NRC Research Press
ARTICLE

To feed or flee: early life-history behavioural strategies of juvenile lake sturgeon (*Acipenser fulvescens*) during risk-sensitive foraging

F. Bjornson, M. Earhart, and W.G. Anderson

Abstract: Balancing foraging opportunities with predation risk can promote complex behavioural strategies in juvenile fishes, particularly in northern temperate environments with short growing seasons. To test how predation experience may influence foraging effort and risk assessment of juvenile lake sturgeon (*Acipenser fulvescens* Rafinesque, 1817), flight response and substrate preference behavioural measurements were taken during critical life periods of early exogenous feeding (~60 days post fertilization (dpf)) and pre-winter (~160 dpf). Lake sturgeon were placed in arenas with partial cover and exposed white plastic bottom. Chemical alarm cue (AC) was introduced to predator naïve individuals in the presence or absence of food over the exposed portion of the arena to simulate risk sensitive foraging over diurnal and seasonal periods. The same protocol was run on predator-experienced individuals, which were classically conditioned to predator cue (PC) prior to the trials. Whole-body cortisol measures were also taken to determine the physiological response to predation experience. Results suggest a propensity to forage in spite of predation risk during the naïve ~60 dpf trials and highlight context-specific anti-predator responses of naïve and experienced lake sturgeon. Elevated basal whole-body cortisol levels and reduced body condition ($p < 0.05$) were observed with increased predator experience.

Key words: lake sturgeon, *Acipenser fulvescens*, foraging, learning, predation, physiology.

Résumé : La recherche d’un équilibre entre les occasions de quête de nourriture et le risque de prédation peut favoriser des stratégies comportementales complexes chez les jeunes poissons, particulièrement dans les milieux tempérés nordiques où les saisons de croissance sont courtes. Pour vérifier comment l’expérience de prédation pourrait influencer l’effort de quête de nourriture et l’évaluation du risque chez les esturgeons jaunes (*Acipenser fulvescens* Rafinesque, 1817) juvéniles, des mesures comportementales de la réaction de fuite et des préférences en matière de substrat ont été prises durant les périodes critiques du début de l’étape d’alimentation exogène (~60 jours après la fécondation (jaf)) et avant l’hiver (~160 jaf). Des esturgeons jaunes ont été placés dans des enceintes partiellement couvertes et dotées d’un fond en plastique blanc exposé. Des spécimens sans expérience de prédateurs ont été exposés à un signal d’alarme chimique en présence ou en l’absence de nourriture dans la partie exposée de l’enceinte afin de simuler une quête de nourriture sensible au risque durant des périodes diurnes et saisonnières. Le même protocole a été appliqué à des spécimens avec expérience de prédateurs, conditionnés de manière classique à un signal de prédateur avant les essais. Des mesures de la concentration corporelle globale de cortisol ont été prises afin de déterminer la réaction physiologique à l’expérience de prédation. Les résultats indiqueraient une propension à la quête de nourriture en dépit de l’existence d’un risque de prédation durant les essais à ~60 jaf sur des poissons naïfs et soulignent le fait que les réactions anti-prédation des esturgeons naïfs et expérimentés dépendent du contexte. Une élévation des concentrations corporelles globales de base de cortisol et une diminution de l’embonpoint ($p < 0,05$) étaient associées à une plus grande expérience de prédateurs. [Traduit par la Rédaction]

Mots-clés : esturgeon jaune, *Acipenser fulvescens*, quête de nourriture, apprentissage, prédation, physiologie.

Introduction

The trade-off between feeding and avoiding potential predation threat is often faced by foraging animals (Elgar 1989). For the individual, it is beneficial to promote avoidance behaviour proportional to the gradient of perceived threat so as to maximize foraging. Avoidance behaviour of the individual can be promoted through intrinsic demands (growth, metabolic rate, body condition, etc.) and extrinsic pressures (predation experience, microhabitat heterogeneity, foraging, etc.) (Polverino et al. 2016). Intrinsic demands and extrinsic pressures vary over age, size, body, or physiological condition of the individual, thus promoting behavioural responses optimal for the current state of the individual and environmental context (Wolf and Weissing 2010; Polverino et al. 2016).

During early life stages, prey animals often have high energy requirements and limited predation experience. Rapid shifts in intrinsic energy demands and extrinsic pressures can occur during this period. Responding appropriately to these stimuli across such a gradient can promote complex early life-history behavioural strategies. For example, at early life stages, fish may adopt a more risky foraging strategy when growth is paramount; however, older and larger individuals may be less prone to take risky foraging strategies in favour of predator avoidance. Such ontoge-

Received 30 July 2019. Accepted 12 March 2020.

F. Bjornson, M. Earhart, and W.G. Anderson. Department of Biological Sciences, University of Manitoba, 50 Sifton Road, Winnipeg, MB R3T 2N2, Canada.

Corresponding author: Forrest Bjornson (email: forrestbjornson@gmail.com).

Can. J. Zool. **98**: 541–550 (2020) dx.doi.org/10.1139/cjz-2019-0181

netic shifts in risk assessment behaviour can rapidly occur in juvenile fishes and are well documented (Golub and Brown 2003; Jones et al. 2003; Marcus and Brown 2003; Harvey and Brown 2004; Lonnstedt et al. 2012). Furthermore, risk assessment behaviour can change over much shorter time frames such as day or night foraging when predation pressures and the ability to avoid predators may greatly change.

In higher latitude temperate environments, fishes must grow and obtain energy reserves within a small window of time, balancing energy allocations towards somatic growth, anti-predator behaviour, and energy storage in preparation for winter. Numerous models (Ludwig and Rowe 1990; Rowe and Ludwig 1991; Houston et al. 1993; Werner and Anholt 1993; Clark 1994) and laboratory experiments (Johansson and Rowe 1999; Anholt et al. 2000; Johansson et al. 2001) demonstrate that prey take greater risks when foraging since this window of time is reduced. Indeed, winter mortality may be more severe than predation mortality in juvenile fishes (Schultz and Conover 1997), which may promote an early life behavioural strategy that maximizes growth and energy reserves in some fishes (Post and Evans 1989; Post and Parkinson 2001; Hurst and Conover 2003). Understanding the extent to which predation threat and time constraints promote risk-taking behaviour is challenging, yet it may prove rewarding since the selective forces promoting this behaviour have large population-level consequences (Biro et al. 2003, 2004).

Lake sturgeon (*Acipenser fulvescens* Rafinesque, 1817), along with Siberian sturgeon (*Acipenser baerii* Brandt, 1869), occupies the most northerly habitat and coldest water of all sturgeon species (Artyuklin 1995). After spring spawning, juvenile lake sturgeon have a single season to grow before they endure their first overwinter challenge, which may last for at least 3 months with limited foraging opportunities and temperatures near 0 °C (McDougall et al. 2014). Such overwintering events have large consequences for survival and subsequent year-class strength as that cohort moves into the following year (Post and Evans 1989; Hurst and Conover 2003; Deslauriers et al. 2018; Yoon et al. 2019). Recent laboratory studies have demonstrated complex behavioural responses in juvenile lake sturgeon (Wishingrad et al. 2014*b*, 2014*c*; Ferrari et al. 2015; Sloychuk et al. 2016; Bjornson and Anderson 2018), but none have attempted to determine the trade-off between foraging and responding to predation threats in juvenile lake sturgeon.

The juvenile lake sturgeon is an ideal species to examine the trade-off between foraging and responding to potential predation threat because the selective forces promoting foraging behaviour in a northern temperate environment may be more pronounced. In particular, juvenile lake sturgeon are routinely stocked into natural habitats as part of stock enhancement programs because they are considered imperiled across their range (Pollock et al. 2015). Understanding the behavioural responses necessary for survival in the natural environment are essential for the survival of stocked progeny. Therefore, the present study aims to test the hypothesis (*i*) that age 0+ lake sturgeon diurnal risk assessment behaviour will shift from favoring foraging behaviour to anti-predator behaviour as they develop (i.e., as they grow and shift intrinsic states); (*ii*) that predation experience (i.e., more predation experience) would further shift their diurnal and ontogenetic risk assessment behaviour from foraging to responding to potential predation threat; (*iii*) that predation experience may reduce fitness through chronic elevation of the endocrine stress response in a heightened predation pressure environment.

Materials and methods

Animal acquisition and care

Unfertilized eggs and sperm were stripped from spawning adults caught below Pointe du Dubois generating station (50°17′52″N, 95°32′51″W) on the Winnipeg River, Manitoba, Canada, in May 2016 following previously published procedures (Genz et al. 2014). Upon gamete collection, all adult fishes were immediately returned to the river. The gametes were stored in a cooler, and brought to the University of Manitoba, Department of Biological Sciences, and artificially fertilized. Eggs from two females were fertilized with sperm from four males to form evenly mixed parentage from the brood stock and account for genetic effects on behaviour. The ratio of eggs to sperm was 15 mL of eggs to 40 μL of sperm and gametes were gently mixed for 2 min in 10 mL of water. They were then rinsed three times with de-chlorinated water and rinsed again in Fuller's clay for 30 min to remove the adhesive coating of the eggs (Genz et al. 2014). Fertilized eggs were then equally distributed between five flow-through MacDonald hatching jars (approximately 75 mL of fertilized eggs per jar) at 16 °C and 100% dissolved oxygen (DO) de-chlorinated tap water with a 14 h light : 10 h dark photoperiod. Dead or fungus-infected eggs were removed twice daily, with mortalities recorded. Upon hatching, the larva spilled out of the jars into one of two 75 L circular tanks in even densities and were supplied with flow-through de-chlorinated tap water at 16 °C and 100% DO with a 14 h light : 10 h dark photoperiod. At 27 days post fertilization (dpf), the lake sturgeon were fed live *Artemia* Leach, 1819 at 0700, 1200, and 1800 CST daily. After 44 dpf, shaved frozen bloodworms were mixed into their diet, and gradually the size and proportion of bloodworm increased until 51 dpf when they were fed bloodworm exclusively. At 67 dpf, the fish were moved into two 230 L tanks supplied with flow-through de-chlorinated tap water at 16 °C and 100% DO with a 14 h light : 10 h dark photoperiod, in similar densities. Sex of the individuals could not be determined, thus sex-based influences on behaviour and physiology could not be measured in these experiments.

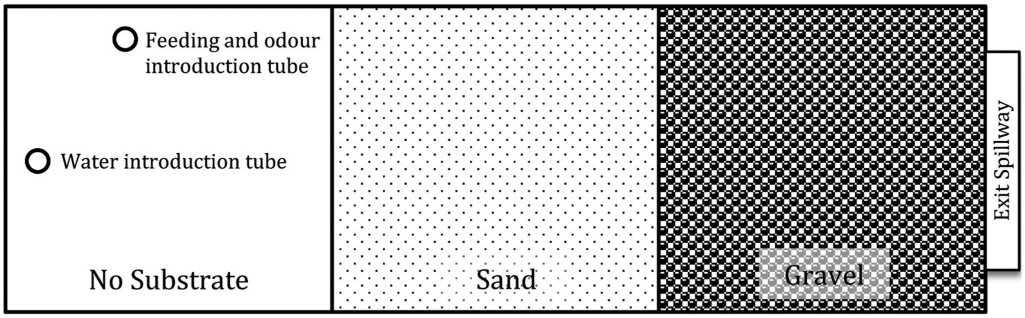
All ensuing experimental procedures were approved by the University of Manitoba Protocol Review Committee (protocol No. F015-007) under the guidelines of the Canadian Council on Animal Care.

Odour preparation

To ensure fresh and age-specific chemical alarm cue (AC) for each experiment, juvenile lake sturgeon AC was prepared at two critical life periods: (1) early exogenous feeding at 55 dpf (approximately 45 days post hatch) when fish have just transitioned fully to a diet of blood worm and freely forage in the water column, and (2) pre-winter at 170 dpf (approximately 160 days post hatch) when fish are again freely foraging in the water column with fully developed scutes. Eighteen juvenile lake sturgeon at 55 dpf (mean total length 27 mm; mass 0.09 g) and three juvenile lake sturgeon at 170 dpf (mean total length 56.5 mm; mass 5.82 g) were sacrificed by cervical dislocation in the absence of MS-222 that may dampen production of AC in the skin and therefore the subsequent response by our focal animals to the skin extract. The gut cavity of each fish was cleared to remove any dietary cues and the remaining tissue, for each separate experimental period, was placed into 100 mL of de-chlorinated water and homogenized with a Polytron™ 2100. The homogenate was then filtered through glass wool, diluted to 3.2 g/L, following the protocol of Wishingrad et al. (2014*a*), and frozen at −20 °C for later use.

Predator odour cue (PC) was prepared from northern crayfish (*Orconectes virilis* (Hagen, 1870)), a known predator of juvenile lake sturgeon (Caroffino et al. 2010), collected below the Pointe du Bois and Slave Falls generating station on the Winnipeg River from 20 to 21 May 2016. A total of 68.3 g of crayfish was collected and frozen for transport back to the University of Manitoba. Predator cue preparation followed a similar protocol as described for the alarm cue, where the homogenized whole-body crayfish were filtered through glass wool and made to a final concentration of 3.2 g/L and frozen at −20 °C for later use. Additionally, a control of distilled water was also frozen for later use in the behavioural trials.

Fig. 1. Behavioural arenas measuring 21 cm long by 14 cm wide and 6.4 cm deep at ~60 days post fertilization (dpf) trials and 59.7 cm long by 42.9 cm wide and 14.9 cm deep in ~160 dpf trials, with each substrate encompassing a third of the area, and each containing randomly assorted substrates (no substrate, sand, and gravel) with feeding tube position over no substrate in all arenas.



Experiment 1: diurnal risk assessment ontogeny

Twelve behavioural arenas measuring 21 cm long by 14 cm wide and 6.4 cm deep were constructed (Fig. 1). Three substrates were positioned randomly in the arena: gravel to present interstitial spaces for cover-seeking individuals, sand for preferred substrate of juvenile lake sturgeon (Chiasson et al. 1997; Benson et al. 2005), and a smooth, white plastic substrate to present an exposed area (described henceforth as no substrate); all positioned at the same elevation. Fine mesh was fastened over the exit spillway to contain the fish, and the arenas were placed in a large splash tray and curtained off to prevent disturbance from observers. A feeding hose and odour hose were positioned over the white plastic substrate of each arena to represent a high-risk feeding area and led over the top of the surrounding curtains so that food and odour could be introduced without disturbing the fish. The arenas were placed in a single splash tray, with a row of six on either side of the splash tray. Three SONY DCR-SR68 Handycam camcorders, each encompassing two arenas in their field of view, were positioned in the middle of the splash tray and were rotated to view each side during the behavioural trials.

One hundred and forty-four lake sturgeon between 52 and 54 dpf were anaesthetized with MS-222, weighed and measured, and introduced into the behavioural arenas in groups of six to create a total of 24 independent trials. The groups of six created a gregarious social context typical of their early life behaviour (Allen et al. 2009; Barth et al. 2009), and ensuing behavioural measurements thus included risk-taking and cover-seeking behaviours within an ecologically relevant group context. Lake sturgeon were fed 0.5 mL of frozen *Artemia* at 0800 CST and again at 2000 CST daily. Behaviours were recorded on the third day during the introduction of 0.5 mL of whole-body homogenate AC, at concentrations used by Wishingrad et al. (2014*a*), into the arenas simultaneously with the presence or absence of 0.5 mL of *Artemia*, at both 0800 and 2000 CST on the same individuals. Thus, a 1 × 2 design was used to measure the behavioural response of lake sturgeon to AC in the presence or absence of food.

At 158–170 dpf, the same protocol was conducted on an additional 144 juvenile lake sturgeon in a total of 24 independent trials to measure potential ontogenetic shifts in behavioural responses. To accommodate growth of these fish, 12 larger arenas measuring 59.7 cm long by 42.9 cm wide and 14.9 cm deep were constructed in a similar fashion as the first risk assessment trial, providing approximately the same space per fish as the earlier trials. Additionally, 5 mL of frozen bloodworm was fed to the lake sturgeon and 3.4 mL of AC was introduced to create similar concentrations as used in the earlier trial. Thus, predator naïve juvenile lake sturgeon were presented with a risk-sensitive foraging paradigm during early exogenous feeding and pre-winter developmental periods. Risk-taking behaviour, as defined as the magnitude of escape response, and cover-seeking behaviour (i.e., increased time spent over gravel and sand substrata) was measured as the number of line crossings in the experimental arena, could be quantified as a function of time of day and developmental stage in the presence or absence of food with $n = 12$ for each treatment.

Experiment 2: predation experience

Between 49 and 67 dpf (early exogenous feeding), lake sturgeon were conditioned to the predator cue in two groups, an acute group and a chronic group, to simulate different levels of predation experience. Acute conditioning took place between 49 and 58 dpf in 7 L flow-through tanks (approximately 20 fish/tank) with an air stone for additional water mixing. A combined stimulus of AC and PC (AC+PC) was introduced into half the tanks, and the other half received a control of PC with water (PC+C) between 0800–1000 CST and 1600–1800 CST for 3 days, such that a total of six conditioning events occurred. During each conditioning event, the water flow was turned off for 10 min, allowing the air stone to mix the odours throughout the tank. At the end of the conditioning period, the lake sturgeon were immediately moved to the same behavioural arenas as used in the risk assessment experiment. The same protocol as the risk assessment experiment was used with the exception of a 2 × 2 experimental approach where predation experienced (AC+PC) and naïve to predation experience (PC+C) lake sturgeon were introduced to PC and water in the presence or absence of food at 2000 and 0800 CST to test how acute predation experience might influence their risk-sensitive foraging behavioural response. This was repeated at 160 to 169 dpf (~160 dpf) to measure any ontogenetic shifts in acute predation experience. A total of 96 trials were run for acute predation experience and naïve to predation experience (288 acute experienced individual fish and 288 control individual fish) for early exogenous feeding and pre-winter life periods in lake sturgeon.

Chronic predation experience involved the same protocol as the acute conditioning, except the duration of exposure to the odours lasted 12 days, providing a total of 24 conditioning events for 55 to 67 dpf (early exogenous feeding) lake sturgeon. Chronic conditioning was not completed for the pre-winter life stage because the numbers of lake sturgeon were limited. A total of 48 trials were run on chronic predator-experienced and naïve to predation experience (144 chronic conditioned and 144 control individuals) lake sturgeon during early exogenous feeding. Thus, differing levels of predator-experienced juvenile lake sturgeon were presented with a risk-sensitive foraging paradigm where risk-taking behaviour (i.e., the magnitude of escape response) and cover-seeking behaviour (i.e., increased time spent over gravel and sand substrata) could be quantified as a function of time of day, developmental stage, and varying extrinsic states with $n = 12$ for each treatment.

Risk assessment and conditioning video coding

All videos were coded with JWatcher version 1.0 (Blumstein et al. 2006) for both experiment 1 and experiment 2 in the same way. Briefly, the data were separated into 8 min pre- and post-stimulus introduction periods so that stimulus-induced behaviour could be measured. Two focal fish were randomly selected from each arena, with flight response and substrate-type preference behaviour being coded. A 1 × 2 grid was superimposed over the arena and the sum of grid lines crossed by the head of the focal individuals allowed estimation of baseline movement and stimulus-induced movement. Thus, potential risk-taking behaviour as a function of differences in grid lines crossed between pre- and post-stimulus introduction periods could be determined (i.e., magnitude of escape response), which has been reported to be a robust measure of anti-predator behaviour in juvenile lake sturgeon (Wishingrad et al. 2014*a*; Sloychuk et al. 2016). Two widthwise grid lines lay on the divide between each substrate type, and the other lay lengthwise, bisecting each substrate. By doing so, it was possible to measure movement between substrate types, as well as within. Additionally, the total amount of time the focal fish spent over each substrate type in each arena during the trial was collected to determine cover-seeking behaviour (i.e., more time spent in gravel substrate) and potential stimulus-induced change.

Whole-body cortisol analysis

In July 2017, lake sturgeon raised in a similar manner as previously described were moved into six 7 L flow-through tanks at 56 dpf, with densities of 20 fish/tank, following the same protocol as experiment 2 for acute and chronic predator conditioning. Treatments of AC+PC, PC+water, and a control group receiving introductions of water only were randomly assigned to two of the six tanks. Acute conditioning began 9 days after chronic-conditioned groups, such that sampling of all individuals occurred at 67 dpf, at the same time of day. Fresh odours were produced and introduced to the treatments using the same protocol as experiment 2. At the end of the conditioning, 10 individuals from each acute and chronic group were immediately sacrificed in an overdose of MS-222 (200 mg/L), measured for total length and mass, and frozen at −80 °C for later whole-body basal cortisol analysis. Terminal sampling of the fish took less than 3 min.

Table 1. Results of generalized linear mixed models (GLMMs) from experiment 1 predicting the effect of alarm cue (AC) or AC with food (AC+Food) on the escape response and substrate-type preference (gravel, sand, no substrate) of juvenile lake sturgeon (*Acipenser fulvescens*) in relation to Period,* Time,† and Season.‡

Model	Predictor variable	Estimate	SE	$\Pr(>\lvert z\rvert)$
Escape response				
AC	Period	0.55	0.17	0.000889
AC	Season	0.52	0.18	0.003687
AC	Time	0.84	0.19	1.1×10^{-5}
AC+Food	Period	−0.89	0.15	5.21×10^{-9}
AC+Food	Season	—	—	—
AC+Food	Time	−0.71	0.16	4.89×10^{-6}
Gravel				
AC	Period	0.59	0.17	0.000625
AC	Season	−1.94	0.27	3.03×10^{-13}
AC	Time	0.76	0.21	0.00029
AC+Food	Period	−0.49	0.23	0.03193
AC+Food	Season	−1.20	0.34	0.000437
AC+Food	Time	—	—	—
Sand				
AC	Period	—	—	—
AC	Season	—	—	—
AC	Time	—	—	—
AC+Food	Period	—	—	—
AC+Food	Season	—	—	—
AC+Food	Time	—	—	—
No substrate				
AC	Period	—	—	—
AC	Season	0.31	0.12	0.0124
AC	Time	—	—	—
AC+Food	Period	—	—	—
AC+Food	Season	0.21	0.11	0.066
AC+Food	Time	—	—	—

Note: Parameter estimates, standard errors (SE), and probability associated with the Wald statistic ($\Pr(>\lvert z\rvert)$) of the negative binomial GLMMs of the eight models all run separately for the presence or absence of food, with fish ID as the random effect nested within tank ID. Em dashes denote the absence of significance of the factor within the model, which was removed from the final Akaike's information criterion (AIC) model.

*Period is from pre- to post-stimulus introduction.

†Time is associated to evening or morning trials.

‡Season is associated to the ~60 days post fertilization (dpf) or ~160 dpf trials.

Individual fish were thawed, weighed, and immediately placed in separate tubes with phosphate-buffered solution (0.1 mol/L Na_2HPO_4 and 0.03 mol/L NaH_2PO_4 at pH 7.4) for whole-body cortisol analysis. The tubes were homogenized, vortexed, and whole-body cortisol was extracted with Sep-Pak C18 cartridges. Three millilitres of methanol was used to elute the cortisol from the Sep-Paks, and the eluted samples were then dried and frozen at −80 °C for later measurement of cortisol using a radioimmunoassay (RIA) as previously described (Zubair et al. 2012). Briefly, samples were reconstituted in 250 μL RIA buffer (90 mL Milli-Q water, 10 mL PBS, 0.9 g NaCl, and 0.5 g bovine serum albumin). One hundred microlitres of 5000 DPM tritiated cortisol (PerkinElmer, Inc., Waltham, Massachusetts, USA) and 100 μL of cortisol-specific antibody (1:6000 dilution) (Fitzgerald Industries, Acton, New Jersey, USA) were added to 100 μL of reconstituted sample in duplicate. Assay tubes were vortexed, then incubated at room temperature for 1 h before an overnight incubation at 4 °C. The following day, the assay was stopped with the addition of 100 μL charcoal separation buffer (1.25 g of charcoal and 0.125 g of dextran in 25 mL RIA buffer) to each assay tube. Samples were allowed to incubate on ice for 15 min, then they were centrifuged at 4 °C for 15 min (rabbit anti-cortisol polyclonal antibody; Fitzgerald Industries). The liquid phase was then decanted into a scintillation vial, 4 mL of scintillation cocktail (Ultima Gold, PerkinElmer, Inc.) was added to each vial, and samples were counted on a liquid scintillation counter (PerkinElmer, Inc.; Tri-Carb 3110 TR) for 5 min. Cortisol concentration for each sample was determined by interpolating against a standard curve, generated with each assay in triplicate. Extraction efficiency was 99.5% ± 1.32% and intra-assay variation and inter-assay variation were 13% and 7%, respectively. Serial dilution of a sample pool showed good parallelism with the standard curve.

Statistical analysis

Generalized linear mixed models (GLMMs) were used in experiment 1 to quantify the relationship between lake sturgeon flight response to AC in the presence or absence of food, and substrate-type preference, during ~60 and ~160 dpf trials. In all models, the identity of the lake sturgeon in the arenas was modeled as a random effect nested within arena to account for the selection of two focal lake sturgeon from each arena and measurement of the same group of individuals during evening and morning trials. The introduced stimuli, AC and AC with food, were modeled separately because the objective was to quantify the behavioural response to each stimulus independently of each other. Analyses were carried out using R (R Core Team 2016) with GLMMs fitted using the glmer function from the lme4 package (Bates et al. 2015). The final model quantifying flight response to AC consisted of all predictor variables (Period, denoting pre- or post-stimulus introduction period; Season, denoting ~60 or ~160 dpf trials; and

Table 2. Results of generalized linear mixed models (GLMMs) from experiment 2 predicting the effect of odour[*] and food[†] on the escape response and substrate-type preference (gravel, sand, no substrate) of juvenile lake sturgeon (*Acipenser fulvescens*) in relation to Period,[‡] Time,[§] and Season,[‖] with Conditioning[¶] and Exposure[**] effects tested for later recognition of the predator odour and potential ensuing risk assessment behaviour.

Model	Estimate	SE	$\Pr(>\|z\|)$
Escape response			
Period	−0.84	0.07	2.0×10^{-16}
Food	0.57	0.09	1.0×10^{-10}
Exposure	—	—	—
Time	0.60	0.09	1.2×10^{-10}
Odour	−0.12	0.09	0.16
Season	−0.32	0.11	0.0047
Conditioning	0.43	0.11	5.0×10^{-5}
Gravel			
Period	−0.45	0.08	6.8×10^{-8}
Food	—	—	—
Exposure	—	—	—
Time	0.56	0.14	5.8×10^{-5}
Odour	—	—	—
Season	1.22	0.18	6.5×10^{-12}
Conditioning	0.59	0.18	0.0014
Sand			
Period	−0.09	0.04	0.016
Food	0.30	0.11	0.006
Exposure	0.25	0.11	0.024
Time	—	—	—
Odour	—	—	—
Season	0.97	0.14	1.1×10^{-12}
Conditioning	0.24	0.14	0.089
No substrate			
Period	0.08	0.06	0.0025
Food	−0.10	0.03	0.0662
Exposure	−0.14	0.06	0.0138
Time	0.08	0.06	0.1519
Odour	—	—	—
Season	−0.61	0.06	2.0×10^{-16}
Conditioning	—	—	—

Note: Parameter estimates, standard errors (SE), and probability associated with the Wald statistic ($\Pr(>|z|)$) of the negative binomial GLMMs of the eight models all run separately for the presence or absence of food, with fish ID as the random effect nested within tank ID. Em dashes denote the absence of significance of the variable within the model, which was removed from the final Akaike's information criterion (AIC) model.

[*] Odour is associated to a control of water or predator odour within the stimulus.

[†] Food is associated to the presence or absence of food within the stimulus.

[‡] Period is from pre- to post-stimulus introduction.

[§] Time is associated to evening or morning trials.

[‖] Season is associated to the ~60 days post fertilization (dpf) or ~160 dpf trials.

[¶] Conditioning associated to naïve fishes (PC+C) or predator-conditioned fishes (AC+PC), where PC is predator cue, C is control water, and AC is alarm cue.

[**] Exposure to odours during conditioning of the acute (6 exposures) or chronic (24 exposures) group.

Table 3. Poisson and negative binomial dispersion estimates of the generalized linear mixed models (GLMM) from the risk assessment experiment predicting the effect of the introduction of alarm cue (AC) in the presence or absence of food on juvenile lake sturgeon (*Acipenser fulvescens*) escape response and substrate-type preference (gravel, sand, no substrate).

Stimulus introduced	Poisson dispersion	Negative binomial dispersion
Escape response		
AC	6.83	0.60
AC+Food	4.69	0.75
Gravel		
AC	0.27	0.58
AC+Food	0.39	0.55
Sand		
AC	0.48	0.58
AC+Food	0.64	0.73
No substrate		
AC	0.45	0.62
AC+Food	0.54	0.71

Table 4. Poisson and negative binomial dispersion estimates of the generalized linear mixed models (GLMMs) from experiment 2 predicting lake sturgeon (*Acipenser fulvescens*) escape response and substrate-type preference (gravel, sand, no substrate) in response to the introduced stimulus of predator cue or a control of water, either in the presence or absence of food.

Model	Poisson dispersion	Negative binomial dispersion
Escape response	157	0.55
Gravel	0.30	0.40
Sand	0.49	0.51
No substrate	0.34	0.48

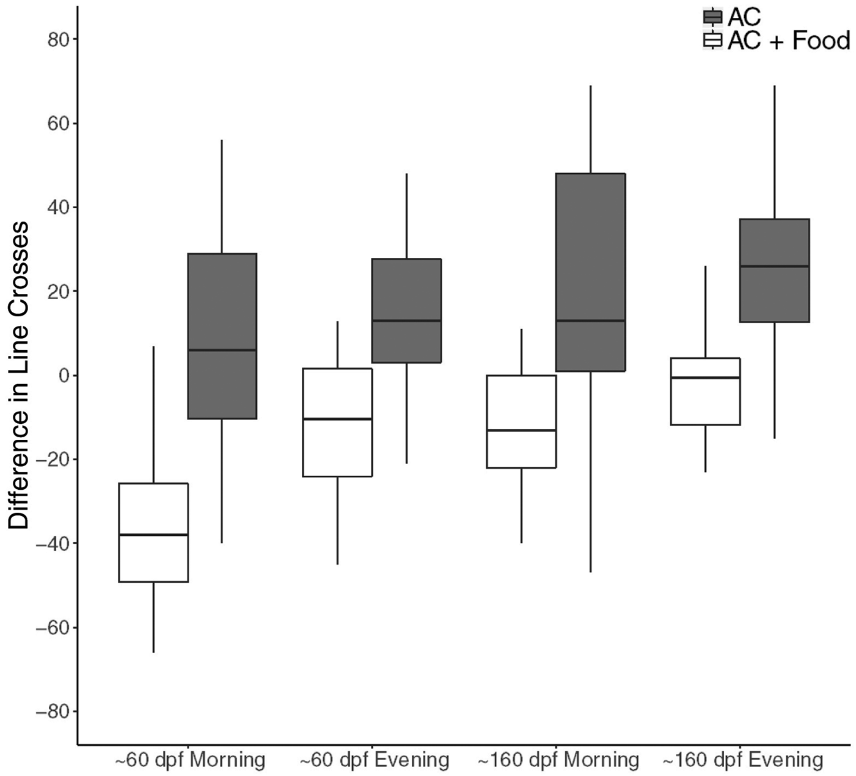
Time, denoting evening or morning trials), and the final model quantifying flight response to AC with food consisted of only Period and Time, based on Akaike's information criterion (AIC) (Table 1). Competing models consisted of all possible subsets of these predictor variables (Table 1).

GLMMs were similarly used in experiment 2 to quantify flight and substrate-type preference response of predator-experienced lake sturgeon to the introduced stimulus. These models consisted of seven possible response variables (Period; Food, denoting the presence or absence of food in the stimulus; Conditioning, denoting predator-experienced or naïve lake sturgeon; Time; Odour, denoting the presence or absence of PC in the stimulus; Season; and Exposure, denoting acute or chronic predator-experienced lake sturgeon). All variables were modeled together because the objective was to examine the main factors that drive juvenile lake sturgeon behaviour with a more complex phenotype consisting of more developed extrinsic experiences and intrinsic pressures.

Model ranking was accomplished with AIC in the function drop1 from the lme4 package (Bates et al. 2015) (Table 2). The response variable for each model was either flight response, sand, gravel, or no substrate, depending on whether flight or cover-seeking response was of interest in the analysis. In all models, a negative binomial distribution outperformed a Poisson distribution (Tables 3 and 4), which was used in the final model. The identity of the lake sturgeon in the arenas was modeled as a random effect nested within arena to account for the selection of two focal lake sturgeon from each arena and measurement of the same individuals during evening and morning trials.

Cortisol data were log-transformed to achieve normality (Shapiro–Wilk's test, $p = 0.40$), and linear regression was used to examine the differences in juvenile lake sturgeon basal whole-body cortisol concentrations between treatments (AC+PC, PC+C, control), season (early exogenous feeding and pre-winter), and predation experience exposure (acute vs. chronic). The Breusch–Pagan test was used to confirm homogeneity of variance in the models ($p > 0.05$ in all models).

Fig. 2. Change in line crosses (post-stimulus minus pre-stimulus magnitude of escape response) during the risk assessment trials of ~60 days post fertilization (dpf) and ~160 dpf lake sturgeon (*Acipenser fulvescens*) to the introduction of alarm cue (AC) in the presence or absence of food, with trials run during the morning and evening. Boxplot hinges are expressed as first and third quartiles around the median, with whiskers representing ±1.5 inter-quartile range.



Total length, mass, and condition factor of the lake sturgeon were tested for normality (Shapiro–Wilk's test; p = 0.05, 0.003, and 0.42, respectively). Given a linear regression's robust nature towards deviations from normality (Lix et al. 1996; Keselman et al. 2008) and the minimal deviations observed in the models (qqnorm plots), linear regression was used to measure potential differences in body metrics between the predator experience treatments and exposure. Length, mass, and condition factor models were tested for homogeneity of variance with the Breusch–Pagan test (p = 0.53, 0.23, and 0.14, respectively).

Results

Risk assessment ontogeny

The introduction of AC resulted in a significant flight response (i.e., magnitude of escape response) in all AC alone trials and it became significantly more pronounced in the pre-winter trials (Fig. 2, Table 1). However, when the combined stimulus of AC with food was introduced, no flight response was observed at either critical life period of early exogenous feeding or pre-winter. Rather, basal movement levels significantly reduced in all AC with food trials. However, the reduction in flight response to AC with food in the pre-winter trials was more moderate, suggesting a potential shift from foraging to anti-predator behaviour. Additionally, the largest difference in response to AC was observed between evening and morning trials, where flight response was significantly higher in the morning (Fig. 2, Table 1). Lake sturgeon flight response did not change over the critical life periods (season) in response to the combined stimulus of AC with food, and thus, was removed from the final model (AIC).

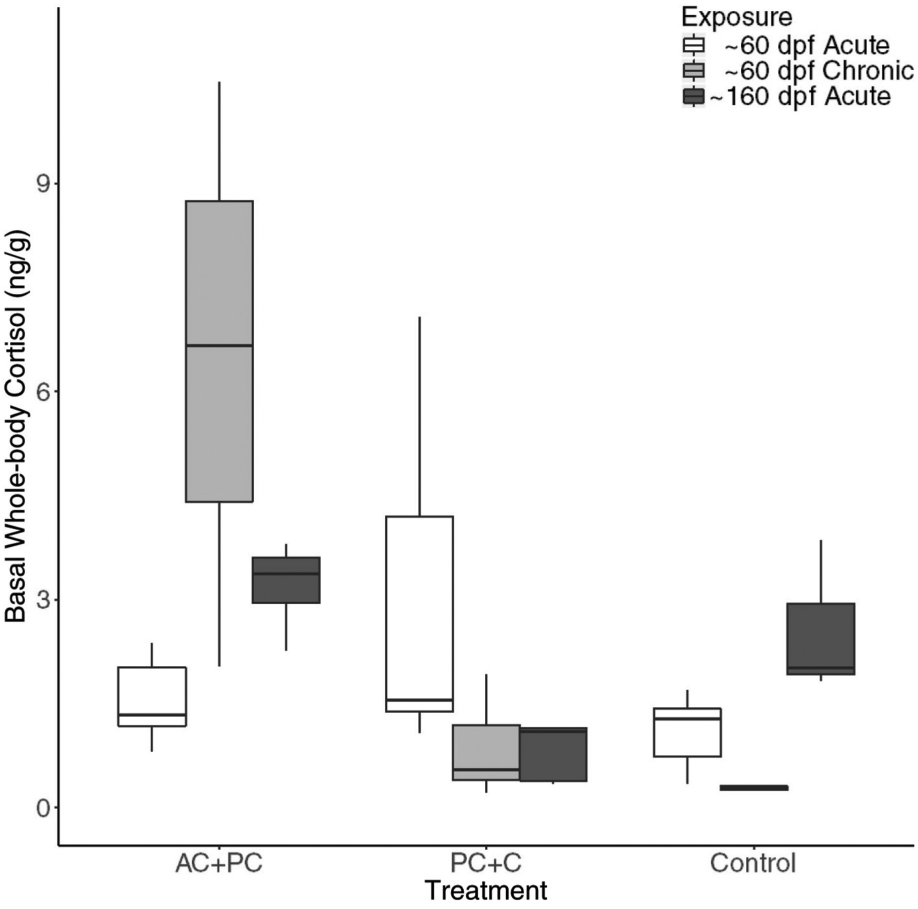
Overall, season had the strongest influence on lake sturgeon gravel and no substrate preference in response to AC, where gravel preference significantly decreased from early exogenous feeding to pre-winter (~60 to ~160 dpf) and no substrate significantly increased (Table 1), suggesting less risk-averse behaviour with development. In response to the combined stimulus, gravel preference significantly declined and the reduction was more pronounced with season. For no substrate preference, season was only positively influenced by the combined stimulus. In all models, no stimulus-induced change in sand preference was observed.

Predator experience

Overall, flight response of the juvenile lake sturgeon significantly decreased from pre- to post-introduction of the stimulus (Table 2). Similar to experiment 1, their flight response significantly decreased from morning to evening trials, suggesting that their responses to foraging and anti-predator behaviour are heightened in the early hours of the day. Season also produced a significant reduction in their flight response behaviour, where more developed fish in the pre-winter trials overall responded with reduced flight response behaviour. Again, the introduction of food with the odour stimulus resulted in reduced flight response behaviour, suggesting that juvenile lake sturgeon continue to respond to foraging opportunities despite developmental stage or heightened predation experience. Interestingly, predator-experienced lake sturgeon flight response behaviour was no different from their naïve counter parts, nor did the predator cue in the introduced stimulus produce any flight behaviour in the fish. However, juvenile lake sturgeon of chronic predation experience had a significantly depressed flight response from their naïve or acute predation-experienced counter parts.

In all models, juvenile lake sturgeon cover-seeking behaviour significantly reduced from pre- to post-stimulus introduction, where time spent in gravel and sand substrates significantly reduced and time in no substrate significantly increased (Table 2).

Fig. 3. Basal whole-body cortisol concentrations of experienced (alarm cue with predator cue (AC+PC)), naïve (predator cue with control water (PC+C)) and control lake sturgeon (*Acipenser fulvescens*) subject to either acute experience (6 exposures to the odours) or chronic experience (24 exposures to the odours) at ~60 days post fertilization (dpf) and ~160 dpf. Data are expressed in nanograms per gram (ng/g) body mass, with boxplot hinges as first and third quartiles around the median and whiskers representing ±1.5 inter-quartile range.



However, cover-seeking behaviour significantly increased with season, where fish in the pre-winter trials spent more time in gravel and sand substrates, with a subsequent reduction in no substrate time. The introduction of food in the stimulus significantly increased the amount of time juvenile lake sturgeon spent over the more "risky" no substrate to forage, with corresponding reduction of time spent over sand and no change over gravel. Interestingly, juvenile lake sturgeon with predation experience spent significantly more time over sand substrate and less time over the more "risky" no substrate. Additionally, juvenile lake sturgeon with chronic predation experience spent significantly more time over the gravel substrate, which represented the most-safe habitat by providing interstitial spaces for the fish to seek cover. Similar to their flight response behaviour, the introduction of PC in the stimulus had no effect on their cover-seeking behaviour.

Cortisol

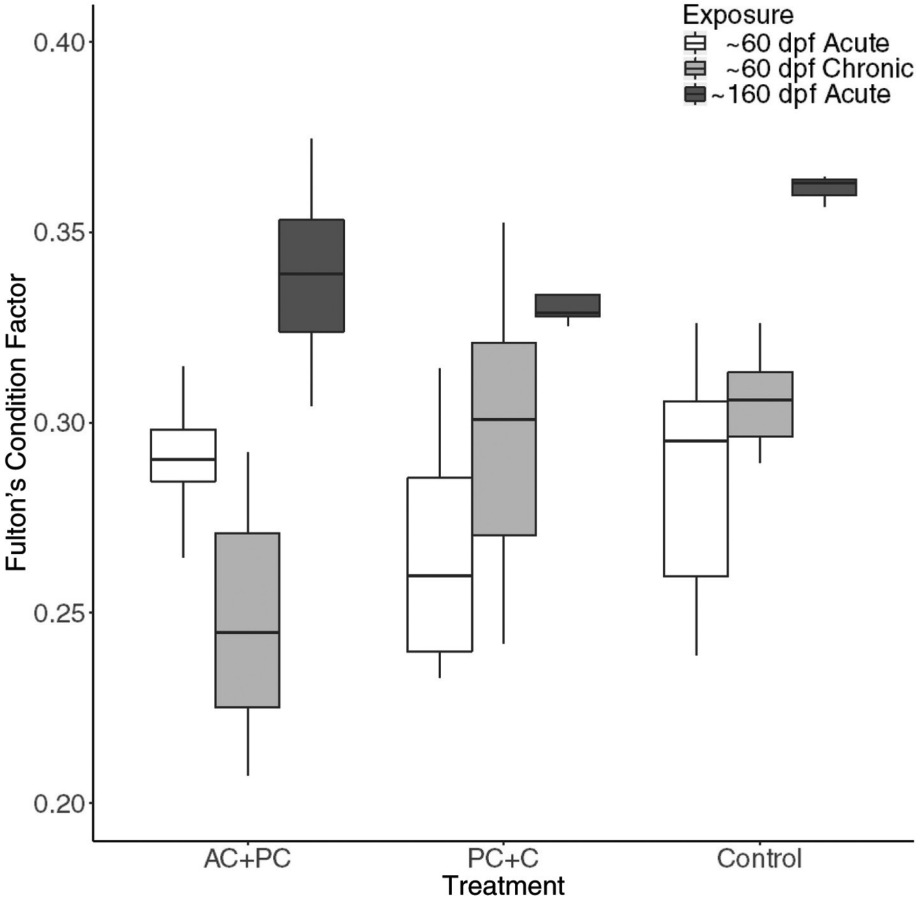
Following predator experience conditioning, basal whole-body cortisol concentrations were significantly higher in juvenile lake sturgeon introduced to AC+PC (Fig. 3), whereas exposure (acute vs. chronic) to the odours and season (early exogenous feeding vs. pre-winter) had no effect on basal cortisol concentrations. However, lake sturgeon chronically exposed to AC+PC had significantly lower Fulton's condition factor ($p < 0.05$) than chronic PC+C and control treatments (Fig. 4), which may impact overwinter survival in juvenile lake sturgeon (Yoon et al. 2019).

Discussion

In the risk assessment experiment, predator-naïve juvenile lake sturgeon responded with increased anti-predator behaviour during the morning trials and with development in response to AC alone. However, the introduction of the combined stimulus of AC with food elicited a strong foraging response, i.e., they moved to the source of food and consumed all available food, despite the presence of potential predation risk, time of day, or critical life stage. This response is not uncommon among fishes, as a number of studies report the costs of leaving an ideal foraging patch may exceed the perceived risk (Stankowich and Blumstein 2005), which is particularly true in temperate environments where overwinter mortality can be more severe than predation mortality in fishes (Schultz and Conover 1997) and where obtaining enough energy reserves to maximize winter survival is paramount (Post and Evans 1989; Post and Parkinson 2001; Hurst and Conover 2003). Studies on other sturgeon species suggest finding properly sized and nutritious food is critical for early life survival (Wildhaber et al. 2007). Similarly, environmental selective pressures on juvenile lake sturgeon may promote an early life behavioural strategy that selects for foraging over anti-predator behaviour during risk-sensitive foraging occurrences.

Juvenile lake sturgeon contextual behavioural responses to predation threat provide insight into the multiple selective forces on their early life behavioural strategy. First documented by Wishingrad et al. (2014*a*) was the ability of juvenile lake sturgeon

Fig. 4. Fulton's condition factor of juvenile lake sturgeon (*Acipenser fulvescens*) treatments (experienced fishes introduced to alarm cue with predator cue (AC+PC), naïve fishes introduced to predator cue with control water (PC+C), and control) after being subject to chronic experience (24 introductions of odourants over 12 days) at ~60 days post fertilization (dpf). Boxplot hinges are expressed as first and third quartiles around the median, with whiskers representing ±1.5 inter-quartile range.



to produce and respond to AC with innate and immediate antipredator behaviour. Shortly after, Sloychuk et al. (2016) demonstrated the ability of juvenile lake sturgeon to rapidly learn predator odours through classical conditioning. These mechanisms are costly to produce and are characteristic of predator–prey arms race relationships in fishes (Ferrari et al. 2010*b*), suggesting that predation pressure poses a strong selective force on juvenile lake sturgeon. Interestingly, predation experience had no effect on their flight response behaviour in our study. Perhaps the design of this study, by contrasting high potential predation risk with protracted foraging opportunities, created a high consequence paradigm that did not allow other effects to be statistically observed. Predator abundance (Hurst 2007) and prey availability can vary markedly (Heck and Crowder 1991) over small spatial scales in freshwater habitats similar to the ones occupied by juvenile lake sturgeon. In such environments of gradient risks and foraging patches, it is likely that more complex risk assessment behaviour could be observed.

Physiological responses to predation threat are necessary. Typically, this involves activation of the hypothalamic–pituitary–interrenal (HPI) axis, which can lead to increased plasma levels of glucocorticoids such as cortisol (Barton 2002) to mobilize energy reserves and fuel a response. Similarly, chronic exposure of AC combined with PC showed a significant increase in basal whole-body cortisol concentrations in juvenile lake sturgeon at 60 dpf. Such physiological responses have been observed in other taxa. For example, Clinchy et al. (2004) observed increased glucocorticoids in Song Sparrows (*Melospiza melodia* (A. Wilson, 1810)) residing in high-predation environments, and Berger et al. (2007) found a similar trend in marine iguanas (*Amblyrhynchus cristatus* Bell, 1825), which experience recent predator introductions.

If glucocorticoid levels are elevated for prolonged periods, then they can have deleterious effects on the well-being of vertebrates (Clinchy et al. 2004; Sapolsky et al. 2000). Indeed, juvenile lake sturgeon exposed to chronic exposures of AC with PC at ~60 dpf had significantly lower mass and Fulton's condition factor than those individuals in the chronic PC with water and control treatments. Such body differences, particularly Fulton's condition factor can influence juvenile lake sturgeon conspecific resource competition and potentially decrease their ability to respond to potential predation threat (Bjornson and Anderson 2018). In addition to physiological responses, juvenile lake sturgeon with chronic predator experience responded behaviorally with significantly less time spent over the no substrate foraging area and more time seeking cover in the gravel interstitial spaces. This response shows the first deviation in juvenile lake sturgeon early life-history strategy of feeding regardless of predation threat. This may suggest that over a gradient of predation pressures, a threshold may be reached where the predation pressures exceed that of the upcoming winter-period pressures, and a shift in risk assessment behaviour during risk-sensitive foraging may occur. However, if high predation pressures continue, then it is likely that overwinter survival may be compromised (Bjornson and Anderson 2018; Deslauriers et al. 2018).

Results indicate the complex and context-dependent early life behavioural strategies of juvenile lake sturgeon during risk-sensitive foraging. During an individual lake sturgeon's early life period, foraging plays a strong role in their risk assessment behaviour and this may be more important than responding to potential predation threats during early life stages. However, our results suggest that juvenile lake sturgeon have the ability to assess multiple risks simultaneously and produce complex behavioural responses optimal for survival. If predation threat is elevated for a sufficient time, then juvenile lake sturgeon may shift from foraging to anti-predator behaviour during risk-sensitive foraging opportunities, with potential implications for winter survival. Such observations have clear implications for conservation management of this species. In particular, a better understanding of juvenile lake sturgeon risk assessment may improve stock enhancement by providing enriched rearing environments that promote more ecologically robust behavioural phenotypes, as well as more appropriate release timing into natural ecosystems that align better with their risk assessment ontogeny.

Acknowledgements

We thank the animal care staff, particularly T. Smith, for their assistance in the care and maintenance of the animals used in this study. Funding for this work was provided by a Natural Sciences and Engineering Research Council of Canada (NSERC) – Manitoba Hydro Industrial Research Chair and NSERC Discovery grant 05348-15 awarded to W.G.A.

References

Allen, P.J., Barth, C.C., Peake, S.J., Abrahams, M.V., and Anderson, W.G. 2009. Cohesive social behaviour shortens the stress response: The effects of conspecifics on the stress response in lake sturgeon *Acipenser fulvescens*. J. Fish Biol. **74**(1): 90–104. doi:10.1111/j.1095-8649.2008.02112.x. PMID:20735526.

Anholt, B.R., Werner, E.E., and Skelly, D.K. 2000. Effect of food and predators on the activity of four larval ranid frogs. Ecology, **81**: 3509–3521. doi:10.1890/0012-9658(2000)081[3509:EOFAPO]2.0.CO;2.

Artyuklin, E.N. 1995. Contributions on biogeography and relationships within the genus *Acipenser*. Sturgeon Quarterly, **3**: 6–8.

Barth, B.C.C., Peake, S.J., Allen, P.J., and Anderson, W.G. 2009. Habitat utilization of juvenile lake sturgeon, *Acipenser fulvescens*, in a large Canadian river. J. Appl. Ichthyol. **25**: 18–26. doi:10.1111/j.1439-0426.2009.01335.x.

Barton, B.A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. **42**: 517–525. doi:10.1093/icb/42.3.517.

Bates, D., Maechler, M., Bolker, B., and Walker, B. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. **67**: 1–48. doi:10.18637/jss.v067.i01.

Benson, A.C., Sutton, T.M., Elliott, R.F., and Meronek, T.G. 2005. Evaluation of sampling techniques for age-0 lake sturgeon in a Lake Michigan tributary. N. Am. J. Fish. Manage. **25**: 1378–1385. doi:10.1577/M04-172.1.

Berger, S., Wikelski, M., Romero, L.M., Kalko, E.K.V., and Rödl, T. 2007. Behavioral and physiological adjustments to new predators in an endemic island species, the Galápagos marine iguana. Horm. Behav. **52**: 653–663. doi:10.1016/j.yhbeh.2007.08.004. PMID:17904141.

Biro, P.A., Post, J.R., and Parkinson, E.A. 2003. From individuals to populations: prey fish risk-taking mediates mortality in whole-system experiments. Ecology, **84**: 2419–2431. doi:10.1890/02-0416.

Biro, P.A., Abrahams, M.V., Post, J.R., and Parkinson, E.A. 2004. Predators select against high growth rates and risk-taking behaviour in domestic trout populations. Proc. R. Soc. B Biol. Sci. **271**: 2233–2237. doi:10.1098/rspb.2004.2861. PMID:15539348.

Bjornson, F., and Anderson, W.G. 2018. Body condition, rather than size, predicts risk-taking and resource holding potential in hatchery reared juvenile lake sturgeon, *Acipenser fulvescens*. J. Fish Biol. **93**: 1188–1196. doi:10.1111/jfb.13840. PMID:30345517.

Blumstein, D.T., Daniel, J.C., and Evans, C.S. 2006. JWatcher. Version 1.0. Available from https://www.jwatcher.ucla.edu.

Caroffino, D.C., Sutton, T.M., Elliott, R.F., and Donofrio, M.C. 2010. Predation on early life stages of lake sturgeon in the Peshtigo River, Wisconsin. Trans. Am. Fish. Soc. **139**: 1846–1856. doi:10.1577/T09-227.1.

Chiasson, W.B., Noakes, D.L.G., and Beamish, F.W.H. 1997. Habitat, benthic prey, and distribution of juvenile lake sturgeon (*Acipenser fulvescens*) in northern Ontario rivers. Can. J. Fish. Aquat. Sci. **54**(12): 2866–2871. doi:10.1139/f97-194.

Clark, C.W. 1994. Antipredator behavior and the asset-protection principle. Behav. Ecol. **5**: 159–170. doi:10.1093/beheco/5.2.159.

Clinchy, M., Zanette, L., Boonstra, R., Wingfield, J.C., and Smith, J.N.M. 2004. Balancing food and predator pressure induces chronic stress in songbirds. Proc. R. Soc. B Biol. Sci. **271**: 2473–2479. doi:10.1098/rspb.2004.2913. PMID:15590598.

Deslauriers, D., Yoon, G.R., Earhart, M.L., Long, C., Klassen, C.N., and Anderson, W.G. 2018. Over-wintering physiology of age-0 lake sturgeon (*Acipenser fulvescens*) and its implications for conservation stocking programs. Environ. Biol. Fishes, **101**(4): 623–637. doi:10.1007/s10641-018-0724-4.

Elgar, M.A. 1989. Predator vigilance and group size in mammals and birds: a critical review of the empirical evidence. Biol. Rev. Camb. Philos. Soc. **64**: 13–33. doi:10.1111/j.1469-185X.1989.tb00636.x. PMID:2655726.

Ferrari, M.C.O., Wisenden, B.D., and Chivers, D.P. 2010*b*. Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. Can. J. Zool. **88**(7): 698–724. doi:10.1139/Z10-029.

Ferrari, M.C.O., Wishingrad, V., Musgrove, A.B., and Chivers, D.P. 2015. Risk in a changing world: environmental cues drive anti-predator behaviour in lake sturgeon (*Acipenser fulvescens*) in the absence of predators. Behaviour, **152**(5): 635–652. doi:10.1163/1568539X-00003246.

Genz, J., McDougall, C.A., Burnett, D., Arcinas, L., Kheeto, S., and Anderson, W.G. 2014. Induced spawning of wild-caught lake sturgeon (*Acipenser fulvescens*): assessment of hormonal and stress responses, gamete quality and survival. J. Appl. Ichthyol. **30**: 1565–1577. doi:10.1111/jai.12548.

Golub, J.L., and Brown, G.E. 2003. Are all signals the same? Ontogenetic change in the response to conspecific and heterospecific chemical alarm signals by juvenile green sunfish (*Lepomis cyanellus*). Behav. Ecol. Sociobiol. **54**: 113–118. doi:10.1007/s00265-003-0629-9.

Harvey, M.C., and Brown, G.E. 2004. Dine or dash? Ontogenetic shift in the response of yellow perch to conspecific alarm cues. Environ. Biol. Fishes, **70**: 345–352. doi:10.1023/B:EBFI.0000035432.12313.87.

Heck, K.L., and Crowder, L.B. 1991. Habitat structure and predator–prey interactions in vegetated aquatic systems. *In* Habitat structure: the physical arrangement of objects in space. Population and community biology series. Vol. 8. *Edited by* S.S. Bell, E.D. McCoy, and H.R. Mushinsky. Springer, Dordrecht, the Netherlands. pp. 281–299.

Houston, A.I., McNamara, J.M., and Hutchinson, J.M.C. 1993. General results concerning the trade-off between gaining energy and avoiding predation. Philos. Trans. R. Soc. B Biol. Sci. **341**: 375–397. doi:10.1098/rstb.1993.0123.

Hurst, T.P. 2007. Causes and consequences of winter mortality in fishes. J. Fish Biol. **71**(2): 315–345. doi:10.1111/j.1095-8649.2007.01596.x.

Hurst, T.P., and Conover, D.O. 2003. Seasonal and interannual variation in the allometry of energy allocation in juvenile striped bass. Ecology, **84**: 3360–3369. doi:10.1890/02-0562.

Johansson, F., and Rowe, L. 1999. Life history and behavioral responses to time constraints in a damselfly. Ecology, **80**: 1242–1252. doi:10.1890/0012-9658(1999)080[1242:LHABRT]2.0.CO;2.

Johansson, F., Stoks, R., Rowe, L., and De Block, M. 2001. Life history plasticity in a damselfly: effects of combined time and biotic constraints. Ecology, **82**: 1857–1869. doi:10.1890/0012-9658(2001)082[1857:LHPIAD]2.0.CO;2.

Jones, M., Laurila, A., Peuhkuri, N., Piironen, J., and Seppa, T. 2003. Timing an ontogenetic niche shift: responses of emerging salmon alevins to chemical cues from predators and competitors. Oikos, **102**: 155–163. doi:10.1034/j.1600-0706.2003.12347.x.

Keselman, H.J., Algina, J., Lix, L.M., Wilcox, R.R., and Deering, K.N. 2008. A generally robust approach for testing hypotheses and setting confidence intervals for effect sizes. Psych. Meth. **13**: 110–129. doi:10.1037/1082-989X.13.2.110.

Lix, L.M., Keselman, J.C., and Keselman, H.J. 1996. Consequences of assumption violations revisited: A quantitative review of alternatives to the one-way analysis of variance *F* test. Rev. Educ. Res. **66**: 579–619. doi:10.2307/1170654.

Lonnstedt, O.M., McCormick, M.I., Meekan, M.G., Ferrari, M.C.O., and Chivers, D.P. 2012. Learn and live: predator experience and feeding history determines prey behaviour and survival. Proc. R. Soc. B Biol. Sci. **279**: 2091–2098. doi:10.1098/rspb.2011.2516. PMID:22237904.

Ludwig, D., and Rowe, L. 1990. Life history strategies for energy gain and predator avoidance under time constraints. Am. Nat. **135**: 686–707. doi:10.1086/285069.

Marcus, J.P., and Brown, G.E. 2003. Response of pumpkinseed sunfish to conspecific chemical alarm cues: an interaction between ontogeny and stimulus concentration. Can. J. Zool. **81**(10): 1671–1677. doi:10.1139/z03-165.

McDougall, C.A., Blanchfield, P.J., and Anderson, W.G. 2014. Linking movements of lake sturgeon (*Acipenser fulvescens* Rafinesque, 1817) in a small hydroelectric reservoir to abiotic variables. J. Appl. Ichthyol. **30**(6): 1149–1159. doi:10.1111/jai.12546.

Pollock, M.S., Carr, M., Kreitals, N.M., and Phillips, I.D. 2015. Review of a species in peril: what we do not know about lake sturgeon may kill them. Environ. Rev. **23**(1): 30–43. doi:10.1139/er-2014-0037.

Polverino, G., Bierbach, D., Killen, S.S., Uusi-Heikkilä, S., and Arlinghaus, R. 2016. Body length rather than routine metabolic rate and body condition correlates with activity and risk-taking in juvenile zebrafish *Danio rerio*. J. Fish Biol. **89**: 2251–2267. doi:10.1111/jfb.13100. PMID:27615803.

Post, J.R., and Evans, D.O. 1989. Size-dependent overwinter mortality of young-of-the-year yellow perch (*Perca flavescens*): laboratory, in situ enclosure, and field experiments. Can. J. Fish. Aquat. Sci. **46**(11): 1958–1968. doi:10.1139/f89-246.

Post, J.R., and Parkinson, E.A. 2001. Energy allocation strategy in young fish: allometry and survival. Ecology, **82**: 1040–1051. doi:10.1890/0012-9658(2001)082[1040:EASIYF]2.0.CO;2.

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from https://www.r-project.org/.

Rowe, L., and Ludwig, D. 1991. Size and timing of metamorphosis in complex life cycles: time constraints and variation. Ecology, **72**: 413–427. doi:10.2307/2937184.

Sapolsky, R.M., Romero, L.M., and Munck, A.U. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. **21**: 55–89. doi:10.1210/edrv.21.1.0389. PMID:10696570.

Schultz, E.T., and Conover, D.O. 1997. Latitudinal differences in somatic energy storage: adaptive responses to seasonality in an estuarine fish (Atherinidae: *Menidia menidia*). Oecologia, **109**: 516–529. doi:10.1007/s004420050112. PMID:28307335.

Sloychuk, J.R., Chivers, D.P., and Ferrari, M.C.O. 2016. Juvenile Lake Sturgeon go to school: life-skills training for hatchery fish. Trans. Am. Fish. Soc. **145**: 287–294. doi:10.1080/00028487.2015.1123183.

Stankowich, T., and Blumstein, D.T. 2005. Fear in animals: a meta-analysis and review of risk assessment. Proc. R. Soc. B Biol. Sci. **272**: 2627–2634. doi:10.1098/rspb.2005.3251. PMID:16321785.

Werner, E.E., and Anholt, B.R. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. Am. Nat. **142**: 242–272. doi:10.1086/285537. PMID:19425978.

Wildhaber, M.L., Papoulias, D.M., DeLonay, A.J., Tillitt, D.E., Bryan, J.L., and Annis, M.L. 2007. Physical and hormonal examination of Missouri River shovelnose sturgeon reproductive stage: a reference guide. J. Appl. Ichthyol. **23**: 382–401. doi:10.1111/j.1439-0426.2007.00878.x.

Wishingrad, V., Sloychuk, J.R., Ferrari, M.C.O., and Chivers, D.P. 2014*a*. Alarm cues in Lake Sturgeon *Acipenser fulvescens* Rafinesque, 1817: potential implications for like-skills training. J. Appl. Ichthyol. **30**: 1441–1444. doi:10.1111/jai.12580.

Wishingrad, V., Chivers, D.P., and Ferrari, M.C.O. 2014*b*. Relative cost/benefit trade-off between cover-seeking and escape behaviour in an ancestral fish: the importance of structural habitat heterogeneity. Ethology, **120**: 973–981. doi:10.1111/eth.12269.

Wishingrad, V., Ferrari, M.C.O., and Chivers, D.P. 2014*c*. Behavioural and morphological defences in a fish with a complex antipredator phenotype. Anim. Behav. **95**: 137–143. doi:10.1016/j.anbehav.2014.07.006.

Wolf, M., and Weissing, F.J. 2010. An explanatory framework for adaptive personality differences. Philos. Trans. R. Soc. B Biol. Sci. **365**: 3959–3968. doi:10.1098/rstb.2010.0215. PMID:21078648.

Yoon, G.R., Deslauriers, D., Enders, E.C., Treberg, J.R., and Anderson, W.G. 2019. Effects of temperature, dissolved oxygen and substrate on the development of metabolic phenotypes in age-0 lake sturgeon, *Acipenser fulvescens*: implications for overwintering survival. Can. J. Fish. Aquat. Sci. **76**(9): 1596–1607. doi:10.1139/cjfas-2018-0399.

Zubair, S.N., Peake, S.J., Hare, J.F., and Anderson, W.G. 2012. The effect of temperature and substrate on the development of the cortisol stress response in the lake sturgeon, *Acipenser fulvescens*, Rafinesque (1817). Environ. Biol. Fishes, **93**(4): 577–587. doi:10.1007/s10641-011-9951-7.