# Swimming performance and larval quality are altered by rearing substrate at early life phases in white sturgeon, *Acipenser transmontanus* (Richardson, 1836)

By D. W. Baker[1], D. S. O. McAdam[2], M. Boucher[3], K. T. Huynh[4] and C. J. Brauner[4]

[1]ICSS, VIU, Nanaimo, BC, Canada; [2]MOE, University of British Columbia, Vancouver, BC, Canada; [3]Freshwater Fisheries Society of BC, Abbotsford, BC, Canada; [4]Department of Zoology, University of British Columbia, Vancouver, BC, Canada

## Summary

To investigate the role of substrate enrichment on larval growth and performance, white sturgeon, *Acipenser transmontanus,* were reared for 12 dph (the pre-feeding stage) in the presence and absence of enriched substrates (i.e. structurally complex media). Following this period, larval sturgeon were transferred to holding tanks with unenriched substrate (lacking structural complexity) and reared for an additional 30 days, during which time health and performance indicators (growth, $U_{crit}$, startle response reaction time) and whole body lipid composition were assessed at 15 and 18°C. Sturgeon reared on unenriched substrates tended to grow more slowly (up to 40% reduced mass at 40 dph) with a lower condition factor (5–15% lower between 8 and 40 dph), but also exhibited delayed gut development and reduced rate of yolksac absorption (at 15 dph) than those reared with enriched substrates. Whole body lipid composition was significantly altered with substrate enrichment, although the biological relevance of these changes is unknown. White sturgeon reared without exposure to enriched substrates at some temperatures and developmental phases demonstrated modest reductions in aerobic (~20–30% lower $U_{crit}$) and startle response performance (~5–10% slower reaction time) at 15 and 30 dph. Overall, most effects were influenced by rearing temperatures and parentage, such that differences were not statistically significant under all conditions. Clearly, however, substrate enrichment plays an important role in development of white sturgeon during early life stages.

## Introduction

White sturgeon, *Acipenser transmontanus*, are native to three major watersheds in western North America (Columbia, Sacramento and Fraser River watersheds). Most Canadian populations are endangered (COSEWIC, 2012) and four of six Canadian populations are listed under the Species at Risk Act (SARA), with the Kootenay River population also listed under the US Endangered Species Act. Three populations in Canada are undergoing chronic recruitment failure and are currently sustained by hatchery inputs. While causes are uncertain, recruitment failure likely results from increased early life phase mortality, which may be caused by altered characteristics of river substrates (McAdam et al., 2005; Paragamian et al., 2009). Changes in rearing substrate (e.g. increased fine substrates in rearing gravels) have been shown to have a negative impact on development of salmonids (Peterson and Martin-Robichaud, 1995; Kihslinger and Nevitt, 2006) and other species (e.g. robust redhorse, *Moxostoma robustum*; Jennings et al., 2010). While the specific mechanisms involved in larval effects are poorly understood, they are highly relevant to understanding habitat-recruitment relationships in fish in general, but white sturgeon in particular.

Altering substrate enrichment (i.e. structural complexity, Batzina et al., 2014) can affect larval survival and quality in white sturgeon as early as in the yolksac stages. Substrate change has been linked with decreased wild recruitment for the Kootenay (Paragamian et al., 2009) and Nechako (McAdam et al., 2005) populations, and manipulative field studies show effects on larval retention, survival and growth (McAdam, 2012; Crossman and Hildebrand, 2014). Laboratory studies confirm this, where the degree of substrate enrichment affects not only growth and survival, but also whole animal energetics as inferred from changes in metabolic scope throughout larval white sturgeon development (Boucher, 2012; Boucher et al., 2014). As reductions in larval growth may also lead to indirect effects on larval condition that diminish survival (Anderson, 1988; Gessner et al., 2009; Boucher, 2012), determining the source of reduced larval condition has important implications for evaluation of the quality of both captive and wild larvae. For example, differentiating whether the mortality of feeding larvae is the result of prior conditions (e.g. substrate effects on yolksac larvae) or subsequent conditions (e.g. food availability effects on feeding larvae) may be critical for directing population recovery efforts, due to the substantial differences in habitat utilization between these two stages.

Negative effects associated with substrate enrichment on physiological performance (such as swimming or escape behaviours) in early life phase sturgeon may manifest before the effects on growth and survival. Previous research has revealed effects of substrate enrichment on larval behaviour (hiding, Gessner et al., 2009; McAdam, 2011; predator avoidance, Gadomski and Parsley, 2005; McAdam, 2011). Swimming performance or predator avoidance responses may be vital – for example, the transition to exogenous feeding increases requirements for prey capture and predator avoidance. When fish are pushed to their physical limits (i.e. exercise), the respiratory, cardiovascular and metabolic pathways

are all forced to operate maximally, and any compromise in physiological performance may be revealed (e.g. as premature fatigue, Randall and Brauner, 1991; Brauner et al., 1994; Jain et al., 1998). In addition to earlier detection, faster swimming and more rapid escape behaviours can be assumed to benefit fish, while the relationship between growth and survival can be complex (Anderson, 1988; Fuiman, 2002). Consequently, maximal sustainable aerobic swimming speed ($U_{crit}$) and startle response reaction time (SRRT, also referred to as escape latency or alarm responsiveness) can be indicative diagnostic tools (Suboski et al., 1990; Domenici et al., 2007; Nendick et al., 2009), and provide valuable proxies for larval quality.

Another avenue for assessing larval condition is through analysis of fatty acid composition. Fatty acids (FA) provide essential molecular precursors, which support energetic demands, superior growth, and developmental requirements of larvae, and FA composition has been evaluated previously for a variety of species as an indicator of larval quality (see Ferron and Leggett, 1993). Deficiencies in some fatty acids have been shown to reduce larval growth, performance and survival in a number of fish species (Navarro and Sargent, 1992; Koven et al., 2001; Tocher, 2003). Also, improved larval escape performance in larvae with higher arachidonic acid (ARA) levels (Fuiman and Ojanguren, 2011) indicates potentially important links between FA composition and survival.

The goal of this study was to investigate the effects of rearing yolksac larvae on enriched substrates from 0 to 12 days post-hatch (dph), on growth and performance to 40 days post-hatch. We measured persistent and carry-over effects on growth and physiological performance ($U_{crit}$ and SRRT) at two temperatures, 15°C and 18°C, the latter close to an upper thermal maxima. We additionally assessed whole body fatty acid (FA) composition and yolksac and gut development. Based on prior observations (Boucher et al., 2014), we predicted that white sturgeon larvae reared with enriched substrates during the yolksac larvae phase would exhibit superior survival, growth and quality (as indicated by enhanced physiological performance) due to decreased stress or overall activity. Overall, this research provides insight into both substrate effects on recruitment, and the potential use of novel quality indicators (swimming performance, FA composition) to assess the condition of white sturgeon larvae.

## Materials and methods

### Animal husbandry

White sturgeon larvae utilized in this study were the progeny of wild caught broodstock derived from the transboundary reach of the Columbia River, spawned at the Kootenay Trout Hatchery in Wardner, BC in 2010 (*Series I*) and 2011 (*Series II*). Embryos were from two distinct genetic crosses, arbitrarily referred to as Family A and B for *Series I*, and Family C and D for *Series II*. Embryos were transferred from the hatchery to the University of British Columbia (UBC, Vancouver, BC) at approximately 6 days post-fertilization in chilled coolers via airfreight (4 h transit time).

In *Experimental Series I*, (June–August 2010) embryos were transferred to individually aerated, drip irrigated, plastic tubs (8-L) with no internal flow, supplied with biofiltered, temperature controlled, recirculating dechlorinated Vancouver City water. In *Experimental Series II* (June–August 2011), embryos and hatching larvae were held in 50-L glass aquaria supplied with static dechlorinated freshwater, with water quality maintained by regular water exchange with de-chlorinated water (~50%) once (1–11 dph) or twice (12–40 dph) daily. In both cases, embryos and larvae were exposed to a photoperiod of 12:12 h light:dark and held at either 15 (±1°C) or 18 (±1°C) (only *Series I*) temperatures. Upon initiating of feeding (12 dph), larvae were fed to satiation 3 times daily with a larval mash (crushed Skretting BioTrout pellets, supplemented with dry krill and Cyclopeeze®). Tanks were cleaned by siphon to remove uneaten food 1 h after each feeding.

In *Series 1*, larval sturgeon from the same families but reared at the Kootenay Trout Hatchery to either 14 dph (referred to as hatchery reared unenriched substrate 15 dph, or HUS15, see Table 1) or 29 dph (referred to as hatchery reared unenriched substrate 30 dph, or HUS30, see Table 1) were transported to UBC in thermostatted (through addition of high thermal inertia packs) coolers via airfreight (4 h transit time). These larval sturgeon were transferred to identical holding tanks within the recirculating system (in *Experimental series I*) or identical glass aquaria (in *Experimental Series II*) with other measured parameters (such as water flow, temperature, and diet) kept similar. The larvae transported to UBC at 15 dph were reared for a further 15 days in identical holding tanks (referred to as hatchery and lab reared unenriched substrate 30 dph, or HLUS30, see Table 1).

Food was withheld 12 h before maximum aerobic swimming performance testing ($U_{crit}$) and startle response assessment to avoid the metabolic effects of digestion. All holding and sampling procedures were approved by the University of British Columbia Animal Care Committee (protocol number A07-0080).

### Experimental protocols

**Experimental series I.** In this experimental series, we investigated the effect of substrate enrichment on mass, $U_{crit}$, startle response reaction time (SRRT) and whole animal FA ratios at two temperatures (15°C and 18°C). Following hatch, sturgeon larvae were transferred to identical tanks (20-L plastic aquaria) that included either unenriched substrate (US, lacking structural complexity) or enriched substrate (ES, having structural complexity). In *Series I*, US treatment consisted of a bare, plastic bottom, and the ES consisted of 100 submerged Bioballs™. Larvae from each of two genetically distinct families were reared at two temperatures (15 and 18°C) for each substrate condition and all family, temperature, and treatment combinations were replicated in duplicate. At 12 dph, substrate was removed from the ES treatment and larvae in all treatments were reared over unenriched substrate for the remainder of the experiment (to 30 dph). Groups were kept separately throughout the course of the

Table 1
Definitions of treatment groups for Series I and Series II

| Label | Definition | Series |
|---|---|---|
| ES | Enriched substrate, up to ~12 dph | Series I (Bioballs[TM])<br>Series II (Gravel) |
| US | Unenriched substrate | Series I (no added substrate)<br>Series II (sand) |
| HUS15 | Hatchery reared unenriched substrate, 15 dph | Series I |
| HUS30 | Hatchery reared unenriched substrate, 30 dph | Series I |
| HLUS30 | Hatchery and lab reared, unenriched substrate, 30 dph | Series I |

| | | Days post hatch (dph) | | | | | |
|---|---|---|---|---|---|---|---|
| Family | Treatment group | 4 | 7 | 11 | 15 | 25 | 29 |

*Series I*: Mass (mg) of white sturgeon larvae from Family A and B reared on enriched (ES) or unenriched (US) substrates at 15 and 18°C up to 29 days post hatch (dph)

| Family | Treatment group | 4 | 7 | 11 | 15 | 25 | 29 |
|---|---|---|---|---|---|---|---|
| 15°C | | | | | | | |
| A | US | $22.3 \pm 0.2$ | $26.9 \pm 0.5$ | $28.4 \pm 0.5$ | $29.3 \pm 0.4$ | $29.3 \pm 1.2$ | $29.4 \pm 0.9$ |
| A | ES | $23.2 \pm 0.4$ | $27.3 \pm 0.4$ | $29.4 \pm 0.3$ | $31.1 \pm 0.5$ | $31.8 \pm 1.0$ | $30.7 \pm 1.4$ |
| B | US | $24.0 \pm 0.5$ | $28.0 \pm 0.3$ | $31.0 \pm 0.3$ | $31.8 \pm 0.7$ | $28.1 \pm 0.9$ | $29.4 \pm 0.6$ |
| B | ES | $23.9 \pm 0.6$ | $28.5 \pm 0.2$ | $30.7 \pm 0.2$ | $33.2 \pm 0.8$ | $30.4 \pm 0.7$ | $28.0 \pm 0.7$ |
| 18°C | | | | | | | |
| A | US | $23.3 \pm 0.5$ | $28.2 \pm 0.4$ | $30.7 \pm 0.4$ | $31.8 \pm 0.6$ | $29.7 \pm 1.0$ | $31.7 \pm 1.4$ |
| A | ES | $23.7 \pm 0.6$ | $28.1 \pm 1.1$ | $30.9 \pm 0.4$ | $33.4 \pm 1.0$ | $30.9 \pm 1.5$ | $31.8 \pm 1.8$ |
| B | US | $25.9 \pm 0.1$ | $29.8 \pm 0.2$ | $33.9 \pm 0.5$ | $32.7 \pm 0.6$ | $33.2 \pm 1.8$ | $31.1 \pm 1.3$ |
| B | ES | $25.4 \pm 0.3$ | $30.5 \pm 0.2$ | $35.9 \pm 0.4$ | $34.4 \pm 0.7$ | $36.6 \pm 1.0$ | $29.7 \pm 1.2$ |
| 15°C | | | | | | | |
| A | HUS15, HLUS30[a] | n/a | n/a | n/a | $42.3 \pm 0.2$ | n/a | $68.7 \pm 0.2$ |
| B | HUS15, HLUS30[a] | n/a | n/a | n/a | $47.2 \pm 0.1$ | n/a | $83.5 \pm 2.1$ |
| A | HUS30[b] | n/a | n/a | n/a | n/a | n/a | $94.9 \pm 0.8$ |
| B | HUS30[b] | n/a | n/a | n/a | n/a | n/a | $111 \pm 3$ |

All sturgeon transferred to tanks with no enriched substrate at approx. 10–12 dph. Superscript 'a' = fish shipped to UBC at 14 dph and weighed at 15 dph and 29 dph. Superscript 'b' = fish shipped to UBC at 28 dph, and weighed at 29 dph. Values expressed as means ± SEM.

experiment (i.e. families, replicates, temperatures and substrate conditions remained separated).

White sturgeon larvae were weighed at 4, 7, 11, 15, 25, and 29 dph. Larvae were collected (n = 6 per treatment) into a 50 ml centrifuge tube and terminally euthanized in MS-222 (0.1 g $L^{-1}$, buffered with 0.2 g $L^{-1}$ NaHCO$_3$). Larvae were then gently blotted with paper towels and transferred to an ultra-fine scale (sensitivity ± 0.1 mg) to obtain mass measurements. Other larvae were frozen in liquid nitrogen for later analysis of lipid composition. Larvae initially reared at the Kootenay Trout hatchery were also assessed in this way at 15 (HUS15) and 30 (HUS30, HLUS30) dph (see Table 1).

**Maximal sustainable aerobic swimming speed (Ramped $U_{crit}$) protocol**

Swim trials were conducted in fresh water at 15 dph and 30 dph. Subjects were collected randomly from actively swimming fish. At each temperature, a total of 64 fish were challenged with ramped-critical swim tests (n = 8 per group; 2 substrates × 2 families × 2 replicates, with the exception of 30 dph sturgeon held at 18°C where survival was too low). Hatchery reared larval sturgeon (HUS15, HUS30, and HLUS30) were subjected to an identical protocol (n = 8 per family). Two Blazka-type (Blazka, 1960) swim tunnels (mini swim tunnel, Loligo Systems; www.loligosystem.com, 26.4 mm diameter, 100.0 mm length) were fitted with plastic flow-straighteners at both ends to facilitate laminar flow and separate fish from the propeller mechanism that produced the flow. Temperature was maintained at ±1°C of rearing temperature (i.e. 15 or 18°C) throughout the swim trials. Fish within each group were distributed equally between the two swim tunnels to avoid bias. An additional plastic mesh was added to the flow straighteners to prevent fish from seeking refuge within the holes of the grid.

Following transfer in water to the darkened swim flume, sturgeon were permitted 5 min to recover from handling before the initiation of any flow. Fish were then allowed 20–30 min to habituate to the tunnel environment at a low flow rate (~0.25 bl $s^{-1}$) before the ramping protocol began. Water velocity was increased by approx. 0.3 cm $s^{-1}$ every minute until fatigue. Fish were considered fatigued following a 10 s period where fish could not escape the down-stream grid, although the initial time that fish laterally contacted the grid was recorded. At fatigue, water flow was stopped and the fish was removed, euthanized with MS-222 (0.1 g $L^{-1}$, buffered with 0.2 g $L^{-1}$ NaHCO$_3$), patted dry, weighed, and the length measured. Fish were then placed in 1.5 ml bullet tubes, rapidly flash frozen in liquid nitrogen, and stored at −80°C for future analysis.

Water velocities (cm $s^{-1}$) were calculated from images obtained from high-speed photography (420 frames $s^{-1}$) of the movement of a coloured dye injected at the upstream

end of the swim tunnel and correlated with the voltage (V) supplied to the propeller motor. Each swim tunnel was calibrated independently; the voltage:water velocity relationships were similar between the two. There was no overall effect of swim tunnel selection (i.e. A vs B) on swim performance at either 15°C or 18°C.

### Startle response reaction time (SRRT) protocol

The SRRT was measured in actively swimming larval white sturgeon that were randomly captured (n = 10 per group; 2 substrates × 2 families × 2 replicates) from holding tanks with a beaker and transferred in water and allowed to recover for 1 h in a Petri dish (10 cm diameter filled with water) located on a well-lit temp. controlled wet table. Fish held at 18°C for 30 dph exhibited poor survival and thus were excluded from this test. To induce the startle response, a metal rod was used to strike the edge of the Petri dish during which a vertically positioned high-speed video camera (420 frames s$^{-1}$) recorded the event. Following this procedure, fish were terminally anaesthetized.

The SRRT was evaluated using image analysis software (ImageJ, version 1.4) by counting the number of frames between the initial strike of the metal rod on the Petri dish and the first visible movement of the head following stimulation of the startle response. Time between stimulus and response (i.e. delay time) was calculated based on the camera's frame capture rate and referred to as startle response reaction time (SRRT).

### Experimental series II.

In this experimental series, we investigated the effect of substrate enrichment on growth, condition factor and fatty acid composition. Following hatch, larval sturgeon were transferred to identical tanks that held either added sand (particulate size < 0.2 cm; treatment group = unenriched substrate, US) or larger gravel (roughly 1–2 cm diameter; treatment group = enriched substrate, ES) through 11 dph. Again, treatments were replicated twice (2 substrate × 2 families). At 12 dph, all added substrates were removed completely from all tanks, and larvae reared in an unenriched substrate environment (i.e. bare glass tank) from 12 to 40 dph. At 12 dph, feeding commenced with the same food and feeding protocol as described in *Series I*.

Sturgeon larvae (n = 10) were collected at 0, 1, 4, 7, 10, 12, 15, 25, 30 and 40 dph from each treatment group (i.e. US and ES), terminally euthanized in MS-222 (0.1 g L$^{-1}$, buffered with 0.2 g L$^{-1}$ NaHCO$_3$) and preserved in 4% paraformaldehyde (0.1 M phosphate buffer, pH = 7.4). Samples were then evaluated for wet weight and length.

### Yolksac and gut development analysis

At 15 dph, five larvae (n = 5) from each treatment in *Series II* were preserved in 4% para-formaldehyde and sent to Wax-it Histology Services Inc. (Vancouver, BC) for sectioning and staining. Longitudinal sections down the midline were stained using hematoxylin and eosin (H & E). Larval sections were evaluated based on the developmental state of their digestive tract: in particular, observations of the presence of a yolksac (indicating incomplete conversion to the gastric stomach) and development of the stomach lining (e.g. improved staining, increased mucosal folds and muscle) were recorded.

### Fatty acid analysis

Fatty acid analysis was performed on larvae from both *Series I* and *II*. In *Series I*, larvae were sampled at 15 (experimental groups and hatchery reared fish), 29 (experimental groups) and 34 dph (hatchery reared fish), while in *Series II*, all larval groups were sampled at 30 and 40 dph. To obtain fatty acid profiles and composition, randomly collected larvae were blotted dry, frozen in liquid nitrogen and stored at −80°C. On the day of the analysis, frozen larvae were homogenized using Tissue Tearor™ homogenizer (model 398; Mexico). Lipids were extracted according to Bligh and Dyer (1959), methylated in 6% HCl/methanol solution at 80°C for a minimum of two hours, and a further extraction was done in hexane. The resulting fatty acid methyl esters (FAMEs) were identified and quantified using HPLC gas chromatography (1 μl injection volume, GC-Varian model 3900, California, USA) with an Agilent column (100 m × 0.25 mm id; CP7420) and a flame ionization detector. FAMEs were eluted from the column with helium as the carrier gas. The column heating protocol was adopted from Grant et al. (2008). An initial temperature of 140°C was raised to 216°C at a rate of 4°C min$^{-1}$, then to 240°C at 2°C min$^{-1}$, with the injector and detector temperature set at 260°C (total running time: 46 min.). FAMEs were identified using GALAXIE WORKSTATION software (Varian; version 1.9) and individual FAME concentration was expressed as percentage of the total identifiable fatty acids. Treatments were compared based on 1) levels of individual FAs and 2) FA ratios including the Essential Fatty Acid Deficiency Index (EFADI, Takeuchi, 1997).

### Calculations and statistical analysis

In *Series I*, maximum sustainable aerobic swimming speed ($U_{crit}$) was calculated according to Brett (1964) using the equation:

$$U_{crit} = U_i + [U_{ii}(T_i T_{ii}^{-1})]$$

with $U_i$ the highest velocity maintained for the whole minute interval, $U_{ii}$ the velocity increment, $T_i$ the time elapsed at fatigue velocity and $T_{ii}$ the interval time (i.e. 60 s). $U_{crit}$ is expressed in body lengths per sec (bl s$^{-1}$).

In *Series II*, condition factor (CF) was calculated as:

$$CF = \frac{weight(g) \times 100}{[length(cm)]^3}$$

and EFADI was calculated as:

$$EFADI = \frac{(C18:1n-7 + C18:1n-9)}{Total\ n-3\ fatty acids}$$

All analyses were conducted using SigmaPlot 10.0 with level of statistical significance of P < 0.05. Values are reported as means ± standard errors of the means (SEM).

In *Series I*, the combined effect of all three factors (temperature, substrate type and genetic origin) on each of mass, $U_{crit}$ and startle response delay were examined using a mixed model, 3-way analysis of variance (3-way ANOVA). SNK *post hoc* tests were used to identify where significant differences occurred.

In *Series II*, comparisons of parameters (e.g. length, mass, condition factor, family, FAs, slope of linear trends) between enriched and unenriched substrate treated groups were tested using Student's paired *t*-test. When more than two groups were compared (US, ES and hatchery-reared), one-way ANOVA was followed by post hoc analysis with the pairwise Holm-Sidak test.

## Results

### Experimental series I: the effect of substrate enrichment on growth, $U_{crit}$, and SRRT

White sturgeon larvae increased in mass over the course of the experiment ($P < 0.05$, see Table 1). Temperature affected growth such that white sturgeon were significantly larger at 18°C than at 15°C at each time point measured after 1 dph. There were also effects of parentage: white sturgeon from Family B were significantly heavier than those of Family A from 1 dph until 25 dph. Larval mass was not significantly different between substrate treatments until 11 dph, although at this sampling time the effect of substrate alone could not be resolved due to a significant interaction term among the three factors ($P = 0.005$). However, from 15 dph up to and including 29 dph, white sturgeon reared in ES were significantly larger than those reared with US.

Rearing conditions at the Kootenay Trout Hatchery appear to be more suited for rapid growth of larval white sturgeon as hatchery reared sturgeon were significantly larger than their lab reared counterparts at both 15 dph and 29 dph (Table 2).

### Maximum swimming speed ($U_{crit}$)

At 18 dph, there was a significant effect of substrate on $U_{crit}$, however, this effect was dependent on temperature. When analyzed separately, at 15°C there was no effect of substrate treatment (i.e. US vs ES) on $U_{crit}$. In contrast, white sturgeon larvae reared at 18°C in US had a significantly lower $U_{crit}$ than those in ES (1.7 bl s$^{-1}$ compared to 2.6 bl s$^{-1}$, respectively). Despite the larger size of family B and faster overall swimming speeds, larvae from family A had significantly higher average $U_{crit}$ relative to body length, which is consistent with expected scaling effects.

At 30 dph, no difference in swimming performance was detected between substrate treatments at 15°C. Larvae from Family A again had a higher overall $U_{crit}$ than Family B. Low larval survival at 18°C did not allow examination of substrate effects on $U_{crit}$. White sturgeon larvae collected from the hatchery had significantly higher mean $U_{crit}$ than UBC reared fish at both 15 and 30 dph (Fig. 1).

Larvae used for $U_{crit}$ exhibited similar growth trends in response to temperature and family differences as those larvae examined solely for growth, indicating that the fish selected (from active swimmers within the tank) accurately represented size and length of fish overall from that treatment. In addition, no differences in condition factor of larvae were observed between treatments at 15 dph (pooled value, CF = 0.43 ± 0.01).

### Startle response reaction time (SRRT)

Overall, no main effect of substrate or temperature on SRRT was detected, but larvae from Family A had a significantly longer overall SRRT than those from Family B. Data were from each temperature separately in order to a) avoid the possible confounding effects of temperature on growth and development, b) increase statistical power, and c) match the statistical protocol of $U_{crit}$ analysis, This approach illustrated that while there was no effect of family or substrate treatment on SRRT at 18°C (Fig. 2), at 15°C there was a significant overall effect of both family and substrate treatment, with larval sturgeon reared on US requiring over 20% longer to respond than ES (Fig. 2). It is worth noting, however, that the statistical power of this examination was low, and so these findings may be conservative.

### Experimental series II: the effect of substrate enrichment on growth and gut development

As in *Series I*, larvae reared with ES were heavier and longer than those reared with US. For example, ES-treated larvae sampled from Family C were significantly heavier at all sampling times from 8 to 40 dph (excluding 15 dph), and significantly longer at all time points from 10 dph to 40 dph (excluding 12 dph); these differences were as great as 39% and 9%, respectively (Fig. 3). Similar patterns were seen for Family D (Fig. 3d).

In general, condition factor was higher in larvae from ES treatments than from US in both families (Fig. 3e,f). All larvae independent of substrate treatments exhibited a sharp decrease in condition factor from about 1.0 at hatch (0 dph) to below 0.6 by 15 dph and subsequently stabilized at 0.5; this, however, is a reflection of developmental changes (e.g. yolksac absorption) rather than indicative of any change in health status.

### Gut development

Gut development appears more advanced in larvae from ES than in US treatments based upon histological findings. For example, increased musculature of the stomach wall and invaginations in the mucosal lining in four of five larvae from ES for Family C (compared to zero of five for US) and five of five larvae from Family D (compared to two of five for US) suggest that development is occurring faster or developmental phases are reached earlier with exposure to ES (Fig. 4). Additionally, the complete lack of yolksacs in larvae from ES relative to the presence in US in some larvae (three of five larvae from Family C and two of five larvae from Family D) support this conclusion.

Table 2
*Series I*: Fatty acid identity and percent of total lipids extracted from white sturgeon larvae collected in *Series I* at 15°C for Families A and B

| | | Fatty acid level (% of total lipids) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Family A | | | | Family B | | | |
| Age (dph) | Fatty acid identity | HUS[1] | HLUS[1] | US | ES | HUS[1] | HLUS[1] | US | ES |
| 15 | 14:0 | $1.4 \pm 0.1^a$ | n/a | $1.8 \pm 0.1^b$ | $1.9 \pm 0.1^b$ | $1.0 \pm 0.1^a$ | n/a | $1.8 \pm 0.1^b$ | $1.8 \pm 0.1^b$ |
| | 16:0 | $19.1 \pm 0.5^a$ | n/a | $17.7 \pm 0.2^a$ | $18.0 \pm 0.4^a$ | $20.3 \pm 0.5^a$ | n/a | $17.9 \pm 0.2^b$ | $17.9 \pm 0.3^b$ |
| | 16:1 n-7 | $4.3 \pm 0.2^a$ | n/a | $5.6 \pm 0.1^b$ | $5.7 \pm 0.1^b$ | $3.6 \pm 0.2^a$ | n/a | $5.7 \pm 0.2^b$ | $5.6 \pm 0.1^b$ |
| | 18:0 | $5.1 \pm 0.2^a$ | n/a | $4.0 \pm 0.1^b$ | $4.1 \pm 0.1^b$ | $5.4 \pm 0.1^a$ | n/a | $3.5 \pm 0.1^b$ | $3.6 \pm 0.1^b$ |
| | 18:1 n-9 | $19.3 \pm 0.3^a$ | n/a | $22.5 \pm 0.1^b$ | $22.2 \pm 0.1^b$ | $19.3 \pm 0.5^a$ | n/a | $23.3 \pm 0.2^b$ | $23.5 \pm 0.2^b$ |
| | 18:1 n-7 | $4.0 \pm 0.1^a$ | n/a | $4.3 \pm 0.1^b$ | $4.3 \pm 0.1^b$ | $3.9 \pm 0.1^a$ | n/a | $4.3 \pm 0.1^b$ | $4.3 \pm 0.1^b$ |
| | 18:2 n-6 | $3.4 \pm 0.1^a$ | n/a | $4.1 \pm 0.1^b$ | $4.2 \pm 0.1^b$ | $2.8 \pm 0.1^a$ | n/a | $4.0 \pm 0.1^b$ | $4.0 \pm 0.1^b$ |
| | 18:3 n-3 | $3.4 \pm 0.2^a$ | n/a | $4.4 \pm 0.1^b$ | $4.4 \pm 0.1^b$ | $2.8 \pm 0.1^a$ | n/a | $4.4 \pm 0.1^b$ | $4.4 \pm 0.1^b$ |
| | 20:4 n-6 | $6.1 \pm 0.2^a$ | n/a | $5.7 \pm 0.1^b$ | $5.5 \pm 0.1^b$ | $7.3 \pm 0.1^a$ | n/a | $5.7 \pm 0.1^b$ | $5.7 \pm 0.1^b$ |
| | 20:5 n-3 | $6.8 \pm 0.3^a$ | n/a | $6.4 \pm 0.1^a$ | $6.4 \pm 0.1^a$ | $7.1 \pm 0.1^a$ | n/a | $6.2 \pm 0.1^b$ | $6.1 \pm 0.1^b$ |
| | 22:1 n-9 | $4.4 \pm 0.5^a$ | n/a | $1.2 \pm 0.3^b$ | $1.2 \pm 0.3^b$ | $5.6 \pm 0.6^a$ | n/a | $1.7 \pm 0.2^b$ | $1.8 \pm 0.2^b$ |
| | 22:5 n-3 | $2.6 \pm 0.1^a$ | n/a | $2.9 \pm 0.1^b$ | $2.9 \pm 0.1^b$ | $2.5 \pm 0.1^a$ | n/a | $3.0 \pm 0.1^b$ | $3.0 \pm 0.1^b$ |
| | 22:6 n-3 | $16.3 \pm 0.5^a$ | n/a | $14.6 \pm 0.2^b$ | $14.5 \pm 0.2^b$ | $16.8 \pm 0.4^a$ | n/a | $13.9 \pm 0.2^b$ | $13.7 \pm 0.2^b$ |
| 29 | 14:0 | n/a | n/a | $1.7 \pm 0.1^a$ | $2.1 \pm 0.2^b$ | n/a | n/a | $2.2 \pm 0.2^a$ | $2.1 \pm 0.3^a$ |
| | 16:0 | n/a | n/a | $20.0 \pm 0.4^a$ | $20.9 \pm 0.7^a$ | n/a | n/a | $21.8 \pm 0.4^a$ | $21.2 \pm 0.4^a$ |
| | 16:1 n-7 | n/a | n/a | $7.2 \pm 0.2^a$ | $8.1 \pm 0.5^a$ | n/a | n/a | $8.0 \pm 0.5^a$ | $8.3 \pm 14^a$ |
| | 18:0 | n/a | n/a | $6.0 \pm 0.2^a$ | $5.8 \pm 0.2^a$ | n/a | n/a | $6.4 \pm 0.2^a$ | $6.8 \pm 0.6^a$ |
| | 18:1 n-9 | n/a | n/a | $14.2 \pm 0.4^a$ | $14.4 \pm 0.6^a$ | n/a | n/a | $11.3 \pm 0.3^a$ | $10.8 \pm 0.2^a$ |
| | 18:1 n-7 | n/a | n/a | $4.3 \pm 0.1^a$ | $4.5 \pm 0.1^b$ | n/a | n/a | $4.2 \pm 0.2^a$ | $4.1 \pm 0.2^a$ |
| | 18:2 n-6 | n/a | n/a | $3.5 \pm 0.1^a$ | $3.8 \pm 0.1^a$ | n/a | n/a | $3.2 \pm 0.2^a$ | $3.3 \pm 0.5^a$ |
| | 18:3 n-3 | n/a | n/a | $2.3 \pm 0.1^a$ | $2.4 \pm 0.1^a$ | n/a | n/a | $1.8 \pm 0.1^a$ | $1.8 \pm 0.4^a$ |
| | 20:4 n-6 | n/a | n/a | $6.4 \pm 0.1^a$ | $5.7 \pm 0.2^b$ | n/a | n/a | $5.9 \pm 0.2^a$ | $6.1 \pm 0.6^a$ |
| | 20:5 n-3 | n/a | n/a | $8.1 \pm 0.1^a$ | $8.1 \pm 0.3^a$ | n/a | n/a | $7.5 \pm 0.2^a$ | $7.7 \pm 0.4^a$ |
| | 22:1 n-9 | n/a | n/a | $5.1 \pm 0.7^a$ | $4.2 \pm 0.4^a$ | n/a | n/a | $6.5 \pm 0.5^a$ | $5.0 \pm 1.0^a$ |
| | 22:5 n-3 | n/a | n/a | $2.2 \pm 0.1^a$ | $2.1 \pm 0.1^a$ | n/a | n/a | $1.7 \pm 0.1^a$ | $1.9 \pm 0.1^a$ |
| | 22:6 n-3 | n/a | n/a | $15.3 \pm 0.2^a$ | $13.9 \pm 0.5^b$ | n/a | n/a | $14.2 \pm 0.4^a$ | $15.0 \pm 0.7^a$ |
| 34 | 14:0 | $2.7 \pm 0.6^a$ | $3.0 \pm 0.5^a$ | n/a | n/a | $3.8 \pm 0.2^a$ | $4.0 \pm 0.2^a$ | n/a | n/a |
| | 16:0 | $21.9 \pm 0.8^b$ | $19.3 \pm 0.4^a$ | n/a | n/a | $22.7 \pm 0.5^b$ | $20.1 \pm 1.0^a$ | n/a | n/a |
| | 16:1 n-7 | $5.2 \pm 0.3^b$ | $10.1 \pm 1.6^a$ | n/a | n/a | $5.3 \pm 0.1^b$ | $12.2 \pm 1.6^a$ | n/a | n/a |
| | 18:0 | $6.7 \pm 0.4^a$ | $5.2 \pm 0.7^a$ | n/a | n/a | $6.1 \pm 0.2^b$ | $4.1 \pm 0.6^a$ | n/a | n/a |
| | 18:1 n-9 | $9.9 \pm 0.5^a$ | $10.1 \pm 0.1^a$ | n/a | n/a | $10.7 \pm 0.2^b$ | $9.7 \pm 0.1^a$ | n/a | n/a |
| | 18:1 n-7 | $4.0 \pm 0.2^b$ | $4.7 \pm 0.2^a$ | n/a | n/a | $4.2 \pm 0.1^b$ | $4.8 \pm 0.1^a$ | n/a | n/a |
| | 18:2 n-6 | $3.3 \pm 0.6^a$ | $4.2 \pm 0.6^a$ | n/a | n/a | $4.4 \pm 0.2^a$ | $5.0 \pm 0.5^a$ | n/a | n/a |
| | 18:3 n-3 | $0.2 \pm 0.2^b$ | $3.5 \pm 0.9^a$ | n/a | n/a | $0.6 \pm 0.0^b$ | $2.7 \pm 0.4^a$ | n/a | n/a |
| | 20:4 n-6 | $4.2 \pm 0.4^a$ | $4.8 \pm 1.4^a$ | n/a | n/a | $3.5 \pm 0.1^a$ | $2.9 \pm 0.3^a$ | n/a | n/a |
| | 20:5 n-3 | $10.2 \pm 0.7^a$ | $8.0 \pm 1.7^a$ | n/a | n/a | $11.4 \pm 0.2^b$ | $9.7 \pm 0.3^a$ | n/a | n/a |
| | 22:1 n-9 | $10.0 \pm 2.6^a$ | $5.4 \pm 1.0^a$ | n/a | n/a | $5.4 \pm 1.1^a$ | $2.9 \pm 0.5^a$ | n/a | n/a |
| | 22:5 n-3 | $2.3 \pm 0.2^a$ | $2.3 \pm 0.1^a$ | n/a | n/a | $2.4 \pm 0.2^a$ | $2.1 \pm 0.1^a$ | n/a | n/a |
| | 22:6 n-3 | $16.7 \pm 0.6^a$ | $14.1 \pm 1.4^a$ | n/a | n/a | $15.5 \pm 0.4^b$ | $11.7 \pm 1.4^a$ | n/a | n/a |

Each sample (n) assessed for US and ES values consisted of a pool of 2–3 larvae. At 15 dph, n = 7 for Family A. For Family B, n = 8 for larvae from Kootney hatchery (HUS) and n = 6 for US and ES groups. At 29 dph, n = 8 for US and n = 7 for ES for Family A, and n = 9 for US and n = 3 for ES for Family B. At 34 dph, n = 5 for HUS and HLUS groups. Values expressed as means ± SEM. For a given family and sampling time, values within a row with different superscript letters = significantly different (P < 0.05).
[1]HUS treatment groups hatchery-reared and shipped live to UBC either 14 dph (sampled 15 dph) or 28 dph (sampled at 34 dph). HLUS were transported to UBC from hatchery at 14 dph, and sampled at 34 dph.

**Experimental series I and II: the effect of substrate enrichment on relative fatty acid composition**

**Series I.** In general, fatty acid profiles were similar between substrate treatments. Specifically, at 15 dph no effect of substrate was detected in either Family A or B (*t*-test, Table 3). In contrast, at 29 dph, larvae from the ES treatment from Family A exhibited significantly lower relative levels compared to their US reared siblings for a number of fatty acids (e.g. ARA, C20:4 n-6, and DHA, C22:6 n-3). While similar trends were observed in Family B, no statistically significant differences were detected (Table 3).

Hatchery-reared white sturgeon generally had relatively higher levels of the fatty acids measured than those reared in the lab environment. Roughly half of the FAs identified in 15 dph larvae were significantly higher in hatchery larvae (HUS15) than in lab-reared larvae (Table 3), and similar patterns were observed when comparing hatchery-reared larvae at 34 dph (HUS30) with lab-reared larval white sturgeon.
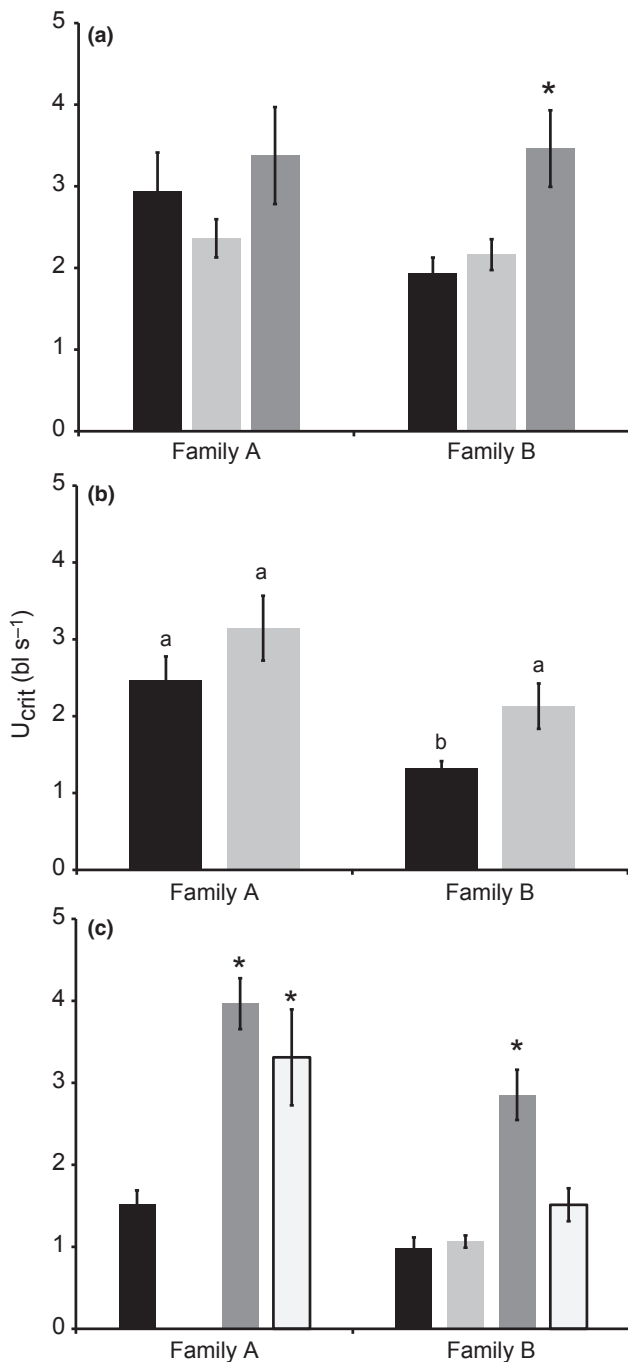
Fig. 1. Differences in maximal sustainable aerobic swimming speed ($U_{crit}$) of larval white sturgeon between substrate treatments [unenriched substrate (US; black bars) vs enriched substrate (ES; medium gray bars)] in *Series I* at (a) 15°C at 15 dph and (b) 18°C at 15 dph, and (c) at 15°C 30 dph. Dark grey and light gray bars = hatchery reared larvae (HUS) and hatchery and lab reared (HLUS) groups, respectively. Values expressed as means ± SEM, N = 8 per group. Asterisk = a statistically significant difference from unenriched substrates within families. Letters that differ = significant differences between all treatment groups.

**Series II.** Relative fatty acid levels in ES larvae were generally higher than those in US larvae, but this finding was dependent to some extent on the family examined. For exam-
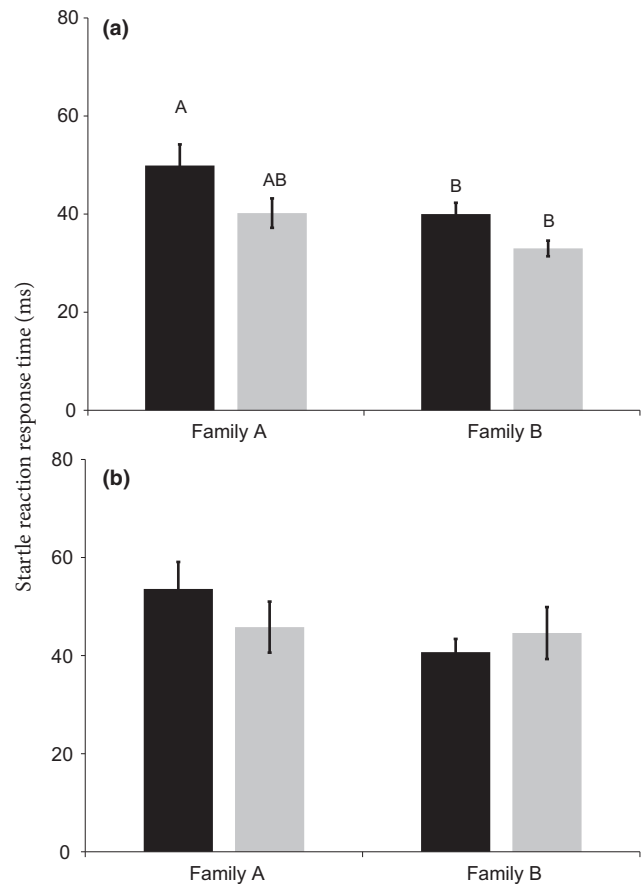


Fig. 2. Differences in startle response reaction time of larval white sturgeon between substrate treatments [unenriched substrate (US; black bars) vs enriched substrate (ES; light gray bars)] in *Series I* at (a) 15°C and (b) 18°C at 15 dph. Values expressed as means ± SEM, N = 10 per group. Letters that differ = significant differences between groups.

ple, in Family C, ES-exposed fish exhibited significantly higher levels for eight of the thirteen fatty acids, including omega 3- and 6- fatty acids, at 30 dph than their US counterparts (Table 3), while in Family D, ES larvae exhibited levels of C14:0 and C18:2 n-6 (linoleic acid, LA), roughly twice that of US larvae (Table 3). Similar respective trends were observed within each family at 40 dph, although differences were less pronounced. Again, ES sturgeon from Family C had levels of C14:0, C16:1 n-7 and LA that were significantly greater than those in US larvae, but in Family D, levels of omega 3 essential fatty acids – EPA, DPA and DHA – were 22, 23 and 17% higher, respectively, in ES larvae (Table 3). Thus, despite significant effects existing between the two treatments, there is no clear pattern.

**Fatty acid ratios**

Fatty acid ratios have been relied on as a means to identify trends and treatment effects based on described biochemical relationships between FAs (e.g. ARA and heneicosapentae-noic acid, HPA). Although relative fatty acid composition
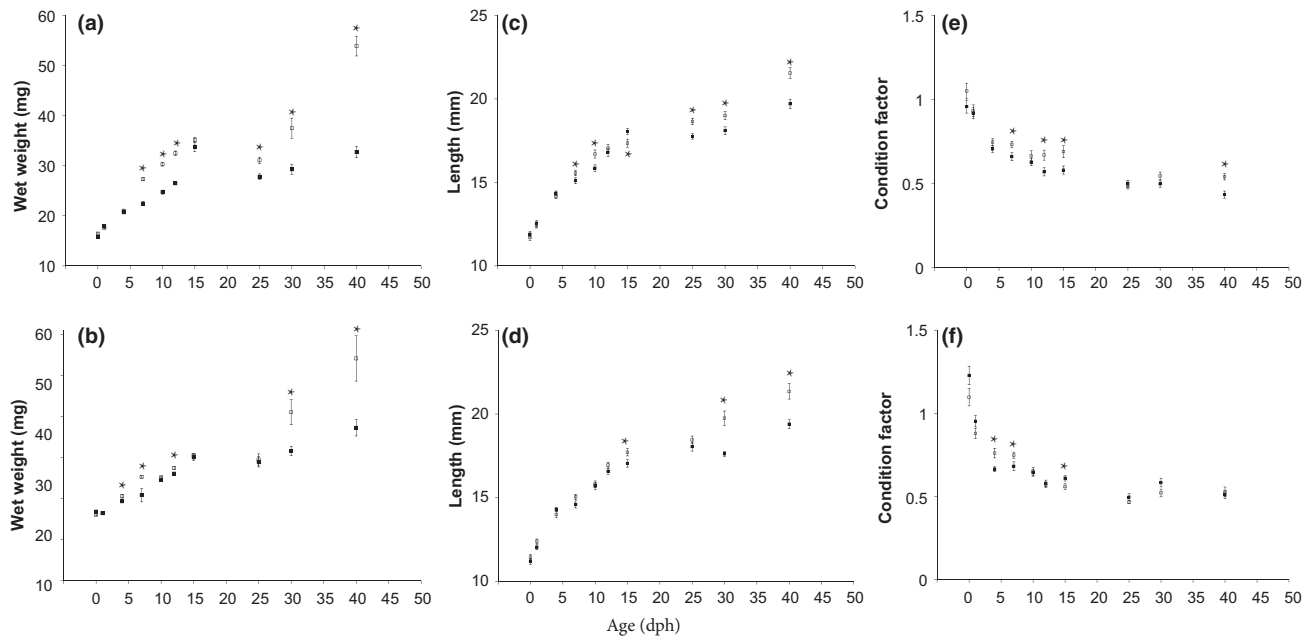
Fig. 3. Effect of substrate (enriched, open symbols vs unenriched, filled symbols) on white sturgeon larval wet weight (a, b), length (c, d) and condition factor (e, f) during development in Family C (squares; a,c,e) and d (diamonds; b,d,f) in *Series II*. Values expressed as means ± SEM (n = 20). For a given time point (dph), an asterisk indicates a statistically significant difference between substrates (P < 0.05).
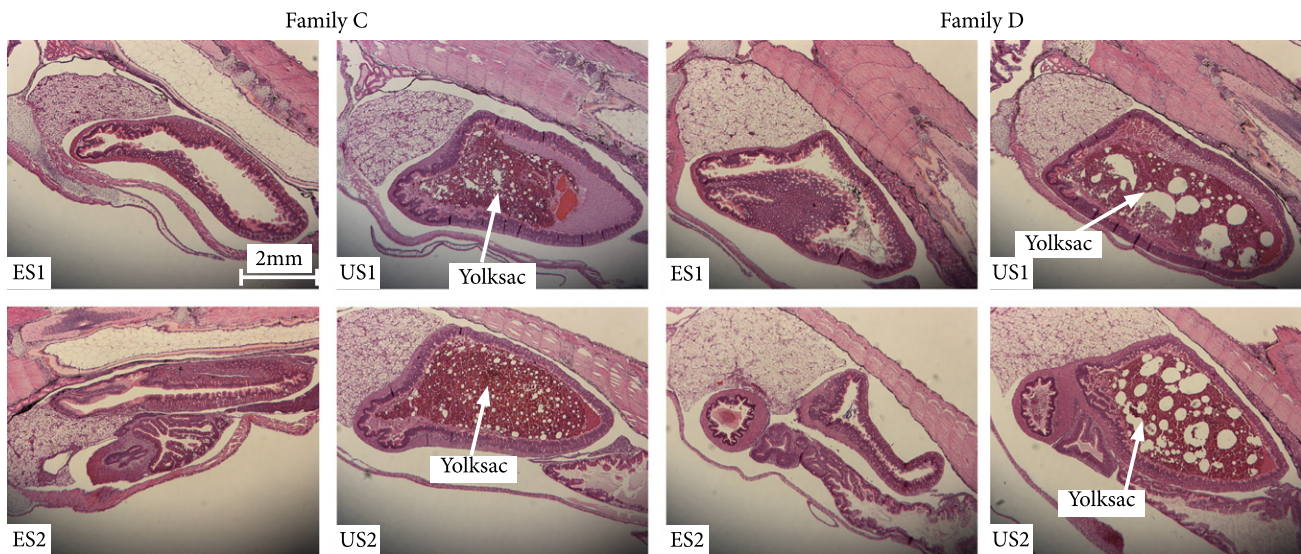


Fig. 4. Representative images of digestive tract morphology of white sturgeon larvae following exposure to enriched (ES) or unenriched (US) substrates at 15 dph in Families C and D in *Series II*. Numbers in labels = individuals (i.e. ES1 is a different animal than ES2). Top left micrograph scale bar = 2 mm. All other micrographs taken at same magnification and scaled equally.

results differ somewhat between *Series I* and *II*, both sets of data were combined to improve statistical confidence and better illustrate overall trends. We acknowledge, however, that these pooled results yield findings that should be interpreted with caution.

Some fatty acid ratios were affected by whether yolksac larvae were exposed to ES or US. For example, while the ratios of both omega 3 to omega 6, and EPA to ARA increased significantly with development in both treatment groups (Fig. 5b,c), the rates of these increases were significantly higher in larval sturgeon reared with ES: that is, there was a significant difference in the slopes of the ratios over time between rearing conditions (*t*-test, Fig. 5b,c). Conversely, the relative amount of DHA to EPA decreased with development (Fig. 5a), but the rate of decrease was not affected by substrate exposure history (Fig. 5a). All larvae irrespective of substrate condition exhibited a decrease in the essential fatty acid deficiency index from roughly 1.0 at

Table 3
*Series II*: Identity and total lipid percentage of fatty acids extracted from white sturgeon larvae collected in *Series II*

| Days post hatch (dph) | Fatty acid identity | Fatty acid level (% of total lipids) | | | |
| | | Family C | | Family D | |
| | | US | ES | US | ES |
|---|---|---|---|---|---|
| 30 | 14:0 | $2.1 \pm 0.3^a$ | $2.9 \pm 0.2^b$ | $1.0 \pm 0.2^a$ | $2.2 \pm 0.4^b$ |
| | 16:0 | $21.0 \pm 0.5^a$ | $22.7 \pm 0.3^b$ | $20.0 \pm 0.6^a$ | $20.8 \pm 0.6^a$ |
| | 16:1 n-7 | $3.7 \pm 0.3^a$ | $5.0 \pm 0.2^b$ | $2.5 \pm 0.2^a$ | $3.8 \pm 0.4^b$ |
| | 17:1 n-7 | $1.3 \pm 0.1^a$ | $1.0 \pm 0.1^b$ | $1.5 \pm 0.1^a$ | $1.4 \pm 0.1^a$ |
| | 18:0 | $7.4 \pm 0.2^a$ | $6.8 \pm 0.2^a$ | $8.3 \pm 0.3^a$ | $7.6 \pm 0.3^a$ |
| | 18:1 n-9 | $9.7 \pm 0.2^a$ | $10.2 \pm 0.1^b$ | $13.0 \pm 0.9^a$ | $12.0 \pm 0.5^a$ |
| | 18:1 n-7 | $3.7 \pm 0.1^a$ | $4.3 \pm 0.1^b$ | $3.1 \pm 0.1^a$ | $3.6 \pm 0.2^a$ |
| | 18:2 n-6 | $2.1 \pm 0.2^a$ | $3.0 \pm 0.2^b$ | $1.4 \pm 0.2^a$ | $2.4 \pm 0.3^b$ |
| | 20:4 n-6 | $7.2 \pm 0.3^a$ | $5.6 \pm 0.3^b$ | $5.6 \pm 0.2^a$ | $4.9 \pm 0.3^b$ |
| | 20:5 n-3 | $7.4 \pm 0.4^a$ | $9.5 \pm 0.2^b$ | $7.1 \pm 0.3^a$ | $8.3 \pm 0.4^b$ |
| | 22:1 n-9 | $11.1 \pm 1.0^a$ | $6.7 \pm 0.6^b$ | $11.9 \pm 0.7^a$ | $9.5 \pm 1.4^a$ |
| | 22:5 n-3 | $1.5 \pm 0.1^a$ | $1.8 \pm 0.1^b$ | $1.5 \pm 0.2^a$ | $1.8 \pm 0.1^a$ |
| | 22:6 n-3 | $18.5 \pm 0.4^a$ | $18.6 \pm 0.5^a$ | $17.9 \pm 0.4^a$ | $17.8 \pm 0.4^a$ |
| 40 | 14:0 | $1.9 \pm 0.3^a$ | $2.8 \pm 0.2^b$ | $1.8 \pm 0.3^a$ | $2.0 \pm 0.3^a$ |
| | 16:0 | $20.1 \pm 0.9^a$ | $20.7 \pm 0.7^a$ | $17.8 \pm 0.8^a$ | $20.7 \pm 0.3^b$ |
| | 16:1 n-7 | $3.7 \pm 0.3^a$ | $4.8 \pm 0.2^b$ | $3.3 \pm 0.3^a$ | $3.9 \pm 0.3^a$ |
| | 17:1 n-7 | $1.1 \pm 0.1^a$ | $0.9 \pm 0.1^a$ | $1.2 \pm 0.1^a$ | $1.2 \pm 0.1^a$ |
| | 18:0 | $6.9 \pm 0.4^a$ | $6.1 \pm 0.2^a$ | $6.8 \pm 0.3^a$ | $6.7 \pm 0.3^a$ |
| | 18:1 n-9 | $9.0 \pm 0.4^a$ | $9.5 \pm 0.3^a$ | $8.6 \pm 0.4^a$ | $10.5 \pm 0.2^b$ |
| | 18:1 n-7 | $4.0 \pm 0.2^a$ | $4.4 \pm 0.2^a$ | $3.5 \pm 0.5^a$ | $4.1 \pm 0.1^a$ |
| | 18:2 n-6 | $2.3 \pm 0.2^a$ | $2.9 \pm 0.2^b$ | $1.9 \pm 0.2^a$ | $2.2 \pm 0.2^a$ |
| | 20:4 n-6 | $4.8 \pm 0.3^a$ | $3.7 \pm 0.1^b$ | $4.0 \pm 0.2^a$ | $3.8 \pm 0.2^a$ |
| | 20:5 n-3 | $8.7 \pm 0.4^a$ | $9.9 \pm 0.4^a$ | $7.7 \pm 0.5^a$ | $9.9 \pm 0.3^b$ |
| | 22:1 n-9 | $8.3 \pm 0.8^a$ | $5.6 \pm 0.4^b$ | $10.8 \pm 1.4^a$ | $6.2 \pm 0.8^b$ |
| | 22:5 n-3 | $1.7 \pm 0.1^a$ | $2.0 \pm 0.1^a$ | $1.6 \pm 0.1^a$ | $2.1 \pm 0.1^b$ |
| | 22:6 n-3 | $17.5 \pm 1.0^a$ | $17.5 \pm 0.8^a$ | $16.1 \pm 0.8^a$ | $19.3 \pm 0.4^b$ |
| | 24:1 n-9 | $0.9 \pm 0.1^a$ | $0.8 \pm 0.1^a$ | $1.0 \pm 0.1^a$ | $0.9 \pm 0.1^a$ |

Fish sampled at 30 and 40 dph for both Families C and D (n = 10). Values expressed as means ± SEM. For a given family and sampling time, values within a row with different superscript letters = significantly different (P < 0.05).

15 dph to 0.6 by 30 dph and then a stabilization at approx. 0.5 by 40 dph until the termination of the experiment (Fig. 5d). As with the condition factor, this is likely a natural trend associated with early development that has not been previously reported for sturgeon.

## Discussion

The findings of this study demonstrate that exposure to enriched substrates during the early life history of white sturgeon was beneficial overall, and so expand on prior findings regarding growth and physiological effects (Boucher, 2012; Boucher et al., 2014). Here we determined that white sturgeon larval growth and condition factor, gut development, $U_{crit}$, SRRT, and FA composition were all altered when reared in the absence of enriched substrates, although temperature and parentage in some cases mediated or exacerbated those effects. The identification of more phenotypic limitations associated with poor substrate conditions in sturgeon provides further support (see also Gessner et al., 2009; Zubair et al., 2012; Boucher et al., 2014) for the causal links between reduced substrate complexity (i.e. loss of interstitial habitats) and recruitment limitations (McAdam et al., 2005; Paragamian et al., 2009; McAdam, 2012).

### Substrate effects on growth and development

Consistent with other studies (Gessner et al., 2009; Boucher et al., 2014), yolksac larvae reared under an enriched substrate regime exhibited enhanced growth and higher condition factors. This effect was, however, family and temperature dependent, as well as more pronounced in *Series II*. Substrate enrichment has been observed to affect growth in salmonids (Peterson and Martin-Robichaud, 1995; Kihslinger and Nevitt, 2006) and other species (e.g. robust redhorse, *Moxostoma robustum*; Jennings et al., 2010). While there are exceptions, in general increased size can confer substantial survival benefits (Fuiman, 2002).

Delayed development of a functional gastric stomach and the initiation of feeding as a result of early rearing conditions may provide a potential mechanism for the high mortality rate commonly observed in lab or hatchery rearing of many sturgeon species (e.g. Asgari et al., 2014). Histology revealed developmental differences in the gastrointestinal system between treatments, including greater development of stomach musculature and more rapid yolk absorption in the ES treatments (Fig. 4). While it is perhaps not surprising that prolonged retention of the yolksac in US was associated with delayed gut development, we believe these findings would likely represent negative effects
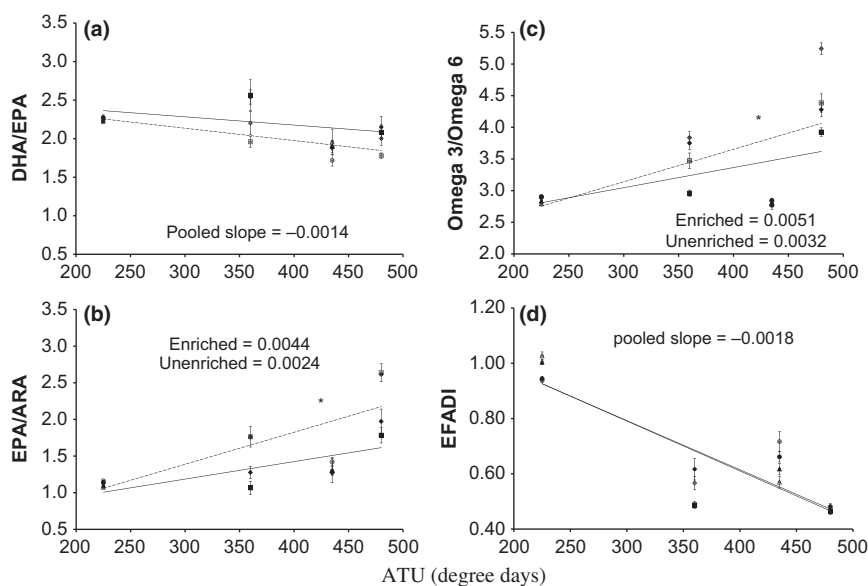
Fig. 5. Effect of substrate (enriched, open symbols, vs unenriched, filled symbols) on fatty acid ratios from both *Series I* and *II* during larval development in Families A (circle), B (triangle), C (square) and D (diamond): (a) docosahexanenoic acid C22:6n-3/eicosapentaenoic acid C20:5n-3 (DHA/EPA), (b) eicosapentaenoic acid C20:5n-3 / arachidonic acid C20:4n-6 (EPA/ARA) (c) Omega 3/Omega 6 fatty acids and (d) essential fatty acid deficiency index. Ratios calculated from fatty acid percentages reported in Tables 1 and 2. Linear regressions calculated using individuals for each substrate condition (n = 63 for enriched and n = 69 for unenriched). Slopes of regression lines reported at the bottom left of each panel, where an asterisk indicates statistically different slopes between substrates (P < 0.05). Values expressed as means ± SEM (n = 3–10).

on larval quality based on interpretation of larval quality indicators observed in other fishes (e.g. Theilacker and Watanabe, 1989). Most prior studies of early larval ontogeny in sturgeon evaluate larvae reared without substrate enrichment. The provision of substrate during the yolksac phase may therefore provide a valuable modification to rearing protocols (Boucher, 2012).

### Substrate effects on $U_{crit}$ and SRRT

White sturgeon reared on US exhibited a $U_{crit}$ that was 25.5% lower at 15 dph when reared at 18°C, but showed no significant effect when reared at 15°C. Recently demonstrated was that sturgeon reared in ES exhibit a greater metabolic scope (difference between maxima and resting metabolic rate; Boucher, 2012) – this increase may help explain our finding of an $U_{crit}$ effect of US only at an elevated temperature (18°C). The addition of a second stressor (e.g. temperature) may exacerbate substrate effects when combined with an exercise challenge. Alternately, given the effect of temperature on the rate of development, the difference in $U_{crit}$ may be related to life history characteristics or ontogeny.

Larvae reared on ES exhibited a 20% shorter SRRT compared to those reared with US at 15°C, but not at 18°C. The basis for the differential effect of temperature is unknown, but may again be related to the different developmental stages of the animals at the different temperatures. It is likely that the SRRT informs on life history relevant traits, such as predator avoidance, however, further studies are required to investigate this directly.

### Substrate effects on fatty acid composition

FA profiles for sturgeon larvae were generally similar to those of other fish, with the functionally important palmitic (C16:0) and oleic (C18:1n-9) fatty acids (Rainuzzo et al., 1997) as the principal saturated and monounsaturated fatty acid and DHA (important in neural activity, Tocher, 2003) as the predominant polyunsaturated fatty acid. White sturgeon larvae exhibited a DHA/EPA ratio of about 2.0, and similar results have been observed in some other sturgeon species (Gershanovich, 1989). Interestingly, EFADI values were not significantly different between US and ES treatments and were similar to those found for other fishes. For example, EFADI was on average 0.44 for wild-caught marine fish (Takeuchi, 1997) and between 0.35 and 1.08 for lab-reared Chilean flounder (Wilson, 2009). In white sturgeon in this study, EFADI declined from 1.03 to 0.57 from 15 to 40 dph. Thus, despite reduced growth associated with US, no indications of energetic limitations associated with feed availability or absorption were observed based on EFADI.

Of particular importance was the finding that relative differences in some FA ratios between larvae reared on enriched substrates became greater, not less, over time (up to 40 dph). Both EPA:ARA and omega 3:omega 6 ratios increased more rapidly in larvae reared on ES during ontogeny (*t*-test), this difference due primarily to lower concentrations of ARA. Variation in the FA ratios is associated with exposure to prior stressors (Sargent, 1995; Tocher, 2003), and elevation of ARA has been associated with elevated cortisol, as ARA-derived eicosanoids are linked to production of corticosteroids (Martins et al., 2012). The presence of elevated cortisol in the absence of substrate enrichment in both

lake sturgeon and white sturgeon (Zubair et al., 2012; Bates et al., 2014) may therefore be associated with slower development and reduced growth. Collectively, these findings suggest that larvae reared on enriched substrates may be less likely to exhibit chronic stress, a conclusion consistent with improved stress tolerance in Persian sturgeon and beluga that were given fatty acid supplementation (Noori et al., 2011).

## Conclusions

Through investigation of the role of substrate on early development of white sturgeon, we have demonstrated that rearing on bare or unenriched substrates over the first 10–12 dph resulted in modest deleterious modifications to size, $U_{crit}$ and SRRT when assessed later during development, although these effects could be mediated by parentage or rearing temperature. In addition, despite no evidence of malnutrition, larvae reared in US exhibited an altered fatty acid profile and slower yolk utilization, although further analysis is required to determine the biological relevance of and mechanisms associated with these differences. Clearly, the structural complexity of substrates play an important role in white sturgeon development during early life phases, and we believe this role may ultimately help explain rearing and recruitment challenges under captive and wild conditions.

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**Author's address:** Daniel Baker, ICSS, VIU, 900 Fifth St., Nanaimo, BC V9R 5S5, Canada.
E-mail: dan.baker@viu.ca