002) and total organic carbon (TOC) (Islam and Weil, 1998).

### 2.4. Water and sediment chemistry

Sediment and POCIS samples were stored at -20 °C. Sediments were processed using microwave assisted solvent extraction (MASE) and extracts were analyzed using gas chromatography–mass spectrometry (GC/MS) Zhang et al. (2015). POCIS were extracted according to previously published protocols Sellin et al. (2009). Reference compounds

and high purity solvents (Optima, Fisher Scientific) were obtained from Thermofisher (St. Louis, MO) or Sigma-Aldrich (St. Louis, MO). Labelled internal standards $^{13}C_3$-atrazine, $^{13}C_3$-deethylatrazine, and $^{13}C_3$-deisopropylatrazine were obtained from Cambridge Isotopes (Tewksbury, MA). A complete list of target compounds is included in Supplemental Table 1. Surrogate compounds, terbuthylazine and butachlor, were added at the beginning of sample processing to help quantify losses of chemically similar target compounds, while internal standards were added near the end of processing for calibration of the instrument response for each compound as described below.

A suite of pesticides was selected for measurement due to regional application practices and their regular detection during the spring pulse in previous studies within the Elkhorn River watershed (Ali and Kolok 2015; Zhang et al. 2015). Included in this suite of pesticides were atrazine, acetochlor, metolachlor all of which are consistently detected within spring to early summer runoff (Lerch et al. 2011a; Lerch et al. 2011b; Knight et al. 2013; Ali and Kolok 2015; Fairbairn et al. 2016).

Extraction of POCIS followed published protocols Alvarez et al. (2004), Kolok et al. (2014) and Sellin et al. (2009). Briefly, samplers were rinsed with reagent water, disassembled, membranes separated and sorbent material quantitatively transferred using ~10 mL of methanol to glass chromatography columns packed with a small plug of glass wool. Target compounds are then slowly eluted from the POCIS sorbent using 50 mL of a 1:9 mixture of methanol and ethyl acetate into glass evaporation tubes (RapidVap, Labconco, Kansas City, MO). The extract was spiked with 2000 ng of surrogate compounds (terbuthylazine and butachlor), evaporated by heating and vortexing under a nitrogen stream at 50 °C until 2–3 mL solvent remained. The concentrated extract was transferred to a glass culture tube and mixed with anhydrous sodium sulfate to remove residual water. The dried extract was quantitatively transferred to a second culture tube with additional ethyl acetate, spiked with 5000 ng labelled internal standards and evaporated to approximately 100 μL and then transferred to a 2 mL autosampler vial fitted with a 300 μL silanized glass insert using ~200 μL of ethyl acetate. Converting the instrument detection limits (IDL = 3 s) based on the variability of the lowest standard (250 ng·mL$^{-1}$), the overall detection limits in the POCIS are conservatively estimated to be near 20 picograms (pg) on column corresponds to 5 ng recovered from the POCIS using a 250 μL final extract volume. Surrogate recovery averaged 52 ± 26% in POCIS extracts. A fortified blank was prepared by spiking 1000 ng of analyte into the evaporation tube and analyzed as a sample. Target compound recovery averaged 77 ± 40%. A laboratory reagent blank containing only surrogate and internal standard compounds contained no analyte above the estimated detection limit.

Sediment samples were extracted using MASE techniques with a MARS XPress (CEM Corporation, Matthews, NC) microwave system. Briefly, 5.0 g of thawed sediment was accurately weighed into a 10 mL Teflon™ microwave tube, mixed with 6 mL of acetonitrile and spiked with 400 ng of surrogate compounds. Samples were mixed by vortexing, and then microwaved at 800 W temperature ramped to 90 °C over 10 min and held at 90 °C for 5 min. After cooling to room temperature and allowing particles to settle, the acetonitrile was transferred to glass evaporation tubes (RapidVap N2, Labconco, Kansas City, KS). An additional 10 mL of acetonitrile was added to the sediment to effect quantitative transfer and mixed by vortexing for 30 s. After settling, the second portion of acetonitrile combined in the evaporation tube and concentrated under nitrogen at 45 °C until ~1–2 mL of extract remained. The concentrated extract was transferred to a glass culture tube, spiked with 1000 ng labelled internal standard, any residual water removed by pipetting, and then dried with anhydrous sodium sulfate. The extracted sample was then transferred to a second culture tube using ethyl acetate, evaporated to 100 μL, and then transferred to a 2 mL autosampler vial fitted with a 300 μL silanized glass insert using ~200 μL of ethyl acetate. Method detection limits (MDL) were determined by extraction of 8 replicates of sand spiked at 4 ng·g$^{-1}$ (Supplemental Table 1).

All extracts were analyzed on an Agilent 5973 GC/MS outfitted with a Leap CombiPAL autosampler with split-less injection using a Restek (Bellefonte, PA) Rtx-1, 30 m × 0.25 mm ID and 0.25 μm film thickness capillary column. Oven temperature was programed to run at 80 °C for 0.75 min, ramp to 170 °C at 40 °C·min$^{-1}$, ramp to 236 °C at 2.5 °C·min$^{-1}$, ramp to 275 °C at 40 °C·min$^{-1}$ and hold for 9.62 min. The injection port temperature was 250 °C and the transfer line interface temperature was 280 °C. Retention times, quantifying ions, and instrument detection limits determined from repeated analysis of the lowest standard are included in Supplemental Table 1. $^{13}C_3$-atrazine was used as the internal standard for all compounds except for DEA and DIA which used their respective labelled analogues.

### 2.5. Relative gene expression analysis

Whole larvae bodies were used for reverse transcription quantitative polymerase chain reaction (RT-qPCR). RNA extraction utilized the SV Total RNA Isolation System (Promega, Sunnyvale, CA, USA) following manufacturer's recommendations. RNA was resuspended and stored in nuclease-free water at −80 °C until analysis. Purity and concentration of RNA were assessed by Nanodrop (NanoDrop Technologies, Wilmington, DE, USA) based on optical densities at 260 nm/280 nm and 260 nm/230 nm. Total extracted RNA samples were diluted to 15 ng/μL in preparation for cDNA synthesis. First-strand cDNA synthesis was performed using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, California, USA) per the manufacturer's recommendations. All PCR reactions were performed on the CFX Connect Real-Time PCR Detection System using the iTaq Universal SYBR® Green Supermix, 2× concentration, (Bio-Rad) per the manufacturer's protocol. Briefly, 2 μL of diluted cDNA template was added to 20 μM forward and reverse primers in a 15-μL volume containing iTaq Supermix. Target genes involved in steroid signaling and synthesis were selected for analysis (primer sequences and their sources are provided in Supplemental Table 2), these included: androgen receptor (AR), insulin-like growth factor 1 (IGF1), 17β-hydroxysteroid dehydrogenase (HSD17B), gonadal aromatase (CYP19A), doublesex and mab-3 related transcription factor 1 (DMRT1) and estrogen receptor subtype 1 (ERα). The reference gene ribosomal protein (RPL8) was used to normalize gene expression (Kolok et al. 2007). All reaction efficiencies were between 85 and 110%.

### 2.6. Statistical analysis

Data were analyzed using JMP 11 software (SAS, Cary, NC, USA). Morphometric data for Exposure 1 ($n$ = 35–36) and Exposure 2 ($n$ = 35–36) larvae were compared between treatment groups using $t$-test. Comparison of relative gene expression between station control and river exposed larvae from Exposure 1 ($n$ = 10–12) and Exposure 2 ($n$ = 16–20) was also conducted using $t$-test. Welch's $t$-test was used when the assumption of equal variances between treatment groups was not satisfied as determined by Bartlett's test. Statistical significance was assumed at $p < 0.05$.

## 3. Results

### 3.1. Water quality and chemistry

The 2015 spring pulse, as determined by atrazine testing, began in early May and coincided with an approximately 2.5-fold increase in Elkhorn River discharge (Fig. 1). Over the course of the 28-d sampling period there was a major discharge event that peaked on May 8, followed by two lesser peaks in river discharge on May 15 and May 26. There was considerable fluctuation in the levels of suspended solids being transported within the Elkhorn River which coincided with periods of elevated discharge.

Within the mesocosms, fathead minnow larvae experienced variation in water quality parameters based on water source (*i.e.* laboratory

or river water). Average values (±SD) for water quality parameters in the river exposed mesocosm are summarized in Table 1. Station control larvae experienced less variation in water pH (7.52 ± 0.32), conductivity (457.1 ± 39.8 μS·cm$^{-1}$), and suspended solids (240.1 ± 184.4 mg·L$^{-1}$) than did the river exposed fish, but larvae in both groups were exposed to natural diel oscillations in water temperature (Fig. 2). Changes in dissolved oxygen levels of station control larvae decreased from 7.78 (±1.15) mg·L$^{-1}$ over the pulse period to 5.86 (±0.95) mg·L$^{-1}$ as temperature increased over the post-pulse period which were comparable to variations measured in the river exposed mesocosm (Table 1).

Analytical chemistry data collected from POCIS confirmed the elevated agrichemical concentrations during the 2015 pulse relative to pre- and post-pulse periods (Table 1). Relative to the pre- and post-periods there was a >5-fold increase in total pesticide concentrations during the period between May 8 through May 22. Atrazine, acetochlor and metolachlor were consistently the most abundant herbicides measured in POCIS before, during and after the agrichemical pulse. Other pesticides analyzed but not detected in POCIS samplers included alachlor, butylate, chlorthalonil, cyanazine, deisopropylatrazine (DIA), s-ethyl-dipropylthiocarbamate (EPTC), norflurazon, pendamethalin, permethrin, prometon, propachlor, simazine, telfluthrin, trifluralin.

### 3.2. Sediment characteristics and chemistry

Physical analysis of the suspended sediment deposited in the larval mesocosm showed greater accumulation of sediment following high flow events with a relatively consistent composition over the 28-d period (Table 2). Two to four times more sediment was accumulated in the river water mesocosm in the first two weeks relative to the second half of the exposure. As would be expected, during weeks with reduced river discharge there was a reduction in sand fraction and an increase in the silt fraction. The percentage of clay and TOC remained stable throughout the entire exposure.

The herbicides atrazine, acetochlor, deethylatrazine and metolachlor were detected in sediment samples collected over the course of the 28-d exposure (Table 2). The highest concentrations of sediment-associated herbicides occurred during the third sampling period (S3) following the pulse period. This increase in sediment associated agrichemicals was discordant with the peak in agrichemicals observed in the water chemistry during the two-week pulse period (Table 1). Other pesticides analyzed but not detected in sediment samples included alachlor, butylate, chlorthalonil, cyanazine, DIA, dimethenamid, EPTC, metribuzin, pendamethalin, permethrin, prometon, propachlor, propazine, simazine, telfluthrin, trifluralin.

### 3.3. Biological effects

Immediately following exposure to the Elkhorn River agrichemical pulse there were differences in growth between station control and river exposed larvae at 19 dph that were reversed by 33 dph (Fig. 3). At the end of Exposure 1 (19 dph), river exposed larvae had a reduced body mass ($t$-test, df = 70; $p < 0.001$) and condition factor ($t$-test, df = 69; $p < 0.001$) relative to station controls. No significant difference in larval body length was detected at 19 dph. Conversely, by the end of Exposure 2 (33 dph) the river exposed larvae were significantly larger than station controls in terms of body mass ($t$-test, Welch's correction, df = 27.52; $p = 0.011$), length ($t$-test, df = 38; $p = 0.038$) and condition factor ($t$-test, df = 38; $p = 0.001$). By the end of the 28-d exposure there was 25% and 8% mortality of station control and river exposed larvae, respectively.

When compared to station controls, river exposed fathead minnow larvae showed significant changes in the whole-body expression of endocrine responsive genes following the pulse (Exposure 1) and post-pulse (Exposure 2) exposures (Fig. 4). At the end of Exposure 1 (5–19 dph), larvae had an upregulation of IGF1 ($t$-test, Welch's correction df = 22; $p = 0.013$) and a downregulation of AR ($t$-test, df = 21; $p = 0.044$) (Table 3). No difference was detected in the expression of CYP19A, DMRT1, ERα and HSD17B at the end of Exposure 1 (Table 3).

At the end of Exposure 2 (5–33 dph), river exposed larvae maintained the upregulation of IGF1 relative to controls at 33 dph (Fig. 4; $t$-test, df = 34; $p < 0.001$). However, by the end of Exposure 2 the river exposed fathead minnow larvae experienced a significant upregulation of AR expression relative to station controls ($t$-test, df = 34; $p < 0.0076$). No differences were detected in the expression of the steroidogenic genes HSD17B and CYP19A, as well as genes DMRT1 and ERα (Table 3).

## 4. Discussion

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. It was hypothesized that one of the short-term agricultural pulses would elicit a down regulation in endocrine gene expression and growth, and that post- pulse the larvae would compensate. Larvae exposed to a 14-d pulse showed suppressed endocrine gene expression and growth. Their response to the pulse induced suppression was an over compensation in both growth and endocrine function.

**Table 1**
Water quality and chemical analysis of river water flowing into the larval mesocosm during the exposure period. Nanograms (ng) of pesticides in extracts from polar organic chemical integrative samplers (POCIS) deployed across the 2015 agrichemical pulse. Analytical detection limits were determined at <5.0 ng in POCIS.

| | April 21–May 5 (Pre-pulse)[a,b] | May 8- May 22 (Pulse) | May 22–June 5 (Post-pulse) | Fold change (Pre-pulse to pulse) | Fold change (Pulse to post-pulse) |
|---|---|---|---|---|---|
| Temperature (°C) | – | 16.70 (2.05) | 20.00 (2.08) | – | 0.8 |
| Dissolved $O_2$ (mg·L$^{-1}$) | – | 8.42 (1.37) | 5.75 (0.73) | – | 1.5 |
| pH | – | 8.23 (0.16) | 8.32 (0.09) | – | 1.0 |
| Conductivity (μS·cm$^{-1}$) | – | 530.0 (78.0) | 584.0 (24.0) | – | 0.9 |
| Suspended Solids (mg·L$^{-1}$) | – | 5426.7 (3376.2) | 4460.7 (4178.5) | – | 1.2 |
| Acetochlor | 244.2 | 1775.1 | 134.6 | 7.3 | 13.2 |
| Atrazine | 229.4 | 1517.4 | 339.9 | 6.6 | 4.5 |
| Dimethenamid | 75.4 | 136.1 | 20.9 | 1.8 | 6.5 |
| Metolachlor | 197.9 | 709.7 | 133.9 | 3.6 | 5.3 |
| Deethylatrazine (DEA) | 20.2 | 34.5 | 24.5 | 1.7 | 1.4 |
| Propazine | 3.6 | 23.2 | 4.3 | 6.4 | 5.4 |
| Simazine | 10.0 | 5.3 | 6.5 | 0.5 | 0.8 |
| Metribuzin | < 5.0 | 14.3 | < 5.0 | – | |

[a] '–a' indicates not measured.
[b] <5.0 - below estimated detection limits.

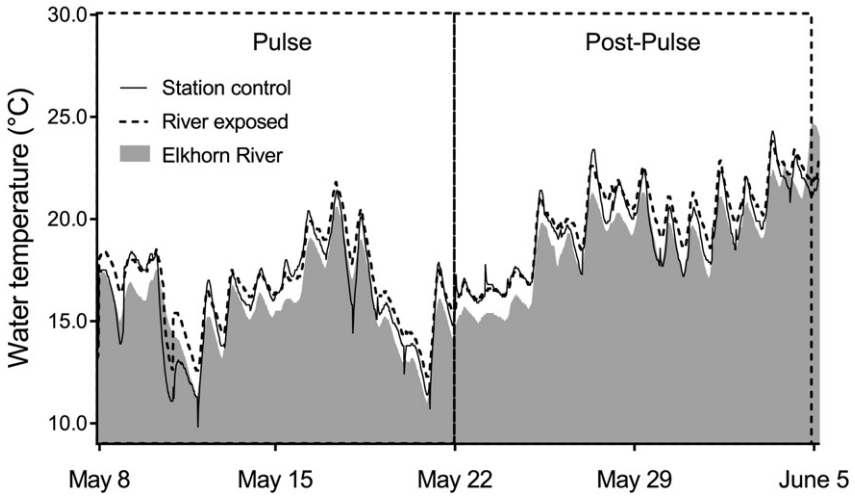



**Fig. 2.** Oscillation in water temperature over the exposure period. Water temperature of the Elkhorn River (solid grey) based on data collected from the USGS Elkhorn River (06800500) gaging Station at Waterloo, Nebraska, USA. Temperature for station control (solid line) and river exposed (dotted line) mesocosms were collected at 30 min intervals using HOBO® data loggers (Bourne, MA).

### *4.1. Agrichemical runoff in the Elkhorn River*

Although the sampling time periods for the POCIS were different than that for the sediment (Fig. 1), both illustrate short term changes in the chemical environment that larvae were exposed to. Changes in aqueous pesticide concentrations observed in this study are consistent with previously published differences in agrichemical concentrations during a discharge event relative to post-discharge. Knight et al. (2013) used POCIS samplers to compare agrichemical concentrations during and after a discharge event. The discharge event led to 1.6 to 28-fold increases in herbicide concentrations relative to post-discharge. Similarly, the present study observed increases (up to 13-fold) in atrazine, acetochlor and metolachlor (Table 2). These three herbicides represent a signature combination of pollutants found in runoff from corn and soybean production (Lerch et al. 2011a; Lerch et al. 2011b; Ali and Kolok 2015; Zhang et al. 2015; Fairbairn et al. 2016). The nearly identical concentration of herbicides before and after the discharge event highlights the ephemeral occurrence of waterborne agrichemicals in this watershed.

As seen in previous studies (Ali and Kolok 2015; Zhang et al. 2015), increases in river discharge were accompanied with increases in waterborne agrichemicals as well as the mobilization of sediments and other suspended solids carrying their own pesticide burden. Chemical analysis of accumulated suspended sediments revealed a mismatch between the peaks of pesticides in water and sediment over the 28-d sampling period (Table 2; Table 3). Specifically, the highest concentrations of waterborne pesticides coincided with the major discharge event whereas sediment-associated pesticide concentrations were the greatest during a lesser spike in river discharge during the post-pulse period, S3 (Fig. 1; Table 3). One plausible explanation for observed differences in aqueous and sediment-associated pesticide concentrations is that a fraction of dissolved pesticides entering the Elkhorn River during the pulse period partitioned into the sediment which was later mobilized as suspended solids during the lesser discharge event (S3). The role of sediments in the kinetics of agrichemicals in the Elkhorn River has been previously documented (Kolok et al. 2014; Zhang et al. 2015).

The aquatic environments of the pulse and post-pulse periods were distinct from one another not only in their pesticide profiles but in the fluctuations of several physicochemical parameters that can affect aquatic biota. This was evident by measured differences in conductance, dissolved oxygen levels, and accumulated mass of suspended solids between the pulse and post-pulse periods (Table 1; Table 2). All of these abiotic factors varied relative to river discharge (Kolok et al. 2014; Kjelland et al. 2015). Biological responses of fish living in this environment would be expected to be a function of these changing physicochemical parameters, as well as diurnal and seasonal (vernal) changes in temperature (Table 1) and photoperiod, (Clark et al. 2005; Blanco-Vives et al. 2011; Ali and Kolok 2015) regardless of the presence of any agrichemicals in the water. This underscores the importance of *in situ* studies for understanding the impacts of natural, composite exposures, as these are events that are not readily simulated under laboratory conditions.

**Table 2**
Physical and pesticide analysis of sediments obtained from within the larval mesocosm over the course of the 2015 field season.

| | | Pulse | | Post-pulse | |
|---|---|---|---|---|---|
| Collection date | | May 13 (S1) | May 20 (S2) | May 27 (S3) | June 3 (S4) |
| Mass (g) | | 2551.27 | 1140.95 | 631.57 | 403.70 |
| Texture [a] | % sand | 10 | 3 | 3 | 8 |
| | % silt | 67 | 73 | 71 | 72 |
| | % clay | 23 | 24 | 26 | 20 |
| Total organic carbon (%) | | 0.18 | 0.21 | 0.23 | 0.15 |
| Pesticides ($ng \cdot g^{-1}$) [b] | | | | | |
| Acetochlor | | < 0.3 | < 0.3 | 3.92 | < 0.3 |
| Atrazine | | 7.32 | 7.50 | 8.74 | 3.12 |
| Deethylatrazine (DEA) | | < 1.4 | < 1.4 | 2.32 | < 1.4 |
| Metolachlor | | 1.49 | 2.94 | 3.47 | 1.09 |

[a] Using standard methods for sieve and hydrometer analysis.
[b] "<" below method detection limits ($ng \cdot g^{-1}$) see Supplemental Table 1.

### *4.2. Biological response of larvae to an episodic exposure*

The present study documented an interesting dynamic between the expression of AR and IGF1 in fathead minnow larvae following exposure to a seasonal discharge event. Specifically, this is of interest due to their endocrine interactions as well as their responsiveness to environmental stimuli, including endocrine disrupting agrichemicals.

At the molecular level, peptide and steroid hormones facilitate the integration of environmental stimuli with the regulatory mechanisms of multiple physiological systems (Bradshaw 2007; Pittman et al. 2013). The peptide hormone, IGF1, has endocrine, paracrine and autocrine activity which is readily upregulated or downregulated in response to environmental factors including changes in salinity, nutritional status, temperature and photoperiod (reviewed by Reinecke 2010). As environmental conditions become optimal for survival and development of an organism, growth will occur. Warmer water temperatures stimulate growth in rainbow trout (*Oncorhynchus*

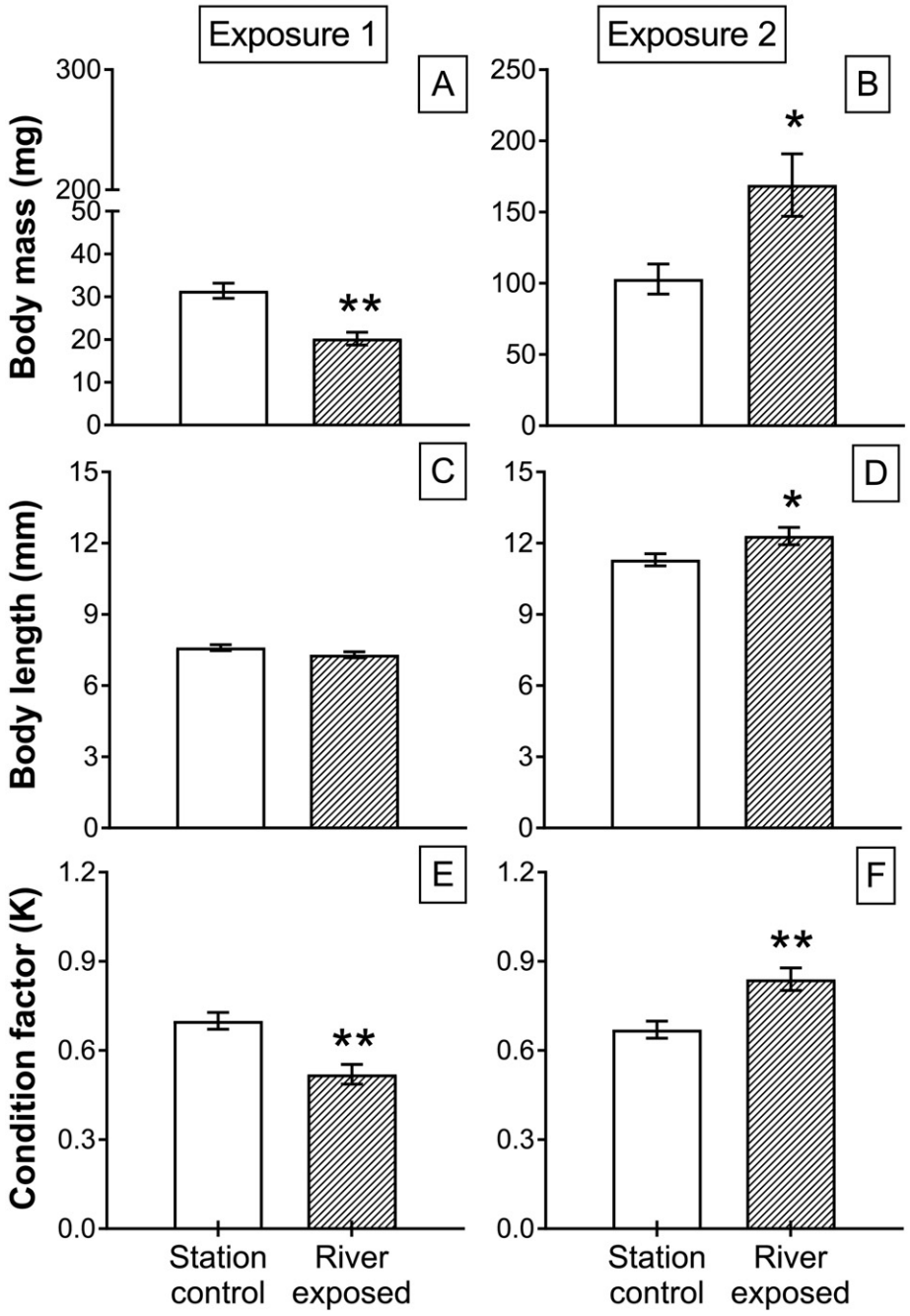



**Fig. 3.** Morphometric results for station control (empty bar) and river exposed (filled bar) fathead minnow larvae at the end of Exposures 1 and 2 (see Fig. 1 for description). Body mass (A), fork length (C) and condition factor (E) for station control and river exposed larvae following Exposure 1 (5–19 dph; $n = 35$–$36$) and body mass (B), fork length (D) and condition factor (F) following Exposure 2 (5–33 dph; $n = 20$). Values presented as mean (±SEM) with significant differences at $\alpha = 0.05$ and $\alpha = 0.01$ denoted by single and double asterisks, respectively.

*mykiss*) through the increased release of growth hormone and subsequent stimulation of IGF1 (Gabillard et al. 2003). The strong association between IGF1 and growth in bony fish has led to the application of IGF1 mRNA as a molecular marker of growth under a variety of environmental manipulations (Vera Cruz et al. 2006; Montserrat et al. 2007; Vera Cruz and Brown, 2009; Reinecke 2010; Picha et al. 2014). However, it has been demonstrated that nuclear receptors also modulate the expression of IGF1 following activation by steroid hormones (Riley et al. 2004; Johns et al., 2011; Norbeck and Sheridan 2011; Cleveland and Weber 2015). Immature coho salmon (*Oncorhynchus kisutch*) injected with 11-ketotestosterone or testosterone had a significant increase of circulating IGF1 protein one to two weeks following treatment (Larsen et al. 2004). More recently, analysis of hepatic gene expression in female rainbow trout injected with either 17β-estradiol or dihydrotestosterone demonstrated that androgens stimulate IGF1 expression whereas estrogens suppressed IGF1 expression (Cleveland and Weber 2015).

The link between IGF1 regulation and sex steroids in fish leaves their early life growth and development susceptible to endocrine disruption by steroidogenic contaminants, such as agrichemicals; however, effects may be confounded by environmental conditions. Sustained *in situ* exposure of juvenile fathead minnows to agricultural ditch water containing a mixture of androgens, estrogens and pesticides lead to increased body size along with male-biased sex ratios at the end of a 6-week study (Leet et al. 2012). During a subsequent 45-day laboratory study, an increase in the body mass and length of fathead minnows when exposed to a simulated mixture of agrichemicals was observed, but

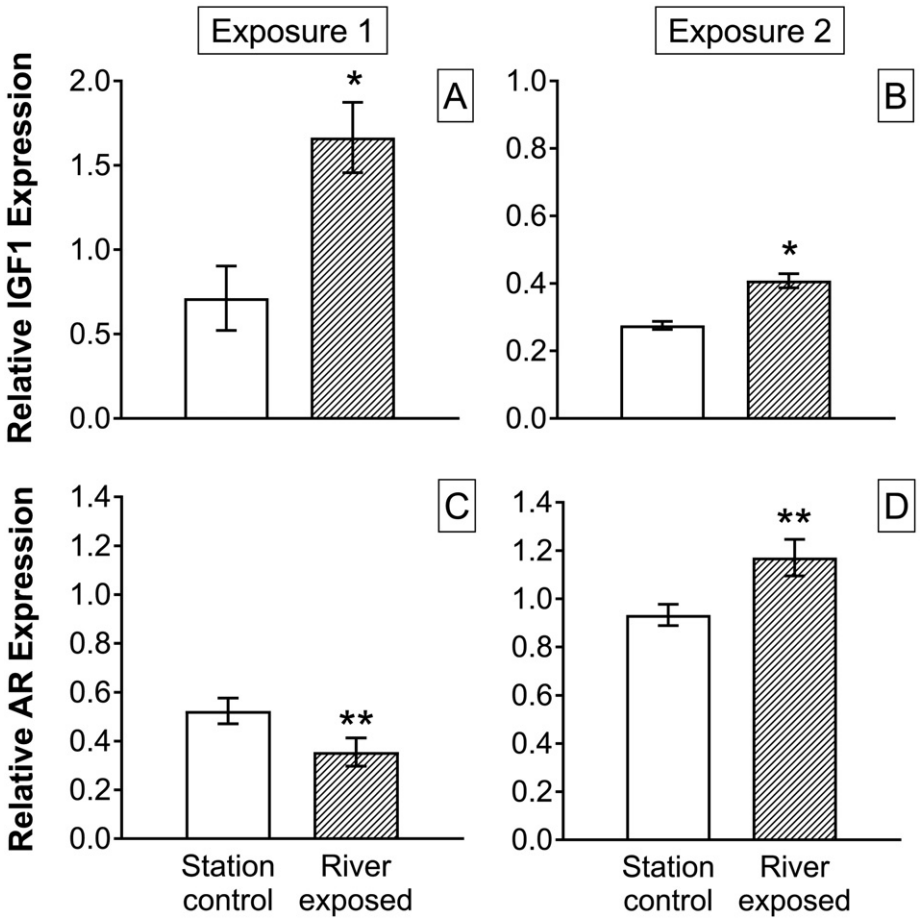



**Fig. 4.** Relative RNA expression in station control (empty bar) and river exposed (filled bar) fathead minnow larvae at the end of Exposures 1 and 2 (see Fig. 1 for description). Mean expression values (±SEM) for insulin-like growth factor 1 (IGF1) following Exposure 1 (A) and Exposure 2 (B). Mean expression values (±SEM) for androgen receptor (AR) following Exposure 1 (C) and Exposure 2 (D). Sample size of 10–12 per treatment group in Exposure 1 and $n = 16$–20 in Exposure 2. Significant differences at $\alpha = 0.05$ and $\alpha = 0.01$ denoted by single and double asterisks, respectively.

changes in steroid responsive genes at 20 dph or final sex ratio (Leet et al. 2015) was not detected. Leet et al. (2015) speculated that the disparity between outcomes from the *in situ* (Leet et al. 2012) and laboratory studies (Leet et al. 2015) was due to the absence of environmental factors such as spikes in temperature or sediment interactions which may contribute to the overall response of larval fish towards endocrine disrupting chemicals. In these studies, endpoint analysis demonstrated an effect of agrichemicals on growth and development, however they overlook the developmental response that occurs with the exposure.

The present study documents compensatory growth in fathead minnow larvae following exposure to a seasonal discharge event. Immediately after the major discharge event (Exposure 1; Fig. 1), river exposed larvae displayed suppressed growth and endocrine gene expression as determined by body mass and androgen receptor expression, respectively (Table 3; Figs. 3 and 4). This suppression of growth and endocrine function was reversed at the end of a depuration period following the main discharge event which allowed river exposed larvae to achieve greater growth and androgenic gene expression (*i.e.* masculinization), relative to station controls. Curiously, river exposed larvae maintained elevated IGF1 expression throughout the study which was discordant with the observed compensation in other biological endpoints. An explanation for this discrepancy is that induction of compensatory growth by the growth hormone-IGF1 axis is not directly mediated by IGF1 (Beckman 2011), rather the induction of compensatory growth is controlled by multiple endocrine and paracrine mechanisms (Won and Borski 2013). To our knowledge, this is the first study to characterize compensation of larval fish following an *in situ* episodic exposure to seasonally occurring agrichemical stressor.

Given that molecular defeminization has been consistently documented in adult fathead minnows following exposure to an agrichemical pulse (Knight et al. 2013; Ali and Kolok 2015; Zhang et al. 2015), our initial question was whether we would see a similar anti-estrogenic profile in larval fathead minnows. Both Knight et al. (2013) and Ali and Kolok (2015) found that the pulse-associated defeminization of adult fish was absent during post-pulse periods with reduced

**Table 3**
Summary of apical and molecular endpoints observed in river exposed larvae relative to their station control counterparts. Significant increases (↑) and decreases (↓) represented by arrows and no significant differences denoted by dash (−) ($\alpha = 0.05$).

| | Exposure 1 (Pulse, 19 dph) | Exposure 2 (Post-pulse, 33 dph) |
|---|---|---|
| Apical Endpoints | | |
| Body mass | ↓ | ↑ |
| Body length | – | ↑ |
| Condition factor (K) | ↓ | ↑ |
| Molecular Endpoints | | |
| Androgen receptor (AR) | ↓ | ↑ |
| Insulin-like growth factor 1 (IGF1) | ↑ | ↑ |
| Estrogen receptor α (ERα) | – | – |
| Doublesex mab-3 related transcription factor 1 (DMRT1) | – | – |
| Gonadal aromatase (CYP19A) | – | – |
| 17β-Hydroxysteroid dehydrogenase (HSD17B) | – | – |

"dph" – days post-hatch.

concentrations of agrichemicals, similar to the post-pulse concentrations seen in this study. In a subsequent study on the Elkhorn River, Zhang et al. (2015) found that agrichemical-laden sediments carried by pulse events were responsible for reductions in hepatic estrogen- and androgen-receptor expression in adult fish. The downregulation of androgen receptor expression in adult fish (Zhang et al. 2015) is consistent with the response observed in the present study immediately following the pulse. It is likely that the absence of defeminization of minnow larvae in the present study is due to either environmental or biological factors. While Zhang et al. (2015) confirmed that sediment was a major source of agrichemical exposure for adult fish, this may not hold true for larvae whose interactions with sediment and water may differ from adult fish. A biological explanation for the absence of defeminization is that the expression profile of estrogen receptors in fathead minnow, namely receptor subtypes α, β and γ, has been shown to vary over the course of ontogeny which would determine what type of response larvae and juvenile fish might have towards an anti-estrogenic mixture (Filby and Tyler 2007; Johns et al. 2011; Leet et al. 2013). Therefore, while subtle differences were observed between the responses of larvae from the present study and those observed in adult fish from previous studies there is evidence for a consistent response of fish towards agrichemical pulses. Future studies are needed to investigate the impacts of similar exposures on multiple levels of endocrine activity and the mechanisms by which compensatory responses occur.

## Acknowledgements

This project was partially supported by National Science Foundation grant award CBET-0966858 to ASK and CBET-0966850 to SLB. JMA was supported by the Robert G. Daugherty Water for Food Global Institute Graduate Assistantship Program, University of Nebraska. JLS was supported by the National Science Foundation Graduate Research Fellowship Program and CBET-0966850. Ashkan Banan and Mariah Rakestraw provided technical assistance for the project. The Elkhorn River Research Station is owned and operated by the Nebraska Watershed Network, Department of Biology, College of Arts and Sciences, University of Nebraska at Omaha.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.03.093.

## References

Ali, J.M., Farhat, Y.A., Kolok, A.S., 2016. Biological impacts in fathead minnow larvae following a 7-day exposure to agricultural runoff: a microcosm study. Bull. Environ. Contam. Toxicol. 96 (4):432–437. http://dx.doi.org/10.1007/s00128-016-1762-2.

Ali, J.M., Kolok, A.S., 2015. On-site, serial exposure of female fathead minnows to the Elkhorn River, Nebraska, USA, spring agrichemical pulse. Environ. Toxicol. Chem. 34 (6):1354–1361. http://dx.doi.org/10.1002/etc.2928.

Ali, M., Nicieza, A., Wootton, R.J., 2003. Compensatory growth in fishes: a response to growth depression. Fish Fish. 4 (2):147–190. http://dx.doi.org/10.1046/j.1467-2979.2003.00120.x.

Alvarez, D.A., Petty, J.D., Huckins, J.N., Jones-Lepp, T.L., Getting, D.T., Goddard, J.P., Manahan, S.E., 2004. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. Environ. Toxicol. Chem. 23 (7): 1640–1648. http://dx.doi.org/10.1897/03-603.

Armstrong, J.B., Schindler, D.E., Ruff, C.P., Brooks, G.T., Bentley, K.E., Torgersen, C.E., 2013. Diel horizontal migration in streams: juvenile fish exploit spatial heterogeneity in thermal and trophic resources. Ecology 94 (9):2066–2075. http://dx.doi.org/10.1890/12-1200.1.

Baroiller, J.F., D'Cotta, H., Saillant, E., 2009. Environmental effects on fish sex determination and differentiation. Sex. Dev. 3 (2–3):118–135. http://dx.doi.org/10.1159/000223077.

Beckman, B.R., 2011. Perspectives on concordant and discordant relations between insulin-like growth factor 1 (IGF1) and growth in fishes. Gen. Comp. Endocrinol. 170 (2):233–252. http://dx.doi.org/10.1016/j.ygcen.2010.08.009.

Biswas, S., Shapiro, C.A., Kranz, W.L., Mader, T.L., Shelton, D.P., Snow, D.D., ... Ensley, S., 2013. Current knowledge on the environmental fate, potential impact, and management of growth-promoting steroids used in the US beef cattle industry. J. Soil Water Conserv. 68 (4):325–336. http://dx.doi.org/10.2489/jswc.68.4.325.

Blanco-Vives, B., Aliaga-Guerrero, M., Cañavate, J.P., Muñoz-Cueto, J.A., Sánchez-Vázquez, F.J., 2011. Does lighting manipulation during incubation affect hatching rhythms and early development of sole? Chronobiol. Int. 28 (4):300–306. http://dx.doi.org/10.3109/07420528.2011.560316.

Blann, K.L., Anderson, J.L., Sands, G.R., Vondracek, B., 2009. Effects of agricultural drainage on aquatic ecosystems: a review. Crit. Rev. Environ. Sci. Technol. 39 (11):909–1001. http://dx.doi.org/10.1080/10643380801977966.

Bradshaw, D., 2007. Environmental endocrinology. Gen. Comp. Endocrinol. 152 (2–3): 125–141. http://dx.doi.org/10.1016/j.ygcen.2006.12.026.

Clark, R.W., Henderson-Arzapalo, A., Sullivan, C.V., 2005. Disparate effects of constant and annually-cycling daylength and water temperature on reproductive maturation of striped bass (*Morone saxatilis*). Aquaculture 249 (1–4):497–513. http://dx.doi.org/10.1016/j.aquaculture.2005.04.001.

Cleveland, B.M., Weber, G.M., 2015. Effects of sex steroids on expression of genes regulating growth-related mechanisms in rainbow trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. 216:103–115. http://dx.doi.org/10.1016/j.ygcen.2014.11.018.

Crawford, C.G., 2001. Factors affecting pesticide occurrence and transport in a large midwestern river basin. J. Am. Water Resour. Assoc. 37 (1), 1–15.

Devlin, R.H., Nagahama, Y., 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture 208 (3–4):191–364. http://dx.doi.org/10.1016/S0044-8486(02)00057-1.

Fairbairn, D.J., Karpuzcu, M.E., Arnold, W.A., Barber, B.L., Kaufenberg, E.F., Koskinen, W.C., ... Swackhamer, D.L., 2016. Sources and transport of contaminants of emerging concern: a two-year study of occurrence and spatiotemporal variation in a mixed land use watershed. Sci. Total Environ. 551–552:605–613. http://dx.doi.org/10.1016/j.scitotenv.2016.02.056.

Filby, A.L., Tyler, C.R., 2007. Cloning and characterization of cDNAs for hormones and/or receptors of growth hormone, insulin-like growth factor-I, thyroid hormone, and corticosteroid and the gender-, tissue-, and developmental-specific expression of their mRNA transcripts in fathead minnow (*Pimephales promelas*). Gen. Comp. Endocrinol. 150 (1):151–163. http://dx.doi.org/10.1016/j.ygcen.2006.07.014.

Gabillard, J., Weil, C., Rescan, P., Navarro, I., Gutiérrez, J., Le Bail, P., 2003. Effects of environmental temperature on IGF1, IGF2, and IGF type I receptor expression in rainbow trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. 133 (2):233–242. http://dx.doi.org/10.1016/S0016-6480(03)00167-9.

Gee, G.W., Or, D., 2002. 2.4 particle-size analysis. In: Dane, J.H., Topp, C.G. (Eds.), Methods of Soil Analysis: Part 4 Physical Methods, SSSA Book Ser. 5.4. SSSA, Madison, WI: pp. 255–293 http://dx.doi.org/10.2136/sssabookser5.4.c12.

Islam, K.R., Weil, R.R., 1998. A rapid microwave digestion method for colorimetric measurement of soil organic carbon. Commun. Soil Sci. Plant Anal. 29 (15–16), 2269–2284.

Jaimes-Correa, J.C., Snow, D.D., Bartelt-Hunt, S.L., 2015. Seasonal occurrence of antibiotics and a beta agonist in an agriculturally-intensive watershed. Environ. Pollut. 205: 87–96. http://dx.doi.org/10.1016/j.envpol.2015.05.023.

Johns, S.M., Denslow, N.D., Kane, M.D., Watanabe, K.H., Orlando, E.F., Sepúlveda, M.S., 2011. Effects of estrogens and antiestrogens on gene expression of fathead minnow (*Pimephales promelas*) early life stages. Environ. Toxicol. 26 (2):195–206. http://dx.doi.org/10.1002/tox.20545.

Kaushal, S.S., Groffman, P.M., Band, L.E., Elliott, E.M., Shields, C.A., Kendall, C., 2011. Tracking nonpoint source nitrogen pollution in human-impacted watersheds. Environ. Sci. Technol. 45 (19):8225–8232. http://dx.doi.org/10.1021/es200779e.

Kjelland, M.E., Woodley, C.M., Swannack, T.M., Smith, D.L., 2015. A review of the potential effects of suspended sediment on fishes: potential dredging-related physiological, behavioral, and transgenerational implications. Environ. Syst. Decis. 35 (3):334–350. http://dx.doi.org/10.1007/s10669-015-9557-2.

Knight, L.A., Christenson, M.K., Trease, A.J., Davis, P.H., Kolok, A.S., 2013. The spring runoff in Nebraska's (USA) Elkhorn River watershed and its impact on two sentinel organisms. Environ. Toxicol. Chem. 32 (7):1544–1551. http://dx.doi.org/10.1002/etc.2220.

Kolok, A.S., Sellin, M.K., 2008. The environmental impact of growth-promoting compounds employed by the United States beef cattle industry: history, current knowledge, and future directions. Rev. Environ. Contam. Toxicol. 195, 1–30.

Kolok, A.S., Sellin Jeffries, M.K., Knight, L., Snow, D.D., Bartelt-Hunt, S.L., 2014. The hourglass: a conceptual framework for the transport of biologically active compounds from agricultural landscapes. American Water Resources Association]–>J. Am. Water Resour. Assoc. 50 (2):266–274. http://dx.doi.org/10.1111/jawr.12158.

Kolok, A.S., Snow, D.D., Kohno, S., Sellin, M.K., Guillette Jr., L.J., 2007. Occurrence and biological effect of exogenous steroids in the Elkhorn River, Nebraska, USA. Sci. Total Environ. 388 (1–3):104–115. http://dx.doi.org/10.1016/j.scitotenv.2007.08.001.

Larsen, D.A., Shimizu, M., Cooper, K.A., Swanson, P., Dickhoff, W.W., 2004. Androgen effects on plasma GH, IGF-I, and 41-kDa IGFBP in coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 139 (1):29–37. http://dx.doi.org/10.1016/j.ygcen.2004.07.007.

Leet, J.K., Lee, L.S., Gall, H.E., Goforth, R.R., Sassman, S., Gordon, D.A., ... Sepúlveda, M.S., 2012. Assessing impacts of land-applied manure from concentrated animal feeding operations on fish populations and communities. Environ. Sci. Technol. 46 (24): 13440–13447. http://dx.doi.org/10.1021/es302599t.

Leet, J.K., Lesteberg, K.E., Schoenfuss, H.L., Olmstead, A.W., Amberg, J.J., Ankley, G.T., Sepúlveda, M.S., 2013. Sex-specific gonadal and gene expression changes throughout development in fathead minnow. Sex. Dev. 7 (6):303–307. http://dx.doi.org/10.1159/000353877.

Leet, J.K., Sassman, S., Amberg, J.J., Olmstead, A.W., Lee, L.S., Ankley, G.T., Sepúlveda, M.S., 2015. Environmental hormones and their impacts on sex differentiation in fathead minnows. Aquat. Toxicol. 158:98–107. http://dx.doi.org/10.1016/j.aquatox.2014.10.022.

Lerch, R.N., Sadler, E.J., Baffaut, C., Kitchen, N.R., Sudduth, K.A., 2011a. Herbicide transport in Goodwater creek experimental watershed: II. Long-term research on acetochlor, alachlor, metolachlor, and metribuzin. J. Am. Water Resour. Assoc. 47 (2):224–238. http://dx.doi.org/10.1111/j.1752-1688.2010.00504.x.

Lerch, R.N., Sadler, E.J., Sudduth, K.A., Baffaut, C., Kitchen, N.R., 2011b. Herbicide transport in Goodwater creek experimental watershed: I. Long-term research on atrazine. J. Am. Water Resour. Assoc. 47 (2):209–223. http://dx.doi.org/10.1111/j.1752-1688.2010.00503.x.

Montserrat, N., Gabillard, J.C., Capilla, E., Navarro, M.I., Gutiérrez, J., 2007. Role of insulin, insulin-like growth factors, and muscle regulatory factors in the compensatory growth of the trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. 150 (3): 462–472. http://dx.doi.org/10.1016/j.ygcen.2006.11.009.

Norbeck, L.A., Sheridan, M.A., 2011. An in vitro model for evaluating peripheral regulation of growth in fish: effects of 17β-estradiol and testosterone on the expression of growth hormone receptors, insulin-like growth factors, and insulin-like growth factor type 1 receptors in rainbow trout (*Oncorhynchus mykiss*). Gen. Comp. Endocrinol. 173 (2):270–280. http://dx.doi.org/10.1016/j.ygcen.2011.06.009.

Pérez-Domínguez, R., Holt, G.J., 2006. Interrenal and thyroid development in red drum (*Sciaenops ocellatus*): effects of nursery environment on larval growth and cortisol concentration during settlement. Gen. Comp. Endocrinol. 146 (2):108–118. http://dx.doi.org/10.1016/j.ygcen.2005.10.006.

Pérez-Domínguez, R., Holt, S.A., Holt, G.J., 2006. Environmental variability in seagrass meadows: effects of nursery environment cycles on growth and survival in larval red drum *Sciaenops ocellatus*. Mar. Ecol. Prog. Ser. 321:41–53. http://dx.doi.org/10.3354/meps321041.

Picha, M.E., Biga, P.R., Galt, N., McGinty, A.S., Gross, K., Hedgpeth, V.S., ... Borski, R.J., 2014. Overcompensation of circulating and local insulin-like growth factor-1 during catch-up growth in hybrid striped bass (morone chrysops × *Morone saxatilis*) following temperature and feeding manipulations. Aquaculture 428–429:174–183. http://dx.doi.org/10.1016/j.aquaculture.2014.02.028.

Pittman, K., Yúfera, M., Pavlidis, M., Geffen, A.J., Koven, W., Ribeiro, L., ... Tandler, A., 2013. Fantastically plastic: fish larvae equipped for a new world. Rev. Aquac. 5 (Suppl. 1): S224–S267. http://dx.doi.org/10.1111/raq.12034.

Reddon, A.R., Hurd, P.L., 2013. Water pH during early development influences sex ratio and male morph in a west african cichlid fish, *Pelvicachromis pulcher*. Zoology 116 (3):139–143. http://dx.doi.org/10.1016/j.zool.2012.11.001.

Reinecke, M., 2010. Influences of the environment on the endocrine and paracrine fish growth hormone-insulin-like growth factor-I system. J. Fish Biol. 76 (6):1233–1254. http://dx.doi.org/10.1111/j.1095-8649.2010.02605.x.

Riley, L.G., Hirano, T., Grau, E.G., 2004. Estradiol-17β and dihydrotestosterone differentially regulate vitellogenin and insulin-like growth factor-I production in primary hepatocytes of the tilapia *Oreochromis mossambicus*. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. 138 (2):177–186. http://dx.doi.org/10.1016/j.cca.2004.07.009.

Schulz, R., 2004. Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution: a review. J. Environ. Qual. 33 (2), 419–448.

Sellin Jeffries, M.K., Abbott, K.I., Cowman, T., Kolok, A.S., 2011a. Occurrence and endocrine effects of agrichemicals in a small Nebraska, USA, watershed. Environ. Toxicol. Chem. 30 (10):2253–2260. http://dx.doi.org/10.1002/etc.615.

Sellin Jeffries, M.K., Conoan, N.H., Cox, M.B., Sangster, J.L., Balsiger, H.A., Bridges, A.A., ... Kolok, A.S., 2011b. The anti-estrogenic activity of sediments from agriculturally intense watersheds: assessment using in vivo and in vitro assays. Aquat. Toxicol. 105 (1–2):189–198. http://dx.doi.org/10.1016/j.aquatox.2011.04.008.

Sellin, M.K., Snow, D.D., Schwarz, M., Carter, B.J., Kolok, A.S., 2009. Agrichemicals in Nebraska, USA, watersheds: occurrence and endocrine effects. Environ. Toxicol. Chem. 28 (11):2443–2448. http://dx.doi.org/10.1897/09-135.1.

Shrimpton, J.M., Zydlewski, J.D., Heath, J.W., 2007. Effect of daily oscillation in temperature and increased suspended sediment on growth and smolting in juvenile Chinook salmon, *Oncorhynchus tshawytscha*. Aquaculture 273 (2–3):269–276. http://dx.doi.org/10.1016/j.aquaculture.2007.10.009.

Smiley, P.C., King, K.W., Fausey, N.R., 2014. Annual and seasonal differences in pesticide mixtures within channelized agricultural headwater streams in central Ohio. Agric. Ecosyst. Environ. 193:83–95. http://dx.doi.org/10.1016/j.agee.2014.04.022.

US EPA, 2004. Silver Lake Research Corp. Watersafe® Pesticide Test. Environmental Technology Verification Report. https://archive.epa.gov/nrmrl/archive-etv/web/pdf/01_vr_silverlake.pdf.

Vecchia, A.V., Gilliom, R.J., Sullivan, D.J., Lorenz, D.L., Martin, J.D., 2009. Trends in concentrations and use of agricultural herbicides for corn belt rivers, 1996–2006. Environ. Sci. Technol. 43 (24):9096–9102. http://dx.doi.org/10.1021/es902122j.

Vera Cruz, E.M., Brown, C.L., 2009. Influence of the photoperiod on growth rate and insulin-like growth factor-I gene expression in nile tilapia *Oreochromis niloticus*. J. Fish Biol. 75 (1):130–141. http://dx.doi.org/10.1111/j.1095-8649.2009.02271.x.

Vera Cruz, E.M., Brown, C.L., Luckenbach, J.A., Picha, M.E., Bolivar, R.B., Borski, R.J., 2006. Insulin-like growth factor-I cDNA cloning, gene expression and potential use as a growth rate indicator in nile tilapia, *Oreochromis niloticus*. Aquaculture 251 (2–4): 585–595. http://dx.doi.org/10.1016/j.aquaculture.2005.06.039.

Villamizar, N., Ribas, L., Piferrer, F., Vera, L.M., Sánchez-Vázquez, F.J., 2012. Impact of daily thermocycles on hatching rhythms, larval performance and sex differentiation of zebrafish. PLoS One 7 (12). http://dx.doi.org/10.1371/journal.pone.0052153.

Won, E.T., Borski, R.J., 2013. Endocrine regulation of compensatory growth in fis. Front. Endocrinol. 4 (JUL). http://dx.doi.org/10.3389/fendo.2013.00074.

Zhang, Y., Krysl, R.G., Ali, J.M., Snow, D.D., Bartelt-Hunt, S.L., Kolok, A.S., 2015. Impact of sediment on agrichemical fate and bioavailability to adult female fathead minnows: a field study. Environ. Sci. Technol. 49 (15):9037–9047. http://dx.doi.org/10.1021/acs.est.5b01464.

**Supplemental Table 1.** Pesticide compounds (include CAS# and molecular weight) measured in POCIS and sediment samples together with instrumental parameters, instrument detection limits (average IDL=20 pg) estimated from repeated injections of a 250 $pg \cdot uL^{-1}$ calibration standard. Sediment method detection limits ($MDL=t_{n-1}s$) were estimated using the standard deviation of 8 replicate analyses of 5 gram of clean matrix fortified at 4 $ng \cdot g^{-1}$. Recoveries = 100 x (measured/fortified). "NM" indicates not measured.

| Compound | CAS# | Molecular Weight ($g \cdot mol^{-1}$) | Quantitation Ion ($m \cdot z^{-1}$) | Retention Time (min) | IDL (pg) | MDL Sediment ($ng \cdot g^{-1}$) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| Acetochlor | 34256-82-1 | 269.767 | 146 | 11.09 | 8.2 | 0.3 | 57.2 |
| Alachlor | 15972-60-8 | 269.767 | 160 | 11.47 | 6.6 | 1.6 | 34.5 |
| Atrazine | 1912-24-9 | 215.68 | 200 | 8.42 | 16.5 | 0.3 | 99.9 |
| Butylate | 2008-41-5 | 217.37 | 146 | 5.14 | 25.5 | 2.8 | 62.6 |
| Chlorthalonil | 1897-45-6 | 265.91 | 266 | 9.40 | 5.9 | 0.9 | 51.1 |
| Cyanazine | 21725-46-2 | 240.69 | 212 | 12.51 | 12.8 | 1.0 | 68.0 |
| Deethylatrazine (DEA) | 6190-65-4 | 187.63 | 172 | 7.17 | 29.3 | 2.3 | 114 |
| Deisopropylatrazine (DIA) | 1007-28-9 | 173.60 | 158 | 7.00 | 26.7 | 1.4 | 81.3 |
| Dimethenamid | 87674-68-8 | 275.79 | 154 | 10.84 | 2.9 | 3.2 | 103 |
| EPTC | 759-94-4 | 189.32 | 128 | 4.59 | 15.9 | 4.8 | 44.2 |
| Metolachlor | 51218-45-2 | 282.80 | 162 | 13.09 | 22.0 | 0.3 | 64.8 |
| Metribuzin | 21087-64-9 | 214.29 | 198 | 10.45 | 9.2 | 7.2 | 105 |
| Norflurazon | 27314-13-2 | 303.67 | 303 | 21.51 | 9.6 | NM | NM |
| Pendamethalin | 40487-42-1 | 281.31 | 252 | 14.68 | 28.5 | 3.1 | 128 |
| Permethrin | 52645-53-1 | 391.29 | 183 | 30.97 | 80.6 | 4.3 | 115 |
| Prometon | 1610-18-0 | 225.29 | 210 | 8.34 | 8.4 | 2.1 | 83.0 |
| Propachlor | 1918-16-7 | 211.69 | 120 | 6.71 | 91.1 | 0.6 | 70.4 |
| Propazine | 139-40-2 | 229.71 | 214 | 8.57 | 7.7 | 0.1 | 35.1 |
| Simazine | 122-34-9 | 201.66 | 201 | 8.20 | 3.9 | 0.7 | 120 |
| Telfluthrin | 79538-32-2 | 418.74 | 177 | 10.07 | 5.4 | 0.3 | 67.6 |
| Trifluralin | 40487-42-1 | 303.67 | 306 | 7.67 | 5.8 | 0.3 | 98.2 |

**Supplemental Table 2**. Primers used for real-time polymerase chain reaction analysis with respective annealing temperature and NCBI ascension number for each gene.

| Gene | Forward sequence 5´- 3´ | Reverse sequence 5´- 3´ | Temp (°C) | Ascension Number |
|---|---|---|---|---|
| AR[a] | GTTTCCGTAACCTGCATGTGG | CGCGCATTAGCGTTCTTGTA | 60.9 | AY727529 |
| CYP19A[b] | GCGGCTCCAGATACTC | ACTCTCCAGAATGTTTAACC | 55.0 | AJ277867 |
| ERα[a] | AGTGAGCAGTCAAGCCGTGTT | GGTCAGGTGGCATGCATAAAG | 63.0 | AY727528 |
| DMRT1[c] | AGGTCGTGGGTGATGTGAAT | GGCCACTGCAGAGCTTAGAG | 65.0 | DT303249.1 |
| HSD17B[d] | ACAGCCAGCCGTAGAC | TCCTAAAGCCAGTGATGAC | 62.6 | DT161033 |
| IGF1[e] | GGCAAAACTCCACGATCCCTA | ATGTCCAGATATAGGTTTCTTTGCTG | 61.4 | AY533140 |
| RPL8[a] | GCCCATGTCAAGCACAGAAAA | ACGGAAAACCACCTTAGCCAG | 59.2 | AY919670 |

[a] Kolok et al. 2007, [b] Wood et al. 2015, [c] Leet et al. 2013, [d] Filby et al. 2006, [e] Beggel et al. 2011

**References**

Beggel, S., Connon, R., Werner, I., & Geist, J. (2011). Changes in gene transcription and whole organism responses in larval fathead minnow (*Pimephales promelas*) following short-term exposure to the synthetic pyrethroid bifenthrin. Aquat. Toxicol.105(1–2), 180–188. doi:10.1016/j.aquatox.2011.06.004

Davis, L. K., Visitacion, N., Riley, L. G., Hiramatsu, N., Sullivan, C. V., Hirano, T., & Grau, E. G. (2009). Effects of o,p'-DDE, heptachlor, and 17β-estradiol on vitellogenin gene expression and the growth hormone/insulin-like growth factor-I axis in the tilapia, *Oreochromis mossambicus*. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol., 149(4), 507–514. doi:10.1016/j.cbpc.2008.11.007

Filby, A. L., Thorp, K. L., & Tyler, C. R. (2006). Multiple molecular effect pathways of an environmental oestrogen in fish. J. Mol. Endocrinol. 37(1), 121–134. doi:10.1677/jme.1.01997

Leet, J. K., Gall, H. E., & Sepúlveda, M. S. (2011). A review of studies on androgen and estrogen exposure in fish early life stages: effects on gene and hormonal control of sexual differentiation. J. Appl. Toxicol. 31(5), 379–398. doi:10.1002/jat.1682

Wood, R. K., Seidel, J. S., & Martyniuk, C. J. (2015). Transcripts involved in steroid biosynthesis and steroid receptor signaling are expressed early in development in the fathead minnow (*Pimephales promelas*). Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol., 182, 64–-72. doi:10.1016/j.cbpb.2014.12.003