Behavioral Ecology
doi:10.1093/beheco/arr009
Advance Access publication 10 March 2011

Original Article

Behavioral and physiological consequences of nest predation pressure for larval fish

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Localized antipredator behaviors have been observed in a wide variety of taxa. Recent work has also shown that animals that provide parental care adjust their behavior when faced with variation in offspring predation pressure. This variation in offspring predation pressure may also influence the antipredator behavior of offspring if improved antipredator behaviors can increase their probability of survival. We tested if a natural gradient in nest predation pressure influenced antipredator behaviors of larval teleost fish (smallmouth bass, *Micropterus dolomieu*). We examined the predator avoidance of wild larvae from 6 populations that differed in nest predation pressure, and we also compared the recovery from a simulated predator attack of 2 populations at the opposite extremes of predation pressure. We found that larvae differed in their ability to avoid the nest predator, but larvae from lakes of low predation pressure responded similarly to larvae from lakes of high predation pressure. Generally, older offspring were not significantly better at avoiding predators relative to younger offspring, but we found a weak and significant positive correlation between the size of young offspring and their predator avoidance behavior. The recovery from a simulated predation event varied relative to predation pressure. Larvae from the site of high nest predation pressure exhibited reduced rates of maximal oxygen consumption and recovered faster than larvae from the low predation pressure site. Thus, variation in nest predation pressure had little influence on the antipredator behavior of offspring, which are provided with parental care but may have important metabolic consequences. *Key words:* antipredator behavior, *Micropterus dolomieu*, parental care, predation pressure, recovery. *[Behav Ecol 22:510–519 (2011)]*

INTRODUCTION

Predator–prey research has often focused on how prey assess and respond to the risk of predation within a particular ecological setting. Spatial and temporal variability in predation pressure can be important for prey decision-making, particularly when making choices that relate to habitat, foraging, or reproduction (reviewed by Lima and Dill 1990). Alternatively, predation pressure can vary across broad-scale landscapes (e.g., populations), which provides opportunities for local adaptations in antipredator behaviors (Magurran et al. 1993). In many cases, these localized antipredator behaviors are believed to be hereditary but are also influenced by the current environment of individuals (Huntingford and Wright 1993). A wide range of animals (e.g., spiders, amphibians, fish, and birds; Giles and Huntingford 1984; Riechert and Hedrick 1990; Relyea 2002; Griesser and Nystrand 2009) have shown local antipredator behavioral adaptations to their predator environments. Recent work on species that provide parental care has shown that parents can also be sensitive to variation in nest predation pressure and will adjust their parental behaviors accordingly (Fontaine and Martin 2006; Gravel and Cooke 2009). Indeed, parents adjust their guarding behavior because differences in nest predation pressure can greatly influence the survival of valuable offspring. Although the interactions between parental care behavior and nest predation pressure have recently been examined in parental birds (Martin et al. 2000; Fontaine et al. 2007) and fish (Gravel and Cooke 2009), variation in predation pressure may also have important consequences for offspring. There are several ecological differences between birds and fish, that make fish an interesting model for which to further examine the effects of nest predation pressure on offspring. First, the most common form of parental care for fish is guarding offspring, which can range from behaviors such as mouth brooding to nest building and the defense from predators (Blumer 1982). These antipredator behaviors may be quite important in the learned behavior of fish offspring (Tulley and Huntingford 1987). Second, the abundance of aquatic nest predators may be greater than the terrestrial environment (Magnhagen 1992), partially due to the fact that fish typically produce a great number of small eggs, and these in turn are consumed by a great variety of predators. Third, larval fish antipredator defenses develop early, are often innate, and are essential to juvenile survival (Fuiman and Magurran 1994). All of these characteristics make the larval fish of parental care–providing species a good model for which to test for the effects of developmental environment on antipredator behavior.

In this study, we used the smallmouth bass (*Micropterus dolomieu*) as a model to evaluate the influence of predation pressure on the antipredator behavior and physiological characteristics of larval fish. Smallmouth bass provide sole-paternal care for approximately 4 weeks, where males court and spawn with females, fan and guard eggs as well as developing offspring from potential nest predators (Ridgway 1988). Smallmouth bass are found across much of eastern

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Received 13 October 2010; revised 20 January 2011; accepted 26 January 2011.

and central North America (Scott and Crossman 1973) and thus inhabit a wide range of habitats, including those that differ in predation pressure (Steinhart et al. 2004, 2005; Gravel and Cooke 2009; Gravel et al. 2010). In some populations, nest predation pressure can be so high that if a nest is left unattended, such as the case when a male is caught by an angler, it may be entirely consumed by predators within minutes (Kieffer et al. 1995; Suski et al. 2003; Steinhart et al. 2004; Gravel and Cooke 2009). For this reason, there is still a need for the early development of antipredator behaviors in offspring even though they may be well cared-for by vigilant males. The importance of antipredator behaviors is indisputable, and the behavioral consequences of predation risk have been well examined in the literature (reviewed by Lima and Dill 1990). Unfortunately, little work has focused on the physiological implications, which may also be associated with variation in predation pressure. Important relationships have been established between an individual's physiology and other ecologically relevant variables, such as prey availability (Kaufman et al. 2006) or migratory style (Wikelski et al. 2003; Pon et al. 2007) but rarely for predation pressure (but see Bell et al. 2010). Of the numerous tools that have been used to quantify the effects of environmental variation on animal behavior, measures of metabolic rate (MR) may be among the most powerful. The flexibility of this tool enables researchers to measure resting and active MR and also measure the MR of individuals that are performing specific activities or recovering from energetically costly challenges such as a simulated predator attack. Furthermore, slight environmental differences (e.g., temperature, pollution) are known to exert strong impacts on organisms' MR (Schmidt-Nielsen 1984; McKenzie et al. 2007) and are generally conserved across species (Gillooly et al. 2001). As such, we propose to examine the influence of predation pressure on the antipredator behavior of larval fish using 2 methods. First, we propose to use a strict behavioral approach to test if populations that differ in nest predation pressure respond similarly to an introduced nest predator. Second, we propose to use a physiological approach to test if predation pressure influences the rates of oxygen consumption following a simulated predator attack (i.e., exercise). Recovery from exercise is often used as an ecologically relevant indicator of survival (Czesny et al. 2003; Suski et al. 2007), can be a good measure of active MR (Soofiani and Priede 1985) and is particularly relevant when individuals must continue to be vigilant to successive predation attempts.

Our first hypothesis tested the influence of variation in predation pressure on the predator avoidance behavior of larval smallmouth bass. We tested the antipredator behavior of larvae at 2 periods of larval development. We predicted that larval smallmouth bass from populations with increased nest predation pressure would have increased predator avoidance behaviors relative to larvae from populations with low predation pressure and that older larvae would better avoid predators (Brown 1984). Our second hypothesis tested if predation pressure could influence the recovery of larvae from a simulated predator attack. These populations could show physiological differences for 2 main reasons. First, larvae native to environments with high predation pressures may simply be in better physiological condition due to genetic and/or environmental training effects and therefore may demonstrate accelerated recovery relative to larvae from low predation environments. Smallmouth bass tend to show some degree of interannual nest-site fidelity (Ridgway, Maclean et al. 1991; Barthel et al. 2008), and, as a result, parents may be producing offspring that are in better physiological condition in areas of high predation pressure for several generations. Larvae native to areas of high predation pressure may also be involved in a greater number of burst swimming events, which may lead to beneficial training effects (Pearson et al. 1990; Davison 1997). For both these reasons, we would expect larvae from sites with high predation pressure to have lower MRs and recovery more quickly from exercise than larvae from areas of low predation pressure. A second possibility is that there are negative sublethal impacts of predation pressure because most prey animals exhibit a physiological stress response to predators (e.g., Cockrem and Silverin 2002; Monclus et al. 2009). If increased predation pressure is viewed as a chronic stressor (as in Scheuerlein et al. 2001), we would expect larvae from high predation pressure environments to have sublethal metabolic costs associated with this chronic stressor such as higher MRs (Lankford et al. 2005) and an impaired ability to recovery from exercise relative to larvae from low predation pressure environments. When taken together, the results of this study will improve our understanding of both how predation pressure can shape behavioral properties of prey fish but also how prey fishes can express differences in physiological characteristics that can lead to improved survival across varying predation levels.

METHODS

Study site and predation pressure

In the springs of 2008 and 2009, snorkelers surveyed the shoreline of 6 lakes on the Rideau River and Gananoque River systems (Upper Rideau Lake, Charleston Lake, Indian Lake, Newboro Lake, Opinicon Lake, and Sand Lake) in eastern Ontario, Canada, to locate smallmouth bass nests. Nests were individually marked using a numbered piece of polyvinyl chloride pipe. Experienced snorkelers estimated male total length (TL), (Suski et al. 2003) and these length estimates were later validated by catching some males by rod and reel (Suski and Philipp 2004, Gravel MA, unpublished data). Larger males attract larger females, which results in larger males receiving a greater number of eggs per mating (Ridgway, Shuter et al. 1991; Philipp et al. 1997; Hanson and Cooke 2009). Male and female size has the potential to influence offspring survival and behavior if larger parents produce larger offspring, which is closely linked to size-selective mortality and performance in juvenile fish (Sogard 1997) and thus must be considered in this study. These lakes were chosen based on their close proximity to each other (within a 50 km radius; Figure 1) and due to previously documented variation in nest predation pressure (Gravel and Cooke 2009). Bluegill (*Lepomis macrochirus*), pumpkinseed (*L. gibbosus*), yellow perch (*Perca flavescens*), black crappie (*Pomoxis nigromaculatus*), largemouth bass (*M. salmoides*), rock bass (*Ambloplites rupestris*), and conspecifics have all been documented as nest predators for smallmouth bass (Scott and Crossman 1973). Our work has shown that the nest predators in this system are almost exclusively *L. spp.*, that nest predation pressure varies greatly among these lakes (Gravel and Cooke 2009) and that nest predation pressure is consistent across years (Gravel MA, unpublished data).

Offspring collection—predator avoidance

In 2008 and 2009, offspring were collected (only from 4 lakes in 2008: Indian Lake, Newboro Lake, Opinicon Lake, and Sand Lake) at approximately 7–8 days posthatch from a subset of nesting males ($n = 9$–13 nests per lake) in each lake where the exact date of egg deposition was known, and the male had not been removed by researchers. This stage is termed "larvae" and it distinguished by a pigmented eyespot, free-swimming, and exogenously feeding larvae that have a much reduced yolk sac (Wallace 1972; Balon 1975). Typically, the

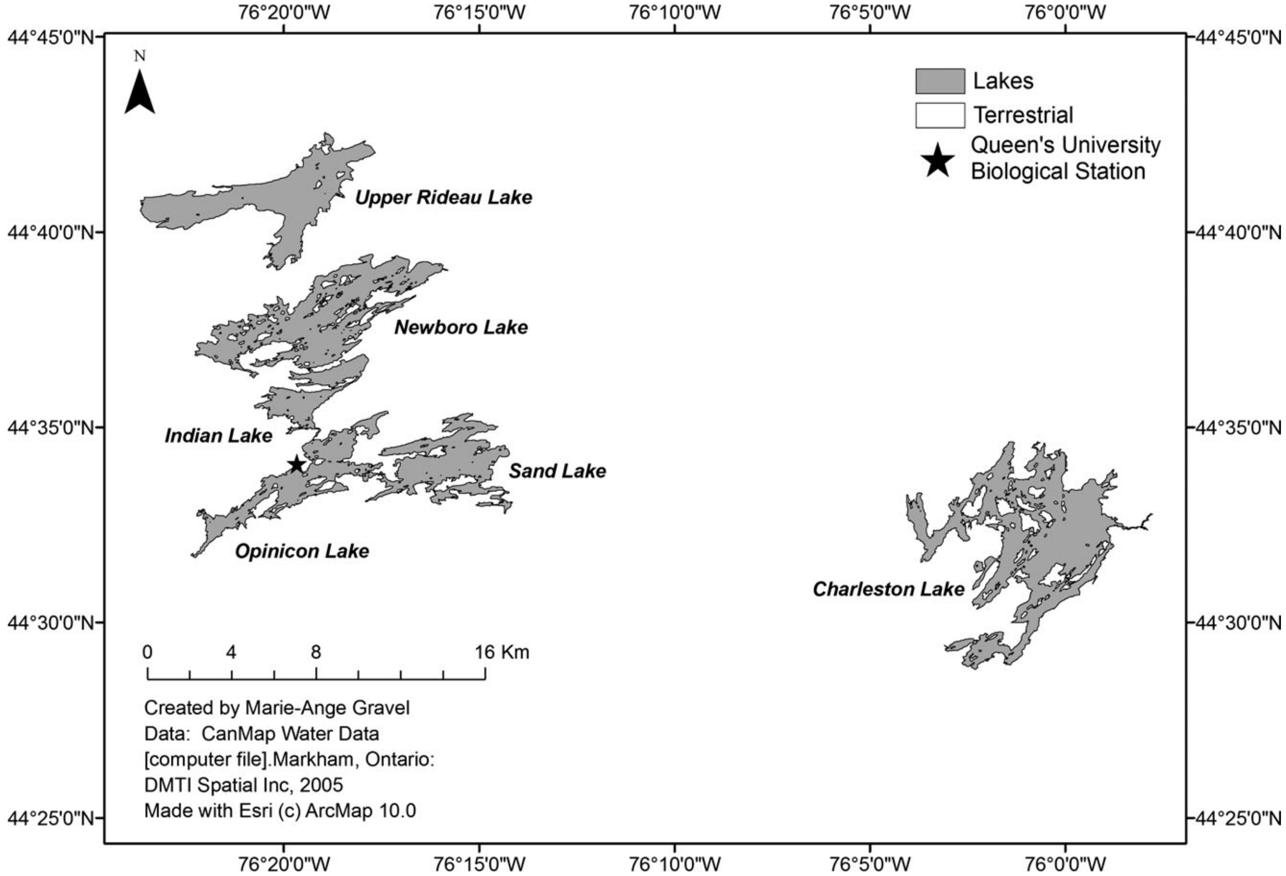

Figure 1
Map of study area showing the 6 lakes and the Queen's University Biological Station.

males had been guarding the developing offspring for 21 days at that point. In 2008 only, juveniles were again collected approximately 15 days posthatch (from the same nests when possible). Juveniles are distinguished by the visible presence of adult smallmouth bass structural elements (Wallace 1972; Balon 1975). This stage corresponded to approximately 28 days of parental care. It is around this time that parental care will soon be terminated, and juveniles will remain in littoral habitats to fend for themselves. Ideally, offspring would be collected at identical ages across all lakes (e.g., degree days [DD] posthatch), but some variation was unavoidable due to the nature of running experiments with wild-caught animals and the fact that lakes warm differentially due to differences in depth and turbidity. Offspring were collected ($n = 50$) with an aquarium net, placed in a glass Mason jar (750 ml) with a mesh screw top, filled with fresh lake water, and kept in a cooler for transportation (up to 6 h). Offspring were brought back to the Queen's University Biology Station (Chaffey's Lock, Ontario) where the mason jars were placed in a bath of temperature-regulated lake water (Opinicon Lake, 17 ± 1 °C), allowing some water exchange between the water in the jars and lake-water bath. Air stones were also added to each mason jar. Offspring were kept overnight, and antipredator behavior trials began the following morning.

Offspring collection—simulated predator attack

For the simulated predator attack, larvae were collected from 2 lakes at the extremes of the predation pressure gradient: Upper Rideau Lake (lowest predation pressure) and Opinicon Lake (highest predation pressure). A random subset of smallmouth bass nests was identified when males were guarding new eggs <3 days old. At that time, the snorkeler also estimated male size. These nests ($n = 8$ for Upper Rideau Lake and $n = 6$ for Opinicon Lake) were monitored by a snorkeler every few days, and larvae were collected ($n = 50$) in a manner identical to the methods described above. The larvae were held in the laboratory overnight in the aerated mason jars at conditions described above, and experiments began the following day.

Because oxygen consumption and MR of fish embryos are strongly influenced by age and development stage (Wieser 1995; Hanel et al. 1996) and because development rate of smallmouth bass embryos is related to water temperature (Shuter et al. 1980), it was crucial that we standardized our sites by thermal age and development stage of larvae to ensure that any differences in MRs were not a result of differing development times. For this, the thermal age of larvae from each site was calculated by measuring DD following egg deposition, which was calculated by summing the mean hourly water temperatures from the day of spawning until the day of larvae collection (modified from Pawiroredjo et al. 2008). In Opinicon Lake, a temperature probe (model 105T thermocouple; Campbell Scientific, Edmonton, Alberta, Canada) measured hourly water temperature at 0.2 and 3.3 m in areas adjacent to nesting smallmouth bass. We generated the mean values from these 2 probes because the depth of smallmouth bass nests is typically somewhere between 0.2 and 3.3 m (Rejwan et al. 1997). Thermal loggers (iButton; Maxim Integrated Products, Sunnyvale, CA) were placed at the nest sites of 2 representative nests (one relatively shallow and one relatively deep) in Upper Rideau Lake when eggs were discovered, and water temperature data from these 2 thermologgers were downloaded soon after larvae collection and used to generate DD for this site. The first 24 h after egg deposition was excluded from degree day calculations because this data was only available from Opinicon Lake. A subset ($n =$ 5) of larvae from each nest was blotted dry and weighed on an analytical balance (±0.0001 g), and a mean larval mass was generated for every nest.

Predator avoidance behavior

All antipredator protocols were run between 830 and 1730 h. Bluegill sunfish ($n = 10$) were caught by rod-and-reel and landed within 10 s on the morning of each trial and placed in a cooler (50 l) with fresh lake water. Individual bluegill were only used for one trial, and total body length ranged between 92 and 141 mm (mean ± standard error [SE]; 105.9 ± 1.0 mm). For this experiment, 35.5 l glass aquaria (25 × 29 × 49 cm) were modified into trial tanks. The aquaria were divided into uneven sections (1/3; predator and 2/3; larvae) by a removable opaque barrier and a permanent perforated transparent barrier. The offspring zone of the tank was subdivided into 3 sections by lines drawn on the inside of the tank and clearly visible to the observer. These lines created a grid and had sections nearest (N), middle (M), and farthest (F) from the predator enclosure. These tanks were covered in brown paper to avoid other visual disturbances and filled with fresh lake water before each trial. Tanks ($n = 4$) were placed alternatively with the predator-side and the prey-side facing the observer to evenly distribute overhead light or other tank effects. Offspring ($n = 10$ from each nest) were placed in the prey-side of a tank with the opaque barrier in place and were left to acclimatize for 10 to 15 min. The observer could run a trial with 4 nests simultaneously. Once offspring were acclimatized, the control trial began. The observer used a scan sampling technique to count the number of offspring in each grid (N, M, and F) at 30-s intervals for a period of 5 min. At the end of the 5 min control, a bluegill was placed in the predator enclosure, and the opaque barrier was carefully removed so as to not disturb the water and the offspring. Scan sampling resumed for another 5 min trial, and the observer noted the location of the offspring within their enclosure at 30-sec intervals. After the termination of the trial, individual bluegill were dipnetted from the aquaria, measured for TL and released back into the lake. Offspring were also measured for TL and weighed. Due to provincial regulations, larvae and juveniles could not be released into lake of origin once exposed to other lake water and were euthanized by anesthetic overdose (200 ppm of buffered MS-222).

Simulated predator attack

Larvae were exercised ($n = 4$ per nest) in Petri dishes (5 × 15 mm) filled with fresh lake water at 17 °C. Larvae were chased manually for 3 min with a micropipette by gently touching the posterior of the tail to mimic a predator attack and induce physiological disturbances resulting from a combination of exercise and the threat of predation (Wieser et al. 1985). Two of the 4 individuals were randomly selected and immediately placed into micro-respirometry chambers. Oxygen consumption was measured using computerized, intermittent flow-through respirometry (Loligo Systems, Hobro, Denmark; Steffensen 1989). The system consisted of 4 glass microchambers (700 μl) outfitted with fiber-optic oxygen probes immersed in a 45 l cooler filled with approximately 10 l of aerated lake water thermostatically maintained at 17.0 ± 1 °C. Change in oxygen concentration (α) for each chamber was calculated as slope ($\Delta O_{2\mathrm{saturation}}/\Delta t$), and oxygen consumption rate ($\dot{M}O_2$, $\mathrm{mg{\cdot}O_2{\cdot}kg^{-1}{\cdot}h^{-1}}$ $\mathrm{individual^{-1}}$) for each chamber was calculated by

$$\dot{M}O_2 = \alpha V_{resp}\beta M_b^{-1},$$

where V_{resp} is the volume of each glass chamber minus the volume of larvae (liter), β is oxygen solubility (adjusted regularly for both temperature and barometric pressure), and M_b is the mass of larvae (kilogram) prior to being placed in the respirometer chamber. The system alternated between a 240-s flush period that added fresh water to each chamber, followed by a 30-s wait period and a 360-s measure period where oxygen concentration in each chamber was measured every 2 s. Several preliminary trials were run prior to the experiment to determine the time necessary for oxygen consumption to decrease within the chambers without negatively impacting larvae (Spoor 1984) and also to define the time required to ensure oxygen concentrations returned to approximately 100% saturation following the flush period. One chamber was randomly assigned to remain empty to correct for background oxygen consumption within the system. To ensure confidence in generated data, only slopes that generated coefficients of determination (R^2 values) that were $\geq$0.99 were used for analyses, and because some R^2 values were slightly below this criteria, sample sizes were reduced to $n = 8$ for Upper Rideau Lake and $n = 6$ for Opinicon Lake. For our experiment, larvae were allowed to recover from exercise for approximately 80 min (5000 s), during which time oxygen consumption was measured continuously (every 0.6–0.8 s). This measurement duration should be sufficient for recovery based on work performed with juvenile fishes (Gingerich et al. 2010). Although it would have been desirable to obtain data on standard MR (i.e., pre-disturbance values) for the same fish used for respirometry experiments, this was not done for several reasons. First, larvae remained active (swimming) within the chambers such that it was unlikely that we would have been able to obtain true standard MR estimates. Second, larvae were sensitive to repeated handling so it was not possible to first attempt to obtain standard MR data on fish and then remove them from the chambers, chase them, and then return them to chambers without resulting in physical injury.

Statistical analysis

All statistical analyses were performed with J.M.P. 7.0.1 (SAS Institute, Cary, NC) and SPSS 15.0 (IBM, Chicago, IL). Data were tested for normality and equality of variances. We used a one-way analysis of variance (ANOVA) to test for differences in male TL among the lakes that were used for the predator avoidance trials. We used *t* tests to test for differences in male TL and larvae mass between the 2 lakes that were used for respirometry. We used a three-way repeated measures ANOVA with lake, time, and larval stage as main effects for the 2008 predator avoidance trials. A two-way repeated measures ANOVA with lake and time as main effects was used to test the predator avoidance behavior of larvae in 2009 and to compare the oxygen consumption of larvae following a simulated predator attack. The Mauchly's test of sphericity (i.e., test for equality of variances for repeated measures analyses) was not significant for all tests. Significance for all tests (α) was evaluated at 0.05, and data are shown as means ± SE unless otherwise indicated.

RESULTS

Predator avoidance behavior

There was no effect of sampling year on parental male TL ($F_{1,3} = 1.1$, $P = 0.30$), and thus, years were pooled for further analysis. Parental males used for the predator avoidance trials differed in TL ($F_{5,86} = 7.4$, $P < 0.0001$; Figure 2). There was

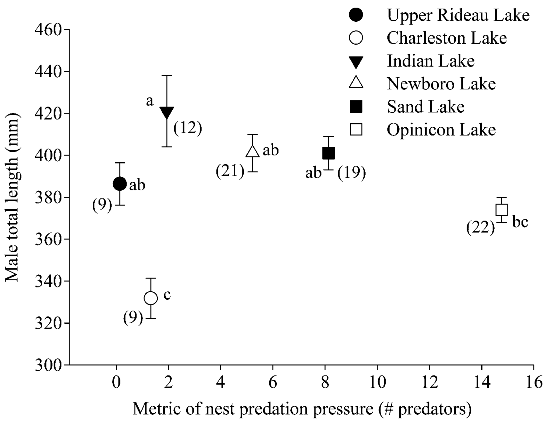

Figure 2
Smallmouth bass parental male TL (mean ± SE) collected from 6 different lakes in eastern Ontario that varied within level of predation pressure. Sample sizes are shown in brackets, and dissimilar letters denote significant differences ($P < 0.05$).

an effect of year on larval length and weight and because all lakes were not sampled in both years, this data were analyzed separately. In 2008, larvae from different lakes differed in length ($F_{3,40} = 6.1$, $P = 0.002$; Figure 3A) and weight ($F_{3,37} = 10.5$, $P < 0.0001$; Figure 3B). Juveniles did not differ in length ($F_{3,39} = 1.6$, $P = 0.20$; Figure 3A) or weight ($F_{3,37} = 2.2$, $P = 0.11$; Figure 3B). Both stages of offspring development spend significantly less time near the predator enclosure once the predator was introduced (Table 1, Figure 3C), but there was no influence of lake or development stage on the predator avoidance behavior of offspring (Table 1). In 2009, larvae used in the antipredator trials also differed in length ($F_{5,54} = 48.3$, $P < 0.0001$; Figure 4A) and weight ($F_{5,54} = 14.0$, $P < 0.0001$; Figure 4B) across lakes. Larvae from all lakes significantly reduced their time in the grid nearest the predator once the predator was introduced (Table 1, Figure 4C). Overall, there was also significant effect of lake on the predator avoidance behavior of larval fish (Table 1, Figure 4), but the data did not follow the predicted pattern because larvae from lakes with the highest predation pressure were not consistently better at avoiding predators than larvae from lakes of low predation pressure. Larvae from Newboro Lake had the greatest decrease in proportion of time spend in grid nearest to the predator once the predator was introduced, followed by Indian Lake and Upper Rideau Lake, which had very similar responses. Larvae from Opinicon Lake, Sand Lake, and Charleston Lake had weaker responses to the predator. Interestingly, there was no significant relationship between male length and larval length ($n = 92$, $R^2 = 0.0002$, $P = 0.91$) or weight ($n = 89$, $R^2 = 0.04$, $P = 0.06$) and between male length and juvenile length ($n = 28$, $R^2 = 0.004$, $P = 0.74$) and weight ($n = 27$, $R^2 = 0.005$, $P = 0.73$). A post hoc test revealed a weak but significant relationship between the TL of larvae and the difference between the proportion of time that larvae spent in the grid nearest the predator in the absence and presence of a nest predator ($n = 104$, $R^2 = 0.08$, $P = 0.004$, Figure 5) but not for juveniles ($n = 43$, $R^2 = 0.08$, $P = 0.07$).

Recovery from simulated predation event

We collected larvae from our low predation site (Opinicon Lake) 24 and 25 days after egg deposition, which corresponded to embryos being 386 DD old. Our high predation site larvae

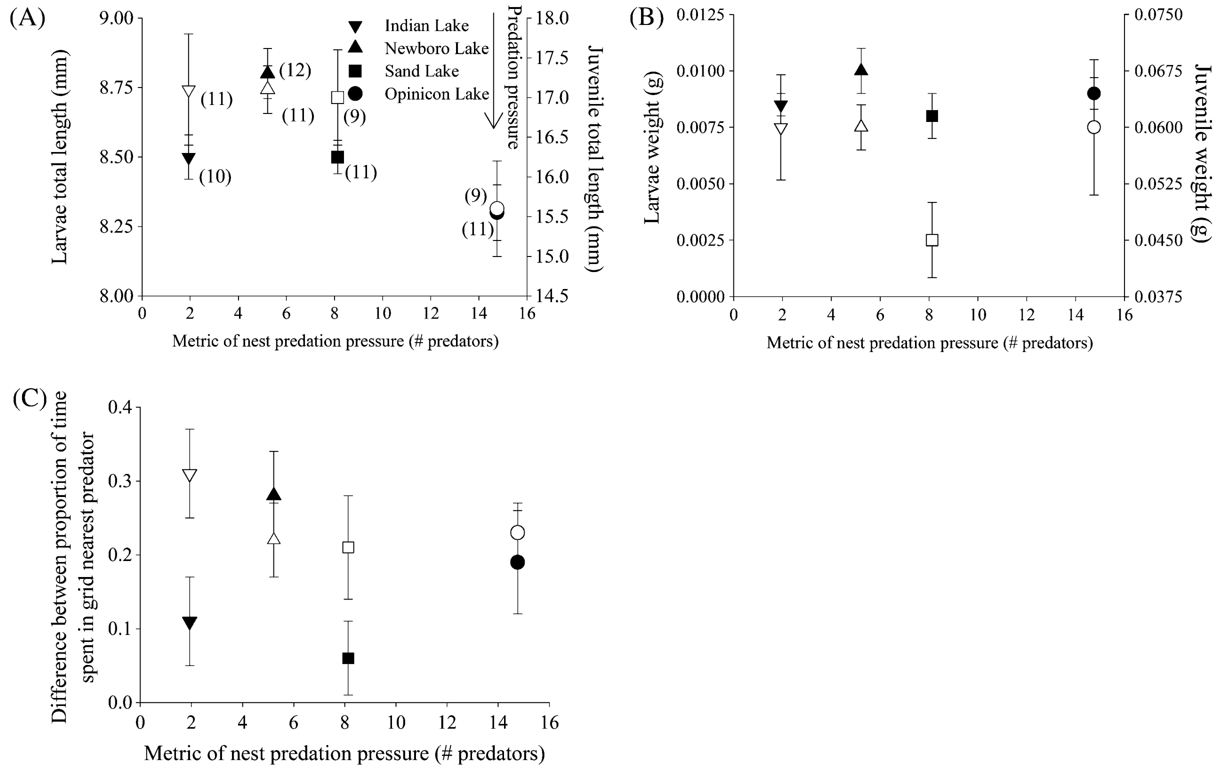

Figure 3
Larvae and juvenile TL (A), total weight (B), and difference (C) between the proportion of time spent in grid nearest predator enclosure in the presence of a predator and in the absence of a predator for larvae (closed symbols) and juveniles (open symbols) from 4 lakes sampled in 2008 which differed in predation pressure. Showing mean ± SE for all parameters. Sample sizes are shown in brackets.

Table 1

Results from repeated measures ANOVA for the antipredator behavior of larvae across a gradient of predation pressure and for the recovery from exercise of larvae from sites of low predation and high predation pressure

Response	Main effects	df	*F*	*P* value
Antipredator behavior (2008)	Stage	1	1.3	0.27
	Lake	3	0.05	0.83
	Time	1	37.1	**<0.0001**
Antipredator behavior (2009)	Lake	5	2.5	**0.04**
	Time	1	95.4	**<0.0001**
	Lake × Time	5	2.0	0.09
Recovery from exercise	Lake	1	12.7	**0.04**
	Time	6	5.4	**<0.0001**
	Lake × Time	6	4.3	**0.001**

Significant differences are shown in bold, df, degrees of freedom.

(Upper Rideau Lake) were collected 24 days following egg deposition, which corresponded to a thermal age of 385 DD. Parental males from the 2 different sites did not differ in TL ($t = -0.71$, $P = 0.49$, mean ± SE, Upper Rideau Lake: 385 ± 10 mm, Lake Opinicon: 377 ± 5 mm), but larvae from the low predation site (Upper Rideau Lake) were approximately 17% heavier than larvae from the high predation site (Lake Opinicon) ($t = -2.54$, $P = 0.021$, mean ± SE, Upper Rideau Lake: 0.012 ± 0.0014 g, Lake Opinicon: 0.0097 ± 0.0006 g) despite being almost identical in thermal age. When MR data for both sites were examined collectively, larvae from the low predation site had higher oxygen consumptions post-exercise compared with larvae from the high predation site (Table 1, Figure 6). When examined on a finer scale, oxygen consumption of larval smallmouth bass following our standardized simulated predation event (i.e., 3 min of exercise) was influenced by time post-disturbance (Table 1). More specifically, oxygen consumption decreased following the disturbance period for both sites (Figure 6), and the rate of recovery was steeper for the low predation site than the high predation site (Table 1, Figure 6). There was no influence of male TL on the oxygen consumption of larvae ($R^2 = 0.135$, $P = 0.22$). Due to small differences in larval size between lakes, we also ran the repeated measures model with larval weight as a covariate, and it did not significantly contribute to the model ($F = 4.16$, degrees of freedom = 1, $P = 0.07$).

DISCUSSION

Local adaptation to variation in predation pressure has been observed in many species. Although parents have also shown localized antipredator behaviors, little work has focused on how offspring respond to variation in nest predation pressure. In circumstances where parents face little risk of predation but offspring predation pressure is high, selective pressures should not only act on the defensive behavior of parents but also on the antipredator behavior of offspring. Our work has shown that a natural gradient of predation pressure has little influence on predator avoidance behavior of larvae but may have important physiological implications.

Predation pressure did not influence the predator avoidance behavior of larval and juvenile smallmouth bass. Offspring

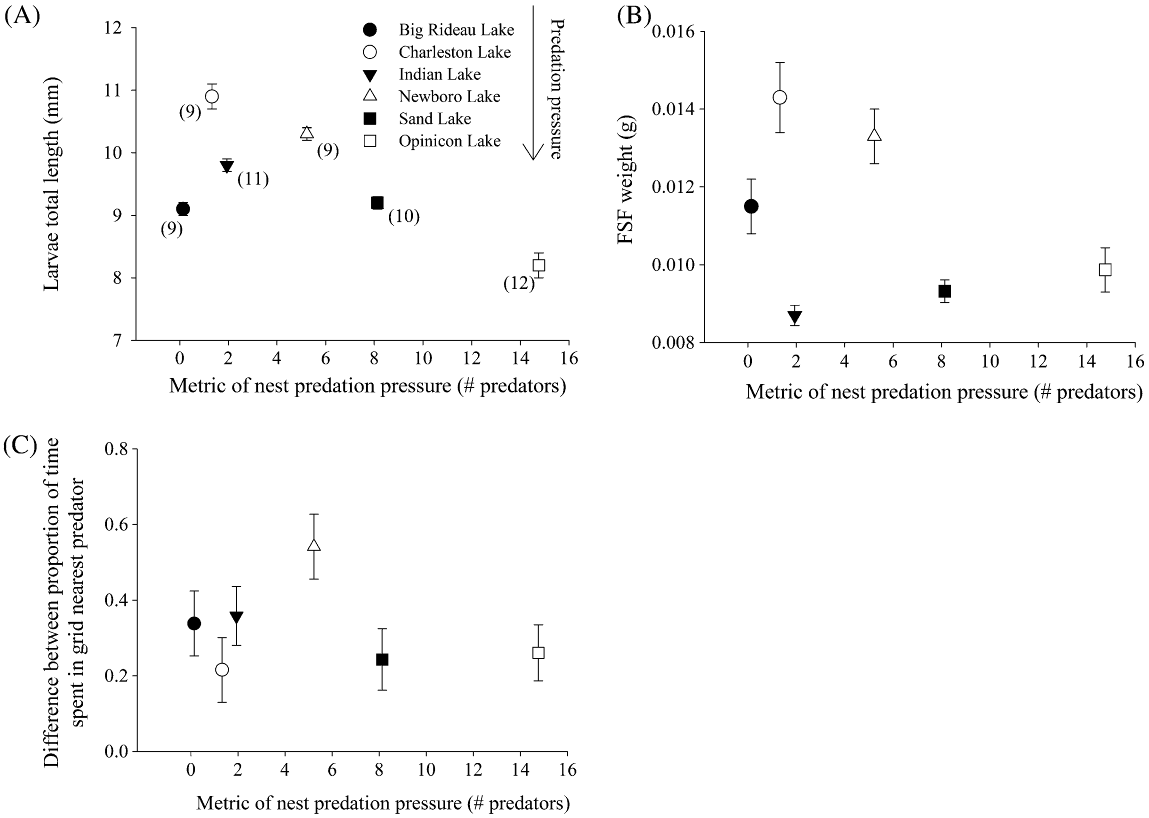

Figure 4
Larvae TL (A), total weight (B), and difference (C) between the proportion of time spent in grid nearest predator enclosure in the presence of a predator and in the absence of a predator for larvae from 6 lakes sampled in 2009 which differed in predation pressure. Showing mean ± SE for all parameters. Sample sizes are shown in brackets.

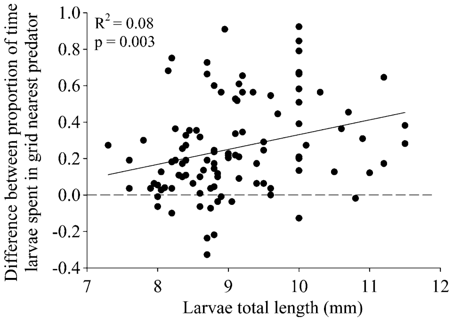

Figure 5
Relationship between larvae TL and the difference between the proportion of time larvae spend in the grid nearest the predator in the absence of predator and in the presence of a predator. Values above the dashed line show larvae from nests, which moved away from the predator, whereas values below the dashed line moved toward the predator.

from the lake with the lowest predation pressure performed similarly to offspring from the lake with the highest predation pressure. Although we demonstrated behavioral differences between lakes that differed in predation pressure, we found no evidence for localized predator avoidance behavior linked to differences in predation pressure. Many taxa have demonstrated population level antipredator tactics (e.g., Giles and Huntingford 1984; Riechert and Hedrick 1990; Relyea 2002; Griesser and Nystrand 2009), particularly when adults of the population were tested. We predicted that in a system where it is the offspring that face immediate predation, we would also detect similar population level differences in offspring behavior. Parents from lakes with high predation pressure could produce offspring that are able to better perform in these environments (i.e., adaptation) or simply because the environment itself enables offspring to become better performers (i.e., acclimation). In contrast to what we predicted, we found that offspring from a gradient of nest predation pressure were equally equipped to avoid predators. We propose 2 alternative hypotheses for this trend. First, in this particular system, offspring survival may not depend on performance. We tested the behavior of offspring when they were still being guarded by a male parent and as they were developing avoidance as well as feeding skills. At this time, the antipredator behavior of the parent may outweigh the ability of offspring to individually avoid predators. We find some support for this notion because parental behaviors are influenced by predation pressure. Nesting male smallmouth bass residing in lakes with high predation pressure spend more time engaged in antipredator activities than males from lakes with low predation pressure and are generally more active (Steinhart et al. 2005; Gravel and Cooke 2009; Gravel MA, unpublished data). A second possibility relates to the cost of developing antipredator skills. The cost of developing and engaging in basic antipredator behavior could be relatively low because offspring from all lakes were able to avoid the predator enclosure to similar degrees. Some of these similarities across populations may also be attributed to the shoaling nature of this behavior because predator avoidance is often facilitated in groups when compared with individual avoidance (Godin 2002). Smallmouth bass offspring demonstrated a clear ability to recognize and avoid potential predators, but this ability was not influenced by the quantity of predators found in the natal rearing environment.

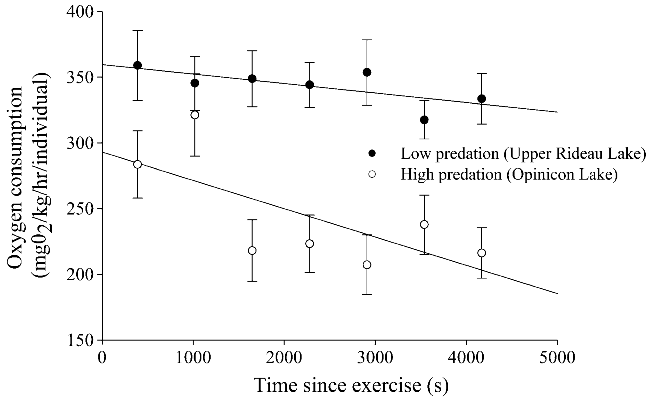

Figure 6
Oxygen consumption (mean ± SE) during postexercise recovery of larval smallmouth bass from lakes with low and high predation pressure following a 3-min chase period. Sample sizes were $n = 8$ for Upper Rideau Lake and $n = 6$ for Opinicon Lake.

Although predation pressure did not clearly influence the antipredator behavior of larval smallmouth bass, we did find a significant positive relationship between the performance of larvae and the TL of larvae. Because we tested the behavior of small fish, body size, which is typically accompanied by further neural development (Wallace 1972), can play an important role in the acquirement of antipredator behaviors. Within this reasoning, we would also expect older offspring (i.e., juveniles) to show increased predator avoidance behavior. Although juveniles usually showed an increased predator avoidance (Figure 3C), there was no statistical distinction between their behavior and larval behavior. Ontogenetic shifts in antipredator behaviors are quite common (e.g., Pongracz and Altbacker 2000; Brown et al. 2002; Dangles et al. 2007) and are particularly relevant for species that provide parental care. Brown (1984) showed that young larvae from species that provide a relatively short period of parental care (e.g., rock bass) showed significantly better antipredator avoidance than young larvae from species that provided relatively lengthy parental care (e.g., largemouth bass). Largemouth bass offspring only began avoiding predators when they were considered juveniles and which corresponded to the period when they would typically be abandoned by their parent. At the early larval stages, younger largemouth bass larvae spend more time foraging than older larvae and are believed to invest strongly into growth instead of predator avoidance, aided by the presence of a vigilant parent (Brown 1985). Our work does not show great support for the same shift for smallmouth bass. Although we found that larval smallmouth bass were able of actively avoiding an introduced predator, there was not a vast improvement in this avoidance from the larval to juvenile stage. There may not be as much room for improvement in the antipredator behavior of smallmouth relative to largemouth bass because Brown (1984) found that larval largemouth bass showed reduced antipredator behaviors.

Our work also showed that predation pressure influences the ability of larval smallmouth bass to recover from exercise. Ideally, our study would have compared the physiological consequences of predation pressure across numerous populations of nesting smallmouth bass (see Gravel and Cooke 2009; Gravel et al. 2010), but the direction of our results clearly supports the notion that individuals from high predation pressure environments recover from exercise more quickly and are potentially in better physiological condition than individuals from the low predation pressure site. Hence, we found no evidence for our alternative prediction, which proposed that there may exist sublethal consequences of predation pressure. Sprint training is known to reduce energy loss during exercise as well as improve the swimming performance of fish and facilitate recovery following exercise (Pearson et al. 1990). In support of this, our work showed that larval smallmouth bass from a lake with high predation pressure had lower MRs during recovery as well as a steeper recovery slope than individuals from a lake with minimal predation pressure. Although 3 min of exercise is probably longer than the length of natural chase event, larvae were not chased to exhaustion and the physiological disturbance they experienced is still physiologically relevant. A possible consequence of this longer chase period may be that the physiological differences between the 2 groups are slightly exaggerated compared to a typical "natural" disturbance. Low MRs (active and standard) are often considered an advantage as they allow for a greater metabolic scope (Cutts et al. 2002), which translates into a greater capacity for activity and recovery. Our experiment did not include the measurement of standard MR for these 2 populations, but standard MR and active MR have been shown to be highly correlated in several species of juvenile fish (Cutts et al. 2002; Hansen and von Herbing 2009). If that is the case with larval bass, we would expect the larvae native to high predation sites to have lower standard MRs, which may permit a greater scope for activity, as it does for other species (Cutts et al. 2002; Hansen and von Herbing 2009). This suggests that larvae from the site of high predation pressure are better physiologically equipped to respond to variation in their environment. Lower maintenance costs may enable fish to use this "surplus" energy in times of need, such as a predation attempt (Priede 1977). A larger scope for activity could translate into behavioral flexibility and allow individuals to perform a wide range of behaviors, which rely on availability of metabolic scope. In contrast, larvae from the site of low predation pressure may be able to invest in higher maintenance costs due to fewer predation events. This may allow them to invest more heavily into growth and reduce the probability of size-selective mortality (Sogard 1997). In general, there is a need for research that examines the relationship between individual variation in behavior and variation in individual physiology to help us better understand the behavioral and ecological consequences of physiological diversity.

Although there was no difference in male size between our sites of low and high predation pressure, there could be other indicators of male quality (i.e., genetic effects) that are contributing to the difference in larval performance (Patterson et al. 2004; Pakkasmaa et al. 2006). Our work has shown that predation pressure is relatively stable across years (Gravel MA, unpublished data) and because smallmouth bass demonstrate some level of nest-site fidelity (Barthel et al. 2008), it is possible that parents from lakes with high predation pressure simply produce offspring better equipped to deal with their environment. Unfortunately, our work does not allow us to tease apart the potential benefits supplied from the predator rich environment (i.e., training effects) and the possible beneficial genetic effects. This type of system also provides little information about maternal effects as female smallmouth bass simply choose males, deposit eggs and then depart, making their collection in wild populations challenging. Consequently, although we know little about female quality as well as the relationship between female quality and offspring size, survival, and/or performance, we have some evidence that maternal effects may differ between these 2 populations due to our differences in offspring size. Larvae from Upper Rideau Lake were 17% heavier than larvae from Opinicon Lake. This could be an indication of investment into larger offspring because these larvae had only recently begun to exogenously feed and still had partial yolk sacs, and it is unlikely that these weight differences could be attributed solely to differences in feeding habits. Unfortunately, this would be contrary to an a priori prediction that relates maternal investment and offspring size. We would expect females from sites of high predation pressure to invest in larger offspring than females from low predation pressure because larger offspring generally are better physical performers and have increased survival (Sogard 1997). Our work shows no support for this hypothesis. It is also possible that females from the site of low predation pressure are larger than females from the site of low predation pressure and simply produce larger eggs and offspring (Kamler 2005).

Our study examined whether a natural gradient in predation pressure would influence the antipredator behavior of young fish that are provided with parental care. We found that predation pressure had little influence on the predator avoidance behavior of larvae but that larvae size contributes to performance. Furthermore, we found that recovery from a simulated predator attack differed between populations at the extremes of the predation pressure gradient. Taken as a whole, our work has shown that ecologically relevant environmental variation such as predation pressure has the ability to influence indicators of performance between populations. Further work that distinguishes between the roles of parental quality and environmental acclimation across these ecological gradients will help us better understand if or how these animals are adapted to these environments.

FUNDING

Natural Sciences and Engineering Research Council (NSERC) Discovery Grant and Canada Research Chair to S.J.C. and by an NSERC CGSD to M.A.G. Additional financial support for this study from the US Department of Agriculture Cooperative State Research Education and Extension Service by McIntire–Stennis funds through project ILLU-875-328 to C.D.S.

The authors would like to thank Zachary Blevins, Laura Chomyshyn, Alison Colotelo, Cody Dey, Andrew Gingerich, Patricia Halinowski, Sean Landsman, Connie O'Connor, Graham Raby, and Samantha Wilson for their help in the lab and field. A special thanks to Jean-Guy Godin for advice on the predator avoidance experiments and to Chris Pullen for building the aquaria. The authors gratefully acknowledge the staff of the Queen's University Biological Station and in particular, Frank Phelan for facilitating this work. The authors would also like to thank 2 anonymous reviewers for constructive criticism and helpful suggestions related to this manuscript. All research was conducted with approval of the Canadian Council on Animal Care as administered through Carleton University and under scientific collection permits provided by the Ontario Ministry of Natural Resources.

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