

COMPETITION AND RISK ASSESSMENT OF JUVENILE LAKE STURGEON
(ACIPENSER FULVESCENS): IMPLICATIONS FOR CONSERVATION
AQUACULTURE

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Abstract

Hatchery supplementation of declining fish populations is often used as part of population enhancement and recovery programs. However, fish produced from the sterile hatchery environment often develop maladaptive behavioural phenotypes that can result in high post-release mortality. Lake Sturgeon (*Acipenser fulvescens*) are reared in hatcheries for conservation purposes, yet little is known of their early life behaviour, making it difficult to determine whether behavioural phenotypes promoted in the hatchery influence post-release survival. Here, I investigate the influence of age 0+ Lake Sturgeon body size on resource holding potential (RHP) and boldness, and the potential for predator conditioning to influence the development of risk assessment and the physiological state of the individuals. In the RHP experiment, Lake Sturgeon were asymmetrically and symmetrically size matched and provided limited resources over seven days to promote feeding motivation. To measure body size mediated bold-shy responses, fishes were asymmetrically size matched in groups of six and a conspecific alarm cue (AC) was introduced to the arena in the presence and absence of food, allowing measurement of their willingness to forage in the presence of potential predation threat (i.e. boldness). Additionally, Lake Sturgeon were acutely (six conditioning events) and chronically (24 conditioning events) conditioned to a novel predator odour, and tested for potential risk assessment when later exposed to the same predator odour in the presence or absence of food. A subset of the conditioned fishes were collected to measure whole body cortisol response to the conditioning. Results indicate condition factor, rather than body size, increases RHP and bold responses in juvenile Lake Sturgeon. Additionally, conditioning did not influence risk assessment behaviour, but rather foraging remained

paramount, despite the presence of potential predation risk. However, chronic conditioning had an effect on whole body cortisol concentrations of Lake Sturgeon, suggesting a sensitive stress response. These results indicate the potential for hatcheries to select condition factor in Lake Sturgeon, and thus individuals of higher RHP and boldness, as well as highlight the complex risk assessment behaviour of juvenile Lake Sturgeon and potential limitations of predator conditioning, both behaviourally and physiologically.

Chapter 1: General Introduction

1.1 Lake sturgeon ecology and life history

Lake sturgeon are one of the largest and most ancestral freshwater fish species of North America (Pollock et al. 2015), and have remained relatively unchanged morphologically, and possibly behaviourally, since the lower Jurassic Period (Sulak and Randall 2002). They belong to the family Acipenseridae, which consists of approximately 25 extant sturgeon species (Birstein and Bemis 1997), and with Paddlefish (*Polyodontidae*) they comprise the most numerous order of all living fossil fishes, the Acipenseriformes (Birstein and DeSalle 1998; Grande and Bemis 1991).

Lake Sturgeon are found throughout the Hudson's Bay, Great Lakes, and Mississippi River drainage basins of North America (Auer 1996a; Scott and Crossman 1998). Their life histories are characteristic of all sturgeons, comprised of long life spans, late age at sexual maturity, and protracted spawning periodicity (Scott and Crossman 1973). Their exclusive freshwater life history is rare among sturgeons (Auer 1996a; Scott and Crossman 1998), as is their life history relative to other freshwater fish species of

North America (Scott and Crossman 1973). It is believed that their longevity increases the number of spawning events they can participate in, ensuring the success of at least one recruitment of a future generation (Duong et al. 2013). Lake Sturgeon follow an r selection approach where females are highly fecund, with approximately 400,000 eggs per female on average in some populations (Bruch et al. 2006), and invest little into their offspring post spawn.

1.2 Species status

Since the late 1800s and early 1900s, Lake Sturgeon populations have been in decline largely due to extensive over fishing, and to date many populations have been extirpated while others dwindle (Pollock et al. 2015). Habitat loss and degradation, as well as unique life history and reproductive traits, inhibit Lake Sturgeon recovery (Beamish et al. 1998). Despite moratoriums or restricted limits on Lake Sturgeon harvest across North America, stocking programs, and recognition as endangered under the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2006), their recovery is slow with only a few successful conservation effort examples (Pollock et al. 2015).

1.3 Conservation efforts and current fish hatchery practices

For decades hatcheries across North America have been raising Lake Sturgeon for stock enhancement of natural populations (Pollock et al. 2015). The approach has been widely used for a number of fish species, and is most well-known for enrichment of Pacific salmonid species (Brown et al. 2013). In the case of Lake Sturgeon, direct gamete

takes from spawning males and females, recently hatched larvae, and or recently fertilized eggs from spawning habitats are collected from wild populations and raised in hatcheries until they outgrow their most vulnerable life stages (i.e. the first few months of life). They are then reintroduced back into the wild as part of population enhancement, recovery programs, and overall conservation efforts (Crossman et al. 2011b).

Although the controlled environment of a fish hatchery removes stochastic factors, such as climate, hydrological conditions and predation, that determine year class strength within the first few months after spawning (Nilo et al. 1997, Verdon et al. 2013, Shaw et al. 2012, Dumont et al. 2011, and Caroffino et al. 2010a, 2010b), it is unknown whether hatcheries provide the necessary means for Lake Sturgeon population recovery (Ceskleba et al. 1985, Olla et al. 1994; Shively et al. 1996; Salvanes and Braithwaite 2006). Only a few studies to date have assessed the efficiency of stock enhancement for Lake Sturgeon. Schram (1999) revealed that the majority of stocked Lake Sturgeon did not venture far from their introduction point. Furthering this concept, Crossman et al. (2011a) found that distribution was significantly greater at 17 weeks of age than younger sturgeon, and that survival could be improved by releasing individuals at night. In addition, Crossman et al. (2011b) suggested genetic diversity of offspring produced by direct gamete takes was lower than that of offspring collected from naturally spawned areas. Though limited, these studies suggest a larger scale, multiyear, and redefined approach incorporating the latest research to properly supplement Lake Sturgeon recovery with stocked animals over their entire range is warranted.

1.4 Social interactions

Agonistic behavior has been typically studied in aggressive, territorial fishes. The results of such interactions often leave the subordinate individual with chronically elevated cortisol concentrations (Sloman et al. 2000a) which lead to reduced appetite, altered feeding behavior, elevated metabolic rates (Gregory and Wood 1999), and reduced growth rates (Barton et al. 1987). Indeed, such social interactions drive growth rate variability within cohorts (Martins et al. 2005). Curiously, Lake Sturgeon are a gregarious species with little to no evidence of intraspecific aggression (Allen et al. 2009), yet growth rates between siblings raised under the same conditions vary hugely (Klassen 2014).

Within growth variation, body size often determines resource holding potential (RHP) (Yue et al. 2006; Jacob et al. 2007) and risk-taking thresholds of the individual (Réale et al. 2007). Given the bearing these traits may have on ecological fitness (Dingemanse and de Goede 2004), a larger fish may occupy optimal feeding areas more frequently, begin to feed more quickly, and be more likely to forage in risky environments (Budaev and Brown 2011; Ioannou et al., 2008), thus acquiring more resources than subordinate fish and accelerating their growth rate. Indeed, Coleman and Wilson (1998) suggested that some pumpkinseed sunfish (*Lepomis gibbosus*) were more willing to take risks exploring novel objects within their pond, and on subsequent gut content examination, these individuals were found to have three times more food in their stomach than their more risk-averse conspecifics.

In the context of a hatchery environment, where there are reliable resources and few repercussions for risky behaviour, size variation may be largely driven by unequal

competitive ability among individuals. As such, behaviour traits correlated with body size may be inadvertently selected for in the hatchery environment. For example, Gregory and Wood (1998) observed growth rate variability was caused by dominant-subordinate interactions in hatchery-reared juvenile Rainbow Trout (*Oncorhynchus mykiss*), and Irwin et al. (2002) found similar results in hatchery-reared Turbot (*Scophthalmus maximus*). As hatcheries typically select larger individuals, dominant-submissive behavioural phenotypes adaptive in the hatchery environment may be released into the wild, where selective pressures may differ greatly. Additionally, hatchery-reared fishes typically exhibit bolder behavioural tactics (Brown et al. 2013), which can result in lower survival post release as a result of predation pressure. Taken together, the implications on growth variability in hatchery-reared Lake Sturgeon necessitate further inquiry to determine if behavioral phenotypes are a result of the hatchery environment, and how they may influence post release fitness of these individuals.

1.5 Chemical mediated learning

Maladaptive behavioural phenotypes arising from hatchery environments are well documented (Olla et al. 1998). Often decreased appetites, poor swimming performance, limited recognition of prey and naïvety towards predation threats characterize hatchery-reared fishes (Olla et al. 1998; C. Brown and Day 2002; Fernö et al. 2011). In particular, the ability for an individual fish to detect and appropriately respond to predation threat is critical because of the fatal repercussions of not doing so. Indeed, it is thought that predator naïvety of hatchery-reared fish results in high post-release predation mortality,

hindering the effectiveness of conservation hatchery programs (Suboski and Templeton 1989; Brown and Smith 1998; Brown et al. 2013a).

One well-documented approach to prepare hatchery-reared fishes for future predation threats faced post release is through chemical-mediated learning, or odour conditioning. Typically, damage to a conspecific releases alarm cues (AC) that aid in predator conditioning, as they are released by mechanical damage during a predator attack and are thus a reliable indicator of predation threats (Chivers et al. 2007, 2012; Ferrari et al. 2010c). When detected by a conspecific, or sympathetic heterospecifics, they respond with a strong and immediate species-specific antipredator behaviour (Ferrari et al. 2010c). By pairing AC with a novel predator odour, fishes can learn to associate risk with that predator odour in later encounters, thus providing hatchery-reared fish with predator recognition prior to release.

Despite considerable potential for conditioning to improve post stock survival of hatchery-reared fishes, only a handful of studies have attempted to examine post stock survival, and have produced mixed results. For example, Berejikian et al. (1999) conditioned Chinook salmon (*Oncorhynchus tshawytscha*) to avoid an ecologically relevant predator in the lab, but did not find improved survival post release. Similarly, Hawkins et al. (2007) found predator-conditioned Atlantic salmon (*Salmo salar*) survival was no better post-release than their naïve counterparts. Conversely, D'Anna et al. (2012) observed a near doubling of post stock survival in predator-conditioned White seabream (*Diplodus sargus*), and Hutchison et al. (2012) a two-to-fourfold increase in predator-conditioned juvenile Murray cod (*Maccullochella peelii*) survival post release. Though limited, these studies suggest the need to redefine the approach incorporating the latest

research on ontogenetic timing, population variation, and retention of conditioning to properly supplement natural populations of fishes with predator-conditioned, hatchery-reared fishes for conservation purposes.

Recently, Sloychuck et al. (2016) demonstrated that five-month-old Lake Sturgeon can learn to associate risk with predator odour after a single prior conditioning event. Sloychuck et al. (2016) further observed population variation in establishing conditioned behaviour, which was mitigated by increasing the number of conditioning events, suggesting some of the complexities to consider when conditioning Lake Sturgeon for conservation purposes. Additionally, Lake Sturgeon undergo rapid development over the first season of life, developing scutes at approximately 25mm total length (Gadomski and Parsley 2005a) and greatly increasing fin area and musculature that improve escape performance (Wishingrad et al. 2014b), which likely alter the gradient and nature of predation pressure. As such, conditioned behaviour may be instilled more effectively, and retained longer, during life periods when predation pressures are likely to be highest and learned behaviour most relevant (Brown et al. 2013). Therefore, by using repeated conditioning events with ontogeny taken into consideration, conditioning may promote post-release survival of hatchery-reared Lake Sturgeon more strongly.

No studies to our knowledge have examined the effects of conditioning on the physiological state of Lake Sturgeon. However, the presence of AC is a reliable indicator of predation threat (Chivers et al. 2007, 2012; Ferrari et al. 2010c), and fishes must respond to adverse situations, such as predation threat, with primary, secondary or tertiary physiological responses (Barton 2002). A primary response is mediated through the neuroendocrine system, where activation of the sympathetic autonomic nervous

system results in chromaffin tissue releasing catecholamines into circulation. This is accompanied by rapid cardiorespiratory changes that prepare the individual for a fight or flight response (Barton 2002). Additionally, the hypothalamic–pituitary–interrenal (HPI) axis is activated and releases glucocorticoids, such as cortisol, into the blood plasma (Barton 2002). Secondary responses may be activated if stressors associated with predation persist. This may lead to changes in metabolic rates caused by increased plasma glucose levels. In extreme events of chronic stress the tertiary response may occur, where severe immunosuppression, inhibition of reproduction, and other symptoms may influence the well-being of the individual as a result of the chronic stress (Barton 2002).

Although no studies have examined the effect of conditioning on the physiological state of Lake Sturgeon, a few studies have examined the physiological response to AC, with mixed results. For example, Rehnberg et al. (1987) observed an increase in plasma cortisol concentration of Pearl Dace (*Semotilus margarita*) when exposed to conspecific AC. Similarly, Henrique et al. (2015) found a significant increase in Nile Tilapia (*Oreochromis niloticus*) plasma cortisol concentrations when exposed to AC. Conversely, Ide et al. (2003) found no change in plasma cortisol concentrations after Red-tailed Brycon (*Brycon cephalus*) were exposed to AC. Additionally, Hare et al. (2015) found no change in plasma cortisol concentration of two-year-old Lake Sturgeon to AC exposure. Given that conditioning techniques may use multiple exposures of AC to establish a learned response, and the potential for this to induce a secondary or tertiary physiological response, it is imperative that the physiological state of the individual is considered during conditioning. Particularly, this must be considered when exposure to cortisol during early development can significantly shape an organism's cortisol stress response

and ultimately fitness in later life (Crespi and Denver 2005; Kapoor et al. 2006; Auperin and Geslin 2008; Breuner 2008).

1.6 Thesis objectives

Little is known regarding the biology of early life stages of Lake Sturgeon, in particular their first year of life (age-0+). Their small size and typically turbid environments present them as a challenging study animal in the wild environment. Laboratory research has opened a promising avenue for examining age-0+ Lake Sturgeon, describing their substrate preferences and dispersal patterns (Hastings et al. 2013), antipredator behaviour (Wishingrad et al. 2014a), social behaviour (Allen et al. 2009; Klassen 2014) and exploration of life-skills training (Sloychuk et al. 2016). Natural populations of Lake Sturgeon in many regions across their range are slow or possibly incapable of recovering in the absence of conservation effort. This coupled with potential further habitat degradation and fragmentation make the need for improved conservation practices evident. This thesis aims to further our knowledge on age-0+ Lake Sturgeon with the ultimate goal to create rearing environments in Lake Sturgeon hatcheries that improve survival and fitness of individuals raised in these facilities and used in stock enhancement programs.

To address the hypothesis that growth variability in juvenile Lake Sturgeon cohorts influences hatchery-related fitness, Chapter 2 was dedicated to examining boldness and RHP. Boldness was quantified by exposing asymmetrically size-matched juvenile Lake Sturgeon to conspecific AC simultaneously with or without food. Resulting antipredator behaviour (i.e. activity and cover seeking behaviour) of the asymmetrically

size-matched fish was then measured against their willingness to feed in the presence of AC, yielding a measure of boldness in the individuals. I hypothesized that larger-bodied Lake Sturgeon would respond with depressed antipredator behaviour to the AC and forage more in the presence of AC than smaller conspecifics (Grant 1990; Réale et al., 2007; Polverino et al., 2016). In the second experiment, asymmetric and symmetrically size-matched juvenile Lake Sturgeon were placed in a resource limited environment with a point source for food introduction. I hypothesized that larger individuals would realize a higher RHP than smaller conspecifics by occupying the feeding area longer and consuming more food (Otronen 1988; Huntingford et al.1990; Olsson 1992; Faber and Baylis 1993).

Given the potential implications of boldness and RHP on Lake Sturgeon conservation aquaculture, Chapter 3 was dedicated to furthering knowledge on juvenile Lake Sturgeon risk assessment and conditioning ontogeny. The first experiment was aimed at measuring risk assessment at two critical life stages, early exogenous feeding and pre-winter, when foraging is critical and vulnerability to predation threat is likely to be highest (Caraffino et al., 2010). Observations were made of groups of six Lake Sturgeon at both life stages to introductions of AC with or without food, to determine the balance of responding to threat (flight and cover seeking behaviour) and foraging opportunities. Measurements were also taken in the evening and morning to explore potential diel variation in risk assessment. I hypothesized that as Lake Sturgeon developed, their escape potential and nutritional status would improve, allowing them to forego foraging in light of predation risk (Wishingrad et al., 2014b). Once the early ontogeny of risk assessment was better understood in Lake Sturgeon, I hypothesized that

predator conditioning would be most effective when Lake Sturgeon were more able and/or willing to respond to risk (i.e. pre-winter) (Lima and Dill 1990; Hawkins et al., 2008; Brown et al., 2013). In addition, I hypothesized that more conditioning events (number of exposures to AC paired with predator odour) would instill a stronger learned response to the potential threat (Sloychuk et al., 2016). As such, experiment two was conducted in a similar manner as experiment one, but instead either naïve fish were tested, or fish conditioned in an acute (six exposures to AC and PC) or chronic (24 exposures to AC and PC) group.

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*. *Journal of Fish Biology* 74:90–104.
- Auer, N.A. 1996a. Importance of habitat and migration to sturgeons with emphasis on lake sturgeon. *Canadian Journal of Fisheries and Aquatic Sciences* 53:152–160.
- Auperin, B., and Geslin, M. 2008. Plasma cortisol response to stress in juvenile rainbow trout is influenced by their life history during early development and by egg cortisol content. *General and Comparative Endocrinology* 158:234–239.

- Barton, B.A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids, *Integrative and Comparative Biology* 42:517–525.
- Barton, B. A., Schreck, C. B. and Barton, L. D. 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. *Diseases of Aquatic Organisms* 2:173–185.
- Beamish, F.W.H., Noakes, D.L.G., and Rossiter, A. 1998. Feeding ecology of juvenile Lake Sturgeon, *Acipenser fulvescens*, in northern Ontario. *Canadian Field-Naturalist*. 112:459–468.
- Berejikian, B. A., Smith, R. J. F., Tezak, E. P., Schroder, S. L., and Knudsen, C. M. 1999. Chemical alarm signals and complex hatchery rearing habitats affect antipredator behavior and survival of Chinook salmon (*Oncorhynchus tshawytscha*) juveniles. *Canadian Journal of Fisheries and Aquatic Sciences* 56:830–838.
- Birstein, V.J., and Bemis, W.E. 1997. How many species are there within the genus *Acipenser*? *Environmental Biology of Fishes* 48:157–163.
- Birstein, V.J., and DeSalle, R. 1998. Molecular phylogeny of Acipenserinae. *Molecular Phylogenetics and Evolution* 9:141–155.
- Brown, C., and R. Day. 2002. The future of stock enhancements: lessons for hatchery practice from conservation biology. *Fish and Fisheries* 3:79–94.
- Brown, G. E., Ferrari, M. C. O., Malka, P.H., Fregeau, L., Kayello, L., and Chivers, D. P. 2013. Retention of acquired predator recognition among shy versus bold juvenile rainbow trout. *Behavioral Ecology and Sociobiology* 67:43-51.

- Brown, G. E., and R. J. F. Smith. 1998. Acquired predator recognition in juvenile rainbow trout (*Oncorhynchus mykiss*): conditioning hatchery reared fish to recognize chemical cues of a predator. *Canadian Journal of Fisheries and Aquatic Sciences* 55:611–617.
- Bruch, R.M., Miller, G., and Hansen, M.J. 2006. Fecundity of lake sturgeon (*Acipenser fulvescens*, Rafinesque) in Lake Winnebago, Wisconsin, U.S.A. *Journal of Applied Ichthyology* 22:116–118.
- Breuner, C. 2008. Maternal stress, glucocorticoids, and the maternal/fetal match hypothesis. *Hormones and Behaviour* 54:485–487.
- Budaev, S., and C. Brown. 2011. Personality traits and behaviour. Pages 135–165 in C. Brown, K. Laland, and J. Krause, editors. *Fish cognition and behavior*, 2nd edition. Wiley-Blackwell, London.
- Caroffino, D.C., Sutton, T.M., Elliott, R.F., and Donofrio, M.C. 2010a. Early life stage mortality rates of lake sturgeon in the Peshtigo River, Wisconsin. *North American Journal of Fisheries Management* 30:295–304.
- Caroffino, D. C., Sutton, T. M., Elliott, R. F., and Donofrio, M. C. 2010b. Predation on Early Life Stages of Lake Sturgeon in the Peshtigo River, Wisconsin. *Transactions of the American Fisheries Society* 139:1846–1856.
- Ceskleba, D.G., AveLallemant, S., and Thuemler, T.F. 1985. Artificial spawning and rearing of lake sturgeon, *Acipenser fulvescens*, in Wild Rose State Fish Hatchery, Wisconsin, 1982–1983. *Environmental Biology of Fishes* 14:79–85.
- Chivers, D. P., Brown, G. E., and Ferrari, M. C. O. 2012. Evolution of fish alarm

- substances. Pages 127–139 in C. Brömark and L.-A. Hansson, editors. *Chemical Ecology in Aquatic Systems*. Oxford University Press, Oxford, UK.
- Chivers, D. P., Wisenden, B. D., Hindman, C. J., Michalak, T. A., Kusch, R. C., Kaminskyj, S. G. W., Jack, K. L., Ferrari, M. C. O., Pollock, R. J., Halbewachs, C. F., Pollock, M. S., Alemadi, S., James, C. T., Savaloja, R. K., Goater, C. P., Corwin, A., Mirza, R. S., Kiesecker, J. M., Brown, G. E., Adrian, J. C., Jr., Krone, P. H., Blaustein, A. R., and Mathis, A. 2007. Epidermal “alarm substance” cells of fishes maintained by non-alarm functions: possible defence against pathogens, parasites and UVB radiation. *Proceedings of the Royal Society of London, Series B* 274:2611–2619.
- Coleman, K., and Wilson, D. 1998. Shyness and boldness in pumpkinseed sunfish: individual differences are context-specific. *Animal Behaviour* 56:927–936.
- COSEWIC. 2006. COSEWIC assessment and update status report on the lake sturgeon *Acipenser fulvescens* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. pp. 107. Available from http://publications.gc.ca/collections/collection_2007/ec/CW69-14-484-2007E.pdf. Accessed 29 Aug 2017.
- Crespi, E.J., and Denver, R.J. 2005. Ancient origins of human developmental plasticity. *American Journal of Human Biology* 17:44–54.
- Crossman, J.A., Scribner, K.T., Yen, D.T., Davis, C.A., Forsythe, P.S., and Baker, E.A. 2011b. Gamete and larval collection methods and hatchery rearing environments affect levels of genetic diversity in early life stages of lake sturgeon (*Acipenser fulvescens*). *Aquaculture* 310:312–324.

- D'Anna, G., Giacalone, V. M., Fernández, T. V., Vaccaro, A. M., Pipitone, C., Mirto, S., Mazzola, S., and Badalamenti, F. 2012. Effects of predator and shelter conditioning on hatchery-reared white seabream *Diplodus sargus* (L., 1758) released at sea. *Aquaculture* 356–357:91–97.
- Dingemanse, N. J., and de Goede, P. 2004. The relation between dominance and exploratory behaviour is context-dependent in wild great tits. *Behavioral Ecology* 15:1023–1030.
- Dumont, P., D'Amours, J., Thibodeau, S., Dubuc, N., Verdon, R., Garceau, S., Bilodeau, P., Mailhot, Y., and Fortin, R. 2011. Effects of the development of a newly created spawning ground in the Des Prairies River (Quebec, Canada) on the reproductive success of lake sturgeon (*Acipenser fulvescens*). *Journal of Applied Ichthyology* 27:394–404.
- Duong, T.Y., Scribner, K.T., Forsythe, P.S., Crossman, J.A., and Baker, E.A. 2013. Interannual variation in effective number of breeders and estimation of effective population size in long-lived iteroparous lake sturgeon (*Acipenser fulvescens*). *Molecular Ecology* 5:1282- 1294.
- Faber, D. B. and Baylis, J. R. 1993. Effects of body size on agonistic encounters between male jumping spiders (Araneae: Salticidae). *Animal Behaviour* 45:289-299.
- Fernö, A., Huse, G., Jakobsen, P. J., Kristiansen, T. S., and Nilsson, J. 2011. Fish behaviour, learning, aquaculture and fisheries. Pages 359–404 in C. Brown, K. Laland, and J. Krause, editors. *Fish cognition and behavior*, 2nd edition. Wiley-Blackwell, London.

- Ferrari, M. C. O., Wisenden, B. D., and Chivers, D. P. 2010c. Chemical ecology of predator–prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology* 88(7):698–724.
- Gadomski, D.M., and Parsley, M.J. 2005a. Laboratory studies on the vulnerability of young white sturgeon to predation. *North American Journal of Fisheries Management* 25:667- 674.
- Grande, L., and Bemis, W.E. 1991. Osteology and phylogenetic relationships of fossil and recent paddlefishes (*Polyodontidae*) with comments on the inter-relationships of Acipenseriformes. *Journal of Vertebrate Paleontology* 11:10–121.
- Grant, J. W. 1990. Aggressiveness and the foraging behaviour of young-of-the-year brook charr (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences* 47:915–920.
- Gregory, T. R. and Wood, C. M. 1999. The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiological and Biochemical Zoology* 72:286–295.
- Hare, A. J., Waheed, A., Hare, J. F., and Anderson, W. G. 2015. Cortisol and catecholamine responses to social context and a chemical alarm signal in juvenile lake sturgeon, *Acipenser fulvescens*. *Canadian Journal of Zoology* 613:605–613.
- Hastings, R. P., Bauman, J. M., Baker, E. a., and Scribner, K. T. 2013. Post-hatch dispersal of lake sturgeon (*Acipenser fulvescens*, Rafinesque, 1817) yolk-sac larvae in relation to substrate in an artificial stream. *Journal of Applied Ichthyology* 29:1208–1213.

- Hawkins, L. A., Armstrong, J. D., and Magurran, A. E. 2007. A test of how predator conditioning influences survival of hatchery-reared Atlantic salmon, *Salmo salar*, in restocking programmes. *Fisheries Management and Ecology* 14:291–293.
- Henrique, F., Sanches, C., Miyai, C. A., Pinho-neto, C. F., and Barreto, R. E. 2015. Stress responses to chemical alarm cues in Nile tilapia. *Physiology and Behavior* 149:8–13.
- Huntingford, F. A. 2004. Implications of domestication and rearing conditions for the behaviour of cultivated fishes. *Journal of Fish Biology* 65:122–144.
- Hutchison, M., Stewart, D., Chilcott, K., Butcher, A., Henderson, A., McLennan, M., and Smith, P. 2012. Strategies to improve post release survival of hatchery-reared threatened fish species. Murray-Darling Basin Authority Publication No. 135/11.
- Ide, L.M., Urbinati, E.C., and Hoffmann, A. 2003. The role of olfaction in the behavioural and physiological responses to conspecific skin extract in *Brycon cephalus*. *Journal of Fish Biology* 63:332–343.
- Ioannou, C. C., Payne, M. and Krause, J. 2008. Ecological consequences of the bold–shy continuum: the effect of predator boldness on prey risk. *Oecologia* 157:177–182.
- Irwin, S., O’Halloran, J. and FitzGerald, R.D. 2002. The relationship between individual consumption and growth in juvenile turbot, *Scophthalmus maximus*. *Aquaculture* 204:65-74.
- Jacob, A., Nussle, S., Britschgi, A., Evanno, G., Muller, R. and Wedekind, C. 2007. Male dominance linked to size and age, but not to ‘good genes’ in brown trout (*Salmo trutta*). *BMC Evolutionary Biology* 7:207–215.

- Kapoor, A., Dunn, E., Kostaki, A., Andrews, M.H., and Matthews, S.G. 2006. Fetal programming of hypothalamo–pituitary–adrenal function: prenatal stress and glucocorticoids. *The Journal of Physiology* 572:31–44.
- Klassen, C. N. 2014. Growth rate and size variability among juvenile lake sturgeon, *Acipenser fulvescens*: Implications for recruitment. Ph.D. Thesis, University of Manitoba 2:22-42.
- Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* 68:619–640.
- Martins, C.I.M., Schrama, J.W., and Verreth, A.J. 2005. The consistency of individual differences in growth, feed efficiency and feeding behaviour in African catfish *Clarias gariepinus* (Burchell 1822) housed individually. *Aquaculture Research* 36:1509-1516.
- Nilo, P., Dumont, P., and Fortin, R. 1997. Climatic and hydrological determinants of year-class strength of St. Lawrence River lake sturgeon (*Acipenser fulvescens*). *Canadian Journal of Fisheries and Aquatic Sciences* 54:774–780.
- Olla, B. L., Davis, M. W., and Ryer, C. H. 1994. Behavioral deficits in hatchery reared fish: potential effects on survival following release. *Aquaculture and Fisheries Management* 25:19–34.
- Olsson, M. 1992. Contest success in relation to size and residency in male sand lizards, *Lacerta agilis*. *Animal Behaviour* 44:386-388.
- Otronen, M. 1988. The effect of body size on the outcome of fights in burying beetles (*Nicrophorus*). *Annales Zoologici Fennici* 25:191-201.
- 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. *Environmental Reviews* 43:30–43.
- Polverino, G., Bierbach, D., and Killen, S. S. 2016. Body length rather than routine metabolic rate and body condition correlates with activity and risk-taking in juvenile zebrafish *Danio rerio*. *Journal of Fish Biology* 49:2251–2267.
- Réale, D., Reader, S. M., Sol, D., McDougall, P. T., and Dingemanse, N. J. 2007. Integrating animal temperament within ecology and evolution. *Biological Reviews* 82:291–318.
- Rehnberg, B.G., and Schreck, C.B. 1987. Chemosensory detection of predators by coho salmon (*Oncorhynchus kisutch*): behavioural reaction and the physiological stress response. *Canadian Journal of Zoology* 65:481–485.
- Salvanes, A. G. V., and Braithwaite, V. 2006. The need to understand the behaviour of fish reared for mariculture or restocking. *ICES Journal of Marine Science* 63:346–354.
- Scott, W.B., and Crossman, E.J. 1998. *Freshwater fishes of Canada*. Fisheries Research Board of Canada, Ottawa.
- Scott, W. B. and Crossman, E. J., 1973. *Freshwater fishes of Canada*. Fisheries Research Board of Canada. 184:966.
- Schram, S. T., Lindgren, J., Evrard, L. M., Schram, S. T., Lindgren, J., and Evrard, L. M. 1999. Reintroduction of Lake Sturgeon in the St. Louis River, Western Lake Superior. *North American Journal of Fisheries Management* 19:815-823.
- Shaw, S.L., Chipps, S.R., Windels, S.K., Webb, M.A.H., McLeod, D.T., and Willis,

- D.W. 2012. Lake sturgeon population attributes and reproductive structure in the Namakan Reservoir, Minnesota and Ontario. *Journal of Applied Ichthyology* 28:168–175.
- Shively, R. S., Poe, T. P., and Sauter, S. T. 1996. Feeding response by northern squawfish to a hatchery release of juvenile salmonids in the Clearwater River, Idaho. *Transactions of the American Fisheries Society* 125:230–236.
- Sloman, K. A., Gilmour, K. M., Metcalfe, N. B. and Taylor, A. C. 2000a. Does socially induced stress in rainbow trout cause chloride cell proliferation? *Journal of Fish Biology* 56:725–738.
- Sloychuk, J. R., Chivers, D. P., and Ferrari, M. C. O. 2016. Juvenile Lake Sturgeon Go To School: Life-Skills Training for Hatchery Fish. *Transactions of the American Fisheries Society* 145:287-294.
- Suboski, M. D., and Templeton, J. J. 1989. Life skills training for hatchery fish: social learning and survival. *Fisheries Research* 7:343–352.
- Sulak, K.J., and Randall, M. 2002. Understanding sturgeon life history: Enigmas, myths, and insights from scientific studies. *Journal of Applied Ichthyology* 18:519-528.
- Verdon, R., Guay, J.C., La Hay, M., Simmoneau, M., Côté-Beher, A., Ouellet, N., and Gendron, M. 2013. Assessment of spatio-temporal variation in larval abundance of lake sturgeon (*Acipenser fulvescens*) in the Rupert River (Quebec, Canada) using drift nets. *Journal of Applied Ichthyology* 29:15–25.
- Wishingrad, V., Sloychuk, J. R., Ferrari, M. C. O., and Chivers, D. P. 2014a. Alarm cues

- in Lake Sturgeon *Acipenser fulvescens* Rafinesque, 1817: potential implications for like-skills training. *Journal of Applied Ichthyology* 30:1441-1444.
- Wishingrad, V., Chivers, D. P., and Ferrari, M. C. O. 2014b. 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.
- Yue, S., Duncan, I. J. H., and Moccia, R. D. 2006. Do differences in conspecific body size induce social stress in domestic rainbow trout? *Environmental Biology of Fishes* 76:425–431.

Chapter 2: Size does not matter: Condition factor, rather than body size, predicts resource holding potential and boldness in juvenile Lake Sturgeon, *Acipenser fulvescens*

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Abstract

Teasing apart the myriad factors that promote an individual's resource holding potential (RHP) and risk assessment is challenging, yet intrinsic pressures such as body size and hunger levels have been shown to be excellent predictors of these processes. In the context of Lake Sturgeon (*Acipenser fulvescens*) conservation aquaculture, where larger body sizes are selected and sterile rearing environments provide no risk experience, understanding how these fishes potentially compete with each other and respond to risk is essential to the program. To test how body size might influence RHP, hatchery-reared juvenile Lake Sturgeon were asymmetrically (~60% size difference) and symmetrically (~3% size difference) size matched in individual tanks. Additionally, asymmetrically size-matched individuals were exposed to conspecific chemical alarm cues with the presence or absence of food to test the influence of body size on potential bold behaviour (taken as the magnitude of antipredator response to alarm cues and foraging in alarm

cues). Results indicate that Lake Sturgeon condition factor, rather than length or body mass, predicts RHP ($p < 0.05$), and that Lake Sturgeon of high condition factor are more likely to endure risk when presented with foraging opportunities. As hatcheries are likely to select higher condition factor individuals, more research is necessary to understand the role condition factor has on their behavioural responses and ensuing fitness post release.

Introduction

Individuals within a population often display consistent differences in behaviour such as activity, risk taking, social, exploratory and aggressiveness (Polverino et al. 2016). Behaviour of an individual may develop under experienced extrinsic factors (predation threats, foraging techniques, etc.), as well as intrinsic (body size, metabolic rate, etc.) demands (Polverino et al. 2016). Under these pressures, consistent behavioural differences between individuals are promoted (Biro and Stamps, 2010; Wolf and Weissing, 2010; Sih et al., 2015). Extrinsic pressures vary over age, size, or body condition of the individual, which influence the cost-benefit ratio of any particular behaviour over time (Wolf and Weissing, 2010). Thus, the optimal behaviour by any one individual at any point in time is promoted through the interplay of extrinsic and intrinsic pressures in light of trade-offs (Carere and Maestripieri, 2013), resulting in phenotypic behavioural differences and creation of behavioural variation in natural populations (Réale et al., 2007; Killen et al., 2013; Sih et al., 2015).

Understanding the role that extrinsic and intrinsic pressures play in shaping an individual's behaviour is challenging, yet some intrinsic factors (i.e. body size, hunger levels, etc.) have been shown to be excellent predictors of behaviour (Krause et al., 1998;

Brown and Braithwaite, 2004; Brown et al., 2007). Typically, body size is a determining factor in resource competition (Otronen 1988; Huntingford et al. 1990; Olsson 1992; Faber and Baylis 1993) and influences the risk-taking threshold (Réale et al., 2007). Given the bearing these traits may have on ecological fitness (Dingemanse and de Goede 2004), large individuals with overall higher resource holding potential (RHP) would likely realize higher fitness than smaller conspecifics. Additionally, risk of predation typically declines with increasing body size (Lorenzen, 2000; Nilsson and Brönmark, 2000), which may promote bolder behaviour (Grant, 1990; Polverino et al., 2016). Indeed, a study by Chiba et al. (2007) suggested that naturally selected northern populations of fast growing Atlantic silverside (*Menidia menidia*) initiate foraging more quickly after a simulated predator attack than slower growth, southern populations.

Nutritional status is also a predictor of resource acquisition and risk taking behaviour (Clark, 1994; Brown et al., 2007; Dial et al., 2008; Filby et al., 2010). Smaller individuals with lower energy reserves may be more motivated to take risks in search of food compared to larger, higher nutritional state individuals (Caraco 1980; Brown and Braithwaite 2004; Killen et al., 2011). Additionally, a smaller individual of poor nutritional status may be more motivated to compete for resources, potentially increasing the probability of antagonistic interaction (Milinski and Parker 1991). However, under a resource-limited context it is suggested body size plays a more prominent role in RHP, despite the potential increase in hunger motivation of lower nutritional state individuals (Wells 1978; Robertson 1986; Given 1988).

The Lake Sturgeon (*Acipenser fulvescens*) is a large, gregarious freshwater fish species of North America (Pollock et al. 2015), that is considered imperiled across their

range (Haxton and Findlay 2008). Unique life history traits, such as protracted spawning periodicity and later age at maturity, make them susceptible to habitat fragmentation and population exploitation (Crouse 1999). As decades of conservation aquaculture have attempted to repatriate this species across their historic range (Pollock et al. 2015), the importance of behaviour has received increasing scrutiny. Often, fish arising from sterile hatchery environments exhibit maladaptive behavioural phenotypes such as limited prey recognition and predator naivety (Taylor et al. 2013). In addition, hatchery environments often select larger body sizes under the assumption that larger fish have improved chances of survival post release, which may lead to behavioural phenotype selection as larger body size is often linked to bolder behaviour in fishes (Krause et al., 1998; Brown and Braithwaite, 2004; Brown et al., 2007). However, little information is available on behavioural responses in relation to size, which is known to vary substantially, in young of the year Lake Sturgeon (Allen et al., 2009; Hare et al., 2015). Therefore, this research aims to answer two questions in age 0+ hatchery reared Lake Sturgeon: 1) do larger-bodied juvenile Lake Sturgeon show relaxed antipredator behaviour in response to predation threat and are they more likely to forage in spite of predation risk (i.e. more bold behaviour) than smaller-bodied conspecifics, and 2) do larger bodied juvenile Lake Sturgeon incur a higher RHP in a resource limited environment than smaller conspecifics. Data produced will indicate if hatcheries select behavioural phenotypes and suggest the potential implications on fitness post release.

Methods

Animal acquisition and care

Lake Sturgeon gametes were collected from four females and five males caught below Pt. du Bois generating station 50°17'52N, 95°32'51W on the Winnipeg River, Manitoba, Canada, on the 22 of May, 2015. Upon gamete collection, all fish 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 were fertilized with approximately 40µl of sperm to 15ml of eggs, to form evenly mixed parentage of the hatchery brood stock, and gently mixed for two minutes in approximately 10ml of water. They were rinsed three times with de-chlorinated city of Winnipeg tap water and rinsed again in clay for 30 minutes to remove the adhesive coating of the eggs. The eggs were then distributed between five flow-through MacDonald hatching jars at 16°C and 100% dissolved oxygen de-chlorinated City of Winnipeg tap water, with 14light:10dark 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 75L circular tubs. At 27 days post fertilization (dpf) the Lake Sturgeon were fed live *Artemia* three times per day. After 44dpf, shaved bloodworms (*Glycera dibranchiate*) were mixed into their diet, and gradually the size and proportion of bloodworms increased until 51dpf when they were fed bloodworm exclusively three times per day. At 67dpf the fish were moved into two 230L tanks to accommodate growth.

Experiment 1 – Boldness

Twelve behavioural arenas measuring 59.7cm long by 42.9cm wide and 14.9cm deep were constructed with formed plastic containers (Figure 2.1). Three substrates were positioned randomly in the arena: gravel, sand, and a smooth, white plastic substrate (described henceforth as no substrate), all positioned at the same elevation. Window screen 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 and lead over the top of the surrounding curtains, such that food and odour could be introduced without disturbing the fish. The arenas were divided into four splash trays, which were set in a row. Six Sony DCR-SR68 Handycam camcorders, each encompassing two arenas in their field of view, were positioned by the outer edge of each splash tray.

At approximately 160dpf, 144 Lake Sturgeon were anaesthetized with MS-222, weighed and measured, and introduced into the behavioural arenas in groups of six Lake Sturgeon to create a social context. Of the six Lake Sturgeon, the largest and smallest focal individuals (body mass and length differences $66 \pm 2.2\%$ and $30 \pm 1.6\%$, respectively) were easily identifiable in the video recordings and were later coded for resulting behaviour. 5ml of frozen bloodworm were fed to the Lake Sturgeon at 8:00hrs and again at 20:00hrs CST daily. On the third day, 3.4ml of whole body grind AC was introduced into each behavioural arena simultaneously with the presence or absence of 5ml of bloodworm, at 20:00hrs CST. AC was prepared as previously described (Wishingrad et al., 2014a), briefly larval Lake Sturgeon of the the same age were

sacrificed and homogenised in 10ml of distilled water using a Polytron 2100. The homogenate was then filtered through glass wool and made up to a final concentration of 3.2g/L. Homogenates were stored at -20°C prior to use. The overhead camcorders recorded eight minutes of pre-stimulus behavior, and a further eight minutes of post-stimulus behaviour. A total of 12 trials with AC introduced with the presence or absence of food were recorded for the Lake Sturgeon. Thus, the Lake Sturgeon were given an opportunity to feed in the presence of potential risk, and the behavioural response of large and small Lake Sturgeon could be observed to help determine if body size influences the magnitude of antipredator behaviour and foraging response in the face of potential predation risk.

Behaviour Coding Experiment 1

All videos were coded with JWatcher version 1.0 (Blumstein et al. 2006). The data were separated into eight minute pre- and post-stimulus introduction periods, so that stimulus-induced behaviour could be measured. The largest and smallest Lake Sturgeon within each arena were coded for activity and substrate type preference. A 1x2 grid was superimposed over the arena, the number of grid lines crossed by the head of the large and small Lake Sturgeon were summed allowing for an estimation of baseline activity and stimulus-induced activity. 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 a substrate.

Additionally, the total amount of time the large and small Lake Sturgeon spent over each

substrate type during the trial was collected to determine substrate type preference and potential stimulus induced change in preference with the introduction of AC.

Experiment 2 – RHP

Aquariums measuring 54.5cm long by 30.5cm wide were labeled, filled to a depth of 12cm with de-chlorinated city of Winnipeg tap water, and an Aquaclear 20 “hang-off-the-back filter” was placed on the back width of the aquarium (Figure 2.2). A blue corrugated plastic sheet measuring 10cm wide by 14cm high was fastened to the bottom and sidewall of the aquarium, 10cm from the front of the aquarium. The blue plastic sheet was placed vertically so that the top emerged over the water line. Finally, a tube was secured to the rim of the aquarium between the blue plastic sheet and front of the aquarium, so that food could be introduced in an enclosed area of the aquarium providing limited access to food without disturbance.

Aquaria were positioned between dividers to prevent fish in neighbouring trials from observing each other and the tanks were surrounded by black curtains during each trial to avoid disturbance. Five individual bloodworms were delivered by syringe via a feeding tube that was positioned over top of the curtain and led to the tank, so as to avoid disturbance to the fish during the trials. Lights were set on a 12:12 hour light:dark schedule, and water temperature was kept at 16°C. Camcorders were positioned above the aquariums so that the fish and aquarium number were clearly identifiable from the video recordings.

Trials began on 9 March, 2016 with eight pairs of symmetrically (mean difference in body mass $6 \pm 2\%$ and length $3 \pm 3\%$) and asymmetrically (mean difference in body

mass $58 \pm 3\%$ and length $25 \pm 1\%$) size-matched juvenile sturgeon, and each pair was randomly assigned to an aquarium. Five bloodworm were then introduced in 1ml of homogenized bloodworm diluted to 0.1g/ml via the feeding tube, thus allowing the sturgeon to detect food while providing them with limited rations compared to their normal daily feed to satiation. This was repeated daily for seven days to strengthen feeding motivation in the fish, as well as to condition them to the food point source. On the seventh day food was introduced into the arena and the overhead digital camcorders captured 10 minutes of behaviour post introduction.

Behaviour Coding Experiment 2

Six minutes of post-food introduction were coded with JWatcher version 1.0 (Blumstein et al. 2006). Behaviour coded for each individual were: 1) bottom behaviour: occurrence of one individual sliding underneath the other to gain optimal feeding access for sturgeon's inferior mouth, 2) pushing: where one individual physically displaced the other, 3) feeding: number of bloodworm consumed, and 4) holding: the proportion of time spent in the feeding area.

Statistical Analysis – Experiment 1

Generalized linear mixed models (GLMMs) were used to quantify the relationship between Lake Sturgeon activity response to AC in the presence or absence of food, and substrate type preference in response to the introduced stimulus. The introduced stimuli, AC and AC with food, were modeled separately as the objectives were to quantify the behavioural response to each stimulus independently of each other. Analyses were carried

out using R (R Core Development Team 2013) with GLMMs fitted using the ‘glmer’ function from the lme4 package (Bates et al. 2015). The full models consisted of two predictor variables (*period*, denoting pre- or post-stimulus introduction period, and Fulton’s *Condition Factor*, *body mass* or *body length* of the individual), and competing models consisted of all possible subsets of these two predictor variables. Model ranking was accomplished with Akaike Information Criterion (AIC) in the function ‘drop1’ from the lme4 package (Bates et al. 2015). The response variable for each model was either activity, sand, gravel, or no substrate, depending on whether activity or substrate type preference was of interest in the analysis. In all models, a negative binomial distribution outperformed a Poisson distribution (Table 2.1), 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 the large and small Lake Sturgeon from each arena.

Experiment 2

Data were tested for normality (Shapiro-Wilks test) and homogeneity of variance (Bartlett’s test). For the RHP experiment, linear regression was used to test for any relationship of the Lake Sturgeons’ body metrics (length, mass, or Fulton’s condition factor) on their holding ability and behaviour (bottom, pushing, feeding, size matching (asymmetric or symmetric)). A linear mixed model with arena as a random effect was also used. However, variance of the random effect approached zero and was thus removed from the model. Pushing behaviour was also removed from the model due to the lack of observations of this behaviour (one individual pushed another twice during a single trial).

Results

Experiment 1 – Activity

The model containing all predictor variables (period and condition factor) had the strongest support for predicting Lake Sturgeon activity response to the introduction of AC and combined stimulus of AC with food (AIC). The parameter estimate showed a positive relationship between stimulus and fish activity in the presence of AC (Table 2.2). A positive relationship between condition factor and stimulus was also observed. However, a large standard error suggests limited predictive value from this estimate, and despite a non-significant p value within the model, condition factor was left in the model based on AIC. The results thus suggest that juvenile small- and large-bodied Lake Sturgeon responded to AC similarly with increased activity. Models containing mass or length were also used, but these metrics were found to not increase the predictive ability of the model (AIC).

A negative stimulus parameter estimate was observed when AC with food was introduced to the Lake Sturgeon, which was statistically significant within the model. Additionally, a negative parameter estimate for condition factor was observed. Though not quite significant ($p = 0.086$), it was again found to improve the predictive ability (AIC) and thus remained in the model.

Experiment 1 - Substrate type preference

In all models, condition factor improved the predictive ability of the model above that of length or body mass (AIC). Period was also found to improve the predictive

ability of all models, with the exception of the sand preference model with AC introduction, which was removed from the final model based on AIC.

A significant increase in gravel preference was observed with the introduction of AC (Table 2.2), which is suggestive of cover-seeking behaviour. The response was not influenced by condition factor, and with the combined stimulus introduction there was no stimulus-induced change in cover-seeking behaviour. No stimulus-induced sand preference, nor no substrate preference was observed. However, condition factor, though not quite significant ($p=0.066$), suggested a trend between condition factor and no substrate type preference in the combined stimulus, suggestive of bold behaviour.

Experiment 2 – Resource Holding Potential

The results of the linear regression ($R^2 = 0.28$) suggest feeding was significantly correlated with holding ($p<0.05$), supporting the study design's assumption that feeding occurred in the feeding area of the arena. Although neither length nor body mass influenced the Lake Sturgeon holding time, condition factor was positively correlated with holding ($p<0.05$), indicating that Lake Sturgeon of higher condition factor occupied the feeding area for longer periods of time (Figure 2.3), despite asymmetric or symmetric size matching.

Discussion

Lake Sturgeon activity significantly increased in response to the introduction of AC, which is typical of their antipredator behaviour as juveniles (Wishingrad et al. 2014a; Sloychuk et al. 2016). Interestingly, neither body length, mass, nor condition

factor had an effect on the magnitude of their activity response. Previous work by Wishingrad et al. (2014b) on similarly-aged Lake Sturgeon suggested that as fins and swimming musculature increase, so would their ability to produce greater escape velocity and sustained activity. It is possible that this difference in observation may be a result of group size; that is, Wishingrad et al. (2014) placed three Lake Sturgeon together in an arena, whereas in this study six were held in an arena. Spottail shiner (*Notropis hudsonius*) tolerate closer predator approach when in groups (Seghers 1981), and on a larger meta-analysis perspective of fish risk assessment, increasing groups size was found to diminish perceived predation risk (Stankowich and Blumstein, 2005). Accordingly, larger Lake Sturgeon may not have exhibited their full flight escape capability in the context of group size in this experiment.

Provided with an optimal feeding patch and copious amount of food, juvenile Lake Sturgeon did not exhibit antipredator behaviour. This response is typical with perception of risk and subsequent response to that risk often being dependent on the quality of the feeding patch, since similar quality patches may be difficult to locate elsewhere (Cooper et al. 2003b). This notion aligns with most life-history optimization models, where overall fitness is a positive function of body size (Rowe and Ludwig, 1991; Abrams and Rowe, 1996). However, a closer look reveals juvenile mortality risk as a function of growth rate to reflect the trade-off between achieving a larger body size and limiting predation on active foragers (Houston et al. 1993; Abrams et al., 1996). Given the short season of opportunity for Lake Sturgeon to develop before winter and the high intrinsic demands of doing so, it is possible that juvenile Lake Sturgeon perceive foraging opportunities to be more advantageous than responding to the potential risk.

Typically, fishes of low energy reserves are more inclined to take risks in search of food than fishes of higher nutritional status (Krause et al., 1998; Brown and Braithwaite, 2004; Killen et al., 2011). However, in this study juvenile Lake Sturgeon of higher condition factor were observed to reduce their activity and increase their no substrate preference in response to the combined stimulus of AC with Food, suggesting they responded more strongly to the foraging opportunity than the potential risk. As the hatchery environment lacks predators, promotion of antipredator behaviour is relaxed, and given the reward for feeding despite potential risks, a link between bolder behaviour and increased condition factor within hatchery-reared Lake Sturgeon may be promoted. Indeed, Wilson et al. (1993) observed bold juvenile pumpkinseed sunfish (*Lepomis gibbosus*) fed sooner in the laboratory than shy ones and had more food in their stomach upon capture in the wild.

A significant relationship between condition factor and holding time was found in Lake Sturgeon. In general, body size is often related to dominance in many taxa (Yue et al. 2006; Jacob et al. 2007). However, in this study the model suggested body size had no influence on holding time of the Lake Sturgeon, despite a ~60% difference in asymmetrically size matched Lake Sturgeon body mass. This is perhaps not surprising given the Lake Sturgeon's protective scutes and lack of weaponry, which would likely result in ineffective conspecific combat. Given the structure of contests, the lack of observed antagonistic behaviour (pushing) in this study aligns with the notion that body size does not provide any obvious advantage in non-weaponized species. Indeed, body size has been shown to be a less important predictor of dominance than nutritional status in non-weaponized species, (Marden and Waage 1990; Kemp and Wiklund 2001). This

may explain the relationship of condition factor and RHP of Lake Sturgeon, rather than length and mass differences.

The results of this study suggest juvenile Lake Sturgeon of high condition factor are likely to be both more bold and have a higher RHP than lower condition factor conspecifics. Sundstrom et al. (2004) observed a similar connection between boldness and RHP in brown trout (*Salmo trutta*). They hypothesized that bold individuals typically find feeding patches first because of their willingness to explore, and subsequently have higher RHP because they must defend their new territory to recover the costs of exploring. Such an explanation for juvenile Lake Sturgeon bold-RHP link would require them to monitor each other, such that shy individuals could follow the exploits of the bold individuals, thus reducing their exposure to potential risks. Whether juvenile Lake Sturgeon monitor each other's behaviour is unknown; however, evidence thus far suggests juvenile Lake Sturgeon shoal in the wild (Barth et al. 2009) and derive benefits from shoaling (Allen et al. 2009). Typically, shoaling fishes share information to avoid predators and synergistically achieve certain tasks, such as foraging (Brabazon et al. 2015). Thus, it is likely that shoaling juvenile Lake Sturgeon share information. However, more research is necessary to determine if information sharing in juvenile Lake Sturgeon may promote a potential bold-RHP link.

Conservation aquaculture for enhancement of natural populations has been widely used on a number of fish species (Brown et al. 2013b), including Lake Sturgeon (Pollock et al. 2015). Often, maladaptive behavioural phenotypes, such as limited recognition of prey and naïvety towards predation threats, characterize hatchery-reared fish (Chivers et al. 2014). To our knowledge, this is the first study suggesting the possibility of

behavioural phenotype selection in hatchery-reared juvenile Lake Sturgeon. The notion that RHP has an effect on growth in nature has been challenged by many studies (Martin-Smith and Armstrong 2002; Harwood et al. 2003; Hojesjo et al. 2004). However, dominance is often linked with exploratory tendencies (Bell 2005), which have been suggested to effect performance in the wild (Adriaenssens and Johnsson 2010). As Lake Sturgeon of higher condition factor are likely to be selected for in the hatchery, it is important to further investigate whether bolder behaviour of these individuals proves to be maladaptive post-release.

Summary

We have shown that condition factor, rather than body size or mass, predicts boldness and RHP in juvenile Lake Sturgeon. Hatcheries are likely to promote such behaviour as they often select for larger juvenile Lake Sturgeon. Risk-prone hatchery-reared Lake Sturgeon may be at higher risk of predation post-release than wild-type conspecifics (Jonsson 1997), potentially reducing the efficiency of the stocking program. These effects should be considered in Lake Sturgeon conservation aquaculture to optimize the program's efficiency and ability to repatriate this species across its historic range.

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References

- Abrams, P.A. and Rowe, L. 1996. The effects of predation on the age and size of maturity of prey. *Evolution* 50:1052–1061.
- Abrams, P. A., Leimar, O., Nylin, S., and Wiklund, C. 1996. The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. *American Naturalist* 147:381–395.
- Adriaenssens, B., and Johnsson, J.I. 2009. Personality and life-history productivity: consistent or variable association? *Trends in Ecology and Evolution* 24:179–180.
- 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*. *Journal of Fish Biology* 74(1):90–104.
- 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. *Journal of Applied Ichthyology* 25:18–26.
- Bell, A.M. 2005. Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). *Journal of Evolutionary Biology* 18:464–473.
- Biro, P. A. and Stamps, J. A. 2010. Do consistent individual differences in metabolic

- rate promote consistent individual differences in behavior? *Trends in Ecology and Evolution* 25:653–659.
- Blumstein, D. T., Daniel, J. C., and Evans, C. S. 2006. JWatcher (Version 1.0)
[http:// www.jwatcher.ucla.edu/](http://www.jwatcher.ucla.edu/).
- Brabazon, A., Cui, W., and Neill, M. O. 2015. Information Propagation in a Social Network: The Case of a Fish Schooling Algorithm. *Intelligent Systems Reference Library* 85:27-51.
- Brown, G.E., Ferrari, M.C.O., Elvidge, C.K., Ramnarine, I., and Chivers, D.P. 2013b. Phenotypically plastic neophobia: a response to variable predation risk. *Proceedings of the Royal Society London, Series B. Biological Sciences* 280:2012-2712.
- Brown, C. and Braithwaite, V. A. 2004. Size matters: a test of boldness in eight populations of the poeciliid *Brachyrhaphis episcopi*. *Animal Behaviour* 68:1325–1329.
- Brown, C., Jones, F. and Braithwaite, V. A. 2007. Correlation between boldness and body mass in natural populations of the poeciliid *Brachyrhaphis episcopi*. *Journal of Fish Biology* 71:1590–1601.
- Caraco, T. 1981. Energy Budgets, Risk and Foraging Preferences in Dark-Eyed Juncos (*Junco hyemalis*), *Behavioural Ecology and Sociobiology* 8:213–217.
- Carere, C. and Maestriperi, D. 2013. *Animal Personalities: Behavior, Physiology, and Evolution*. Chicago, IL: University of Chicago Press.
- Clark, C.W. 1994. Antipredator behaviour and the asset-protection principle. *Behavioral Ecology* 5:159–170.

- Coleman, K., and Wilson, D. 1998. Shyness and boldness in pumpkinseed sunfish: individual differences are context-specific. *Animal Behaviour* 56:927–936.
- Cooper Jr., W. E., Perez-Mellado, V., Baird, T., Baird, T. A., Caldwell, J. P. and Vitt, L. J. 2003. Effects of risk, cost, and their interaction on optimal escape by nonrefuging Bonaire whiptail lizards, *Cnemidophorus murinus*. *Behavioural Ecology* 14:288–293.
- Cooper Jr, W. E. 2003b. Shifted balance of risk and cost after autotomy affects use of cover, escape, activity, and foraging in the keeled earless lizard (*Holbrookia propinqua*). *Behavioural Ecology and Sociobiology* 54:179–187.
- Crouse, D. T. 1999. The consequences of delayed maturity in a human dominated world. In *Life in the slow lane: ecology and conservation of long-lived marine animals*. Edited by Musick, J.A. *American Fisheries Society Symposium* 23:195–202.
- Dial, K. P., Greene, E. and Irschick, D. J. 2008. Allometry of behaviour. *Trends in Ecology and Evolution* 23:394–401.
- Dingemanse, N. J., and de Goede, P. 2004. The relation between dominance and exploratory behaviour is context-dependent in wild great tits. *Behavioral Ecology* 15:1023–1030.
- Faber, D. B. and Baylis, J. R. 1993. Effects of body size on agonistic encounters between male jumping spiders (Araneae: Salticidae). *Animal Behaviour* 45:289–299.
- Filby, A. L., Paull, G. C., Bartlett, E. J., Van Look, K. J. and Tyler, C.R. 2010. Physiological and health consequences of social status in zebrafish (*Danio rerio*). *Physiology and Behavior* 101:576–587.

- Given, M. F. 1988. Territoriality and aggressive interactions of male carpenter frogs, *Rana virgatipes*. *Copeia* 2:411-421.
- Grant, J. W. 1990. Aggressiveness and the foraging behaviour of young-of-the-year brook charr (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences* 47:915–920.
- Harwood, A. J., Armstrong, J. D., Metcalfe, N. B., and Griffiths, S. W. 2003. Does dominance status correlate with growth in wild stream-dwelling Atlantic salmon (*Salmo salar*)? *Behavioural Ecology* 14:902–908.
- Haxton, T. J., and Findlay, C. S. 2009. Variation in large-bodied fish-community structure and abundance in relation to water-management regime in a large regulated river. *Journal of Fish Biology* 74: 2216–2238.
- Houston, A. I., Mcnamara, J. M., and Hutchinson, J. M. C. 1993. General results concerning the trade-off between gaining energy and avoiding predation. *Philosophical Transactions of the Royal Society London Series B* 341:375–397.
- Hojesjo, J., Johnsson, J., and Bohlin T. 2004. Habitat complexity reduces the growth of aggressive and dominant brown trout (*Salmo trutta*) relative to subordinates. *Behavioural Ecology and Sociobiology*. 56:286–289.
- Huntingford, F. A., Metcalfe, N. B., Thorpe, J. E., Graham, W. D. and Adams, C. E. 1990. Social dominance and body size in Atlantic salmon parr, *Salmo salar* L. *Journal of Fish Biology* 36:877-881.
- Jacob, A., Nussle, S., Britschgi, A., Evanno, G., Muller, R. and Wedekind, C. 2007. Male dominance linked to size and age, but not to ‘good genes’ in brown trout (*Salmo trutta*). *BMC Evolutionary Biology* 7:207–215.

- Jackson, C. D., and G. E. Brown. 2011. Differences in antipredator behaviour between wild and hatchery-reared juvenile Atlantic salmon (*Salmo salar*) under seminatural conditions. *Canadian Journal of Fisheries and Aquatic Sciences* 68:2157–2165.
- Jonsson, B. 1997. A review of ecological and behavioural interactions between cultured and wild Atlantic salmon. *ICES Journal of Marine Sciences*. 54:1031–1039.
- Kemp, D. J. and Wiklund, C. 2001. Fighting without weaponry: a review of male-male contest competition in butterflies. *Behavioral Ecology and Sociobiology* 49:429-442.
- Killen, S. S., Marras, S., Metcalfe, N. B., McKenzie, D. J. and Domenici, P. 2013. Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology and Evolution* 28:651–658.
- Krause, J., Loader, S. P., McDermott, J. and Ruxton, G. D. 1998. Refuge use by fish as a function of body length-related metabolic expenditure and predation risks. *Proceedings of the Royal Society of London B* 265:2373–2379.
- Lorenzen, K. 2000. Allometry of natural mortality as a basis for assessing optimal release size in fish-stocking programmes. *Canadian Journal of Fisheries and Aquatic Sciences* 57:2374–2381.
- Marden, J. H. and Waage, J. K. 1990. Escalated damselfly territorial contests are energetic wars of attrition. *Animal Behaviour* 39:954-959.
- Martin-Smith, K. M., and Armstrong, J. D. 2002. Growth rates of wild stream-dwelling Atlantic salmon correlate with activity and sex but not dominance. *Journal of Animal Ecology*. 71:413–423.

- Milinski, M. and Parker, G. A. (1991). Competition for resources. In Behavioural Ecology, an Evolutionary Approach (Krebs, J. R. & Davies, N. B., eds), pp. 137–168. Oxford: Blackwell Scientific Publications.
- Nilsson, P. A. and Brönmark, C. 2000. Prey vulnerability to a gape-size limited predator: behavioural and morphological impacts on northern pike piscivory. *Oikos* 88:539–546.
- Otronen, M. 1988. The effect of body size on the outcome of fights in burying beetles (*Nicrophorus*). *Annales Zoologici Fennici* 25:191-201.
- Olsson, M. 1992. Contest success in relation to size and residency in male sand lizards, *Lacerta agilis*. *Animal Behaviour* 44:386-388.
- 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. *Environmental Reviews* 43:30–43.
- Polverino, G., Bierbach, D., and Killen, S. S. 2016. Body length rather than routine metabolic rate and body condition correlates with activity and risk-taking in juvenile zebrafish *Danio rerio*. *Journal of Fish Biology* 49:2251–2267.
- Polverino, G., Ruberto, T., Staaks, G. and Mehner, T. 2016. Tank size alters mean behaviours and individual rank orders in personality traits of fish depending on their life stage. *Animal Behaviour* 115:127–135.
- Réale, D., Reader, S. M., Sol, D., McDougall, P. T. and Dingemanse, N. J. 2007. Integrating animal temperament within ecology and evolution. *Biological Reviews* 82:291–318.

- Robertson, J. G. M. 1986. Male territoriality, fighting and assessment of fighting ability in the Australian frog *Uperoleia rugosa*. *Animal Behaviour* 34:763-772.
- Rowe, L. and Ludwig, D. 1991. Size and timing of metamorphosis in complex life cycles: Time constraints and variation. *Ecology* 72:413–427.
- Seghers, B. H. 1981 Facultative schooling behavior in the spottail shiner (*Notropis hudsonius*): possible costs and benefits. *Environmental Biology of Fish* 6:21–24.
- Sih, A., Mathot, K. J., Moiron, M., Montiglio, P. O., Wolf, M. and Dingemanse, N. J. 2015. Animal personality and state-behaviour feedbacks: a review and guide for empiricists. *Trends in Ecology and Evolution* 30:50–60.
- Stankowich, T., and Blumstein, D. T. 2005. Fear in animals: a meta-analysis and review of risk assessment. *Proceedings of the Royal Society B*. 272:2627–2634.
- Sundstrom, L.F., Petersson, E., Hojesjo, J., Johnsson, J., and Jarvi, T. 2004. Hatchery selection promotes boldness in newly hatched brown trout (*Salmo trutta*): implications for dominance. *Behavioural Ecology* 15:192–198.
- Taylor, P., Brown, G. E., Ferrari, M. C. O., and Chivers, D. P. 2013. Adaptive forgetting: why predator recognition training might not enhance poststocking survival. *Fisheries* 38:16–25
- Wells, K. D. 1978. Territoriality in the green frog (*Rana clamitans*): vocalizations and agonistic behaviour. *Animal Behaviour* 26:1051-1063.
- Wolf, M. and Weissing, F. J. 2010. An explanatory framework for adaptive personality differences. *Philosophical Transactions of the Royal Society B* 365:3959–3968.
- Wilson, D. S., Coleman, K., Clark, A. B., and Biederman, L. 1993. The shy-bold

- continuum in pumpkinseed sunfish (*Lepomis gibbosus*): An ecological study of a psychological trait. *Journal of Comparative Psychology* 107:250–260.
- Wishingrad, V., Sloychuk, J. R., Ferrari, M. C. O., and Chivers, D. P. 2014a. Alarm cues in Lake Sturgeon *Acipenser fulvescens* Rafinesque, 1817: potential implications for like-skills training. *Journal of Applied Ichthyology* 30:1441-1444.
- Wishingrad, V., Chivers, D. P., and Ferrari, M. C. O. 2014b. Relative cost/benefit trade-off between cover-seeking and escape behaviour in an ancestral fish: the importance of structural habitat heterogeneity. *Ethology* 95:973–981.
- Yue, S., Duncan, I. J. H. and Moccia, R. D. 2006. Do differences in conspecific body size induce social stress in domestic rainbow trout? *Environmental Biology of Fishes* 76:425–431.

Tables and Figures

Table 2.1. Poisson and negative binomial dispersion estimates from the generalized linear mixed models (GLMMs) predicting the effect of Alarm Cue (AC) and AC with food on juvenile Lake Sturgeon activity and substrate type preference.

Model	Poisson Dispersion	Negative Binomial Dispersion
<i>Activity</i>		
AC	8.38	0.57
AC + Food	4.56	0.75
<i>Gravel</i>		
AC	0.44	0.68
AC + Food	0.40	0.66
<i>Sand</i>		
AC	0.69	1.00
AC + Food	0.74	0.79
<i>No Substrate</i>		
AC	0.72	0.91
AC + Food	0.65	0.83

Table 2.2. Generalized linear mixed models (GLMM) results predicting the effect of alarm cue (AC) or AC with food on the activity and substrate type preference response of juvenile Lake Sturgeon and the influence of condition factor on this response, with the parameter estimates, standard errors (SE), and probability associated with the Wald statistic ($\Pr(>|z|)$) of the negative binomial GLMMs of the eight models: Activity, Sand, Gravel, and No Substrate, all run separately for the presence or absence of food, with fish ID as the random effect.

Model	Predictor Variable	Estimate	SE	Pr(> z)
<i>Activity</i>				
AC	Period	1.48	0.37	7.11e-5
AC	Condition Factor	7.69	8.88	0.39
AC + Food	Period	-0.13	0.15	5.2e-9
AC + Food	Condition Factor	-7.47	4.35	8.6e-2
<i>Gravel</i>				
AC	Period	0.85	0.31	6.0e-3
AC	Condition Factor	8.57	6.62	0.20
AC + Food	Period	0.04	0.49	0.94
AC + Food	Condition Factor	-11.74	11.56	0.31
<i>Sand</i>				
AC	Period	NA	NA	NA
AC	Condition Factor	-0.83	0.81	0.31
AC + Food	Period	-0.18	0.18	0.29
AC + Food	Condition Factor	4.02	6.01	0.50

Extension of Table 2.2

Model	Predictor Variable	Estimate	SE	Pr(> z)
<i>No Substrate</i>				
AC	Period	0.19	0.17	0.27
AC	Condition Factor	0.07	5.73	0.99
AC + Food	Period	0.10	0.14	0.47
AC + Food	Condition Factor	-1.91	1.04	6.6e-2

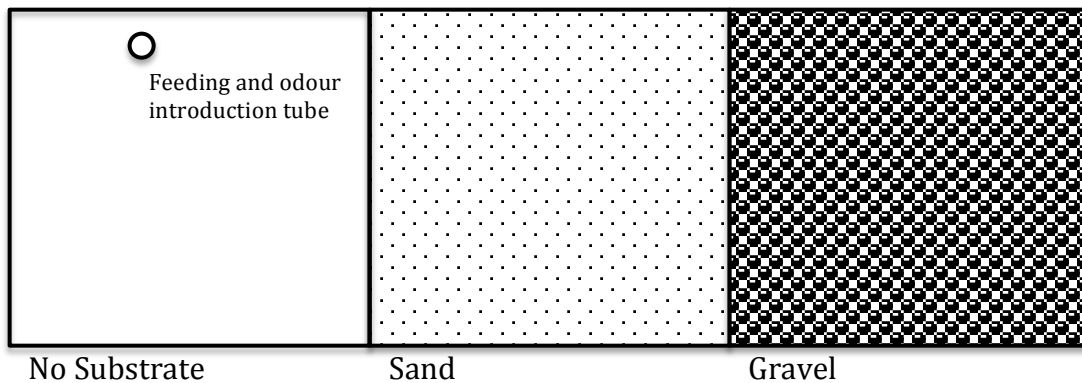


Figure 2.1. Behavioural arenas measuring 21cm long by 14cm wide and 6.4cm deep at ~60 days post fertilization (dpf) trials and 59.7cm long by 42.9cm wide and 14.9cm deep in ~160dpf trials, with each substrate encompassing a third of the area, and each containing randomly assorted substrates (no substrate, sand, and gravel) with feeding tube position over no substrate in all arenas.

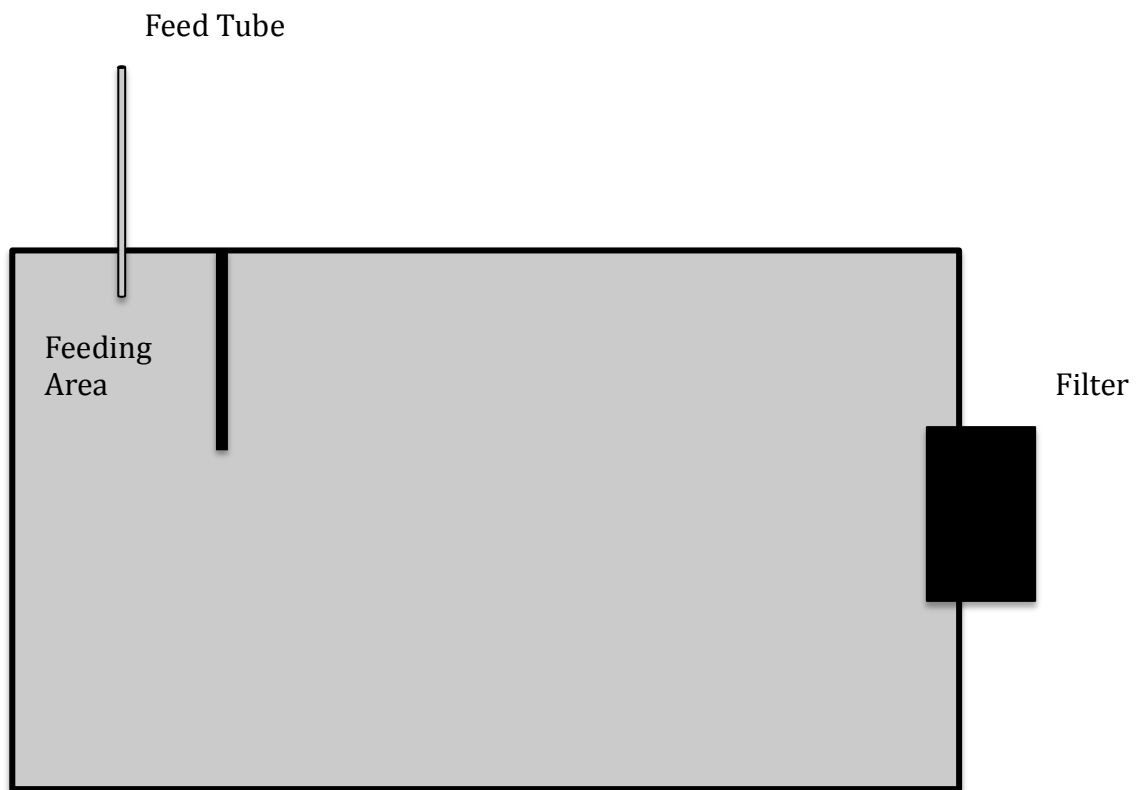


Figure 2.2. Overhead view of aquariums measuring 54.5cm long by 30.5cm wide and 12cm deep, with feeding area only accessible from one direction, used to determine the resource holding potential of asymmetrically and symmetrically size matched juvenile Lake Sturgeon with a point source of food in the feeding area and one direction to access the feeding area, creating a resource limited environment with limited access to those resources.

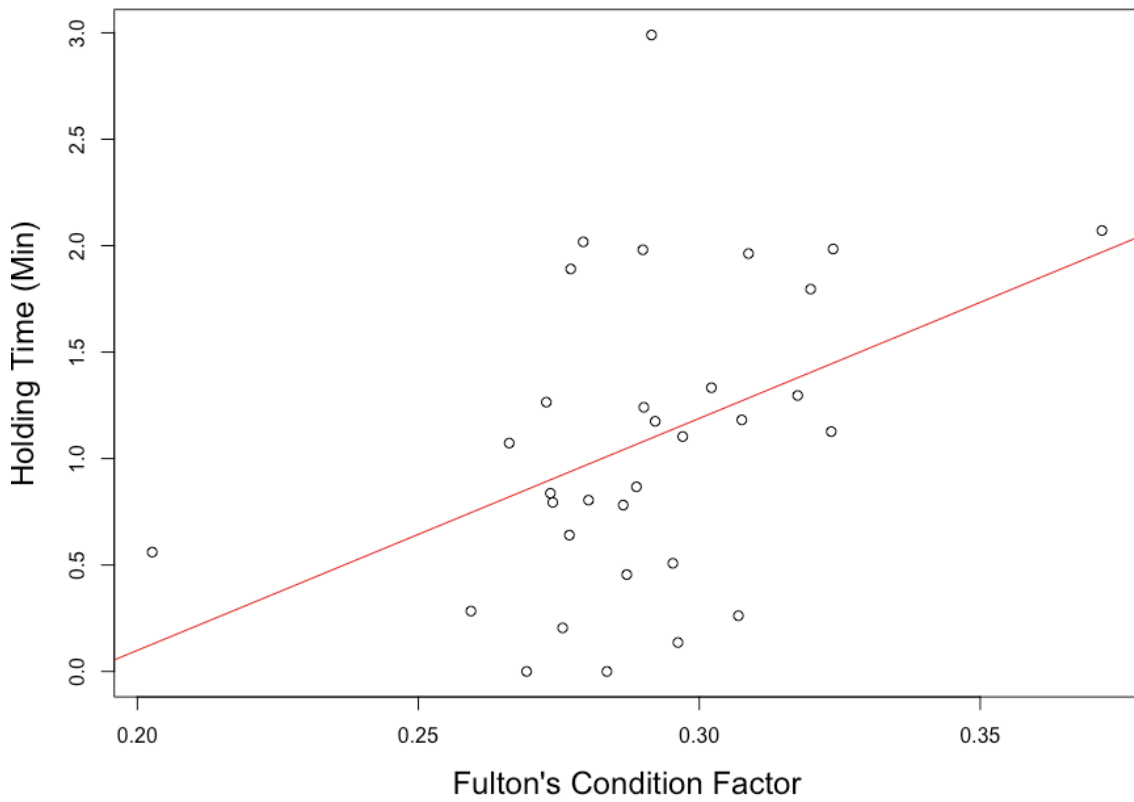


Figure 2.3. Relationship ($R^2 = 0.28$) between Fulton's condition factor and holding time of symmetrically and asymmetrically size-matched juvenile Lake Sturgeon during feeding in limited resources ($n=32$); with all data points having a Cook's Distance <0.5 .

Chapter 3: Diurnal foraging effort and risk assessment of naïve and predator-conditioned Lake Sturgeon (*Acipenser fulvescens*) during critical early life periods

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This manuscript will be submitted to the *Journal of Applied Ichthyology*. F. Bjornson conducted all experiments, data analysis and draft of the manuscript; M. Earhart conducted sample analysis for the cortisol data presented; W.G. Anderson assisted in experimental design, data interpretation and editing the manuscript draft.

Abstract

Responding appropriately to foraging opportunities and predation risk can be the difference between life and death in animals. In particular, this decision may be most costly to predator-naïve, hatchery-reared fish released for conservation purposes. This study examines diurnal foraging effort and risk assessment of naïve and predator-conditioned Lake Sturgeon (*Acipenser fulvescens*) during two critical life periods; early exogenous feeding (~60dpf) and pre-winter (~160dpf). Using an information theoretic approach (AIC), the analysis indicated the most parsimonious model of naïve Lake Sturgeon risk assessment to include the presence of food and time of day, and the conditioned Lake Sturgeon model to include presence of food, time of day, the presence of predator odour, life period, and the conditioning protocol (acute or chronic exposure to alarm cue, predator cue or a combination of both). Results suggest the importance of foraging during the early life stage of Lake Sturgeon and highlight context specific anti-

predator responses of naïve and conditioned fish. Additionally, whole body cortisol analysis revealed significantly ($p > 0.05$) higher cortisol in conditioned Lake Sturgeon, particularly in chronically-conditioned fish, suggesting a heightened responsiveness of the hypothalamic pituitary inter-renal axis as a result of predator odour conditioning.

Introduction

The tradeoff 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. However, the promoted behaviour of the individual can be further subdivided by intrinsic demands (growth, metabolic rate, body condition) and experienced extrinsic pressures (predation experience, micro-habitat heterogeneity, foraging) (Polverino et al. 2016). These pressures vary over age, size, body or physiological condition of the individual, thus promoting behavioural differences optimal for the given circumstances (Wolf and Weissing 2010).

Relying on intrinsic demands and experienced extrinsic pressures to promote behaviour advantageous for survival is crucial in fishes, particularly during development when they have high energy requirements and limited predation experience. For hatchery-reared fishes, lack of experience with natural foraging conditions, microhabitat variability, and predation threats during early development distinctly differs from wild counterparts (Olla et al. 1998; C. Brown and Day 2002; Fernö et al. 2011). As such, hatchery-reared fishes that are routinely stocked into natural waterways as part of population enhancement, recovery or conservation efforts (Brown and Laland 2001;

Salvanes and Braithwaite 2006; Fraser 2008) may experience reduced growth rates, increased predation risk, and/or reduced fitness post release (Huntingford 2004; Fernö et al. 2011). Indeed, it is thought that conservation hatchery programs are hindered by low post-release survival resulting from predator naivety of the released fish (Suboski and Templeton 1989; Brown and Smith 1998; Brown et al. 2013a).

Providing hatchery-reared fishes with predator recognition prior to release has received considerable research effort (Sloychuck et al. 2016). The practice typically involves exposing fish to a predator cue (PC) (odour or visual) combined with chemical alarm cue (AC) released from an injured conspecific. The technique, referred to as conditioning, is a highly efficient form of learning in fishes (Chivers and Smith 1994; Ferrari et al. 2010). However, examining post-release survival of conditioned hatchery-reared fishes has yielded mixed results (Brown et al. 2013a), bringing into question retention of conditioning information and ontogenetic timing most suitable for conditioning. Additionally, exposing fishes to AC may induce a physiological stress response (Henrique et al., 2015), which could impact the health of the individual (e.g. severe immunosuppression) if repeated exposures to AC are used to establish a conditioned response. Therefore, it is necessary to better understand the intrinsic and extrinsic pressures placed on fishes during conditioning.

Since the late 1800s, Lake Sturgeon (*Acipenser fulvescens*) populations have been in decline (Pollock et al. 2015), and they are currently recognized as endangered under the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2006). Habitat loss and degradation, as well as unique life history and reproductive traits, impede Lake Sturgeon recovery (Beamish et al. 1998). For decades hatcheries across

North America have been raising Lake Sturgeon for stock enhancement of natural populations (Pollock et al. 2015). Only a few studies to date have assessed the efficiency of stock enhancement for Lake Sturgeon, with findings suggesting low dispersion from the introduction site (Schram 1999), dispersion increased with age, and release at night improving survival (Crossman et al. 2011a). From these findings, Sloychuck et al. (2016) suggested Lake Sturgeon make an ideal organism for conditioning as local predator odours can be selected. Sloychuck et al. (2016) further demonstrated that five-month-old Lake Sturgeon could be conditioned to predator odours, thus providing a means for hatcheries to improve their rearing practices.

Understanding the intrinsic demands and extrinsic pressures that promote juvenile Lake Sturgeon behaviour is crucial for improving conditioning techniques for stock enhancement. In particular, understanding the ontogeny of risk assessment behaviour in juvenile Lake Sturgeon may improve their post-release survival, as releasing them when they are more able or willing to respond to risk appropriately can reduce predation mortality, as well as influence the effectiveness of conditioning. Additionally, and what has remained largely unexamined in the literature, is the physiological response of the individual to conditioning. Here we investigate 1) the ontogeny of age 0+ hatchery-reared Lake Sturgeon risk assessment behaviour 2) whether predator conditioning can provide extrinsic experiences that influence their risk assessment behaviour such that it may optimize survival and 3) how conditioning protocols (number of exposures to AC and PC) might influence the physiological state of the individuals.

Methods

3.0 Lake Sturgeon acquisition and care

Lake Sturgeon gametes were collected from four females and five males caught below Pt. Dubois generating station on the Winnipeg River, Manitoba, Canada, on 22 May 2015. Upon gamete collection, all fish 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 were fertilized with approximately 40 μ l of sperm to 15ml of eggs, to form evenly mixed parentage of the hatchery brood stock, and gently mixed for two minutes. They were rinsed three times with de-chlorinated city of Winnipeg tap water and rinsed again in clay for 30 minutes to remove the adhesive coating of the eggs. The eggs were then distributed between five flow-through MacDonald hatching jars at 16°C and 100% DO de-chlorinated City of Winnipeg tap water, with 14 hour light:10 hour 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 75L circular tubs. At 27 days post fertilization (dpf) the Lake Sturgeon were fed live *Artemia* at 07:00, 12:00 and 18:00 CST daily. After 44dpf, shaved bloodworms were mixed into their diet, and gradually the size and proportion of bloodworm increased till 51dpf when they were fed bloodworm exclusively. At 67dpf the fish were moved into two 230L tanks to accommodate growth.

3.1 Odour preparation

12 juvenile Lake Sturgeon at 44dpf (average length and weight 46mm and 311mg) were sacrificed by cervical dislocation, and their gut cavity was cleared to remove any dietary cues. The remaining 1.7g of tissue was placed into 100ml of de-chlorinated water and homogenized with a polytron 2100. The homogenate was then filtered through glass wool, and diluted to 3.2g/l, following the protocol of Wishingrad et al. (2014a), and frozen at -20°C for later use.

Predator odour was prepared from Northern Crayfish (*Orconectes virilis*), a known predator of juvenile Lake Sturgeon (Caroffino et al. 2010), collected below the Pt. Dubois and Slave Falls generating station on the Winnipeg River from May 20 to 21, 2015. A total of 68.3g of crayfish were collected, and frozen for transport back to the University of Manitoba. Predator cue preparation followed the protocol of Wishingrad et al. (2014a), and was frozen at -20°C for later use. Additionally, a control of distilled water was also frozen for later use in the behavioural trials.

3.2 Risk Assessment Experiment

Twelve behavioural arenas measuring 21cm long by 14cm wide and 6.4cm deep were constructed with formed plastic containers (Figure 3.1). Three substrates were positioned randomly in the arena: gravel, sand, and a smooth, white plastic substrate (described henceforth as no substrate), all positioned at the same elevation. Window screen 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 and lead over the top of the surrounding curtains, such 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.

144 Lake Sturgeon at ~60 days post fertilization (dpf) were anaesthetized with MS-222, weighed and measured, and introduced into the behavioural arenas in groups of six to create a social context, as juvenile Lake Sturgeon are suggested to shoal (Barth et al. 2009). 0.5ml of frozen *Artemia* were fed to the Lake Sturgeon at 08:00hrs and again at 20:00hrs CST daily. On the third day, 0.5ml of the prepared AC was introduced into the arena simultaneously with the presence or absence of 0.5ml of *Artemia*, at 20:00hrs and 8:00hrs CST on the same individuals. The overhead camcorders recorded eight minutes of pre-stimulus behavior to gather baseline behaviour before the introduction of the stimuli, and a further eight minutes of post-stimulus behaviour to gather any stimuli-induced behaviour. Thus the Lake Sturgeon were given an opportunity to feed in the presence of potential risk, and their behavioural response could be observed to help determine their diel response to potential risk, and how this response may be balanced with the opportunity to forage. A total of 12 trials with AC introduced with the presence or absence of food were recorded for the Lake Sturgeon in the evening and morning.

At ~160dpf, trials were again conducted to determine the risk assessment of juvenile Lake Sturgeon to measure potential ontogenetic shifts in behaviour. To accommodate growth, 12 larger arenas measuring 59.7cm long by 42.9cm wide and 14.9cm deep were constructed with formed plastic containers in a similar fashion as the

first risk assessment trial. The arenas were divided into four splash trays, which were set in a row. Six Sony DCR-SR68 Handycam camcorders, each encompassing two arenas in their field of view, were positioned by the outer edge of each splash tray. The same protocol from the ~60dpf trials was used, with the exception of 5ml of frozen bloodworm being fed to the Lake Sturgeon and 3.4ml of AC introduced to create similar concentrations as used by Wishingrad et al. (2014a).

3.3 Conditioning Experiment

Between 49 and 67dpf, Lake Sturgeon were conditioned in two groups, an acute and chronic group. Acute conditioning took place in four 7l flow-through tanks (approximately 20 fish/tank) with an air stone over a three-day period. During this time, AC and PC were introduced to two of the tanks between 8:00-10:00 and 16:00-18:00 daily, and the other two received PC and water as a control group, such that a total of six conditioning events occurred. During each conditioning event, the water flow was turned off for 10 minutes, allowing the air stone to disperse the odours throughout the tank. At the end of the acute conditioning period, the Lake Sturgeon were moved to the same behavioural arenas as used in the risk assessment experiment, and the same protocol was used to observe their behaviour, with the exception of PC and water, each with the presence or absence of food, being introduced to the arena at 20:00 and 08:00 to test recognition of the predator odour. This was repeated at ~160dpf to measure any ontogenetic shifts in conditioning. A total of six trials were run for conditioned and control (288 acute conditioned and 288 control) Lake Sturgeon for PC and water introductions in the presence or absence of food at both life stages of ~60dpf and

~160dpf. The stimulus introduced thus allowed the Lake Sturgeon to respond with antipredator behaviour to the PC if conditioning was successful, and their potential behavioural response could be contrasted with the control water stimulus. As well, risk assessment behaviour could be observed for conditioned fish given the opportunity of foraging in the presence of PC.

Chronic conditioning involved the same protocol as acute, except the duration lasted 12 days, providing a total of 24 conditioning events for ~60dpf Lake Sturgeon. Chronic conditioning was not completed for the ~160dpf life stage as numbers of Lake Sturgeon were limited. Directly after the conditioning period, the Lake Sturgeon were moved to the behavioural arenas and their behavioural response to the stimuli introduction was recorded under the same protocol as the acutely conditioned group. A total of six trials were run for conditioned and control (288 acute conditioned and 288 control) ~60dpf Lake Sturgeon PC and water introduced in the presence or absence of food.

3.4 Risk assessment and conditioning video coding protocol

All videos were coded with JWatcher version 0.9. The data were separated into pre- and post-stimulus introduction periods, so that stimulus-induced behaviour could be measured. Two focal fish were randomly selected from each arena, with activity and substrate type preference behaviour being coded. A 1x2 grid was superimposed over the arena, the sum of grid lines crossed by the head of the focal fish allowed estimation of baseline activity and stimulus-induced activity. 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, total time the focal fishes spent over each substrate type was collected to determine substrate type preference and potential stimulus-induced preference.

Whole-body cortisol analysis

Directly following completion of one of the acute conditioned tanks, 59dpf, 14 Lake Sturgeon were sampled from the AC+PC treatment, 14 from the PC+water treatment, and 14 from the parental tank naïve to the odours, totaling 42 individuals. Of the 14 individuals from each treatment, seven were immediately sacrificed by immersion in an overdose of MS-222 (200mg.L⁻¹) and frozen at -80°C for later whole-body basal cortisol analysis. The remaining seven were subject to five minutes of a standardized chasing protocol followed immediately by sacrifice with MS-222, and frozen at -80°C for later elevated whole body cortisol analysis. Upon completion of the chronic conditioning, 67dpf, the same protocol was used to assess basal and peak whole-body cortisol of the Lake Sturgeon with the exception that 16 individuals, evenly split between the treatments, were sacrificed.

For completion of the whole-body cortisol analysis, individual Lake Sturgeon were later thawed, weighed and immediately placed in separate tubes with phosphate buffer solution (0.1MNa₂HPO₄ and 0.03 M NaH₂PO₄ pH 7.4). The tubes were sonicated, vortexed and whole-body cortisol was extracted with Sep-Pak C18 cartridges. 3ml of ethanol was used to elute them from the Sep-Paks, and the eluted samples were then dried

and frozen at -80°C for later cortisol measurement with a radioimmunoassay following the protocol of Zubair et al. (2012).

At 173dpf Lake Sturgeon were again sampled for basal and peak whole-body cortisol. The same protocol from the ~60dpf trials was used on acute conditioned Lake Sturgeon with the exception of 10 individuals sacrificed from the AC+PC treatment, 10 from the PC+C treatment, and 6 from the parental tank. Numbers from the acute conditioning were reduced and the chronic conditioning group was not completed during this life stage due to limited numbers of available Lake Sturgeon.

Statistical Analysis – Experiment 1

Generalized linear mixed models (GLMMs) were used to quantify the relationship between Lake Sturgeon activity response to AC in the presence or absence of food, and substrate type preference, during ~60dpf and ~160dpf trials. The introduced stimuli, AC and AC with food, were modeled separately as the objective was to quantify the behavioural response to each stimuli independently of each other. Analyses were carried out using R (R Core Development Team 2013) with GLMMs fitted using the ‘glmer’ function from the lme4 package (Bates et al. 2015). The full model quantifying activity response to AC consisted of three predictor variables (*period*, denoting pre- or post-stimulus introduction period; *season*, denoting ~60dpf or ~160dpf trials; and *time*, denoting evening or morning trials), and the final model quantifying activity response to AC with food consisted of only *period* and *time*. Competing models consisted of all possible subsets of these predictor variables. Additionally, models quantifying activity and substrate type preference response of conditioned Lake Sturgeon to the introduced

stimulus consisted of seven possible response variables (*period*, *food*, denoting the presence or absence of food in the stimulus; *treatment*, denoting conditioned or naïve Lake Sturgeon; *time*; *odour*, denoting the presence or absence of PC in the stimulus; *season*; and *conditioning*, denoting acute- or chronic-conditioned Lake Sturgeon). All variables were modeled together as the objective was to examine the main factors that drive juvenile Lake Sturgeon behaviour in a more complex environment consisting of extrinsic experiences and intrinsic pressures.

Model ranking was accomplished with Akaike Information Criterion (AIC) in the function ‘drop1’ from the lme4 package (Bates et al. 2015). The response variable for each model was either activity, sand, gravel, or no substrate, depending on whether activity or substrate type preference was of interest in the analysis. In all models, a negative binomial distribution out performed a Poisson distribution (Table 3.1 and 3.3), 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 near normality (Shapiro Wilkes test ($p = 0.026$)), and Levene’s test was used to confirm homogeneity of variance. Graphical examination (qqplot) of the transformed data revealed three extreme values that caused the data to stray from normality. Given an ANOVA’s robust nature towards deviations from normality (Keselman et al. 2008; Lix et al. 1996), and the minimal deviation observed in the data, a two-way ANOVA was used to examine the differences in the Lake Sturgeon plasma cortisol concentration between basal and peak measurements of

acute, chronic and control treatments from ~60dpf and ~160dpf, with chronic treatment removed from ~160dpf trials due to limited fish numbers.

Results

Risk Assessment

Overall, the most dramatic difference in response to AC was observed between morning and evening trials, where activity increased significantly from evening to morning (Figure 3.2; Table 3.2). The response to AC also involved a significant increase in activity, which became more pronounced in the ~160dpf trials. However, in the presence of the combined stimulus of AC with food, activity dropped significantly (Table 3.2). This effect was observed over the morning and evening trials, where the reduction in activity was significantly higher in the morning than evening (Table 3.2). Lake Sturgeon activity did not change over the season in response to the combined stimulus of AC with food, and was thus removed from the 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 ~60dpf to ~160dpf and no substrate significantly increased. In response to the combined stimulus, cover-seeking behaviour significantly reduced, 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.

Conditioning

The most dramatic difference in response was observed from the pre- to post-stimulus introduction period (Figure 3.3; Table 3.4), suggesting the stimuli introduction overall significantly decreased juvenile Lake Sturgeon activity regardless of days post fertilization. Following period, food and time significantly increased activity, with activity remaining higher in the absence of food and during morning trials. Additionally, activity significantly decreased from ~60dpf to ~160dpf trials, and Lake Sturgeon from the ~60dpf chronic conditioning group maintained significantly higher activity than ~60dpf acutely conditioned Lake Sturgeon. Also, treatment was removed from the model (AIC), suggesting conditioning Lake Sturgeon to PC did not influence their later response to the stimuli.

In all models, substrate type preference significantly changed from pre- to post-stimulus introduction. However, season had the strongest influence on substrate type preference, with gravel and sand preference significantly increasing in ~160dpf. The absence of food resulted in a significant increase in sand preference and a significant decrease in no substrate preference. Interestingly, Lake Sturgeon conditioned to PC showed a significant increase in gravel preference and a significant decrease in no substrate. As well, chronic conditioned Lake Sturgeon significantly preferred sand and gravel substrates to that of acutely conditioned Lake Sturgeon.

Cortisol

Following conditioning, baseline whole body cortisol concentrations were significantly higher in conditioned fish than control fish (Table 3.5; Figure 3.4). This

trend was further observed in the treatments, where baseline whole body cortisol concentrations were significantly higher in chronically conditioned Lake Sturgeon than the acutely conditioned group (Table 3.5; Figure 3.4). However, baseline whole body cortisol concentrations significantly decreased in the ~160dpf period. Surprisingly, no difference was observed between baseline and peak whole body cortisol concentrations (Table 3.5; Figure 3.4). Additionally, a significant interaction between conditioning, level, and treatment was observed (Table 3.5; Figure 3.4).

Discussion

In the risk assessment experiment, we showed the importance of foraging to juvenile Lake Sturgeon, despite the presence of potential predation risk, time of day or life stage. This response is not uncommon among fishes, as a number of studies report the costs of leaving an ideal patch may exceed the perceived risk (Stankowich and Blumstein 2005). In particular, finding properly sized and nutritious food is critical during early exogenous feeding in other sturgeon species (Wildhaber et al. 2007), suggesting the observed strong response to foraging opportunities in this study may also be necessary for Lake Sturgeon early life survival.

Interestingly, the response to foraging over AC persisted with development, despite the elevated antipredator response to the AC alone stimulus in the ~160dpf trials. Indeed, given the improved nutritional status and higher escape potential of these individuals, the costs of responding to threat may be reduced, allowing them to forego feeding in light of potential predation risk. Such ontogenetic shifts in antipredator behaviour are well documented in fishes (Golub and Brown 2003; Jones et al. 2003;

Marcus and Brown 2003; Harvey and Brown 2004). However, in temperate environments where overwinter mortality can be more severe than predation mortality in fishes (Schultz and Conover, 1997), obtaining enough energy reserves to maximize winter survival is paramount (Post and Evans, 1989; Post and Parkinson, 2001; Hurst and Conover, 2003). Similarly, given the short growing season of juvenile Lake Sturgeon in this study, survival may be maximized by facilitating growth and enriching energy reserves through foraging rather than responding to potential predation risk.

Recently, Sloychuk et al. (2016) demonstrated the potential for predator conditioning in juvenile Lake Sturgeon. Interestingly, conditioning had no effect on the Lake Sturgeon risk assessment behaviour in this study, nor was any predator recognition observed in the predator only stimulus trials. It is possible that retention of learned predator recognition might play a role in these results, as three days were given in this experiment and 24 hours in Sloychuck et al. (2016). Retention of learned predator recognition varies significantly among fishes (Ferrari et al. 2010). For example, Fathead Minnows (*Pimephales promelas*) retained their response with no loss in intensity for at least two months after a single conditioning event to a novel predator odour (Chivers and Smith 1994). Furthermore, hatchery-reared Rainbow Trout retained a detectable response to a novel predator odour after a single conditioning event for 21 days (Brown and Smith 1998). Given that the juvenile Lake Sturgeon in this study received either six or 24 conditioning events (acute and chronic conditioning), it seems unlikely that the potential learned response would be retained for less than three days. Sloychuk et al. (2016) also demonstrated population differences in the number of conditioning events necessary for juvenile Lake Sturgeon to form a learned response to a predator odour. Perhaps the

variability in conditioning juvenile Lake Sturgeon plays a role in our results. Additionally, other examples of limited learned predator recognition suggest that hatcheries select bolder individuals (Sundström et al. 2004), which are prone to retain information less readily than their shy counterparts due to their reduced value placed on predator avoidance (Tymchuk et al. 2007). Finally, another explanation may be that conditioning had an effect on the juvenile Lake Sturgeon behaviour but that it was outweighed by the effects of the other variables in the model, resulting in its exclusion from the final model. These hypotheses may help shed light on the lack of observed predator recognition in our study; however, further research is necessary to understand the role information retention, conditioning variability, hatchery selection of behavioural phenotypes, or potential model limitations play in our results.

Physiological responses to predation threat are necessary in fishes. 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). Our results indicate such a physiological response of juvenile Lake Sturgeon to repeated introductions of AC combined with PC. Particularly, this response was sensitive in the chronic conditioning group basal whole body cortisol concentrations, which received a total of 24 introductions of the stimulus. Such physiological responses have been observed in other taxa. For example, Clinchy et al. (2004) observed increased glucocorticoids in song sparrows residing in high-predation environments, and Berger et al. (2007) found a similar trend in marine iguanas (*Amblyrhynchus cristatus*) which experience recent predator introductions. If cortisol levels are elevated for a long enough period they can have deleterious effects on the well-being of vertebrates (Clinchy et al.

2004; Sapolsky et al. 2000), and thus conditioning juvenile Lake Sturgeon over long periods may not be ideal for instilling a learned response. Indeed, our results suggest heightened antipredator behaviour before the introduction of the stimulus, which is suggestive of elevated perception of risk and is costly to maintain with no apparent benefits. However, Fischer et al. (2014) found reduced waterborne cortisol levels in populations of Trinidadian guppies habituated to high-predation environments compared to other populations of low predation pressure. They hypothesized that this could be an adaptation to high predation environments as a means to reduce the effects of chronic stress. As such, exposing hatchery-reared juvenile Lake Sturgeon to repeated introductions of AC and PC may provide them with intrinsic and extrinsic pressures, beyond predator recognition, necessary for survival post-release if the environment of their release is known to have high predation pressure.

Summary

Our results indicate the complexity and context dependent behavioural response of juvenile Lake Sturgeon to potential predation risk, and their strong preference for foraging. During an individual Lake Sturgeon's early life period, foraging plays a strong role in their risk assessment behaviour, and we suggest that it may be more important than responding to potential predation threats at this life stage. This can impact the efficiency of predator conditioning, as juvenile Lake Sturgeon may respond to foraging opportunities rather than predation cues they may be conditioned to. This may occur despite potential elevated perception of risk, as observed in the chronic conditioning group in our study. However, further information on the retention of conditioned

behaviour and the potential hatchery selection of behavioural phenotypes is necessary to better understand the effectiveness of conditioning in juvenile Lake Sturgeon.

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References

- 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. *Journal of Applied Ichthyology* 25:18–26.
- Barton, B. A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative Comparative Biology* 42:517–525.
- Bates. D., Maechler. M., Bolker. B., and Walker. B. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67:1-48.
- 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. *Hormones and Behaviour* 52:653–663.

- Brown, G. E., M. C. O. Ferrari, and D. P. Chivers. 2013a. Adaptive forgetting: why predator recognition training might not enhance poststocking survival. *Fisheries* 38:16–25.
- Brown, C., and R. Day. 2002. The future of stock enhancements: lessons for hatchery practice from conservation biology. *Fish and Fisheries* 3:79–94.
- Brown, C., and K. Laland. 2001. Social learning and life skills training for hatchery reared fish. *Journal of Fish Biology* 59:471–493.
- Brown, G. E., and J. F. Smith. 1998. Acquired predator recognition in juvenile Rainbow Trout (*Oncorhynchus mykiss*): conditioning hatchery-reared fish to recognize chemical cues of a predator. *Canadian Journal of Fisheries and Aquatic Sciences* 55:611–617.
- 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. *Transactions of the American Fisheries Society* 139:1846–1856.
- Chivers, D. P., and R. J. F. Smith. 1994. Fathead Minnows, *Pimephales promelas*, acquire predator recognition when alarm substance is associated with the sight of unfamiliar fish. *Animal Behaviour* 48:597–605.
- 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. *Proceedings of the Royal Society B*. 271:2473–2479.
- COSEWIC. 2006. COSEWIC assessment and update status report on the lake sturgeon *Acipenser fulvescens* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. pp. 107. Available from <http://publications.gc.ca/>

- collections/collection_2007/ec/CW69-14-484-2007E.pdf. Accessed on 29 Aug 2017.
- Crossman, J.A., Forsythe, P.S., Scribner, K.T., and Baker, E.A. 2011a. Hatchery rearing environment and age affect survival and movements of stocked juvenile lake sturgeon. *Fisheries Management and Ecology* 18:132–144.
- Elgar, M. A. 1989. Predator vigilance and group size in mammals and birds: a critical review of the empirical evidence. *Biological Reviews* 64:13-33.
- Ferrari, M. C. O., Brown, G. E., Jackson, C. D., Malka, P. H., and Chivers, D. P. 2010. Differential retention of predator recognition by juvenile rainbow trout. *Behaviour* 147:1791-1802.
- Ferrari, M. C. O., B. D. Wisenden, and D. P. Chivers. 2010. Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology* 88:698–724.
- Fernö, A., G. Huse, P. J. Jakobsen, T. S. Kristiansen, and J. Nilsson. 2011. Fish behaviour, learning, aquaculture and fisheries. Pages 359–404 in C. Brown, K. Laland, and J. Krause, editors. *Fish cognition and behavior*, 2nd edition. Wiley-Blackwell, London.
- Fraser, D. J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evolutionary Applications* 1:535–586.
- Fischer, E. K., Harris, R. M., Hofmann, H. A., and Hoke, K. L. 2014. Hormones and Behavior Predator exposure alters stress physiology in guppies across timescales. *Hormones and Behavior* 65:165–172.

- Golub, J. L., and G. E. Brown. 2003. Are all signals the same? Ontogenetic change in the response to conspecific and heterospecific chemical alarm signals by juvenile Green Sunfish (*Lepomis cyanellus*). *Behavioral Ecology and Sociobiology* 54:113–118.
- Harvey, M. C., and G. E. Brown. 2004. Dine or dash?: Ontogenetic shift in the response of Yellow Perch to conspecific alarm cues. *Environmental Biology of Fishes* 70:345–352.
- Henrique, F., Sanches, C., Miyai, C. A., Pinho-neto, C. F., and Barreto, R. E. 2015. Stress responses to chemical alarm cues in Nile tilapia. *Physiology and Behavior* 149:8–13.
- Huntingford, F. A. 2004. Implications of domestication and rearing conditions for the behaviour of cultivated fishes. *Journal of Fish Biology* 65:122–144.
- 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.
- Jones, M., A. Laurila, N. Peuhkuri, J. Piironen, and T. Seppa. 2003. Timing an ontogenetic niche shift: responses of emerging salmon alevins to chemical cues from predators and competitors. *Oikos* 102:155–163.
- 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. *Psychological Methods* 13:110–129.
- 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. *Review of Educational Research* 66:579–619.

- Marcus, J. P., and G. E. Brown. 2003. Response of Pumpkinseed sunfish to conspecific chemical alarm cues: an interaction between ontogeny and stimulus concentration. *Canadian Journal of Zoology* 81:1671–1677.
- Olla, B. L., M. W. Davis, and C. H. Ryer. 1998. Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. *Bulletin of Marine Science* 62:531–550.
- 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. *Environmental Reviews* 23: 30–43.
- Polverino, G., Bierbach, D., and Killen, S. S. 2016. Body length rather than routine metabolic rate and body condition correlates with activity and risk-taking in juvenile zebrafish *Danio rerio*. *Journal of Fish Biology* 49:2251–2267.
- Post, J. R., Evans, D. O. 1989. Size-dependent overwinter mortality of young-of-the-year yellow perch (*Perca flavescens*): laboratory, in situ enclosure, and field experiments. *Canadian Journal of Fisheries and Aquatic Sciences* 46:1958–1968.
- Post, J. R., Parkinson, E. A. 2001. Energy allocation strategy in young fish: allometry and survival. *Ecology* 82:1040–1051.
- R Core Team 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Salvanes, A. G. V., and V. Braithwaite. 2006. The need to understand the behaviour of fish reared for mariculture or restocking. *ICES Journal of Marine Science*, 63:346–354.

- Sapolsky, R. M., Romero, L. M., and Munck, A. U. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* 21:55–89.
- Schram, S. T., Lindgren, J., Evrard, L. M., Schram, S. T., Lindgren, J., and Evrard, L. M. 1999. Reintroduction of lake sturgeon in the St. Louis River, western Lake Superior. *North American Journal of Fisheries Management* 19:815–823.
- Schultz, E.T., 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.
- Sloychuk, J. R., Chivers, D. P., Ferrari, M. C. O. 2016. Juvenile Lake Sturgeon go to school: life-skills training for hatchery fish. *Transactions of the American Fisheries Society* 145:287–294.
- Stankowich, T., and Blumstein, D. T. 2005. Fear in animals: a meta-analysis and review of risk assessment. *Proceedings of the Royal Society B*. 272:2627–2634.
- Suboski, M. D., and J. J. Templeton. 1989. Life skills training for hatchery fish: social learning and survival. *Fisheries Research* 7:343–352.
- Sundström, L. F., E. Petersson, J. Höjesjö, J. I. Johnsson, and T. Järvi. 2004. Hatchery selection promotes boldness in newly hatched brown trout (*Salmo trutta*): implications for dominance. *Behavioral Ecology* 15:192–198.
- Wildhaber M.L., D.M., Papoulias, A.J. DeLonay, D.E. Tillitt, J.L. Bryan, and M.L. Annis. 2007. Physical and hormonal examination of Missouri River shovelnose sturgeon reproductive stage: a reference guide. *Journal of Applied Ichthyology* 23:382-401.

Wishingrad, V., D. P. Chivers, and M. C. Ferrari. 2014a. 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.

Wolf, M. and Weissing, F. J. 2010. An explanatory framework for adaptive personality differences. *Philosophical Transactions of the Royal Society B*. 365:3959–3968.

Tymchuk, W. E., L. F. Sundström, and R. H. Devlin. 2007. Growth and survival trade-offs and outbreeding depression in rainbow trout (*Oncorhynchus mykiss*). *Evolution* 61:1225–1237.

Tables and Figures

Table 3.1. Poisson and negative binomial dispersion estimates of the generalized linear mixed models (GLMM) predicting the effect of the introduction of alarm cue (AC) in the presence or absence of food on juvenile Lake Sturgeon activity and substrate type preference.

Stimulus Introduced	Poisson Dispersion	Negative Binomial Dispersion
<i>Activity</i>		
AC	6.83	0.60
AC + Food	4.69	0.75
<i>Gravel</i>		
AC	0.27	0.58
AC + Food	0.39	0.55
<i>Sand</i>		
AC	0.48	0.58
AC + Food	0.64	0.73
<i>No Substrate</i>		
AC	0.45	0.62
AC + Food	0.54	0.71

Table 3.2. Generalized linear mixed models (GLMMs) results predicting the effect of alarm cue (AC) or AC with food on the activity and substrate type preference of juvenile Lake Sturgeon from the pre- to post-stimulus introduction period (Period), over a diel (Time) and seasonal (Season) period of time, with the parameter estimates, standard errors (SE), and probability associated with the Wald statistic ($\Pr(>|z|)$) of the negative binomial GLMMs of the eight models: Activity, Sand, Gravel, and No Substrate, all run separately for the presence or absence of food, with fish ID as the random effect. Blank lines denote the absence of significance of the factor within the model, which are thus were removed from the final model (AIC).

Model	Predictor Variable	Estimate	SE	$\Pr(> z)$
<i>Activity</i>				
AC	Period	0.55	0.17	0.000889
AC	Season	0.52	0.18	0.003687
AC	Time	0.84	0.19	1.1e-5
AC + Food	Period	-0.89	0.15	5.21e-9
AC + Food	Season	-	-	-
AC + Food	Time	-0.71	0.16	4.89e-6
<i>Gravel</i>				
AC	Period	0.59	0.17	0.000625
AC	Season	-1.94	0.27	3.03e-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	-	-	-

Extension of Table 3.2

Model	Predictor Variable	Estimate	SE	Pr(> z)
<i>Sand</i>				
AC	Period	-	-	-
AC	Season	-	-	-
AC	Time	-	-	-
AC + Food	Period	-	-	-
AC + Food	Season	-	-	-
AC + Food	Time	-	-	-
<i>No Substrate</i>				
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	-	-	-

Table 3.3. Poisson and negative binomial dispersion estimates of the generalized linear mixed models (GLMMs) predicting Lake Sturgeon activity and substrate type preference 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
Activity	157	0.55
Gravel	0.30	0.40
Sand	0.49	0.51
No Substrate	0.34	0.48

Table 3.4. Generalized linear mixed models (GLMMs) results predicting the effect of control water or predator odour (Odour) with the presence or absence of food (Food) on the activity and substrate type preference of juvenile Lake Sturgeon from pre-stimulus to post-stimulus introduction period (Period) over a diel (Time) and seasonal (Season) period of time, with naïve and conditioned fishes (Conditioning), that were either acutely or chronically conditioned (Treatment), tested for later recognition of the predator odour and potential ensuing risk assessment behaviour. Parameter estimates, standard errors (SE), and probability associated with the Wald statistic ($\Pr(>|z|)$) of the negative binomial GLMMs of the eight models: Activity, Sand, Gravel, and No Substrate, all run separately for the presence or absence of food, with fish ID as the random effect. Blank lines denote the absence of significance of the factor within the model, which are thus removed from the final model (AIC).

Model	Estimate	SE	Pr(> z)
<i>Activity</i>			
Period	-0.84	0.07	2e-16
Food	0.57	0.09	1.0e-10
Treatment	-	-	-
Time	0.60	0.09	1.2e-10
Odour	-0.12	0.09	0.16
Season	-0.32	0.11	0.0047
Conditioning	0.43	0.11	5.0e-5

Extension of Table 3.4

Model	Estimate	SE	Pr(> z)
<i>Gravel</i>			
Period	-0.45	0.08	6.8e-8
Food	-	-	-
Treatment	-	-	-
Time	0.56	0.14	5.8e-5
Odour	-	-	-
Season	1.22	0.18	6.5e-12
Conditioning	0.59	0.18	0.0014
<i>Sand</i>			
Period	-0.09	0.04	0.016
Food	0.30	0.11	0.006
Treatment	0.25	0.11	0.024
Time	-	-	-
Odour	-	-	-
Season	0.97	0.14	1.1e-12
Conditioning	0.24	0.14	0.089
<i>No Substrate</i>			
Period	0.08	0.06	0.0025
Food	-0.10	0.03	0.0662
Treatment	-0.14	0.06	0.0138
Time	0.08	0.06	0.1519
Odour	-	-	-
Season	-0.61	0.06	2.0e-16
Conditioning	-	-	-

Table 3.5. Results of the two-way ANOVA on the base and peak whole body cortisol concentration of ~60 days post fertilization (dpf) and ~160dpf life stage Lake Sturgeon either acutely (six exposures to alarm cue with predator cue (AC+PC)) or chronically conditioned (24 exposures to AC+PC), naïve (Predator cue with control water (PC+C)) and control (C) individuals.

Variable	P-Value
Conditioning (AC+PC, PC+C, C)	2.58e-06*
Level (Base to Peak)	0.33521
Treatment (Acute, Chronic)	2.34e-10*
Season (early exogenous feeding, pre-winter)	0.00063*
Conditioning*Level	0.42217
Conditioning*Treatment	0.18592
Conditioning*Season	0.28744
Level*Treatment	0.41100
Level*Season	0.82227
Conditioning*Level*Treatment	0.04929*
Conditioning*Level*Season	0.08537

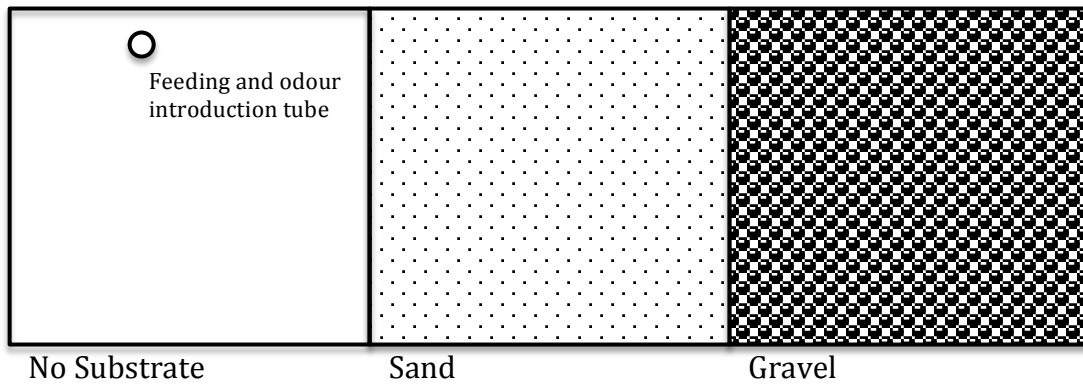


Figure 3.1. Behavioural arenas measuring 21cm long by 14cm wide and 6.4cm deep at ~60 days post fertilization (dpf) trials and 59.7cm long by 42.9cm wide and 14.9cm deep in ~160dpf trials, with each substrate encompassing a third of the area, and each containing randomly assorted substrates (no substrate, sand, and gravel) with feeding tube position over no substrate in all arenas.

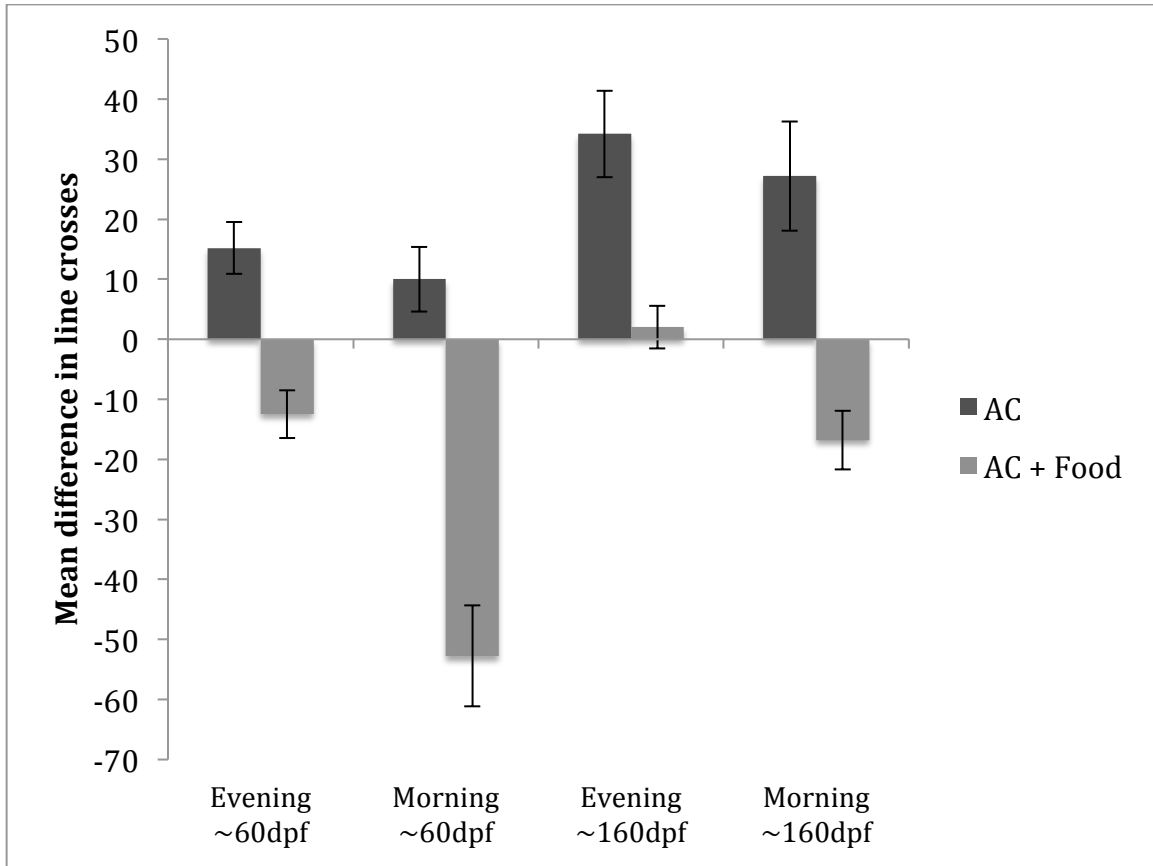


Figure 3.2. Mean (\pm SE) change in line crosses (post-stimulus minus pre-stimulus) during the risk assessment trials of ~60 days post fertilization (dpf) and ~160dpf Lake Sturgeon to the introduction of alarm cue (AC) in the presence or absence of food, with trials run during the morning and evening.

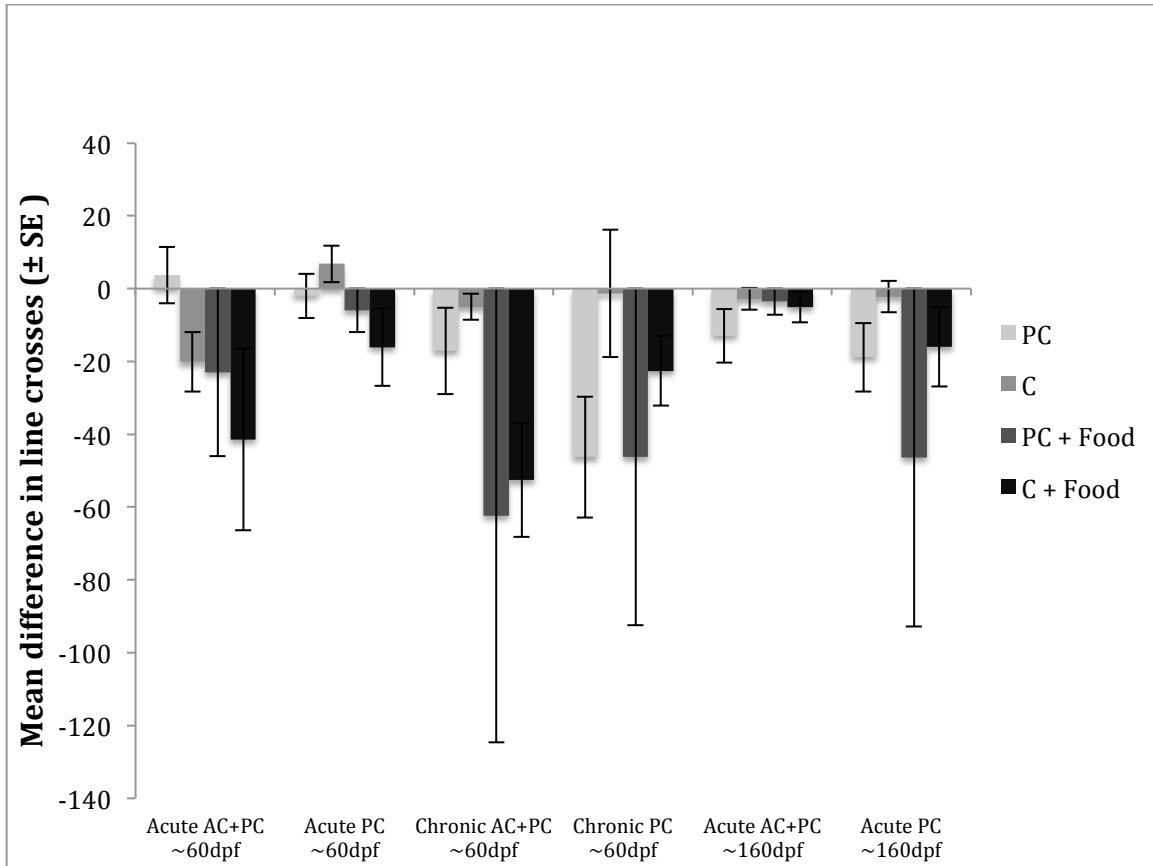


Figure 3.3. Mean (\pm SE) change in line crosses (post-stimulus minus pre-stimulus) during evening recognition trials of ~60dpf (acute and chronic conditioned) and ~160dpf (acute conditioned only) conditioned (alarm cue with predator cue (AC+PC)) and naïve (predator cue with control water (PC+C)) Lake Sturgeon to the introduction of predator cue (PC) or control (C) of water in the presence or absence of food.

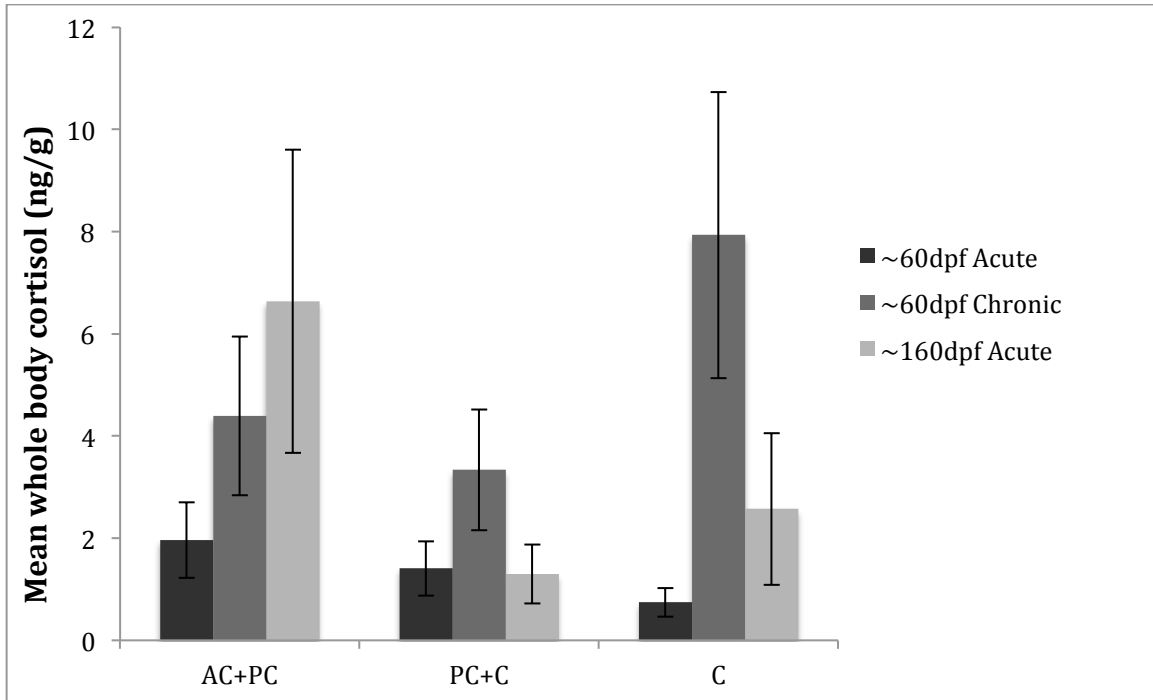


Figure 3.4. Mean basal whole-body cortisol concentrations of conditioned (alarm cue with predator cue (AC+PC)), naïve predator cue with control water (PC+C)) and control (C) Lake Sturgeon subject to either acute conditioning (six exposures to AC+PC) or chronic conditioning (24 exposures to AC+PC) at ~60 days post fertilization (dpf) and ~160dpf.

Chapter 4: General Discussion

The first objective of this thesis was to determine whether body size mediated risk assessment and RHP behaviour. Size variation has been observed within sturgeon cohorts (Gisbert et al. 2000; Nathanailides et al. 2002), including Lake Sturgeon (Klassen 2014). In an attempt to determine whether absolute body size drove growth variability in Lake Sturgeon, Klassen (2014) used a two-patch foraging system (good and poor), and introduced symmetrically or asymmetrically size matched Lake Sturgeon. From the competition, no significance was observed of absolute size on the time spent in the optimal foraging patch, suggesting Lake Sturgeon body size did not influence resource competition. Conversely, in Chapter 1 I observed condition factor, rather than body size, drove RHP in a resource-limited environment, which is typical of non-weaponized species in limited resources (Marden and Waage 1990; Kemp and Wiklund 2001). The difference in observed behavioural responses of the two experiments may have been driven by resource availability. As foraging opportunities improve, the rewards of holding an ideal foraging patch are reduced, and eventually competing will no longer be adaptive in such environments. As Klassen (2014) used a two-patch competition system, resources were more abundant than the limited resource used in Chapter 1, potentially resulting in the lack of body metric mediated resource competitive behaviour observed by Klassen (2014).

Behaviour such as resource competition (Otronen 1988; Huntingford et al. 1990; Olsson 1992; Faber and Baylis 1993) and risk-taking thresholds (Réale et al. 2007) are often correlated with growth rate, and in turn can influence growth rate. Questions regarding the cause and effect of these behaviours are often debated (i.e. are individuals

good competitors because they are big or big because they are good competitors?). Only a few studies have measured the feeding interactions of juvenile Lake Sturgeon (Sbikin and Budayev 1991; Kynard and Horgan 2002; Allen et al. 2009; Klassen 2014), and it remains unclear whether body size or underlying behavioural phenotype drive foraging interactions. In Chapter 2, I demonstrated that condition factor, rather than body size, drives such interactions in a resource-limited environment. However, the study was insufficient for determining whether condition factor was promoted through an initially higher condition factor or the individual's underlying behavioural phenotype. Providing information on this question is paramount in conservation aquaculture programs, which are a prevalent tool used to supplement natural populations (Schram et al. 1999; Jackson et al. 2002; Auer 2002), as condition factor, along with body size, is a metric likely to be selected for as a measure of success the hatcheries. If condition factor promotes certain behaviours, hatchery programs may inadvertently select behavioural phenotypes in Lake Sturgeon, which can reduce phenotypic variability of the released progeny, potentially reducing fitness of the hatchery-reared cohort.

Research pertaining to genetic interactions of hatchery- and wild-reared fishes has received much attention (Waples 1991; Araki et al. 2008; Fraser 2008; Christie et al. 2011). However, negative interactions between numbers of hatchery-reared fishes released and wild conspecific survival has come under recent attention (ecological interactions between wild). For example, hatchery- and wild reared chum salmon (*Oncorhynchus keta*) in inlet and near shore habitat partitioned diets and habitat resources, suggesting competitive interactions between hatchery- and wild-reared individuals (Sturdevant et al. 2012). Additionally, salmonid competitive interactions at

sea are resulting in depressed growth and survival of North Pacific Salmon, which is likely exacerbated by release of hatchery-reared pink (*Oncorhynchus gorbuscha*) and chum salmon (Kaeriyama et al. 2012). Ecological interactions of hatchery- and wild-reared Lake Sturgeon are unknown. However, in Chapter 1 I suggest that hatcheries are likely to select individuals of higher RHP, and previous work from Barth et al. (2009) suggest the shoaling nature of Lake Sturgeon in the wild, which may result in negative ecological interactions between hatchery- and wild-reared individuals.

In Chapter 2 I suggested that conservation aquaculture of Lake Sturgeon may lead to behavioural phenotype selection of bolder individuals by selecting fish of higher condition factor. As bolder behaviour is often associated with less effective retention of learned predator information due to their lesser value placed on predator avoidance (Tymchuk et al. 2007), conditioning may be less effective on hatchery-reared Lake Sturgeon. Indeed, an elaborate experiment was conducted by Brown et al. (2013) to test such a hypothesis in juvenile hatchery-reared Rainbow Trout (*Oncorhynchus mykiss*). In their design, fish were classified as shy vs. bold based on their latency to leave a shelter into a large, unknown environment, and conditioned to recognize a novel predator odour. When testing for recognition 24hr later, no difference in antipredator intensity was observed. However, when recognition trials were run nine days post conditioning, bold trout showed no recognition of the learned response. Taken together, these findings suggest that conditioning is a highly complex and adaptive learning mechanism, and given the potential propensity for hatcheries to select bolder behavioural phenotypes in Lake Sturgeon, it may not provide any functional benefit post release (Brown et al. 2013).

Given the lack of predator recognition I observed in Chapter 3 of predator conditioned Lake Sturgeon, there still remains potential for certain applications of conditioning. Sloychuck et al. (2016) observed that different populations of juvenile Lake Sturgeon could learn to recognize a novel predator odour when exposed to predator odour alone. In chapter 3 I suggest that responding to predator conditioning is likely context dependent, and that foraging is paramount in the early life of Lake Sturgeon. In the natural environment, there are likely ample opportunities for juvenile Lake Sturgeon to encounter predation risk with no immediate foraging opportunities. In such contexts, predator conditioning may improve survival of Lake Sturgeon, potentially resulting in overall higher survival of hatchery-reared individuals. This may be particularly applicable to situations where wild gamete collections are limited and stocking numbers are low; thus providing hatchery-reared fish with any improved chance of survival may result in some individuals being recruited into the natural population.

In Chapter 3 I demonstrated the potential for chronic conditioning of Lake Sturgeon to potentially activate a secondary or tertiary physiological response. Such a response may result in depression of the well-being of the individual (Barton 2002), and potentially reducing the putative fitness gained by predator recognition prior to release. To address this issue, it may be necessary to increase the group size of the conditioned fishes to ameliorate their physiological response. Indeed, a study by Allen et al. (2009) observed a two-fold shorter cortisol stress response following a stressor in one-year-old Lake Sturgeon when held in groups compared to individuals held in isolation; although, no difference was observed in basal or magnitude of cortisol stress response between the grouped fish and fish held in isolation (Allen et al. 2009). As the density and number of

Lake Sturgeon held in the conditioning tanks in Chapter 3 was lower than the parental tank of their rearing, and likely lower than observed in wild populations (Barth et al. 2009), the full physiological benefits of their gregarious nature may not have been realized. As such, it is necessary to examine the influence of differing group sizes on the physiological response of Lake Sturgeon to acute and chronic conditioning in an attempt to maximize the post release fitness of hatchery-reared individuals.

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*. *Journal of Fish Biology* 74:90–104.
- Araki, H., Berejikian, B. A., Ford, M. J., Blouin, M. S. 2008. Fitness of hatchery-reared salmonids in the wild. *Evolutionary Applications* 1:342–355.
- 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. *Journal of Applied Ichthyology* 25:18–26.
- Barton, B. A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids, *Integrative and Comparative Biology* 42:517–525.
- Brown, G. E., Ferrari, M. C. O., Malka, Fregeau, P. H., L., Kayello, L., and Chivers, D. P. 2013. Retention of acquired predator recognition among shy versus bold juvenile rainbow trout. *Behavioral Ecology and Sociobiology* 67:43-51.

- Christie, M. R., Marine, M. L., French, R. A., and Blouin, M. S. 2011. Genetic adaptation to captivity can occur in a single generation. *Proceedings of the National Academy of Science* 109:238–242.
- Faber, D. B. and Baylis, J. R. 1993. Effects of body size on agonistic encounters between male jumping spiders (Araneae: Salticidae). *Animal Behaviour* 45:289-299.
- Gisbert, E., Williot, P. and Castello-Orvay, F. 2000. Influence of egg size on growth and survival of early stages of Siberian sturgeon (*Acipenser baeri*) under small scale hatchery conditions. *Aquaculture* 183:83-94.
- Fraser, D. J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evolutionary Applications* 1:535–586.
- Huntingford, F. A., Metcalfe, N. B., Thorpe, J. E., Graham, W. D., and Adams, C. E. 1990. Social dominance and body size in Atlantic salmon parr, *Salmo salar L.* *Journal of Fish Biology* 36:877-881.
- Jackson, J. R., VanDeValk, A. J., Brooking, T. E., vanKeeken, O. A. and Rudstam, L. G. 2002. Growth and feeding dynamics of lake sturgeon, *Acipenser fulvescens*, in Oneida Lake, New York: results from the first five years of a restoration program. *Journal of Applied Ichthyology* 18:439-443.
- Kaeriyama, M., Seo, H., Kudo, H., and Nagata, M. 2012. Perspectives on wild and hatchery salmon interactions at sea, potential climate effects on Japanese chum salmon, and the need for sustainable salmon fishery management reform in Japan. *Environmental Biology of Fishes* 94:165-177.

- Kemp, D. J. and Wiklund, C. 2001. Fighting without weaponry: a review of male-male contest competition in butterflies. *Behavioral Ecology and Sociobiology* 49:429-442.
- Klassen, C. N. 2014. Growth rate and size variability among juvenile lake sturgeon, *Acipenser fulvescens*: Implications for recruitment. Ph.D. Thesis, University of Manitoba 2:22-42.
- Kynard, B., and Horgan, M. 2002. Ontogenetic behavior and migration of Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus*, and shortnose sturgeon, *A. brevirostrum*, with notes on social behavior. *Environmental Biology of Fishes* 63:137-150.
- Marden, J. H., and Waage, J. K. 1990. Escalated damselfly territorial contests are energetic wars of attrition. *Animal Behaviour* 39:954-959.
- Nathanailides, C., Tsoumani, M., Papazogloy, A. and Paschos, I. 2002. Hatching time and post-hatch growth in Russian sturgeon *Acipenser gueldenstaedtii*. *Journal of Applied Ichthyology* 18:651-654.
- Olsson, M. 1992. Contest success in relation to size and residency in male sand lizards, *Lacerta agilis*. *Animal Behaviour* 44:386-388.
- Otronen, M. 1988. The effect of body size on the outcome of fights in burying beetles (*Nicrophorus*). *Annales Zoologici Fennici* 25:191-201.
- Réale, D., Reader, S. M., Sol, D., McDougall, P. T., and Dingemanse, N. J. 2007. Integrating animal temperament within ecology and evolution. *Biological Reviews* 82:291–318.
- Schram, S. T., Lindgren, J., Evrard, L. M., Schram, S. T., Lindgren, J., and Evrard,

- L. M. 1999. Reintroduction of Lake Sturgeon in the St. Louis River, Western Lake Superior. *North American Journal of Fisheries Management* 19:815-823.
- Sbikin, Y. N. and Budayev, S. V. 1991. Some aspects of the development of feeding relationships in groups of young sturgeons (*Acipenseridae*) during artificial rearing. *Journal of Ichthyology* 31:23-30.
- Sloychuk, J. R., Chivers, D. P., and Ferrari, M. C. O. 2016. Juvenile Lake Sturgeon Go To School: Life-Skills Training for Hatchery Fish. *Transactions of the American Fisheries Society* 145:287-294.
- Sturdevant, M.V., Fergusson, E., Hillgruber, N., Reese, C., Orsi, J., Focht, R., Wertheimer, A., and Smoker, B. 2012. Lack of trophic competition among wild and hatchery juvenile chum salmon during early marine residence in Taku Inlet, Southeast Alaska. *Environmental Biology of Fishes* 94:101-116.
- Tymchuk, W. E., Sundström, L. F., and Devlin, R. H. 2007. Growth and survival trade-offs and outbreeding depression in rainbow trout (*Oncorhynchus mykiss*). *Evolution* 61:1225–1237.
- Waples, R. S. 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. *Canadian Journal of Fisheries and Aquatic Sciences* 48:124–133.