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Early exposure to nonlethal predation risk by size-selective predators increases somatic growth and decreases size at adulthood in threespined sticklebacks

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Abstract

Predation has an important influence on life history traits in many organisms, especially when they are young. When cues of trout were present, juvenile sticklebacks grew faster. The increase in body size as a result of exposure to cues of predators was adaptive because larger individuals were more likely to survive predation. However, sticklebacks that had been exposed to cues of predators were smaller at adulthood. This result is consistent with some life history theory. However, these results prompt an alternative hypothesis, which is that the decreased size at adulthood reflects a deferred cost of early rapid growth. Compared to males, females were more likely to survive predation, but female size at adulthood was more affected by cues of predators than male size at adulthood, suggesting that size at adulthood might be more important to male fitness than to female fitness.

Introduction

Individual organisms often plastically modify their growth trajectories in response to predation risk in ways that appear to be adaptive. For example, when foraging activity increases predation risk, a common response of prey is to reduce foraging activity and hence growth rate in the presence of predators (Werner & Anholt, 1993; Gotthard, 2000; Biro *et al.*, 2004). When increased growth is associated with increased mortality risk from predation, we expect a negative effect of predation risk on growth rate. On the other hand, a positive relationship between predation risk and growth rate might be favoured if predators preferentially consume small individuals (Urban, 2007). When small individuals are more vulnerable to predation, there is an advantage to rapid growth to attain a size refuge from mortality risk (Williams, 1966). Size-selective predation on small individuals is common and can result from several different mechanisms including gape limitation of the predator (Mittelbach, 1981). In contrast to the growth-mortality hypothesis, size-selective predation on small individuals predicts that animals should plastically increase their growth rate in response to cues of predators (Table 1).

Although there is widespread evidence that prey plastically alter a diversity of morphological defences in response to predation pressure, there is little direct experimental evidence that exposure to cues of predators causes prey to plastically increase their somatic growth rate in response to size-selective predation on small individuals. We know that exposure to cues of predators causes animals to accelerate transitions out of vulnerable stages. For example, larval amphibians (Werner, 1986), *Daphnia* (Spitze, 1991; Tollrian, 1995; Beckerman *et al.*, 2007) and snails (Crowl & Covich, 1990) accelerate development rate to sexual maturity when young individuals are more vulnerable, and many studies have shown that exposure to predators can induce morphological defences against predation (e.g. Krueger &

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Table 1 Predicted effects of predation on prey growth and size at maturity according to different hypotheses.

Hypothesis	Effect on prey growth	Effect on prey size at maturity
Growth-mortality tradeoff	Decrease	Decrease?
Size-selective predation on large individuals	Decrease	Decrease
Size-selective predation on small individuals	Increase	Increase or decrease

Dodson, 1981; Bronmark & Miner, 1992; Van Buskirk, 2002). In addition, there is correlative evidence that developmental exposure to cues of predators is associated with a rapid increase in body size in juveniles (Belk, 1995; Johnson & Belk, 1999; Magnhagen & Heibo, 2004), and that strong predation pressure can favour greater willingness to forage under predation risk (Fraser & Gilliam, 1987). However, most studies report negative effects of predation on somatic growth rate (see above), and few studies have shown that size-selective predation on small individuals is directly associated with an immediate increase in somatic growth rate (Schmidt & Van Buskirk, 2005).

In addition to the effects of predation on growth rate, theory predicts that size-selective predation shapes the life history of prey, especially age and size at sexual maturity (Law, 1979; Stearns & Crandell, 1981; Kozlowski, 1992; Taylor & Gabriel, 1992; Abrams *et al.*, 1996). Life history theory predicts that strong predation pressure on large individuals favours early reproduction at a small size (Crowl & Covich, 1990), and there is empirical support for this prediction (Edley & Law, 1988; Belk, 1995; Tollrian, 1995; Ball & Baker, 1996; Reznick & Bryga, 1996; Rodd *et al.*, 1997; Johnson & Belk, 1999; Johnson, 2001; Peckarsky *et al.*, 2001; Jennions & Telford, 2002; Arendt & Reznick, 2005; Gosline & Rodd, 2008; Walsh & Reznick, 2009). However, when small individuals are more vulnerable to predation, and both growth and age/size at maturity are plastic, the predicted effects on age and size at maturity are less clear (Abrams & Rowe, 1996). One possibility is that strong predation pressure on small individuals should have opposite effects on life history traits compared to strong predation pressure on large individuals, i.e. size-selective predation on small individuals favours rapid growth out of vulnerable stages, so that prey are relatively large at sexual maturity (Stearns & Koella, 1986). This hypothesis has had some empirical support (Crowl & Covich, 1990; Wellborn, 1994; Belk, 1995; Sparkes, 1996; Johnson & Belk, 1999; Gosline & Rodd, 2008). However, theoretical work has shown that there are several other important factors that can generate the opposite prediction, such as seasonality (Rowe & Ludwig, 1991; Abrams *et al.*, 1996), size thresholds (Day & Rowe, 2002), asymptotic growth (Kozlowski, 1992; Taylor & Gabriel, 1992), the plasticity of age or size at maturity (Abrams & Rowe, 1996), and whether there are indirect effects of predators on growth via decreased prey density (Abrams & Rowe, 1996). Similarly, the predicted effects of growth–mortality tradeoffs on size at maturity are not straightforward. If predation risk suppresses growth rate, then it might also suppress size at maturity. However, if there is a critical threshold size at maturity, then individuals under predation risk might postpone maturity until reaching the critical size. The predicted effects of growth–mortality tradeoffs and size-selective mortality on growth and size at maturity are summarized in Table 1.

Variation in life history traits among populations of the same species has generated insights into the mechanisms underlying life history tradeoffs and the adaptive significance of predator-induced changes in life history (e.g. Reznick, 1983; Reznick & Bryga, 1996). Similarly, a comparison of males and females of the same species can also reveal the important factors that influence the costs and benefits of life history transitions. For example, if body size has a stronger influence on male fitness than female fitness, then we might expect males to invest in mechanisms that allow them to buffer against deviations from optimal body size.

In this paper, we report the results of a study that takes an experimental approach to test the effects of early exposure to cues of predators on growth rate and size at adulthood, and the relationship between body size and survivorship under predation risk in threespined sticklebacks (*Gasterosteus aculeatus*). We applied nonlethal cues of rainbow trout (*Oncorrhynchus mykiss*) to juvenile sticklebacks, which allowed us to assess whether predation risk affects growth and life history, independent of effects on prey density (Arendt & Reznick, 2005). If high growth rate is associated with high predator-induced mortality, we predicted that sticklebacks exposed to nonlethal cues of predators would reduce their growth rate compared to a control group. In contrast, if predators preferentially consume small individuals, we predicted that sticklebacks exposed to cues of predators would increase their growth rate compared to a control group. We also predicted that exposure to cues of predators would cause changes in adult life history traits such as size at adulthood.

Methods

Threespined sticklebacks

Threespined sticklebacks are small (3–7 cm standard length at sexual maturity) fish renowned for their extensive geographic variation in behaviour, physiology, morphological traits and life history (Bell & Foster, 1994). Some of the variation among freshwater populations of sticklebacks can be attributed to differences in predation pressure. Sticklebacks are subject to predation by a wide range of predators including birds, snakes, odonate larvae

and piscivorous fishes such as trout and are especially vulnerable to predation when they are young and small (Reimchen, 1994). Previous comparisons of natural variation among populations of sticklebacks have suggested that strong predation pressure by piscivorous fishes is associated with larger body size at sexual maturity (Moodie & Reimchen, 1976; McPhail, 1977; Reimchen, 1991). Sticklebacks posses a variety of anti-predator defences such as lateral plates, dorsal and ventral spines and exhibit strong behavioural reactions to predation risk (Huntingford *et al.*, 1994). As adults, sticklebacks are sexually dimorphic (Wootton, 1984). Sticklebacks are seasonal breeders; during the reproductive season, male sticklebacks defend nesting territories and provide sole parental care for the developing eggs and fry. Female sticklebacks produce several clutches of eggs during the breeding season. Sticklebacks continue to grow after sexual maturity (Smith & Wootton, 1995), and previous studies have shown that large body size has positive effects on both female (Kraak & Bakker, 1998) and male (Kraak *et al.*, 1999) fitness. The sticklebacks used in this study were from Putah Creek, CA. Sticklebacks from this population behaviourally respond to the threat of predation and exhibit fully developed spines and partial plating (Bell & Stamps, 2004; Bell, 2005). Stickleback in this population become sexually mature within 1 year and typically live for up to 2 years in the field.

Overview of the experiment

The experiment evaluated the effect of predation on growth and life history traits in sticklebacks and addressed three questions. First, does exposure to cues of trout cause sticklebacks to decrease growth rate, as predicted if there is a tradeoff between growth and mortality, or to increase growth rate, as predicted by size-selective predation on small individuals? To address this, we compared the growth of juvenile sticklebacks that had been exposed to cues of rainbow trout (*Oncorhunchus mykiss*, hereafter nonlethal risk) to sticklebacks that had not been exposed to cues of predators (hereafter, nonlethal risk control). Second, does vulnerability to predation by rainbow trout depend on the size and sex of the individual stickleback? We allowed trout to prey upon sticklebacks, and tested for the effect of body size and sex on the probability of survival. Fish exposed to lethal predation by rainbow trout (lethal risk) were compared to a control treatment that was not exposed to lethal risk (lethal risk control). This allowed us to test whether it is adaptive to increase growth to escape size-selective predation (because small, slow growing individuals were consumed), or whether there is a growth-mortality tradeoff (because fast growing individuals were more likely to be consumed). Third, what are the consequences of early developmental exposure to cues of predators on growth and size at adulthood in males and females? We monitored the growth of the individually marked sticklebacks through adulthood.

Collecting sticklebacks

Wild threespined sticklebacks were collected via dip nets and minnow traps in Putah Creek, CA, in November 2006. The animals were shipped overnight to the University of Illinois, where they were maintained at 13 °C on a natural photoperiod, i.e. changed over the course of the 1.5-year experiment, in a flow-through recirculating system and fed approximately 10% of body weight of a mixed diet [frozen bloodworm, frozen artemia, frozen Cyclopeez (*Argent*), frozen mysis shrimp] daily. We observed that all of the food was eaten during each feeding.

Breeding and marking sticklebacks

Between March 2–23, 2007, the wild caught fish were crossed via artificial fertilization in a North Carolina II breeding design (Lynch & Walsh, 1998). Specifically, 16 males and females were used in twice-replicated 3×3 and 5×5 designs, giving 34 full and half-sib families in total. The purpose of this breeding design was to enable estimation of genetic variation in behavioural and morphological traits (e.g. Dingemanse *et al.*, 2009). Because the fish were not all spawned at exactly the same time, families vary slightly in age throughout the experiment (average age difference = 10 days, range = 0–20 days). When the fish were approximately 4 weeks old, the density of each family's tank was standardized to 15 sticklebacks per tank. At approximately 75 days of age, fish were marked by injecting an elastomer tag that fluoresces under UV light (*Northwest Marine Technologies*) at four different locations under the dorsal spines with a fine syringe (29-gauge), while the fish were anesthetized, and the fish were photographed. The length of the second dorsal spine was estimated from photographs. The fish were assigned to a mixed family group of 50 individuals. Each individual in a group received a unique mark by combining three different colours (red, orange and yellow) in different combinations of four. By individually marking each fish, we were able to follow the growth trajectories of individual animals in the experiment.

The twelve mixed family groups of approximately 50 juveniles included one or two representatives from each family in each group. The individuals were maintained in the same group throughout the rest of the experiment. Groups were housed in recirculating flow through tanks ($107 \times 33 \times 24$ cm, 83.28 L). The total number of juveniles at the start of the experiment was 590, with 7–10 members of each family. Groups were randomly assigned to one of four treatments: (i) nonlethal risk control/lethal risk control; (ii) nonlethal risk/lethal risk control; (iii)

nonlethal risk control/lethal risk; and (iv) nonlethal risk/lethal risk (Fig. 1). There were two groups in the nonlethal risk/lethal risk control, nonlethal risk control/lethal risk control treatments, and four groups in the nonlethal risk/lethal risk and nonlethal risk control/lethal risk treatments. We designed the experiment so that there would be an equal number of individuals in both the lethal risk and lethal risk control treatments at the end of the experiment, i.e. there were twice as many fish in the lethal risk treatments because trout ate half of the sticklebacks.

Applying predator cues (nonlethal risk)

When the fish were approximately ninety days of age, the nonlethal risk treatment was applied for 6 days. Fish in the nonlethal risk control treatment were not exposed to predator cues. The nonlethal risk treatment consisted of exposure to olfactory, visual and tactile cues of rainbow trout. This treatment involved exposure to multiple cues to simulate ecologically -relevant predation risk. Although exposure to predation risk was applied only to juveniles, and it is likely that sticklebacks under high predation pressure in the field are episodically exposed to cues of predation risk throughout their lifetime, this treatment allowed us to assess the effects of early exposure to predation risk on growth and development. Determining whether continual exposure to predation risk results in similar effects is an obvious topic for future study. Olfactory cues consisted of exposure to the scent of trout and the scent of dead sticklebacks. Olfactory cues of trout were obtained by collecting water from a tank containing live rainbow trout. Olfactory cues of dead stickleback were obtained by ablating the skin of 10 freshly killed sticklebacks and then soaking the carcasses in one litre of water overnight. Water samples containing olfactory cues were kept frozen in 50 mL aliquots.

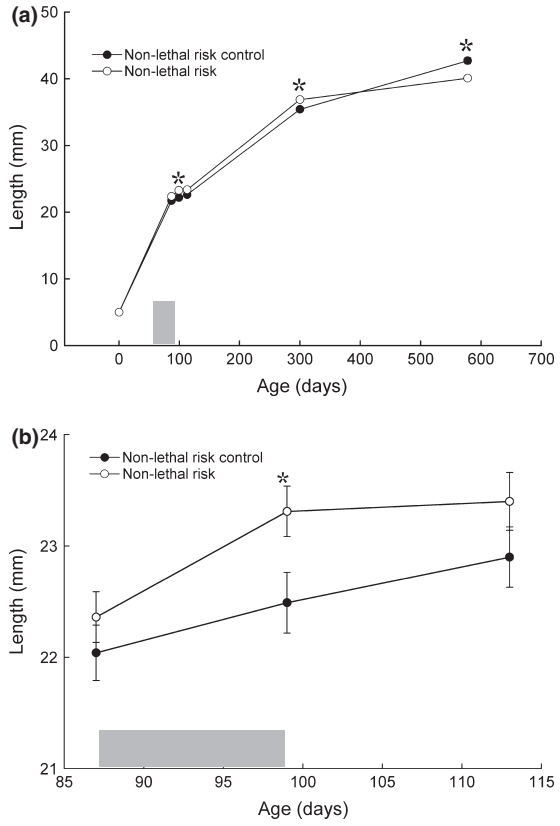

Fig. 1 (a) Growth trajectories of threespined sticklebacks that had either been exposed to cues of predators as juveniles ('nonlethal risk') or had not ('control'). X-values were plotted as the average age of sticklebacks at that measurement (see text for details). (b) Close-up of the growth trajectories of sticklebacks in the control and nonlethal risk treatment during and immediately following exposure to predator cues. Hatched indicates approximates interval of exposure to cues of trout across all fish; however, note that individual tanks were only exposed to cues for 6 days. Standard error bars around marginal means corrected for the random effects of tank and individual are shown. Length at hatching was estimated as 5 mm (Wootton, 1984). *Statistically significant difference.

The aliquots were defrosted, and 50 mL of each cue (trout and dead stickleback) was added to the nonlethal risk treatment on each of the 6 days of treatment. Visual cues were applied daily by adding a model of a rainbow trout at the same location as the olfactory cues for 30 s. The models (n = 2, 205 mm standard length) were made by hand, painted and sprayed with nontoxic materials modelled after a preserved rainbow trout. A stainless steel rod attached to the middle of the model trout allowed us to move the model trout from above, with minimal disturbance to neighbouring tanks. On the second, fifth, and sixth days of treatment, the model trout was added to the tank and held still for 30 s and then quickly moved towards the corner of the tank containing the majority of the sticklebacks, and then to the side and back again on a diagonal. On day six, the same procedure was followed except the sticklebacks were chased for an entire minute.

The nonlethal risk control treatment received 100 mL of defrosted ice cubes made of clean aquarium water during the same period and were not exposed to the trout model. On the second, fifth and sixth days of treatment, the water in the nonlethal risk control treatment was briefly disturbed by splashing the surface to control for the effect of disturbance during chasing.

Lethal risk

Four days after the nonlethal risk treatment, when the fish were approximately 100 days of age and had been in mixed groups for 25 days, fish were transported in buckets by car to the Jake Wolf Memorial Fish Hatchery, Topeka, IL. Sticklebacks exhibit fully developed armour and spines at this age (Sillett & Foster, 2000). Each group was added to a separate 1.5-m-diameter

circular tank with refuges that were sufficient to accommodate all 50 individuals within the tank. The refuges consisted of 20 cm × 20 cm grey plexiglass sheets mounted on 2.54-cm supports and prevented the trout from accessing sticklebacks under the refuge. The refuges cast a shadow but were sufficiently transparent to allow us to see the fish underneath. Fish were fed once a day with their regular diet of frozen bloodworms. Fish were exposed to a natural (0700–2030 photoperiod) and kept in 16 °C flowing well water.

After transport to the hatchery, sticklebacks were allowed to adjust to the new surroundings overnight. The following morning, three randomly selected, hungry trout were added to the experimental tanks. While they were in the tanks with stickleback prey, trout did not receive additional food. Predation rates were determined by twice-daily visual inspections of the tanks. The trout were removed when approximately 50% (±1%) of the sticklebacks had been eaten (average = 5.7 days, range = 3–7 days after introduction of the trout). If half of the sticklebacks had not been predated after 4 days, another two trout were added. Similarly, if there were still more than 50% of the stickleback remaining on day 5, another five trout were added, bringing the maximum number of trout per tank to ten trout in each tank. Different trout were used for all the tanks to ensure that none of them had previous experience with their stickleback prey. In total, 70 different trout were used with an average standard length of 204.5 mm (±11.8 mm). Other studies have shown that trout of this size are especially efficient predators on juvenile sticklebacks (McPhail, 1977; Reimchen, 1991).

Groups in the nonlethal risk, lethal risk control and nonlethal risk control, lethal risk control treatments were held in separate tanks at the hatchery that were identical to the other tanks except that trout were not added to the those tanks. There was no mortality in the lethal risk control treatments.

Measuring body size

The standard length and mass of the individually marked stickleback were measured on five occasions. L1: Prior to exposure to nonlethal risk. The fish were approximately 87 days of age (range = 69–100 days); L2: After exposure to nonlethal risk. The fish were approximately 99 days of age (range = 81–112); L3: After exposure to lethal risk. The fish were approximately 108 days of age (range = 93–122); L4: Subadulthood. The fish were approximately 300 days of age (range = 285–312); L5: Adulthood. The fish were approximately 578 days of age (range = 568–593) and were likely sexually mature. We do not know precisely the age and size at which sticklebacks became sexually mature. Therefore, we refer to this last measurement of body size as 'size at adulthood' rather than 'size at maturity'.

To determine the sex of the sticklebacks, tissue samples were taken from some of the animals for DNA analysis using a sex-specific genetic marker (Peichel *et al.*, 2001). We confirmed sex at the end of the experiment. Sexing information is available for 403 individuals.

Data analysis

We focused our analysis on three specific questions. (1) What is the effect of exposure to predator cues on growth and body size? (2) Is body size related to survivorship under lethal predation risk? (3) What are the long-term consequences of plastic changes in growth for size at adulthood? Where appropriate, we also test for sex differences in growth and body size. We found that exposure to nonlethal risk affected growth and life history and that survival under lethal risk was nonrandom with respect to body size (see Results). As a result, the survivors of the lethal risk treatment represent a biased sample. Therefore, we only consider individuals in the 'lethal risk control' group to address questions 1 and 3. Individuals from all treatment groups are included in the survivorship analysis (Fig. S1).

We evaluated the effect of exposure to predator cues on growth by comparing fish that were either exposed to predator cues or not using general linear mixed models. We constructed different models to evaluate differences between treatments. First, we examined growth over the entire course of the experiment by testing for the effect of nonlethal risk (fixed effect), tank (random effect, nested within treatment), measure (fixed effect, 1–5), individual (random effect, nested within tank) and the interaction between nonlethal risk and measure on ln-transformed standard length. A significant interaction between treatment (nonlethal risk) and measure indicates differences in growth between treatments. Standard length was ln-transformed for this analysis because of unequal variances across measures. We did not consider sex as a fixed factor in this analysis because sexing data were not available for all individuals, and the goal of the initial analysis was to describe the overall effect of nonlethal risk on growth. We consider sex in subsequent analyses of particular intervals of interest. We tested for the effect of nonlethal risk (fixed effect), sex (fixed effect), measure (fixed effect) and tank (random effect, nested within treatment) and the first-order interactions between nonlethal risk, sex and measure on length at each measurement. Nonsignificant interactions were removed sequentially. We report marginal means corrected for the main effects. For illustrative purposes, we report differences in growth rates between treatments as the difference in length between two measurements divided by the number of days that elapsed between measurements. Length and weight were tightly correlated ($r > 0.9$ at all ages); here, we focus on length because weight fluctuates with feeding.

To identify the predictors of survivorship under lethal predation risk, we used logistic regression. Because lethal risk was applied at the tank level, we standardized survival and standard length prior to exposure to selection within each tank. Then, we tested for the effect of body size (L2, length), spine length and sex (categorical) and all the interactions on survival using backwards logistic regression based on likelihood ratios. Our experimental design precluded us from testing for the effect of exposure to nonlethal risk on the probability of survival because selection was applied at the tank level; half of the fish in each tank were consumed by trout, so there was, by definition, no difference in rates of survival between treatments. Because we assume that the fish were all approximately the same size at hatching and were approximately the same age when their standard length prior to exposure to lethal risk was measured, standard length prior to lethal risk is equivalent to growth rates prior to this measurement. To facilitate comparisons with published estimates of the strength of selection, we calculated the standardized selection differential (Endler, 1986). Results are presented as means ± standard errors, unless otherwise noted, and all statistical tests are two-tailed. Analyses were performed in SPSSv18.

Results

Overall description of growth

Sticklebacks showed asymptotic growth over the course of the experiment (Fig. 1a). Considering the control group only, the specific growth rate was highest between hatching and the first measurement, when the fish grew by 0.201 ± 0.003 mm per day. Specific growth rate was lowest between the fourth and fifth measurement (0.027 ± 0.003 mm per day), as the fish approached asymptotic size.

The effect of nonlethal risk on growth and body size

The model testing for the effect of individual, nonlethal risk, tank, measure and the interaction between nonlethal risk and measure on growth over the course of the experiment is provided in Table 2. There was a significant effect of nonlethal risk on growth, as evidenced by the interaction between nonlethal risk and measure.

When we look more closely at growth during particular intervals, we found that during the 6-day period when the fish were exposed to predator cues, stickleback in the nonlethal risk treatment accelerated their growth rate relative to stickleback in the nonlethal risk control treatment (Fig. 1b). The specific growth rate of the nonlethal risk treatment during exposure to predator cues was 0.075 ± 0.01 mm per day vs. 0.036 ± 0.01 mm per day in the nonlethal risk control treatment, so that there was a statistically significant difference in body size between treatments at the end of the 6-day exposure period (Fig. 1b). Following exposure to predator cues, there was no difference in standard length between males and females (males: 23.18 ± 0.362 mm; females: 23.27 ± 0.361 mm).

Table 2 Results of GLM analysis of five measures of standard length.

Source	d.f.	*F*	*P*-value
Measure	4, 572	1524.3	< 0.001
Individual [Tank (Nonlethal risk)]	195, 572	6.374	< 0.001
Nonlethal risk	1, 2.2	3.536	0.189
Tank (Nonlethal risk)	2, 203.8	0.721	0.488
Measure × Nonlethal risk	4, 572	7.311	< 0.001

After predator cues were removed, the acceleration in growth rate of fish in the nonlethal risk treatment attenuated (Fig. 1). Fish in the nonlethal risk treatment grew by 0.008 ± 0.016 mm per day, whereas fish in the nonlethal risk control treatment grew by 0.055 ± 0.016 mm per day. During the third measurement of body size, there was no difference in body size between the fish that had been exposed to nonlethal risk and the nonlethal risk controls (Fig. 1a). During the fourth measurement, fish that had been exposed to nonlethal risk were slightly larger than fish in the nonlethal risk control group (36.87 ± 0.293 mm vs. 35.43 ± 0.338 mm), but this effect was primarily driven by the effect on males (Fig. 2b). At adulthood (L5), fish that had been exposed to predator cues earlier in life were smaller compared to controls (Fig. 1a), but this was primarily driven by the effect of nonlethal risk on females (Fig. 2a,b; see below).

Therefore, although fish grew quickly early in life when they were exposed to predator cues, they ultimately ended up at a smaller size at adulthood (Fig. 3).

Sex differences in growth trajectory

Males and females did not differ on growth rate early in life in either the nonlethal risk control treatment or nonlethal risk treatment (Fig. 2a,b). However, at adulthood, females were larger than males in the nonlethal risk control treatment (44.23 ± 0.671 mm vs. 41.73 ± 0.71 mm).

The effect of predator cues on size at adulthood appears to have been especially strong for females: whereas control females were larger at adulthood than females that had been exposed to predator cues (44.23 ± 0.671 mm vs. 40.09 ± 0.628 mm, Fig. 2a), there was a smaller difference between the size at adulthood of males in the nonlethal risk control vs. nonlethal risk treatments (41.73 ± 0.71 mm vs. 40.06 ± 0.489 mm, Fig. 2b).

Effect of size and sex on survival

Sticklebacks that were larger were more likely to survive predation (Table 3, Fig. 4). The average (±1 standard

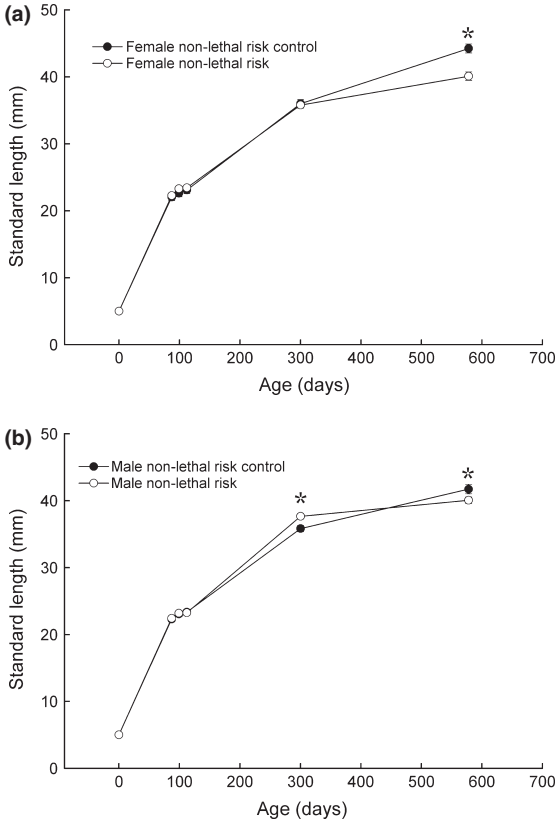

Fig. 2 (a) Growth trajectories of females in the nonlethal risk and nonlethal risk control treatments; (b) Growth trajectories of males in the nonlethal risk and nonlethal risk control treatments. Standard error bars around marginal means corrected for the random effects of tank and individual are shown. *Statistically significant difference.

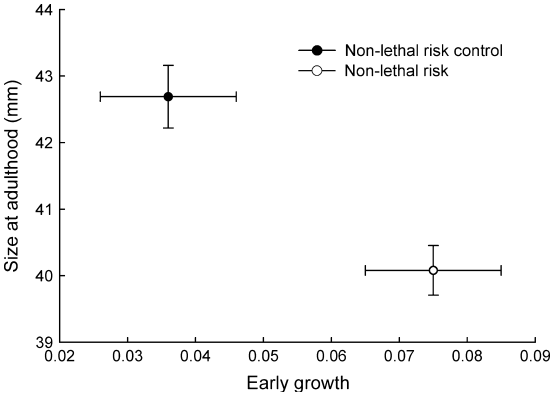

Fig. 3 Tradeoff between early growth rate (daily) in response to predator cues (difference in body size before and after exposure to predator cues, divided by the duration of the treatment period) and size at adulthood in the nonlethal risk and control treatments. Standard error bars are shown.

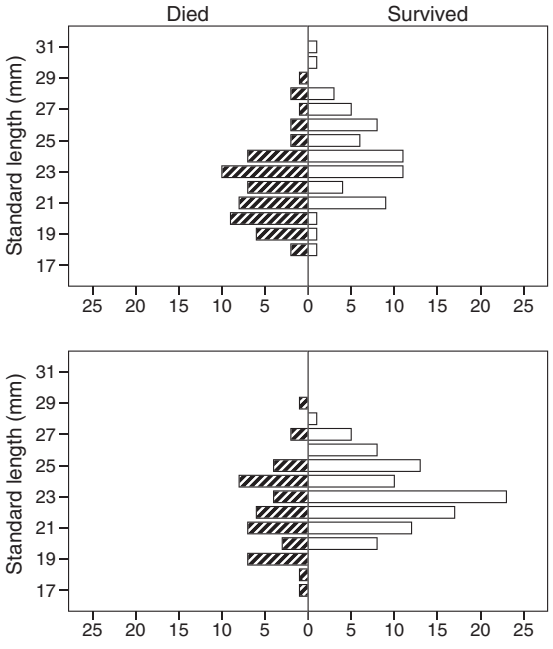

Fig. 4 Distribution of standard lengths for males (top panel) and females (bottom panel) that either were or were not consumed by trout; count is on the *X*-axis. The standard length of males that were consumed by trout was 22.18 mm, SD = 2.5, n = 57, whereas the standard length of males that survived was 24.02 mm, SD = 2.56, n = 62. The standard length of females that were consumed by trout was 22.14 mm, SD = 2.6, n = 44, whereas the standard length of females that survived predation was 23.21 mm, SD = 1.97, n = 97.

Table 3 Logistic regression model identifying predictors of survivorship under lethal predation risk. The final model adequately fit the data according to the Hosmer and Lemeshow Chi-square test of goodness of fit (χ^2 = 12.82, 8 d.f., P = 0.118, n = 261).

Predictor	β	SE	Wald	d.f.	P-value
Length	0.603	0.156	15.05	1	0.000
Sex	0.815	0.281	8.438	1	0.004
Constant	0.065	0.199	0.107	1	0.743

deviation) size of those that survived was 23.48 ± 2.28 mm (n = 197), whereas the average size of those that died was 22.16 ± 2.49 mm (n = 180). The largest stickleback that was eaten was 30 mm. The estimated standardized selection differential was 0.303. Spine length and all the interactions did not influence survival and were omitted from the model.

Females were more likely to survive predation than males: 56% of the animals that were consumed by trout were male (P = 0.004, Table 3). Importantly, this was not because females were bigger than males; there was no sex difference in body size at this age (females: 22.86 ± 0.19 mm; males: 23.13 ± 0.25 mm, Fig. 2).

Discussion

Growth- and size-dependent predation risk

Contrary to the growth-mortality tradeoff hypothesis, exposure to cues of predators caused individual sticklebacks to accelerate juvenile growth. Although many studies have shown that exposure to size-selective predation on small individuals increases development rate (Beckerman *et al.*, 2007), few have shown direct effects on juvenile somatic growth rate. Therefore, it is remarkable that the change in somatic growth in this experiment was immediate and closely matched the timing of exposure to cues of predators. During the 6-day period when juveniles were exposed to cues of predators, the sticklebacks grew faster, such that they were larger than the controls at the end of the exposure period. Then, the fish immediately slowed down their growth once the cues were removed. Given the close synchrony between the timing of exposure (beginning and end) and changes in growth rate, we infer that sticklebacks immediately responded to the presence of predator cues in their environment, and recognized that predator cues were no longer present at the end of the exposure period.

The increase in growth in response to cues of predators was adaptive because bigger fish were more likely to survive predation. Indeed, a fairly small difference in body size (1.32 mm) made the difference between life and death. Although the strength of selection as indicated by the standardized selection differential might appear modest (0.303), it is close to the median reported for selection on body size in fishes [0.37 (Perez & Munch, 2010)]. It is also interesting to note that exposure to cues of predators increased body size by approximately 1 mm. Previous work on this system suggests that small individuals are more vulnerable to predation by salmonid predators both because trout selectively consume small individuals and because smaller individuals are less able to escape capture (Reimchen, 1991). Altogether, our results suggest that size-selective predation on small individuals favours increased growth rate under predation risk in this system, and do not support the growth-mortality tradeoff hypothesis.

We do not know the mechanism underlying the increase in growth rate in response to cues of trout. It is unlikely that sticklebacks exposed to predator cues grew faster by consuming more food because sticklebacks in both treatments were fed the same amount and type of food. Instead, it is likely that sticklebacks exposed to cues of trout diverted more resources to growth by reducing energy expenditure, such as reduced activity, increased shelter use and less time fighting (Johansson & Andersson, 2009). Alternatively, the fish might have improved their efficiency at extracting energy from food (McPeek *et al.*, 2001).

Size at adulthood

Correlative studies of natural variation among stickleback populations experiencing different size-selective predation regimes suggest that strong predation pressure by piscivorous fishes is associated with larger body size at sexual maturity (Moodie & Reimchen, 1976; McPhail, 1977; Reimchen, 1991). Therefore, we predicted that exposure to cues of size-selective predators would cause sticklebacks to become sexually mature at a larger size. However, we observed the opposite: fish that had been exposed to cues of trout as juveniles were smaller as adults, when cues of predators were no longer present.

There are several competing explanations for this result. One possibility is that although we found that bigger fish were more likely to survive under threat of predation, there is considerable overlap in the distribution of body sizes of stickleback that survived and died, i.e. there is not a clear size threshold that distinguishes vulnerable from invulnerable size classes of stickleback (Fig. 4). Other studies have shown that large sticklebacks can be eaten by large piscivorous salmonids (Moodie, 1972). Therefore, trout might not be strictly size selective. Indeed, if adult sticklebacks are also vulnerable, early maturity at a small size might be favoured. In his comparison of natural variation among stickleback populations in size at maturity, McPhail (1977) observed that the size range of predators might be a critical factor. He suggested that when large predators are common, it might not be possible for stickleback prey to attain a size refuge, so the optimal strategy is to breed as close to the physiological minimum size as possible (McPhail, 1977).

Theoretical work suggests other mechanisms that could favour small size at maturity. For example, when there is not an indirect effect of predators on prey food supply, size at maturity decreases in response to increased predation (Abrams *et al.*, 1996). We know that the effects observed in our experiment were not mediated by changes in resource availability because the density of sticklebacks was the same in the nonlethal risk and nonlethal risk control treatments. In addition, Abrams *et al.* (1996) showed that when there are strong seasonal constraints, growth becomes faster and size smaller with less time available in the season. This condition might also be met in our experiment because sticklebacks breed seasonally. However, the breeding season in this population is long (approximately March–September), and individuals can, potentially, breed multiple times over the course of the season (Wootton, 1984).

Finally, an alternative explanation for our results is that the smaller adult size was a deferred cost of early rapid growth [or of deviating from a consistent growth trajectory (Mangel & Stamps, 2001; Stamps, 2007)]. Given that larger body size at maturity has positive effects on both male and female fitness (Dufresne *et al.*, 1990; Kraak *et al.*, 1999), the reduced size at maturity in

animals that grew quickly in response to cues of predators was costly. Another study has also found that rapid growth in juvenile fish is associated with a smaller size at sexual maturity (Morgan & Metcalfe, 2001), and the authors interpreted the smaller size at maturity as a deferred cost of early rapid growth. Our data do not suggest that the reduced age at adulthood reflects a deferred cost of growth depression or compensatory growth (Metcalfe & Monaghan, 2001); sticklebacks did not show depressed growth in the presence of predator cues and then overcompensation. Instead, the predator-induced fast growth was immediate. If future studies provide further evidence that smaller size at adulthood is a common cost of early rapid growth, then this mechanism has broad implications because it suggests that some of the variation in size/age at maturity that has been associated with predation pressure in natural populations could reflect a cost of early rapid growth.

One limitation of this study is that we do not know the precise age at which sticklebacks in this experiment became sexually mature. In this species, it is difficult to estimate the precise time at which individuals, especially males, become reproductively capable because reproductive maturity in males is not always accompanied by observable phenotypic changes in coloration, and the onset of nuptial coloration is highly dependent on the social context. It is possible that fish that had been exposed to cues of predators became sexually mature at a younger age, and then decreased growth rate owing to a tradeoff with reproduction (Roff, 1982). However, sticklebacks continue to grow after sexual maturity (Wootton, 1984; Smith & Wootton, 1995), so it is unlikely that the observed size difference in adulthood reflects a difference in the timing of maturity. Moreover, even if the difference in size at adulthood reflects a difference in the timing of sexual maturity, this does not qualitatively change the interpretation of our results because the predicted effects of size-selective predation on age vs size at maturity are often concordant (Abrams & Rowe, 1996; Abrams *et al.*, 1996).

Sex differences

We found sex differences both in the probability of surviving predation and in the long-term consequences of exposure to cues of predators. Females were more likely to survive predation by trout than males, even though there were no sex differences in body size or behaviour (unpublished data) during the period of exposure. We do not know whether the trout preferred to eat males, or whether females were better able to escape predation. Therefore, we do not have an explanation for this result, although other studies have found that females have higher survivorship under predation (Quinn & Kinnison, 1999; Quinn & Buck, 2001; Calvete *et al.*, 2005). Importantly, control males were not more likely to die than control females.

We also have suggestive evidence that although females were at an advantage in terms of survival under predation pressure, early exposure to predation risk had a stronger effect on their adult size: the difference in age at maturity between the nonlethal risk and nonlethal risk control treatment was larger for females than it was for males. Other studies have also found sex differences in the effects of exposure to predation (Ball & Baker, 1996), and sex differences in the cost of growth, with greater costs generally for females (Leimar *et al.*, 1994; Martin-Smith & Armstrong, 2002). Studies on stickleback suggest that although larger size is favoured in both males and females, the cost of being small is greater for males than females. For example, one study found that female sticklebacks can breed when as small as 32 mm (Poizat *et al.*, 1999), whereas males smaller than 40 mm will virtually never breed (Dufresne *et al.*, 1990; Kraak & Bakker, 1998; Kraak *et al.*, 1999). While an increase in body size from 40 to 50 mm doubles the clutch size in females (Kraak & Bakker, 1998), the same increase in body size leads to a larger increase in reproductive success in males (Kraak *et al.*, 1999). Therefore, large adult body size might be more important for male fitness than for female fitness, and males might have mechanisms for buffering against deviations from large adult body size. However, it is worth noting that small males might obtain fitness benefits via other means such as arriving early on the breeding grounds (Candolin & Voigt, 2003).

Conclusions

While there is good evidence that exposure to predation risk causes prey to decrease growth rates, to induce behavioural and morphological defences against predation, and to accelerate development out of vulnerable stages, this study shows that prey can also increase somatic growth rate in response to exposure to cues of a predator that selectively consumes small individuals. We show that a relatively brief exposure to cues of predation risk when animals are young can have long-term consequences later in life. The degree to which those long-term consequences reflect adaptive, facultative adjustments in response to the environment vs. a deferred cost of mounting defence against predation risk early in life remains to be determined.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Overview of the two × two factorial experimental design to test for the effect of nonlethal risk and lethal risk on growth and survivorship.

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