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The effects of long-term predator exposure on body composition and condition of young Arctic charr (*Salvelinus alpinus*)

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Non-lethal energetic and physiological costs of severe predation risk in aquatic prey are poorly understood. Knowledge of this matter would be especially valuable in life-skills training programs for hatchery-reared fish, where long-term predator conditioning is one potential method to improve the antipredator skills of naïve fish. In this study I examined the effects of long-term (72 days) predator exposure on the body composition (whole-body lipid and water content) and body condition of hatchery-bred Arctic charr (*Salvelinus alpinus*) young. The chemical cues from charr-fed pikeperch (*Sander lucioperca*) were used as the exposure stimuli. My results revealed that the predator-exposed charr had a lower body condition and water content but higher lipid content than the non-exposed conspecifics, demonstrating that long-term predation risk causes serious physiological changes. Since predator conditioning can evidently result in marked physiological changes in prey fish, future studies should take into further consideration not only behavioural but also physiological effects of life-skills training.

Introduction

North-temperate fish species face two conflicting energy allocation strategies during their first growing season: whether to allocate their surplus energy to somatic protein growth or lipid deposition (Biro *et al*. 2005). The energetic content of lipids (38 kJ g^{-1}) is much higher than that of proteins (24 kJ g^{-1}) or carbohydrates (17 kJ g^{-1}) (Jobling 1993), which makes lipids perhaps the most significant energy store in fish (Sheridan 1994). By allocating energy into lipids, young fish can better survive the harsh winter conditions when food availability becomes reduced (Jonsson & Jonsson 1998, Post & Parkinson 2001, Biro *et al*. 2004). On the other hand, by having rapid protein growth the prey can escape predation pressure from gape-limited predators (Zaret 1980, Miller *et al*. 1988, Walls *et al*. 1990). Furthermore, a large body size is advantageous in competition (Johnsson 1993). Recent field studies have supported the hypothesis that it could be an adaptive strategy for young fish to allocate only a little energy into lipids during the summer (i.e. when very small) but to allocate more energy into lipids during the autumn (i.e. when larger) (e.g. Post & Parkinson 2001, Biro *et al*. 2005).

The presence of predators, however, can cause physiologically costly effects for prey that in the long term may jeopardize the adaptive energetic status of an individual. Brief encounters with predators typically decrease the feeding activity (i.e. energy intake) of prey, cause other behavioural antipredator responses, and/or elicit primary and secondary stress responses (e.g. the release of stress hormones into the bloodstream, increased heart rate) (Lima & Dill 1990, Milinski 1993, Godin 1997, Johnsson *et al*. 2001, Woodley & Peterson 2003). The short-term behavioural and physiological responses, for example modifying the cardiorespiratory system, are evidently adaptive in enhancing the probability of survival from sudden predator attacks. It is evident that predation pressure results in selection of behavioural as well as physiological traits that enable fish to respond appropriately to predatory threat (Brown *et al*. 2005). If the presence of predators is occasional and the prey returns to its normal physiological and behavioural state quickly after encounters, there may only be minor effect of these encounters on the energetic status of the prey (Cooke *et al*. 2003). Conversely, if the presence of predators is prolonged, continuous behavioural as well as physiological stress responses may become ultimately energetically expensive and lose their adaptive significance. Tertiary stress responses induced by various chronic environmental stressors include reduced growth, body condition, resistance to diseases, reproductive success, and also a reduced capacity to tolerate additional stressors (*see* Donaldson 1990, Goebe & Barton 1990, Wedemeyer *et al*. 1990, Wendelaar Bonga 1997). Ultimately, these long-term costs can decrease the fitness and survival of a stressed individual (Schreck 2000). However, it is not known how energy allocation patterns (i.e. body composition) and the condition of young fish are affected when the risk of predation ultimately becomes severe, i.e. when the presence of predators is constant and chronic predator-induced stress is possible. The value of this information would be to better understand the costs of non-lethal predator–prey interactions in the aquatic environment.

In addition to natural conditions, the effects of predators on the energetic status and body condition of fish would be especially valuable to explore in life-skills training programs. The effectiveness of long-term predator conditioning on behavioural or physiological survival skills of fish has not previously been tested. To date, a number of short-term training methods have been suggested to improve the survival and antipredator behavioural skills of naïve hatchery fish prior to release into the wild (Brown & Laland 2001, Brown & Day 2002, Kelley & Magurran 2003). Chemical cues of predators and eaten/injured prey are perhaps the most frequently used in these successful training methods (e.g. Brown & Smith 1998, Berejikian *et al*. 2003, Vilhunen *et al*. 2005, Vilhunen 2006). The short-term training techniques lasting only a few minutes are unlikely to result in long-term physiological changes in prey, whereas long-term pre-training may have unwanted physiological effects on prey. As for example poor body condition typically weakens the survival skills of vertebrate prey (Mesa *et al*. 1994, Wirsing *et al*. 2002), long-term predator conditioning might actually weaken the survival of prey after release to the wild, which is an extremely important topic for study.

In this study I tested the effects of long-term predator odour exposure on the body condition and composition (whole-body lipid content and whole-body water content) in young-of-the-year Arctic charr (*Salvelinus alpinus*). I used chemical cues from charr-fed pikeperch (*Sander lucioperca*) as the predator stimulus. Vilhunen (2005) showed recently that prior repeated conditioning to chemical cues from charr-fed pikeperch increased the survival of young charr in real encounters with pikeperch. Therefore, the use of these chemical cues provides an effective training method that could also be applied in long-term training techniques. Five full-sib hatchery-bred Arctic charr families were used in the experiment. One half of each family was exposed to the predator odour and the other half to odourless control water. Altogether, I exposed the charr to the predator cues for 72 days (25 days in the eyed-egg stage and 47 days after hatching), after which I analyzed the body condition, lipid and water content of each individual fish.

Materials and methods

The test fish and the predator exposures

Five dams and five sires from second hatchery generation of Saimaa Arctic charr were used in artificial fertilizations (7–11 November 2003) to form five full-sib families. After fertilization the eggs were incubated in standard hatchery troughs ($215_l \times 41_w \times 15_h$ cm, water level 12 cm). On 10 March 2004 the eyed eggs of each full-sib family were randomly divided into two groups (300 eggs per group) and transferred into two hatchery troughs. The eggs of each family were kept in identical net boxes and the eggs could not be mixed between the families. The eggs in one trough were exposed to the predator odour (predator treatment) starting on 11 March and exposure lasted 25 consecutive days. The eggs in the other trough (control treatment) were exposed at the same time to only pure lake water from lake Ylä-Enonvesi. In the predator treatment the water to the hatchery trough flowed from an aquarium (volume 74 l) that contained two pikeperch (weight 360 g and 600 g). The pikeperch were transferred into the aquarium one week before the exposure started. The pikeperch were fed twice per week with a single one-year-old Arctic charr juvenile (mean weight 8.15 g) before the exposure started and during the exposure period. The charr juveniles had been killed with a single blow to the head and immediately frozen at –25 °C until used. When used as food, charr were first thawed and then cut into five pieces and added to the aquarium. The predator treatment trough received water from the aquarium at a flow rate of 5 l min^{-1} in addition to 5 l min^{-1} pure lake water. In the control treatment, water flow to the trough comprised 10 l min^{-1} pure lake water only.

On 7 April 2004 predator exposure was stopped and families within each treatment (predator *vs*. control) were assigned randomly to separate hatchery troughs (i.e. altogether ten troughs) for hatching. The physical layout of these hatchery troughs were similar to those described above. The first alevins hatched on 18 April and predator exposure continued after all alevins had hatched (9 May) in a similar way to that for the eyed-egg stage. The only exceptions were that the pikeperch were fed three times per week and from the beginning of June they were fed two charr at a time (due to the increased water temperature and consequent increased energy demand of the pikeperch). The hatched young charr were reared at a water temperature of 10–13 °C and subjected to a simulated natural photoperiod.

The environmental conditions (e.g. water temperature, lighting, availability of food, density of fish) were similar for all groups to minimize possible tank effects. Furthermore, during the experiment there were no differences in oxygen concentrations between the predator treatment and the control troughs (two-sample *t*-test: $t = -1.717$, df = 10, $P = 0.117$). The young charr were fed commercial pellets *ad libitum* daily and the troughs were cleaned three times per week. Each trough was partially covered with a plastic plate to provide shadow for the young charr. Altogether, three pikeperch pairs were used in the exposures after hatching. The mean (S.D.) weight of the pikeperch was 406 (129) g, and there were no significant differences in weight between these pairs (Kruskall-Wallis test: $H_2 = 1.143$, $P = 0.565$).

On 21 June parasites (*Ichtyobodo* sp.) were detected in one hatchery trough (predator treatment) and all exposures were interrupted. To be on the safe side the fish in all troughs were successfully treated by bathing with formalin (1:4000). The predator exposures continued on 9 July and each family was exposed to the predators for a further four days before a sample of fish was taken to determine the condition and perform whole-body lipid and water content analysis. I assume that the possible effects of parasites on body composition and condition of fish were minimal since parasites were killed before a full outbreak of infection.

Altogether, 11 to 15 similarly-sized fish per family and per treatment were sampled for the analysis. The average length (S.D.) and weight (S.D.) of the sampled fish was 44.5 (2.79) mm and 0.776 (0.154) g, respectively. The sampled fish were transferred to two flow-through aquaria (volume 31 l) to settle for one day. On the following day the antipredator responses of predator-exposed and naïve charr were tested against chemical cues from non-fed pikeperch in a Y-maze fluviarium (*see* Vilhunen 2006 for

description of the Y-maze). After each trial the fish was netted and killed using an overdose of an anaesthetic (MS-222), whereupon the wet weight and the length were measured. Then the fish were frozen (–70 °C) for subsequent water content and whole-body lipid analysis.

Whole-body lipid and water analysis

To determine their water content and dry weight, fish were thawed, weighed and then dried at 70 °C for 24 h. The dried samples were reweighed to obtain the water content (%) of each individual fish. After this each fish was ground with a mortar and pestle, and approximately 0.1 g of dried sample was analysed for lipids using the sulpho-phospho-vanillin method (*see* Frings & Dunn 1970). This method made it possible to measure the lipid content of individual fish. In brief, the dried sample was extracted with 10 ml chloroform-methanol reagent (volumes 2:1), and centrifuged for 30 min at 3000 rpm. 50 μl of the supernatant was taken into a new tube and dried at 50 °C for one hour. After this, 500 μl of concentrated sulphuric acid was taken into a tube and the tube was heated for 10 min in a boiling water bath. After heating the sample was cooled in a cold water bath for 5 min and 2 ml of phosphovanillin reagent (0.6 g vanillin/100 ml distilled water + 400 ml of concentrated phosphoric acid) was added to each tube. The tubes were incubated for 15 min at 37 °C and cooled for 5 min at room temperature. The absorbance of the sample was measured in a spectrophotometer (Uvidec-340, double-beam spectrophotometer) at 570 nm wavelength. The lipid concentrations were calculated by comparing the sample absorbances to the corresponding absorbance values of standards (containing 50, 100 and 150 μg of lipid). The stock standard (1 mg ml^{-1} lipids) was produced by mixing 10 mg of olive oil with 10 ml of chloroform-methanol reagent. The lipid content of an individual fish was calculated as the percentage of wet weight.

Statistical analyses

I used the residuals of the wet weight to length regression to calculate the body condition of the fish. Both weight and length were ln-transformed [ln(variable + 1)] before the analysis. Using these residuals as a measure of fish condition is less biased than conventional condition factors, as the effect of body size on the relationship between body weight and length can be excluded (Goebe & Barton 1990, Jakob *et al.* 1996, Kotiaho 1999). The weight at length is described by the equation ln(weight + 1) = 1.289 ln(length + 1) – 4.347, $r^2 = 0.866$, $F = 892.16$, $P < 0.001$.

Both whole-body lipid content (%) and water content (%) were significantly correlated with body weight (Pearson correlation: $r = 0.338$, $P < 0.001$ and $r = –0.644$, $P < 0.001$, respectively), and therefore the residuals of the lipid to weight and water to weight regressions were used in the statistical analysis. All variables were ln-transformed [ln(variable + 1)] before the analysis. By using the residuals the effects of size differences between individuals from different families could be controlled for in the analyses. There were significant differences in length (ANOVA: $F_{4,139} = 9.79$, $P < 0.001$) and weight (ANOVA: $F_{4,139} = 6.24$, $P < 0.001$) between the families. There were no differences between the treatments in size (ANOVA: length, $F_{1,139} = 0.479$, $P = 0.490$; weight, $F_{1,139} = 0.575$, $P = 0.450$). The lipid content is described by the equation: ln(lipid content (%) + 1) = 0.522 ln(weight + 1) + 1.596, $r^2 = 0.115$, $F = 17.914$, $P < 0.001$, and the water content by the equation: ln(water content (%) + 1) = –0.072 ln(weight + 1) + 4.418, $r^2 = 0.441$, $F = 108.989$, $P < 0.001$.

I used the paired sample *t*-test to compare the effects of predator treatment on body condition, whole-body lipid and water content of young charr. Mean values of each full-sib family per treatment were used in these analyses (i.e. $N = 5$ per treatment). All statistical analyses were performed using SPSS 11.0.1 (SPSS Inc. 2001).

Results

The paired sample *t*-test revealed that the predator-exposed charr were in poorer condition ($t = 2.841$, df = 4, $P = 0.047$) and contained significantly more lipids ($t = –8.454$, df = 4, $P = 0.001$) and significantly less water ($t = 4.896$,

df = 4, $P = 0.008$) than the non-exposed young charr (Fig. 1).

Discussion

My results clearly demonstrated that long-term predator exposure caused serious physiological changes in the young charr. Furthermore, the results of my study are not in accordance with the adaptive energy allocation strategy recently presented by Biro *et al*. (2005). These authors reported that it could be an adaptive strategy for young fish to allocate only a little energy into lipid deposition during the summer under risk of predation. Their hypothesis was supported by an extensive field study. In the light of this information I hypothesize that the lipid allocation pattern and reduced body condition detected in the predator-exposed young charr are maladaptive responses and the result of either: (1) severe chronic predator-induced physiological stress, (2) reduced feed (energy) intake due to predator exposure, or (3) reduced overall swimming activity (i.e. reduced energy consumption).

The first explanation for the observed alterations in the body composition and body condition due to predator exposure could be that the energetic demand of young charr was increased due to chronic predator-induced stress. Under chronic stress the metabolic demand of an animal remains constantly elevated and stress hormones (especially cortisol) furnish energy by stimulating the catabolism of glygocen, protein and lipid reserves (Wendelaar Bonga 1997, Van Weerd & Komen 1998). However, as Czesny *et al*. (2003) pointed out, the dietary status and body reserves may affect whether lipids or proteins are primarily mobilized under long-term stress. For example, in the juvenile walleye (*Stizostedion vitreum*) lipids were primarily mobilized due to predator exposure (Pratt & Fox 2002). Although I did not analyze the whole-body protein content in the present study, the reduced water content and body condition in the exposed fish possibly also indicates a lower protein:lipid ratio in these fish (*see* Gardiner & Geddes 1980). Therefore, it seems likely that the higher lipid content in the exposed charr could primarily result from the mobilization of protein stores and the use of proteins to supply energy under predator-induced stress. The poor condition and low water content of the exposed young charr also support this hypothesis. A decline in body condition has been detected in fish subjected to chronic stress from

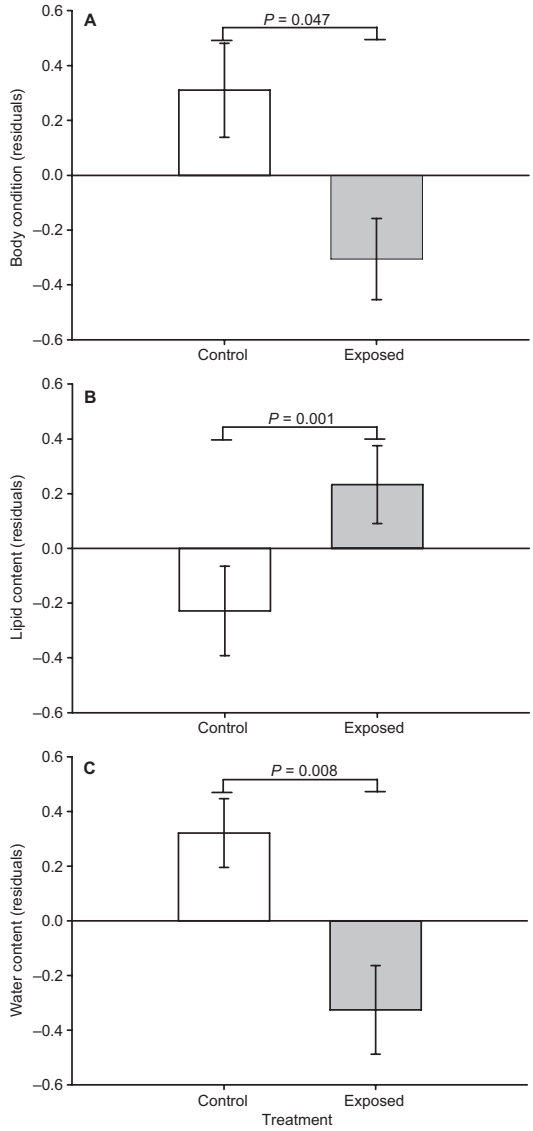

Fig. 1. The mean (± S.E.) of (**A**) body condition, (**B**) whole-body lipid content and (**C**) whole-body water content of young Arctic charr after 72-day exposure to pure lake water (control) or predator odour (exposed). The variables are described by residuals of the weight to length, lipid and water content regressions. $N = 5$ both in control and in predator treatment. P values above bars are paired sample t-test statistics.

high rearing densities or environmental pollution, and is typically interpreted as a depletion of body energy reserves (fat, protein and/or liver glycogen) (Goebe & Barton 1990). The present study, however, is the first to record this tertiary stress response due to long-term predator exposure in fish species. Scheuerlein *et al*. (2001) recently reported similar findings in a bird species, the tropical stonechat (*Saxicola torquata axillaris*). In territories with predators, stonechat males had a lower body condition and higher plasma corticosterone levels than males living in territories without predators, indicating clear chronic stress responses.

It is possible that the differences in body composition and condition between predator-exposed and non-exposed young charr could also be related to predator-induced behavioural responses. For example, decreased foraging activity (i.e. energy intake) due to predators could have affected the results (*see* Jachner 1997, Cooke *et al*. 2003, Woodley & Peterson 2003). The fact that the young Arctic charr seem to innately respond to the chemical cues of pike-perch by reducing their swimming activity and moreover by freezing (Vilhunen & Hirvonen 2003), which no doubt restricts their energy intake, supports this hypothesis. However, the metabolic rate and therefore the energetic consumption of fish may actually decrease due to the reduced swimming activity (Holopainen *et al*. 1997, Huuskonen & Karjalainen 1997). The high lipid content in the exposed fish could result from reduced energetic demand due to the lowered activity. However, the decline in body condition conflicts with this hypothesis, and it is thus more likely that the exposed young charr were suffering from chronic predator-induced stress and/or had reduced their feeding activity (i.e. energy intake). Further studies, however, are needed to verify the issue.

One possible way for prey to adapt to the originally threatening long-term or repeated predation risk, before the energetic costs of antipredator actions affect the fitness and even survival of the individual, is to behaviourally and/or physiologically habituate to the presented stimulus. Response attenuating in fish species has been reported in a number of antipredator behavioural studies (e.g. Magurran & Girling 1986, Jachner 1997, Berejikian *et al*. 2003), as well as in studies examining various environmental stressors (Wendelaar Bonga 1997, Barton 2002). In my study, the young charr were never actually attacked by the predators and did not even see them, so the basis for habituation to the chemical cues due to long-term exposures definitely existed. However, the fact that the predator-exposed young charr were in poorer condition and had a higher whole-body lipid content and lower whole-body water content than the non-exposed charr clearly demonstrates that the young charr were not physiologically habituated to the presented stimulus. Vilhunen (2005) suggested that the chemical alarm cues of eaten or injured conspecifics combined with predator cues may provide such intense and reliable indicators of the dangerous predation risk, especially in populations with a long history of co-evolution with the predators (e.g. Saimaa Arctic charr and pikeperch), that the possible habituation to these cues is slow. The results of my study support this hypothesis.

Long-term predator conditioning is one potential method to improve the antipredator survival skills of hatchery-reared naïve fish before release into the wild. However, it is also important to determine the effects of long-term conditioning on the physiology of fish. In the present study the exposed fish, equipped with a high lipid content, would perhaps survive better or longer after release than the non-exposed fish if food availability in the wild was poor (Biro *et al*. 2004). However, the poor body condition of these fish could have the opposite effect on their survival (*see* Wirsing *et al*. 2002 for a mammalian example). A prey individual that is in substandard condition may be unable to integrate the processes necessary for active-flight predator evasion (Mesa *et al*. 1994). For example, their maximal acceleration performance (i.e. startle response) may be radically reduced (Mesa *et al*. 1994). Analysis of the behavioural data of the present study will reveal how conditioning affected the behavioural antipredator skills of young charr. In future studies it would be essential to evaluate the survival of long-term predator-conditioned fish in real encounters with predators (survival tests) to determine how survival skills are actually affected. Ultimately, the avoidance of pre-

dation is an interaction of both behavioural and physiological capabilities of the prey.

In conclusion, my study showed that presence of predators has far reaching non-lethal costs for prey. In future studies it would be highly valuable to measure both behavioural (i.e. feed intake and overall activity) and physiological responses (i.e. primary and secondary stress responses) during long-term predator exposures, to better understand the energy allocation mechanisms and energetic costs of prey under severe risk of predation. Since predator conditioning can evidently have a dramatic influence on fish physiology, the next phase in life-skills training would be to adjust the duration and timing (i.e. possible sensitive periods) of predator conditioning to obtain both physiologically and behaviourally adaptive fish for reintroductions.

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