Ethology

Behavioural and Growth Responses of a Territorial Fish (Atlantic Salmon, *Salmo salar*, L.) to Multiple Predatory Cues

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Abstract

Antipredatory behaviours are important fitness components. The probability of survival decreases if animals fail to respond to signs of danger, but in contrast, energetic costs increase if the response to the threat is exaggerated. We conducted a laboratory experiment designed to examine the behavioural and growth responses of a territorial fish (Atlantic salmon fry, *Salmo salar*, L.) to different predatory cues (no predatory cues, chemical cues alone, physical cues alone and combined chemical and physical cues). We evaluated the response of Atlantic salmon, focussing on behaviours linked to predator avoidance and to other fitness-enhancing activities (territory defence and energy acquisition) both during the day and the night. The cost of such responses in terms of growth was assessed and we compared the relative contributions of behaviours in explaining individual growth rate, according to each predation treatment. We demonstrated that the magnitude and nature of behavioural modification varied according to the response variables we considered. An index of predator avoidance and the distance from the food source were affected in an additive fashion by predatory cues (interaction term, $p = 0.469$ and $p = 0.888$ for the index of predator avoidance and the distance from the food source respectively); the effect of physical cues was stronger than the effect of chemical cues and the effect of the combined cues was highest. An index of territoriality was affected in a threshold-like fashion (interaction term, $p = 0.040$); chemical or physical cues alone had no effect but when both cues were combined, Atlantic salmon significantly reduced their territorial defence. An index of foraging activity was not significantly affected by predatory cues (alone or combined). We detected no effect on the growth rate of Atlantic salmon ($p = 0.328$). Finally, we found that the relative contribution of behaviours in explaining individual growth rate changed according to the treatments we considered. Overall, these results demonstrated that fish were able to accurately integrate multiple predatory cues and that this information was used to modulate their antipredatory response. Behaviours involved in the response were relatively independent of each other, allowing fish to adopt behavioural tactics that maximized the ratio of net energy gain to predator avoidance.

Introduction

In natural communities, prey often share their environment with many predators unequally distributed in space and time. Ecologists have recently incorporated this predator diversity into their studies, focussing on the response of prey to multiple and simultaneously occurring predators. In these cases, the simplest expectation concerning prey mortality is that predators will have independent linear effects on prey, and the effect of two combined predators would be the sum of their individual effects (i.e. an additive effect). Sih et al. (1998) described an emergent multiple predator effect as one that explains any divergence from this expectation (enhancement or reduction of the effect relative to the additive expectation).

To limit the risk of predation, animals generally increase predator avoidance activity (e.g. time spent hidden in a refuge) while decreasing the time allocated to other fitness-enhancing activities (e.g. energy acquisition, territory defence, courtship and/or parental care; Sih et al. 1985; Lima & Dill 1990; Kats & Dill 1998; Lind & Cresswell 2005). To accurately respond to predation threats and to optimize the balance between predator avoidance and other fitness-enhancing activities, potential prey generally obtain information from cues emanating from predators and/or other prey (Kats & Dill 1998; Schoeppner & Relyea 2005). According to the threat sensitivity hypothesis (Helfman 1989), prey should respond in a graded manner as the threat posed by a predator increases (but see Helfman & Winkelman 1997; Brown et al. 2006). Inappropriate responses to the threat of predation may decrease energy acquisition of animals that spend too much time hiding, but may also decrease the probability of survival if animals fail to respond to a dangerous stimulus.

Faced with many predators, multiple cues should contribute in an additive way to determine the degree of risk-sensitive behaviour (Helfman 1989; Smith & Belk 2001). The behavioural response of prey to multiple cues has been documented in taxonomically diverse animals (e.g. Eklov 2000; Krams 2000; Smith & Belk 2001; Amo et al. 2004; Stapley 2004; Mikheev et al. 2006). For instance, the antipredatory responses of wall lizards (*Podarcis muralis*) was greater when they were exposed to a combination of visual and chemical cues emitted by the smooth snake (*Coronella austriaca*) than when exposed to any one of these cues (Amo et al. 2004).

Antipredation responses have rarely been tested in territorial animals where individuals must simultaneously deal with complex trade-offs between foraging activity, risk of being predated upon and territory defence. Moreover, in most of the studies involving antipredation responses and multiple predatory cues, authors have generally focussed on a single family of behavioural responses (e.g. behaviours related to predator avoidance, Amo et al. 2004 or behaviours related to foraging activity, Mikheev et al. 2006). However, the balance between predator avoidance activity and other fitness-enhancing activities has been overlooked. The antipredatory response is a composite of many behaviours and the 'single-behaviour approach' may lead to incomplete conclusions about the fitness consequences of avoiding predation (reviewed in Lind & Cresswell 2005; see also Ajie et al. 2007). For instance, for territorial animals, one would predict that as the perceived risk of predation increases, predation avoidance activity would increase and both foraging activity and territory defence would decrease in a linear fashion (i.e. all the behaviours are threat-sensitive). In contrast, antipredation behaviours may not co vary in this fashion and nonlinear responses may occur in some behaviours to improve individual lifetime fitness (Lind & Cresswell 2005). For example, some behaviours (e.g. predator avoidance) may additively respond to predation threats while others (e.g. feeding activity) may respond in a non-additive fashion to compensate for the fitness lost by the adjustment of other behaviours (Lind & Cresswell 2005; Ajie et al. 2007).

In this study we used a 'multiple-behaviours approach' to evaluate the antipredatory response of a territorial animal to multiple predatory cues. We first tested the hypothesis that all behaviours involved in the antipredatory response react in an additive fashion to an elevated risk of predation, as predicted by the threat sensitivity hypothesis. We then tested the hypothesis that this response should be costly in terms of individual growth rate, a trait that is indirectly related to fitness (Lind & Cresswell 2005; Ajie et al. 2007). Indeed, if all the behaviours respond in an additive way, we would predict that foraging activity, and thus growth rate, should decrease as predation threat increases. Finally, we evaluated the relative contribution of each behaviour in explaining individual growth rate at different levels of predation risk to assess the variation in individual behavioural tactics.

To address these issues, we used a territorial fish, the Atlantic salmon (*Salmo salar*) as the model system. As in many salmonids, juvenile Atlantic salmon are sit-and-wait predators that feed on invertebrate drift and defend a territory from hetero- and/or conspecifics (Klemetsen et al. 2003). Territory acquisition provides direct fitness benefits as it allows fish to acquire energy and to decrease the risk of being preyed upon by hiding in a refuge (Fausch 1984). Moreover, as in many fishes, the quality of the defended habitat can have indirect repercussions on life-history strategies (Metcalfe et al. 1989; Hoffmann et al. 1999). Finally, salmonids share their environment with many potential predators, such as birds, mammals, water snakes, piscivorous fish and crayfish (e.g. Martel & Dill 1995; Ludwig et al. 2002; L'Abee-Lund et al. 2002). Several studies have demonstrated that salmonids modify their behaviours (antipredatory activity, foraging activity and/or territory defence) when exposed to single predators or their associated cues (e.g. Martel & Dill 1995; Reinhardt 1999; Hirvonen et al. 2000).

We conducted laboratory expts to assess the effects of two predatory cues (chemical cues aimed at simulating a fish predator and physical cues aimed at simulating a piscivorous bird) on the behaviour and growth of Atlantic salmon fry. Atlantic salmon fry were exposed to four different 'cue' treatments (no cues, chemical cues or physical cues alone or the two cues together). We compared behaviours related to predator avoidance, foraging activity and territory defence among these treatments. These behavioural comparisons were done during both the day and the night, as predators are known to strongly influence the diel activity of prey (Reebs 2002; Kronfeld-Schor & Dayan 2003). At the end of the expts, we measured the individual growth rate of Atlantic salmon to estimate the fitness costs of such behavioural responses. Finally, we compared the relative contribution of behavioural traits in explaining individual growth rate according to the four treatments.

Materials and Methods

Experimental Animals

Atlantic salmon used in the expt. were young-of-the-year obtained from the Tadoussac provincial hatchery (Ministère des Resources Naturelles et de la Faune, Tadoussac, QC, Canada). These samples were offspring of wild spawners caught during the 2003 reproductive migration in the Malbaie River (QC, Canada). After 4 mo at the hatchery, fish were transferred to the Laboratoire Régional des Sciences Aquatiques (LARSA) at the University Laval (QC, Canada). Fish were raised in one common holding tank and fed ad libitum on commercial fish food pellets. Water temperature and photoperiod in the holding tank were similar to the conditions prevailing during the expts (see the following section). These fish have been shown to display identical behaviours as wild fish hatched and sampled in the Malbaie R. (Blanchet, S., Paez, D. P., Bernatchez, L. and Dodson, J. J., unpubl. data).

Experiments were conducted using 12 artificial channels fitted with a re-circulating water system. The channels were arranged in two independent-blocked staircase designs. Each block consisted of a biological filter, two large collecting basins (720 l.), three external pumps and six artificial channels (Fig. 1). A PVC tube (5 cm in diameter) linked the two basins and fed water to the biological filter. Two pumps supplied water from one of the basins to four of the six channels at a rate of 2500 cm^3/s per channel.

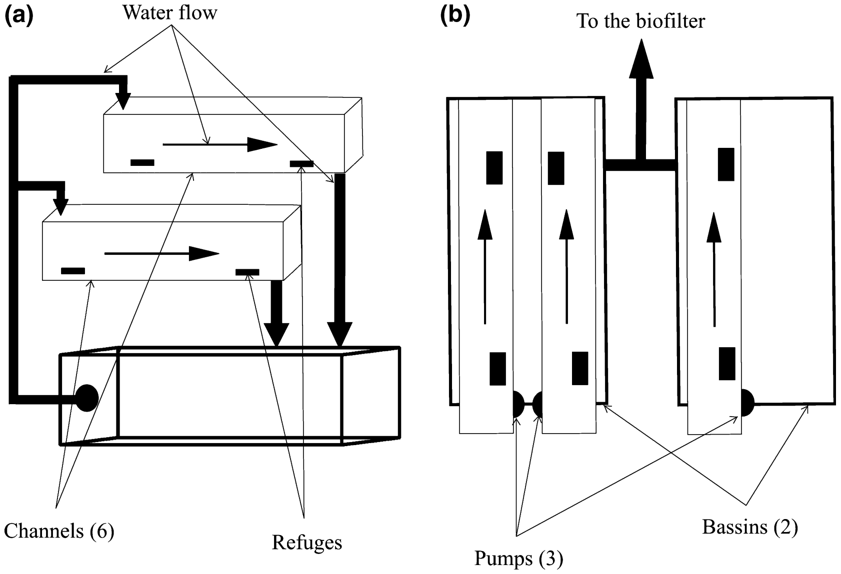

Fig. 1: Simplified view [side view (a) and top view (b)] of a blocked staircase design. Two such independent staircases were used in the expt., providing 12 experimental channels. Each block contains one biofilter, two large basins (720 l), three external pumps and six channels. See the text for a full description of the set-up.

The other pump supplied the same water discharge from the second basin to the two other channels (Fig. 1). Each channel was made of transparent Plexiglas and was 1.90 m long (only approx. 1.50 m was available for fish), 0.30 m wide and 0.30 m deep. The water depth in each channel was 12 cm and average current velocity was 8 cm/s. A single layer of river cobbles (2–3 cm in diameter) covered the entire surface of the arena. Two 60-W light bulbs above each channel served to simulate day light (80% of the available intensity), dawn, dusk and night (7% of the available intensity). Light:dark cycle was 9:14 h plus 30 min of dawn and dusk. Light intensity and photoperiod were automatically set with a photoperiod monitor (SunMatch; Aquabiotech Inc., Coaticook, QC, Canada). Water temperature was maintained constant at 14 ± 1°C which is close to the water temperature of the river Malbaie in mid-Sep. (13.5 ± 3.3°C). Daily food ration (3% of the initial wet body weight, i.e. approx. 3 g pellets per day) was dispensed at the upstream end of the channel by an automatic feeder. Between 0 and 10 pellets were released at 30-min intervals for 24 h. The inner side of each channel was marked along its length to define 15 equal zones (zone 1 being upstream, directly below the automatic feeder) to allow recording of horizontal distribution and individual fish movements. Two half-bricks (12 × 5 × 0.8 cm), each glued to four cobbles, were placed in zones 3 and 13 (i.e. one in the first and one in the second-half of each channel) to serve as refuges.

On 7 February 2005, 48 fry were selected from the holding tank to produce 12 groups of four fish. After 24 h of food deprivation, they were anaesthetized (with clove oil), measured [mean fork length: 65.89 mm ± 1.77 (SD)] and weighed [$\bar{x}$: 2.64 g ± 0.22 (SD)]. Neither size (One-way ANOVA, $F_{11,36} = 0.94$, $p = 0.516$) nor weight (One-way ANOVA, $F_{11,36} = 0.62$, $p = 0.797$) varied among groups, and fish were selected to limit variability within each group. Fish were then individually marked using Visible Implant Elastomer tags (Northwest Marine Technology, Shaw Island, WA, USA). Each group was haphazardly allocated to an experimental channel for a 30-d expt.

The experimental design consisted of four treatments (three replicates/treatment); (1) a control (i.e. no predatory cues), (2) presence of chemical cues (to simulate a fish predator), (3) presence of physical cues (to simulate a bird predator) and (4) combination of both types of cues. The chemical cues, aimed at simulating the presence of a fish predator, were obtained by introducing a large rainbow trout in each of the collecting basins of only one of the blocked staircases for the 30-d period. These hatchery-reared rainbow trout measured 29.4 and 33.3 cm in basins 1 and 2, respectively, and were fed with a piece of a freshly dead Atlantic salmon fry (i.e. roughly the head of the fish) twice a day. Salmonids are able to perceive chemical cues released by predators (i.e. kairomones) and/or by conspecifics attacked by a predator (i.e. alarm cues) (Chivers & Smith 1998; Kats & Dill 1998; Mirza & Chivers 2001; Brown 2003). In this study, water marked with chemical cues continuously flowed through six of the channels. To enhance the effects of chemical cues, we also introduced once every 3 d 20 ml of a distilled solution of crushed Atlantic salmon fry (see Leduc et al. 2004 for the methodology concerning the solution) upstream of the six channels. The physical cues aimed at simulating an attack of an aerial predator were achieved by plunging a wooden bird bill, mounted on a fine wooden arm, into six of the channels: three of these channels also contained water marked with chemical alarm cues (treatment 4) whereas the remaining contained water free of chemical cues (treatment 3). Specifically, three times a day, the wooden bill was slowly placed above the channel after which we conducted a simulated attack on all the fish that were out of a refuge in the first 75 cm of the upstream end of the channel. If fish were perturbed during the stages before the attack, the observer waited for few minutes until fish came back to their initial position. The simulated attacks were halted when all fish were hidden and/or away from the 75 cm upstream end of the channel. No attack was simulated in the remaining 75 cm of the downstream end. Similar simulated avian predation has been successfully used in several studies (e.g. Loot et al. 2002; Griffiths et al. 2004).

Behavioural observations were made during both day-time and night-time after 2 d of acclimation. During day-time (between 9 AM and 1 PM), direct visual observations were carried out for a period of 20 min every 3 d. In this case, six different channels were haphazardly selected each day. At the end of the expt., each channel was observed for five periods of observation. Night-time observations (between 9 PM and 11 PM) were done every 5 d and four channels per night were observed for 10 min each. Night-time observations were carried out using a spotlight with a red filter so as not to disturb fish (Reebs 2002; Hansen & Closs 2005). In total, each channel was observed for two periods of observation. For both day-time and night-time periods, observations began 5 min after the observer's arrival to habituate the fish to his presence, and they were done so that each observation session coincided with a feeding event. Seven behaviours were scored during each trial. Three of them were related to territorial activity: the number of aggressive interactions (chase, displays and nip, Kalleberg 1958; Keenleyside & Yamamoto 1962) initiated by each fish, the number of movements and the distance of each movement. A movement was scored only when a fish moved a distance greater than its body length and when this fish remained at its new site for at least 10 s in a fixed position. A movement was not scored when it occurred during an aggressive act. Three other behaviours were related to foraging activity: the distance from the feeder, the number of food items consumed (i.e. feeding rate) and the percentage of time spent in a feeding position (a fish was considered in a feeding position when it was out of a refuge, facing the current and propped up on its pectoral fins). Finally, the percentage of time hiding under a refuge was a behaviour related to predator avoidance.

Individual performance was evaluated as the daily instantaneous growth rates (G) over the course of the expt. using the following formula:

$$G_{ij} = \frac{\ln(W_{i_{t2}}) - \ln(W_{i_{t1}})}{(t_2 - t_1)}$$

where G_{ij} is the daily growth rate of individual i in the channel j, W_{it1} is the weight of this fish at the beginning of the considered growth period, W_{it2} is the weight of the individual at the end of the expt. and $(t_2 - t_1)$ equalled 30 d.

Statistical Analysis

As experimental design differed between day-time and night-time observational periods, data were analysed separately. In all analyses, we used each individual fish as the replicate unit.

Principal component analyses (PCAs) were used to summarize the behaviours related to territory defence and feeding activity and express them as two indexes: an index of territorial activity and an index of foraging activity. We used the percentage of time spent hiding under a refuge as a direct index of predator-avoidance activity. Principal component analysis reduces dimensionality and eliminates co-linearity between behaviours. The projected scores on the first principal component (PC1) were used as a synthetic independent variable reflecting the index of territoriality or the index of foraging activity of each fish (see Sloman et al. 2002; Bell 2005). Preliminary analysis showed that the distance from the food source contributed little to the first axis of the PCA used to calculate the index of foraging activity (Blanchet S., pers. obs.). To avoid losing information, this behaviour was analysed independently and the index of foraging activity was reduced to the time spent in a feeding position and the number of food items consumed. Table 1 summarizes the results of the PCAs and the behaviours that were considered in the study. For both indexes, a high PC1 score (i.e. strong positive values) indicated highly territorial fish and highly active fish (in terms of foraging activity).

We first used mixed-linear models to test for the effect of predatory cues treatments on each of the four dependent factors (index of territoriality, index of foraging activity, distance from the food source and index of predator avoidance). We used the individual value for each period of observation (five periods during the day and two periods during the night) as the replicate unit, to test for a possible effect of habituation to the predatory cues. If habituation occurred, one would expect that the effects of predatory cues should decrease over the expt. To deal with potential spatial (within a channel) and temporal (between observation periods) dependency among replicate units, we used 'individual' nested within 'period of observation' nested within 'channel' as the random factor (Pinheiro & Bates 2000). 'Period of observation', 'predatory cues treatment' and the resulting interaction were the fixed factor.

Table 1: Summary of the behaviours analysed to describe the anti-predatory response of Atlantic salmon when facing single or combined predatory cues. The results of principal component analyses used to summarize the behaviours related to territorial defence and feeding activity as two indexes are also presented. Loadings represent the strength of the correlation between a trait and a canonical axis

Behaviour	Loading
(a) Index of territory defence	
Number of given aggression	0.806
Number of movements	0.884
Size of the movements	0.721
Cumulative variance explained	65.13%
(b) Index of feeding activity	
Feeding rate	0.772
Time spent in a feeding position	0.772
Cumulative variance explained	59.56%
(c) Distance from the feeding source	–
(d) Index of predator avoidance	
Time spent under a refuge	–

For day-time data and for each behaviour, we additionally computed mixed-linear models using 'chemical cues' and 'physical cues' as two fixed factors, to test the additivity of the combined predator-cue effects. A significant interaction between both factors would indicate that the effect of the two combined predatory cues was not the sum of their individual effects (i.e. deviation from the null expectation, Sih et al. 1998). In these models, we did not included 'period of observation' as a fixed factor as previous analyses provided little support for a habituation effect (see Results). Thus, individual values were averaged over all observation periods, and 'individual' was nested within 'channel' and used as the random factor (Pinheiro & Bates 2000).

To evaluate the cost of the behavioural responses on a secondary fitness trait, we computed a mixed-linear model to compare the effect of the different combination of predatory cues on the growth rate of Atlantic salmon. We included the 'initial body weight', 'predatory cues treatment' and the resulting interaction as fixed factors. 'Individual', nested within 'channel', was the random factor (Pinheiro & Bates 2000).

Finally, we used hierarchical partitioning (Chevan & Sutherland 1991) to assess the relative importance of each behaviour in explaining the individual growth rate of Atlantic salmon fry. A single model was constructed for each predation treatment and we used the individual as the replicate unit. As individual recognition was not possible during the night, only day-time data were used in these analyses. In hierarchical partitioning, all possible models in a multiple regression setting are jointly considered to identify the most likely causal factor. This process involved computation of the increase of the fit of all models with a particular factor compared with the equivalent model without that factor (for more details and examples, see Chevan & Sutherland 1991; Mac Nally 2000; Pont et al. 2005). Hierarchical partitioning provides an independent explanatory power (I) for each variable. Statistical significances of the independent contributions of variables were tested by a randomization routine which yielded Z-scores for the generated distribution and a measure of statistical significance based on an upper 0.95 confidence limit.

The index of predator avoidance was arcsine transformed to meet the assumption of homoscedascticity (Bartlett's test, Zar 1999). All statistical analyses were performed using R version 2.2.1. (R Development Core Team 2005).

Ethical Note

No fish were injured during the expts. This study was carried out according to legislation in Canada under license No. 2004-140.

Results

Behaviour

During the day-time and night-time, we found no evidence that fish habituate to the exposition to physical or chemical cues, as 'period of observation' and the interaction term did not significantly influence any of the four behaviours (Table 2a,b).

During the day, we found that three of the four behaviours (index of territoriality, distance from the food source and index of predator avoidance) were affected by predatory cues treatment (Table 2a; Fig. 2). For these three significant variables, we found that the effect of the combined predatory cues was stronger than the effects of one predatory cue alone and significantly different from the control treatment (see Fig. 2). The effect of combined predatory cues significantly decreased the index of territoriality, increased the distance between fish and the food source and the time fish spent under a refuge (i.e. increased the index of predator avoidance). When each predatory cue was considered as a single independent factor, we found the index of foraging activity was not altered by any of the predatory cues (Table 3). Chemical cues alone affected the index of territoriality and the index of predator avoidance (Table 3). Physical cues had a significant effect on the distance from the food source and the index of predator avoidance (Table 3). The significant interaction found between chemical and predatory cues for the index of territoriality indicated that this behaviour was non-additively altered by the combination of cues (Table 3; Fig. 2). In contrast, distance from the food source and the index of predator avoidance were altered additively by the combination of cues (see Table 3; the interaction terms were nonsignificant for both behaviours).

During the night, only the index of territoriality was affected by predatory cues (see Table 2b; the index of territoriality was significantly higher in the control treatments, results not shown).

Growth

At the end of the expt., growth ranged from 0.0149 to 0.0107 according to the treatment we considered

Table 2: Mixed-models analyses of behavioural responses to predators by Atlantic salmon fry during both the day (a) and night (b). Analysis included the fixed effects of period of observation predatory treatment. During the night, the response 'Index of predator avoidance' was not included as no fish were observed under a refuge

		Response variables			
		Index of territoriality	Index of foraging activity	Position from the food source	Index of predator avoidance
(a) Day-time					
Predatory cues treatment	$F_{3,8}$	5.10	0.93	4.65	10.62
	p	0.029	0.469	0.036	0.003
Period of observation	$F_{4,32}$	0.94	0.81	0.71	0.71
	p	0.455	0.526	0.589	0.588
Period × predatory cues treatment	$F_{12,32}$	1.47	0.52	0.29	0.42
	p	0.183	0.881	0.986	0.942
(b) Night-time					
Predatory cues treatment	$F_{3,8}$	6.32	1.84	2.51	–
	p	0.017	0.218	0.132	–
Period of observation	$F_{4,32}$	2.18	2.36	1.55	–
	p	0.177	0.134	0.248	–
Period × predatory cues treatment	$F_{12,32}$	0.57	3.04	0.68	–
	p	0.651	0.092	0.584	–

(results not detailed). We detected no statistical effect of the initial body weight of the fish ($F_{1,30} = 0.56$, $p = 0.458$) and of the predatory cues treatments ($F_{3,8} = 1.32$, $p = 0.328$) on growth rate. The interaction was not significant ($F_{3,30} = 0.58$, $p = 0.627$).

Relative Contribution of Behaviours

Using hierarchical partitioning, we demonstrated that the independent effect of the index of territoriality was high (between 40% and 50%) and significant in explaining the individual growth rate in the control, the physical cues and the chemical cues treatments (Fig. 3a,b,c). The three other behaviours had a low and nonsignificant independent explanatory power. In contrast, in the combined predatory cues treatment, the explanatory power of the index of territoriality was low and nonsignificant while most of the total variance (more than 50%) was explained by the index of foraging activity alone (Fig. 3d).

Discussion

In this study, we evaluated the effects of multiple predatory cues on several antipredatory behaviours of a territorial animal. We demonstrated that the nature and magnitude of behavioural modification varied according to the response variables we considered. Indeed, three of the four behaviours we considered (i.e. index of territoriality, index of predator avoidance and distance from the food source) were significantly altered by at least one of the predatory cues while foraging activity was not altered. Moreover, of the three altered behaviours, two of them (i.e. index of predator avoidance and distance from the food source) were altered in an additive way, while the index of territoriality was affected in a non-additive way. Although many studies have investigated the antipredatory response of prey when faced with multiple predatory cues (e.g. Hartman & Abrahams 2000; Chivers et al. 2001; Smith & Belk 2001; Amo et al. 2004 ; Stapley 2004; Mikheev et al. 2006), few of them have incorporated more than one behavioural component. Smith & Belk (2001) studied the effects of diet and hunger level of the predator *Lepomis cyanellus* on two antipredatory behaviours (predator avoidance and predator inspection) of the western mosquitofish (*Gambusia affinis*). In accordance with our findings, they found that the nature of behavioural alteration (additive or non-additive) depended on the response variable that was examined. Here, by considering a larger set of behavioural components, we generalized the conclusion that the behaviours related to the antipredatory response are relatively independent of each other.

Two of the behavioural responses we observed (the index of predator avoidance and the distance from the food source) varied in accordance with the threat sensitivity hypothesis (Helfman 1989), indicating that territorial vertebrates are able to integrate multiple sensory inputs to accurately assess the risk of predation and respond in an additive manner to

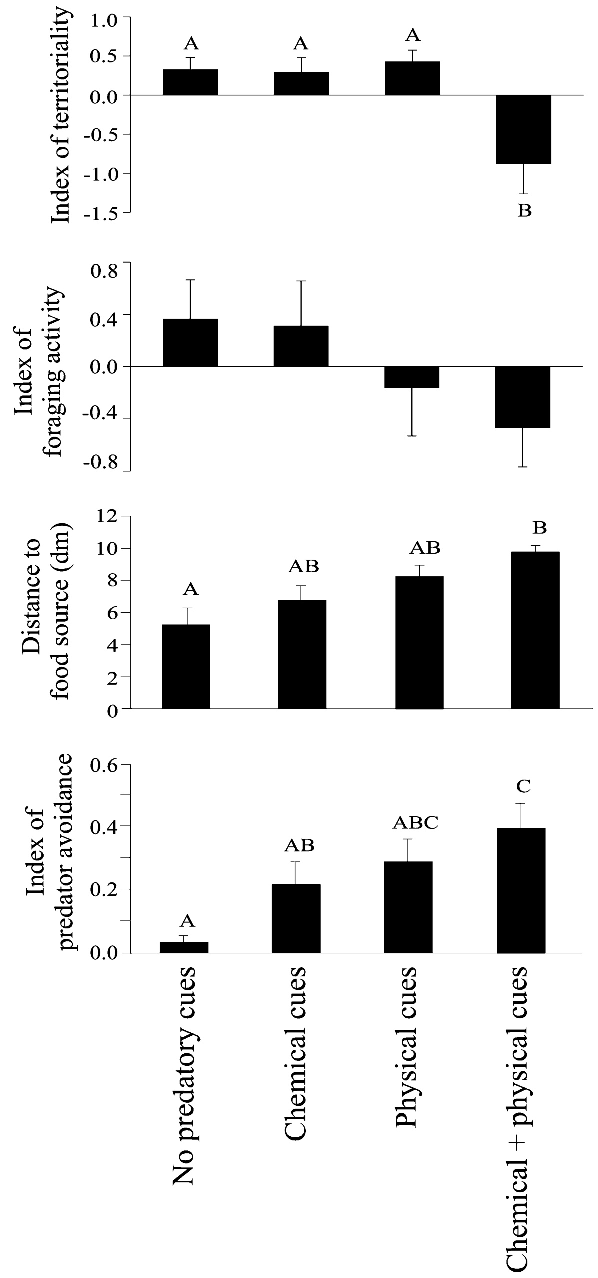

Fig. 2: Patterns of the day-time antipredation response of Atlantic salmon when reared under four different experimental treatments ('no predatory cues', 'chemical cues', 'physical cues' and 'chemical + physical cues' means fish reared with no predatory cues; with chemical cues emitted by a piscivorous rainbow trout fed with freshly dead salmon; with physical cues mimicking a bird bill; with combined chemical and physical cues respectively). Four behaviours were considered: an index of territoriality, an index of foraging activity, the distance from the food source and an index of predator avoidance. Figures identified by letters (A, B or C) indicated the behavioural traits significantly affected by treatments. Two bars significantly identical ($p > 0.05$, contrast tests) are identified by the same letter. Data are $\bar{x}$ (±SE).

the threat posed by the predator(s). Smith & Belk (2001) similarly found that for predator avoidance, western mosquitofish integrate multiple cues in an additive way. For these two behaviours, the effect of physical cues was stronger than the effect of chemical cues and the effect of the combined cues was higher. In our expt., physical cues probably signalled a more proximate danger and therefore elicited a stronger behavioural response. Similarly, Weightman & Arsenault (2002) concluded that the sea pen *Otilosarcus gurneyi* (Cnidaria) was more sensitive to physical contact with the predator sea star *Dermaterias imbricate* than to chemical cues emanating from this predator. However, our result contrasted with the results of Watson et al. (2004), who found that the salamander *Plethodon angusticlavius* was more receptive to chemical cues emanating from the snake *Diadophis punctatus* than to a physical attack by that predator. The authors acknowledged that the relative weakness of the response to physical attack may be related to the attack having occurred away from the foraging area (Watson et al. 2004). Overall, these results suggest that prey are more sensitive to direct cues (i.e. physical cues) rather than indirect cues (i.e. chemical cues).

In contrast to these behaviours, the index of territorial defence was affected in a non-additive manner to the simulated cues. Indeed, Atlantic salmon did not modify their activity of territoriality when exposed to chemical or physical cues, but they significantly reduced their activity when exposed to the combined cues. This threshold-like response, also called 'hypersensitive response' (Helfman & Winkelman 1997; Brown et al. 2006), indicated that Atlantic salmon modified their territorial defence only when the perceived risk of predation was maximal (i.e. when cues were combined). It may be that the fitness benefit of defending a territory is higher than the risk of being preyed upon, until the risk of predation becomes too severe. Indeed, in salmonids as in many territorial species, a territory provides access to a food source, but also offers a refuge to fish faced with a predator (Fausch 1984; Nakano 1995). Moreover, when predation risk is important, the risk of losing a territory to other congeners is probably lowered because the general activity of the fish decreases.

The foraging activity of Atlantic salmon was not altered by any cue. As previously stated, fish moved further away from the food source as predation risk increased. However, they were able to maintain a relatively high foraging activity, indicating that this activity probably imparted an important fitness

Table 3: Mixed-models analyses of behavioural response to predators by Atlantic salmon fry during day-time. Analyses included the fixed effects of the two simulated predators (Physical cues and Chemical cues) to test the non-additivity of antipredator response

		Response variables			
Source of variation		Index of territoriality	Index of foraging activity	Position from the feeding source	Index of predator avoidance
Chemical cues	$F_{1,8}$	6.34	0.54	2.07	10.26
	p	0.035	0.485	0.187	0.012
Physical cues	$F_{1,8}$	4.41	2.11	11.97	22.88
	p	0.068	0.184	0.009	0.001
Chemical × physical cues	$F_{1,8}$	5.96	0.14	0.02	0.58
	p	0.040	0.716	0.888	0.469

advantage that was at least as important as directly avoiding predators (Lind & Cresswell 2005; Ajie et al. 2007).

We demonstrated that predation only slightly affected behaviours during the night, indicating that the effect of predation was low during this period. None of the fish were observed hiding under a refuge during the night, indicating that fish did not try to avoid predators. Several authors have observed that prey species lower their day-time activity, relative to activity during the night, in the presence of diurnal predators (reviewed in Reebs 2002; Kronfeld-Schor & Dayan 2003). These results are in accordance with our finding as they suggested that prey are generally less sensitive to predators during the night. However, as pointed out by Reebs (2002), most of these studies used light intensity (or moon phase) as a surrogate of predation risk; here we provide the first direct evidence that predation risk had little effect on antipredation responses during the night.

Our predatory manipulations were probably exaggerated compared to what occurs in natural systems. Indeed, there is little chance that, in a natural stream or lake, a fish remains continuously near a predator if it can detect its proximity or if it is regularly attacked by this predator. Thus, we must acknowledge that the behavioural responses we described here are probably the maximum that might be expected in natural systems. However, despite the strength of these responses, we found no evidence that the growth rate of Atlantic salmon was altered. This result is consistent with other studies finding no evidence for an effect of predatory cues on prey growth rate (Van Buskirk & Yurewicz 1998; Nyström & Abjornsson 2000; Teplitsky et al. 2003; Schoeppner & Relyea 2005; but see Skelly 1992 ; Relyea 2003; Teplitsky et al. 2004). This result demonstrates that Atlantic salmon adopted a behavioural tactic that aimed to maximize the energy gain while limiting the risk of being eaten. Indeed, behavioural tactics changed according to the risk of predation. When the risk of predation was perceived as relatively low (i.e. during the control, the chemical cues and the physical cues treatments), the activity of territory defence explained an important amount of the total variance observed in individual growth rate. We propose that when the risk of predation was low, it was beneficial for Atlantic salmon to defend their territory as it provided both a source of food and a refuge in case of predator attack. However, when the risk of being preyed upon was higher (i.e. during the combined cues treatment), feeding activity was the better predictor of individual growth rate. According to Biro et al. (2003), these results suggest that fish are behaving in a manner that growth rate was maximized, while adjusting behaviours that are not directly related to growth. Some authors have speculated that fish may favour night-time activity to meet basal energy requirements and thus avoid activity during the day, the time at which the risk of predation is predicted to be higher (Metcalfe et al. 1999). However, we found that feeding rate was approx. five times lower during the night than during the day (in average: 0.054 items consumed/fish/10 min during the night and 0.285 items consumed/fish/10 min. during the day, results not shown), indicating that most of the feeding activity took place during the day (see also Fraser & Metcalfe 1997).

The antipredatory response is a composite of many behaviours and for the first time, we simultaneously included behaviours that were related to predator avoidance and to other fitness-enhancing activities in the presence of multiple predatory cues. We found evidence that Atlantic salmon were able to discriminate between chemical and physical cues, and to integrate this information as a surrogate of predation risk. According to that information, the magnitude and the nature of the response was

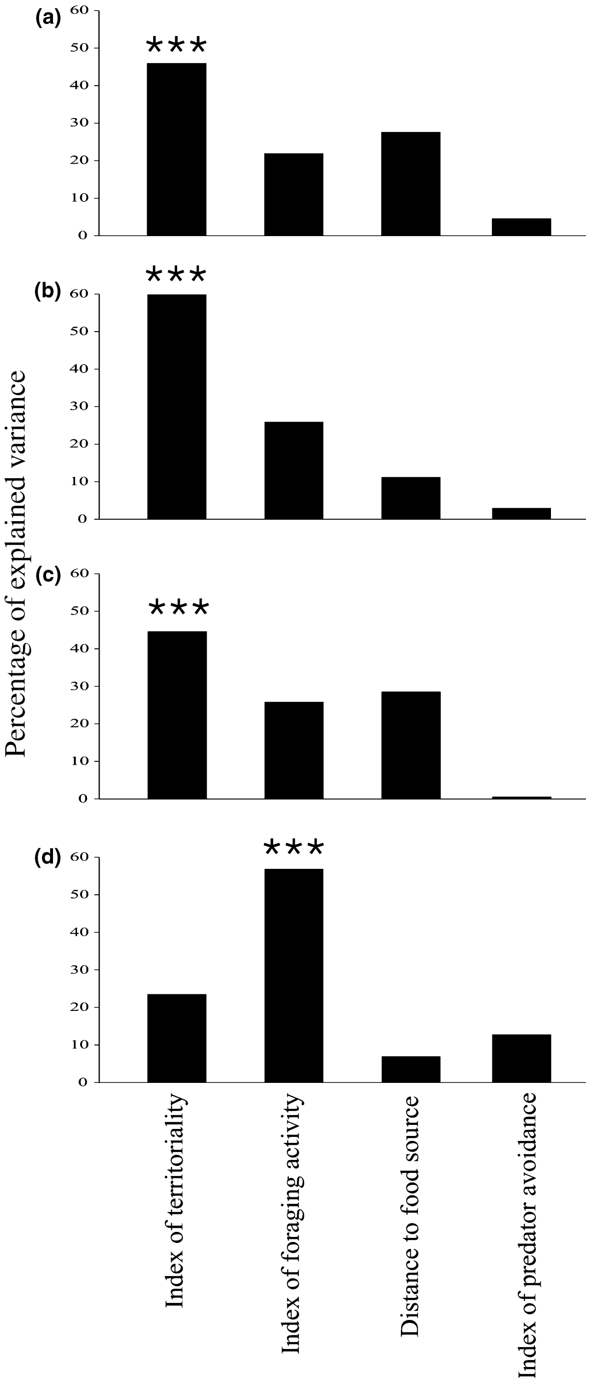

Fig. 3: The independent contributions (given as the percentage of the total explained variance) of the predictor variables for the individual growth rate of Atlantic salmon (a) when reared with no predatory cues; (b) when reared with chemical cues emitted by a piscivorous rainbow trout fed with freshly dead salmon; (c) when reared with physical cues mimicking a bird bill; (d) when reared with combined chemical and physical cues. Only day-time behaviours were considered in the analysis. Significant independent effects are indicated by ***.

strongly dependent upon the behaviour that was considered. Some behavioural responses were unaltered by the presence of predatory cues whereas others were altered in a non-additive or an additive way. Behaviours that conferred a growth advantage in a given situation were only slightly altered by the presence of simulated predator(s). In contrast, the behaviours that directly allowed fish to avoid predators were more sensitive to predation risk. This behavioural plasticity appeared to be adaptive as it allowed fish to maintain growth while decreasing their risk of being preyed upon.

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Literature Cited

Ajie, B. C., Pintor, L. M., Watters, J., Kerby, J. L., Hammond, J. I. & Sih, A. 2007: A framework for determining the fitness consequences of antipredator behaviour. Behav. Ecol. **18**, 267—270. doi: 10.1093/beheco/arl064.

Amo, L., Lopez, P. & Martin, J. 2004: Wall lizards combine chemical and visual cues of ambush snake predators to avoid overestimating risk inside refuges. Anim. Behav. **67**, 647—653. doi: 10.1016/j.anbehav.2003.08.005.

Bell, A. M. 2005: Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). J. Evol. Biol. **18**, 464—473. doi: 10.1111/j.1420-9101.2004.00817.x.

Biro, P. A., Post, J. R. & Parkinson, E. A. 2003: From individuals to populations: prey fish risk-taking mediates mortality in whole-system experiments. Ecology **84**, 2419—2431.

Brown, G. E. 2003: Learning about danger: chemical alarm cues and local risk assessment in prey fishes. Fish Fisheries **4**, 227—234.

Brown, G. E., Bongiorno, T., DiCapua, D. M., Ivan, L. I. & Roh, E. 2006: Effects of group size on the threat-sensitive response to varying concentrations of chemical alarm cues by juvenile convict cichlids. Can. J. Zool. **84**, 1—8. doi: 10.1139/Z05-166.

Chevan, A. & Sutherland, M. 1991: Hierarchical partitioning. Am. Stat. **45**, 90—96.

Chivers, D. P. & Smith, R. J. A. 1998: Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. Ecoscience **5**, 338—352.

Chivers, D. P., Mirza, R. S., Bryer, P. J. & Kiesecker, J. M. 2001: Threat-sensitive predator avoidance by slimy sculpins: understanding the importance of visual versus chemical information. Can. J. Zool. **79**, 867—873. doi: 10.1139/cjz-79-5-867.

Eklov, P. 2000: Chemical cues from multiple predator–prey interactions induce changes in behaviour and growth of anuran larvae. Oecologia **123**, 192—199.

Fausch, K. D. 1984: Profitable stream position for salmonids : relating specific growth rate to net energy gain. Can. J. Zool. **64**, 441—451.

Fraser, N. H. C. & Metcalfe, N. B. 1997: The costs of becoming noctural: feeding efficiency in relation to light intensity in juvenile Atlantic salmon. Funct. Ecol. **11**, 385—391.

Griffiths, S. W., Brockmark, S., Höjesjö, J. & Johnsson, J. I. 2004: Coping with divided attention: the advantage of familiarity. Proc. R. Soc. Lond. B **271**, 695—699. doi: 10.1098/rspb.2003.2648.

Hansen, E. A. & Closs, G. P. 2005: Diel activity and home range size in relation to food supply in a drift-feeding stream fish. Behav. Ecol. **16**, 640—648. doi: 10.1093/beheco/ari036.

Hartman, E. J. & Abrahams, M. V. 2000: Sensory compensation and the detection of predators: the interaction between chemical and visual information. Proc. R. Soc. Lond. B **267**, 571—575.

Helfman, G. S. 1989: Threat-sensitive predator avoidance in damselfish–trumpetfish interactions. Behav. Ecol. Soc. **24**, 47—58.

Helfman, G. S. & Winkelman, D. L. 1997: Threat sensitivity in bicolor damselfish: effects of sociality and body size. Ethology **103**, 369—383.

Hirvonen, H., Ranta, E., Piironen, J., Laurila, A. & Peuhkuri, N. 2000: Behavioural responses of naive Arctic charr young to chemical cues from salmonid and non-salmonid fish. Oikos **88**, 191—199.

Hoffmann, H. A., Benson, M. E. & Fernald, R. D. 1999: Social status regulates growth rate: consequence for life-history strategies. Proc. Natl. Acad. Sci. USA **96**, 14171—14176.

Kalleberg, H. 1958: Observations in a stream tank of territoriality and competition in juvenile salmon and trout (*Salmo salar* L. and *S. trutta* L.). **39**, 55—98, Institute of Freshwater Research, Drottningholm, Sweden.

Kats, L. B. & Dill, L. M. 1998: The scent of death: chemosensory assessment of predation risk by prey animals. Ecoscience **5**, 361—394.

Keenleyside, M. H. & Yamamoto, F. T. 1962: Territorial behaviour of juvenile Atlantic salmon (*Salmo salar* L.). Behaviour **19**, 139—169.

Klemetsen, A., Amundsen, P. A., Dempson, J. B., Jonsson, B., Jonsson, N. & Mortensen, E. 2003: Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Artic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. Ecol. Freshw. Fish. **12**, 1—59.

Krams, I. 2000: Length of feeding day and body weight of great tits in a single-and a two-predator environment. Behav. Ecol. Soc. **48**, 147—153.

Kronfeld-Schor, N. & Dayan, T. 2003: Partitioning of time as an ecological resource. Annu. Rev. Ecol. Evol. Syst. **34**, 153—181.

L'Abee-Lund, J. H., Aass, P. & Saegrov, H. 2002: Long-term variation in piscivory in a brown trout population: effect of changes in available prey organisms. Ecol. Freshw. Fish. **11**, 260—269.

Leduc, A. O. H. C., Kelly, J. M. & Brown, G. E. 2004: Detection of conspecific alarm cues by juvenile salmonids under neutral and weakly acidic conditions: laboratory and field tests. Oecologia **139**, 318—324. doi: 10.1007/s00442-004-1492-8.

Lima, S. L. & Dill, L. M. 1990: Behavioural decisions made under the risk of predation: a review and prospectus. Can. J. Zool. **68**, 619—640.

Lind, J. & Cresswell, W. 2005: Determining the fitness consequences of antipredation behavior. Behav. Ecol. **16**, 945—956. doi: 10.1093/beheco/ari075.

Loot, G., Aulagnier, S., Lek, S., Thomas, F. & Guégan, J. F. 2002: Experimental demonstration of a behavioural modification in a cyprinid fish, *Rutilus rutilus* (L.), induced by a parasite, *Ligula intestinalis* (L.). Can. J. Zool. **80**, 738—744. doi: 10.1139/Z02-043.

Ludwig, G. X., Hokka, V., Sulkava, R. & Ylonen, H. 2002: Otter *Lutra lutra* predation on farmed and free-living salmonids in boreal freshwater habitats. Wildlife Biol. **8**, 193—199.

Mac Nally, R. 2000: Regression and model-building in conservation biology, biogeography and ecology: the distinction between and reconciliation of 'predictive' and 'explanatory' models. Biodivers. Conserv. **9**, 655—671.

Martel, G. & Dill, L. M. 1995: Influence of movement by coho salmon (*Oncorhynchus-kisutch*) parr on their detection by common mergansers (*Mergus merganser*). Ethology **99**, 139—149.

Metcalfe, N. B., Huntingford, F. A., Graham, W. D. & Thorpe, J. E. 1989: Early social status and the development of life-history strategies in Atlantic salmon. Proc. R. Soc. Lond. B **236**, 7—19.

Metcalfe, N. B., Fraser, N. H. C. & Burns, M. D. 1999: Food availability and the nocturnal vs. diurnal foraging trade-off in juvenile salmon. J. Anim. Ecol. **68**, 371—381.

Mikheev, V. N., Wanzenbock, J. & Pasternak, A. F. 2006: Effects of predator-induced visual and olfactory cues on 0+ perch (*Perca fluviatilis* L.) foraging behaviour. Ecol. Freshw. Fish. **15**, 111—117. doi: 10.1111/j.1600-0633.2006.00140.x.

Mirza, R. S. & Chivers, D. P. 2001: Are chemical cues conserved within salmonid fishes? J. Chem. Ecol. **27**, 1641—1651.

Nakano, S. 1995: Competitive interactions for foraging microhabitats in a size-structured interspecific dominance hierarchy of two sympatric stream salmonids in a natural habitat. Can. J. Zool. **73**, 1845—1854.

Nyström, P. & Abjornsson, K. 2000: Effects of fish chemical cues on the interactions between tadpoles and crayfish. Oikos **88**, 181—190.

Pinheiro, J. C. & Bates, D. M. 2000: Mixed-effects Models in S and S-PLUS. Springer-Verlag, New York.

Pont, D., Hugueny, B. & Oberdorff, T. 2005: Modelling habitat requirement of European fishes: do species have similar responses to local and regional environmental constraints? Can. J. Fish. Aquat. Sci. **62**, 163—173. doi: 10.1139/F04-183.

R Development Core Team 2005: R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, available at: http://www.R-project.org..

Reebs, S. G. 2002: Plasticity of diel and circadian activity rhythms in fishes. Rev. Fish Biol. Fish. **12**, 349—371.

Reinhardt, U. G. 1999: Predation risk breaks size-dependent dominance in juvenile coho salmon (*Oncorhynchus kisutch*) and provides growth opportunities for risk-prone individuals. Can. J. Fish. Aquat. Sci. **56**, 1206—1212.

Relyea, R. A. 2003: How prey respond to combined predators: a review and an empirical test. Ecology **84**, 1827—1839.

Schoeppner, N. M. & Relyea, R. A. 2005: Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. Ecol. Lett. **8**, 505—512. doi: 10.1111/j.1461-0248.2005.00744.x.

Sih, A., Crowley, P., McPeek, M., Petranka, J. & Strohmeier, K. 1985: Predation, competition, and prey communities – a review of field experiments. Annu. Rev. Ecol. Evol. Syst. **16**, 269—311.

Sih, A., Englund, G. & Wooster, D. 1998: Emergent impacts of multiple predators on prey. Trends Ecol. Evol. **13**, 350—355.

Skelly, D. K. 1992: Field evidence for a cost of behavioral antipredator response in a larval amphibian. Ecology **73**, 704—708.

Sloman, K. A., Wilson, L., Freel, J. A., Taylor, A. C., Metcalfe, N. B. & Gilmour, K. M. 2002: The effects of increased flow rates on linear dominance hierarchies and physiological function in brown trout, *Salmo trutta*. Can. J. Zool. **80**, 1221—1227. doi: 10.1139/Z02-105.

Smith, M. E. & Belk, M. C. 2001: Risk assessment in western mosquitofish (*Gambusia affinis*): do multiple cues have additive effects? Behav. Ecol. Soc. **51**, 101—107. doi: 10.1007/s002650100415.

Stapley, J. 2004: Do mountain log skinks (*Pseudemoia entrecasteauxii*) modify their behaviour in the presence of two predators? Behav. Ecol. Soc. **56**, 185—189. doi: 10.1016/j.anbehav.2004.02.008.

Teplitsky, C., Plenet, S. & Joly, P. 2003: Tadpoles' responses to risk of fish introduction. Oecologia **134**, 270—277. doi: 10.1007/s00442-002-1106-2.

Teplitsky, C., Plenet, S. & Joly, P. 2004: Hierarchical responses of tadpoles to multiple predators. Ecology **85**, 2888—2894.

Van Buskirk, J. & Yurewicz, K. L. 1998: Effects of predators on prey growth rate: relative contributions of thinning and reduced activity. Oikos **82**, 20—28.

Watson, R. T., Mathis, A. & Thompson, R. 2004: Influence of physical stress, distress cues, and predator kairomones on the foraging behavior of Ozark zigzag salamanders, *Plethodon angusticlavius*. Behav. Process. **65**, 201—209.

Weightman, J. O. & Arsenault, D. J. 2002: Predator classification by the sea pen *Ptilosarcus gurneyi* (Cnidaria): role of waterborne chemical cues and physical contact with predatory sea stars. Can. J. Zool. **80**, 185—190. doi: 10.1139/Z01-211.

Zar, J. H. 1999: Biostatistical Analysis, 4th edn. Prentice Hall International, Upper Saddle River, NJ.