Cues from Introduced Fish Alter Shelter Use and Feeding Behaviour in Adult Alpine Newts

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Abstract

Amphibians are particularly affected by alien fish introductions and are declining worldwide. However, the behavioural mechanisms behind the observed cases of coexistence and exclusion patterns between adult amphibians and fish are poorly understood. In the present study, we aimed at testing the hypothesis that adult newts display different feeding and space use behaviour in the presence of fish cues (i.e. access less food resources and use more shelters than when fish cues are absent). To achieve this we measured behavioural patterns in 100 adult Alpine newts (*Mesotriton alpestris*) in a replicated laboratory design (20 tanks × 7 replicates across time). Half of trials involved individuals in indirect (visual and olfactory) contact with goldfish (*Carassius auratus*), a non-predatory species for adult newts. In the presence of fish, significantly more newts hid under shelters than in their absence, but this difference decreased over time. A lower number of newts fed in comparison with controls. These results show that newts responded to fish presence even in the absence of direct contact, but the differences were small. Newts decreased vital activities such as exploration of open areas and feeding. They also adjusted shelter use over time, suggesting a process of habituation or a risk assessment in the absence of direct risk. These results reveal that exploring behavioural patterns can aid in understanding the causes of exclusion and coexistence patterns between fish and amphibians.

Introduction

The ability to respond adequately to predatory risk is of primary importance for species survival (Lima 1998; Brown & Chivers 2005). By adopting anti-predator strategies at the behavioural, developmental or morphological level, prey can persist in the same environment as predators (Lima & Dill 1990; Relyea 2001). However, anti-predator behaviours (risk effects) often carry high costs, such as reduced growth (Relyea 2002; Teplitsky et al. 2003) and fecundity (DeWitt et al. 1998). Therefore, avoiding costs associated with unnecessary reactions to non-predators is particularly adaptive (Smith et al. 2008b; Gall & Mathis 2010). Accordingly, prey should be able to differentiate between predatory and non-predatory heterospecifics (Schmitz 2005). Predator recognition and effective anti predator responses can result from co-evolution of prey and predator (Lima & Dill 1990). However, worldwide introductions of alien species into natural environments can significantly alter systems, as native prey either may not recognize the new species as predators or react to introduced non-predatory species inaccurately by an unnecessary anti-predatory effort (Cox & Lima 2006; Gall & Mathis 2010).

Species in inland waters are particularly vulnerable to effects of alien species (Francis 2012) because they often experience greater limitations in habitat availability than terrestrial organisms (Gherardi 2010). In such habitats, introduced species have been identified as one of the main causes of species declines, especially in amphibians (McGeoch et al. 2010). Previous research has shown the inability of salamander larvae to recognize an introduced predatory fish as a treat and thus to respond by an anti-predator behaviour (Pearson & Goater 2009; Gall & Mathis 2010). The larvae responses in this case were not different from responses to a native non-predatory fish (Gall & Mathis 2010). Similarly, Pearson & Goater (2009) observed that larvae demonstrated anti-predator behaviour when they were exposed to minnows (non-predatory introduced fish) and by reducing the foraging opportunities, larvae suffered from mortality and decreased growth rate. Several studies highlighted amphibian population extirpation in the presence of introduced fish (Kats & Ferrer 2003; Denoël et al. 2005) and resilience after alien fish removal (Knapp et al. 2001, 2007; Vredenburg 2004). The main identified mechanism was predation on either the adult or larval stage. Both exclusion and coexistence patterns have also been identified between fish spe-

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cies that are predator of amphibian larvae, but not adults (Monello & Wright 1999; Denoël & Lehmann 2006; Denoël et al. 2009). This is true for goldfish (*Carassius auratus*), which is the predominant introduced ornamental fish species in the world (Savini et al. 2010). It is well established that goldfish can consume anuran tadpoles, salamander eggs and larvae (Monello & Wright 2001) and despite an observed coexistence between these species, previous studies showed a strong reduction of the abundance of European newts (*Mesotriton alpestris*, *Lissotriton helveticus* and *Lissotriton vulgaris*) in ponds containing goldfish (Denoël & Lehmann 2006; Denoël & Ficetola 2008; Denoël et al. 2009).

Investigating behavioural mechanisms of interspecific interactions is particularly relevant for understanding species coexistence, but also in the framework of behavioural conservation by determining how species react to anthropogenic pressures (Shuster & Wade 2003; Caro 2007). Most of the studies of anti-predator behaviour in amphibians have used subjects at the larval stage (Orizaola & Brana 2003; Mathis et al. 2008; Dayton & Fitzgerald 2011), both in anurans (Horat & Semlitsch 1994; Relyea 2001; Smith et al. 2010) and urodeles (Van Buskirk & Schmidt 2000; Orizaola & Brana 2005; Schmidt & Van Buskirk 2005). Despite known post-exposure effects (Barbasch & Benard 2011), very little is known about exposure at the adult stage. In the presence of a potential predator, the first reaction of aquatic amphibian prey is often to reduce activity (Mathis et al. 2003; Smith et al. 2008b; Gall & Mathis 2010) and change habitat, such as using more shelters (Hecnar & MCloskey 1997; Orizaola & Brana 2003; Teplitsky et al. 2003). This is particularly the case for the smaller organisms, which are more vulnerable to predation (Brown & Taylor 1995; Eklov 2000; Laurila et al. 2006). Finally, a fundamental component of an effective anti-predatory response is the ability of prey to distinguish between dangerous and safe species or habitats (Brown & Chivers 2005). The ability to recognize and distinguish predator and dangerous location from non-predator and safe location is innate: naïve preys respond adaptively to threats (Laurila 2000; Epp & Gabor 2008; Gall & Mathis 2010). It can also be learned by associating alarm cues with a potential predator or a specific place (Ferrari & Chivers 2011). An effective anti-predator response implies correct risk assessment through the detection of varied cues (Stynoski & Noble 2012) and an adjustment of response (Ferrari et al. 2005). How an animal uses alarm cue information to choose a response is a very important issue (Mathis 2003; Warkentin 2005). Even if multimodal cues (chemical, acoustic, visual and/or tactil) improve detection and provide more accurate information for the prey organism (Partan & Marler 2005), chemical cues of predators alone can alter the behaviour and/or growth of newt embryos (Orizaola & Brana 2004), newt larvae or tadpoles (Eklov 2000; Mathis & Vincent 2000), and adult aquatic salamanders (Woody & Mathis 1998; Epp & Gabor 2008; Mathis & Unger 2012). In contrast to chemical cues, visual cues alone are usually not enough to allow amphibians to distinguish between a predator and a non-predatory species; because of poor visibility in turbid pond waters, individuals receive chemical information more quickly and accurately than visual information (Mathis & Vincent 2000).

Using this framework, we aimed to determine the potential effects of an introduced alien species, the goldfish (predator of amphibian larvae, but not adults), on newt behaviour. Laboratory experiments have revealed that amphibian tadpoles were able to respond efficiently to the presence of goldfish (indirect contact) by shift in activity and habitat, but the response of adult amphibians has yet to be investigated (Takahara et al. 2003; Smith et al. 2008b). In particular, we investigated the effect of indirect contact with goldfish on the adult stage of the Alpine newt *M. alpestris*. Goldfish were not present in the pond of our newts sample but Alpine newts are known to coexist with introduced goldfish at other nearby locations (Denoël & Ficetola 2008). As the newts of our study were completely naïve of goldfish and may then identify them as a threat, we hypothesized that they would respond to this introduction by avoiding open habitats, and thus reduce feeding in such habitats. On the other hand, we expected that newts would reduce their anti-predator response over time in the absence of direct threats from fish.

Materials and Methods

Study Organism

One hundred adult Alpine newts *M. alpestris* (50 males and 50 females; formerly *Triturus alpestris*) were caught on a road in Bassenge the 14th and 15th of March, 2011 (Province of Liege, Belgium, 50°45'N–5°36'E, 70 m elevation a.s.l.). Males differ from females in their secondary sexual traits (cloacal shape, coloration, dorsal crest). Males are also smaller than females ((mean ± SE) total length: 8.36 ± 0.54 and 9.69 ± 0.66 cm respectively). Individuals were captured during their migration from their hibernation place to their breeding pond. Alpine newts are aquatic during the breeding season (March–May) and terrestrial throughout the rest of the year. Because newts were captured before they entered the pond, they had not yet reproduced that year. After the capture, newts were brought directly to the laboratory (20 min drive) in refrigerated boxes (5–10°C; 3 l) containing wet towels.

Laboratory Maintenance

We distributed the newts in 20 identical tanks with five individuals per tanks (60 × 60 cm, 40 cm water level; 135 l). Tanks were open on the top and equipped with an oxygen diffuser. We kept males and females separately so their sexual behaviour did not interfere with the present analysis. The bottoms of the tanks were covered with slates. We provided one large shelter (18 × 25 cm), which constituted a stone placed obliquely along one of the walls of each tank (Fig. 1). Water temperature was maintained at an average of 14.79°C (SE = 0.35°C). We established a photoperiod that reflected the natural cycle of the capture location: beginning at 12 h light – 12 h dark and ending at 13 h light – 11 h dark at the end of the experiment. Subjects were fed every day, during the afternoon, with 50 mg of *Chironomus* larvae per newt. This food amount corresponded to the natural feeding rate of this species (Denoël & Joly 2001). Finally, all newts were released into their capture habitat after the experiment (May 2011).

Goldfish

The goldfish came from an animal store. We bought them more than a month before the beginning of the experiment. They were stored in four tanks (60 × 60 cm, 40 cm high). After allowing 1 wk for the newts to habituate to their new environment, we placed goldfish in ten of the experimental newt tanks (one individual goldfish per tank). They were each placed in a transparent tank (30 × 20 × 22 cm, 9 l) that was
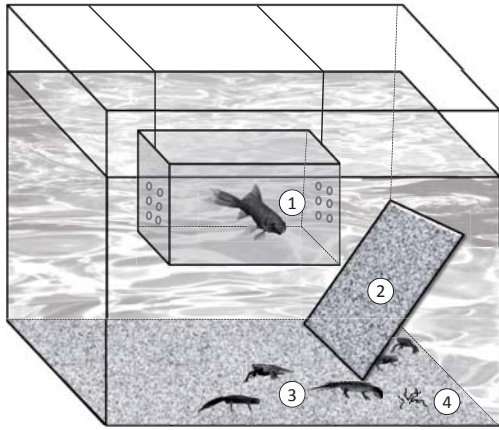
Fig. 1: Experimental tank: 1. Submerged goldfish tank, 2. Shelter (behind an oblique stone), 3. Newts (five per tank), 4. Food (*Chironomus* larvae).

submerged in the newt tank (Fig. 1). Small holes allowed water contact between newt and fish tanks. Thus, we allowed no direct contact between fish and newts, but indirect ones through visual and chemical cues. Goldfish had a mean (±SE) total length of 10.23 ± 1.02 cm. From the day of their arrival to the end of the experiment, they were each fed every 2 d with 400 mg of *Chironomus* larvae. In this way, they received the same food as newts to avoid detection and effects of fish diet cues (Chivers & Smith 1998).

Experimental Procedure

Visual observations began 2 d after fish introduction and were conducted by only one observer. Observations were replicated every 3 d during 6 wk for a total of 14 d of data collection. We analyzed two behavioural patterns: shelter use and feeding. Shelter use data collection consisted of an observation session early in the afternoon. We used a scan sampling method (Martin & Bateson 2007), based on previous Alpine newt research (Winandy & Denoël 2011). We scanned each aquarium subsequently and repeated this ten times per session (a scan every 5 min). These ten scans assessed the average percentage of hidden newts (i.e. under the shelter) in each aquarium. As only two micro-habitats were provided, the number of newts in the open area was automatically deduced from this number and the total of newts in each aquarium. Feeding data collection consisted of a focal observation in the late afternoon. *Chironomus* larvae (250 mg) were placed on the bottom of each aquarium where they were visible from all newts in the aquarium (Fig. 1). We assessed feeding behaviour by determining the percentage of newts who fed on *Chironomus* larvae, the time from food delivery to the first feeding act of a newt (=the minimum latency to forage), and the group mean latency to forage (i.e. the mean of time taken by each newts in the tank to start feeding). These two variables are dependent because the first feeding act can attract other newts to eat (Martin 1982). We simultaneously observed several aquariums for each 20-min focal sampling period.

Statistical Analysis

We used a generalized linear model that accounted for repeated measures to test the effect of fish on shelter use and percentage of feeding across time. The behavioural scores were computed on the basis of 20 scans, i.e. two successive series of ten scans to achieve normality. So we had a total of seven pe-

riods of time (replicate 1 = days 0–6; replicate 2 = days 7–12; replicate 3 = days 13–18; replicate 4 = days 19–24; replicate 5 = days 25–30; replicate 6 = days 31–36; replicate 7 = days 37–42). Because of dependence between the minimum latency to forage and group mean latency to forage, we first used a repeated measures multivariate ANOVA (MANOVA) to assess the effect of fish on the general latency to forage. Secondly, we used a generalized linear model that accounted for repeated measures to test separately the effect of each latency variable. For all analyses, we chose an *a priori* level of significance of 0.05. We conducted all statistical analyses in STATISTICA 10 (Statsoft-France 2011).

Results

Shelter Use

There was no significant main effect of fish on shelter use (Mean ± SE = 34.32 ± 5.76% and 25.95 ± 4.92% for fish and control treatment, respectively; Table 1). Nevertheless, there was a significant effect of fish over time (Table 1). Only during the first period (days 0–6), newts in the fish treatment were significantly more hidden than newts in the control treatment ($F_{3,16}$ = 4.68, p = 0.02; Mean ± SE = 41 ± 5.67% and 16.7 ± 3.26%, respectively; Fig. 2). At this time 47% of the difference between groups was explained by fish presence.

*Feeding*

There was a significant main effect of fish presence, sex and time on the percentage of newts feeding, as well as a significant interaction effect between fish presence, sex, and time (Table 2 and Fig. 3a). Whatever time period and sex, in the presence of fish significantly fewer newts ate prey items in comparison to the control group (Mean ± SE = 33.8 ± 6.32% and 43.7 ± 5.4%, respectively). Thirty-two percent of the difference between groups was explained by fish presence. The latencies of foraging differed significantly in terms of fish presence (MANOVA: λ = 0.56, $F_{2,15}$ = 5.96, p = 0.01) and sex (MANOVA: λ = 0.42, $F_{2,15}$ = 10.41, p < 0.01) but neither effect of time and interaction were significant (p > 0.1). There was a marginally-significant effect of sex and time on the minimum latency to forage (p = 0.06) and no significant interaction with time and sex were found (Table 2 and Fig. 3b). The minimum latency to forage was at a mean ± SE of 767.09 ± 119.42 s and 651.84 ± 113.84 s for the fish treatment and the control group, respectively. Sex and its interaction with time had a significant effect on the

Table 1: Effect of fish on shelter use in Alpine newts: generalized linear model with repeated measures evaluating the effect of fish presence on shelter use and its interaction with sex and time

Behaviour	Factors	df	F	p
Shelter use	Fish	1,16	2.33	0.15
	Sex	1,16	1.25	0.28
	Fish × sex	1,16	0.06	0.81
	Time	6,96	3.51	**<0.01**
	Time × fish	6,96	2.55	**0.03**
	Time × sex	6,96	0.43	0.86
	Time × fish × sex	6,96	2.55	0.20

Significant values are highlighted in bold.
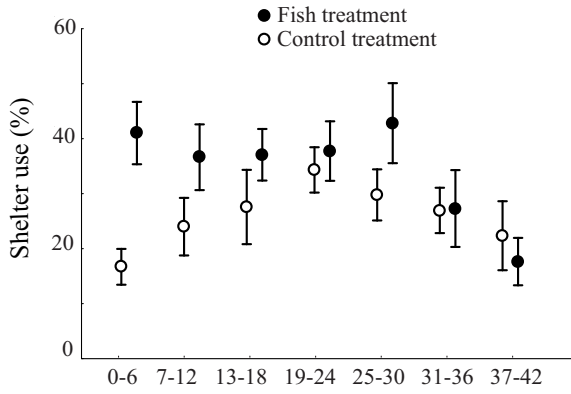
Fig. 2: Effect of fish on shelter use in Alpine newts (Mean ± SE values). See Table 1 for statistical results.

group mean latency to forage but there was no significant effect of fish and its interaction with time and sex (see Table 2 and Fig. 3c) between the two groups (Mean ± SE = 743.2 ± 69.2 s and 681.87 ± 72.66 s for fish treatment and control group respectively).

Discussion

In this study, we assessed the response of naive adults Alpine newts to a non-predatory fish, the goldfish. We found that indirect contact with goldfish can alter adult newt behaviour: the newts reduced their activity level outside shelters, and thereby had lower access to food. These results show that adult newts respond to fish cues by exhibiting an anti-predator response, suggesting that they identify a goldfish as a potential predator.

Newts occupied their habitat differently in the presence of fish by using shelters more often, and thus open areas less. Shelter or vegetation use (Hecnar & MCloskey 1997; Orizaola & Brana 2003; Teplitsky et al. 2003; Stuart-Smith et al. 2008) and the decrease of activity (Mathis et al. 2008; Gall & Mathis 2010; Smith et al. 2010) are common anti-predator responses. Nevertheless, fish introduction often coincides with habitat modification and reduction of vegetation (Richardson et al. 1995; Hartel et al. 2007), making coexistence more difficult. By minimizing activity outside the shelter, prey organisms reduce the chance of being detected and attacked by predators (Stauffer & Semlitsch 1993; Skelly 1994). However, this anti-predatory freezing (Stynoski & Noble 2012) implies a reduction of time spent on other important activities, such as feeding (Lima & Dill 1990; Bridges 2002). This is particularly the case when predatory risk is high (Lima & Dill 1990; Lima 1991). Such decreases in feeding rates could have detrimental long term effects by impairing individual fitness through reduction in growth, development and fertility (Semlitsch 1987). Therefore organisms must often make trade-offs between foraging and the risk of being preyed upon (Horat & Semlitsch 1994; Bridges 2002). During all experiments, in the fish treatment, a significantly lower proportion of newts fed. Moreover there was a significant effect of fish presence on general latency to forage: control newts fed on *Chironomus* larvae faster than newts in fish treatment. In particular, the minimum latency to forage was faster in the control group (marginally-significant effect). The depression of feeding behaviour is probably the result of both increases in shelter use and decreases in activity (Bridges 2002).

Table 2: Effect of fish on feeding behaviour in Alpine newts: general linear model with repeated measures evaluating the effect of fish presence on feeding behaviours and its interaction with sex and time

Feeding behaviours	Factors	df	F	p
Percentage of feeding newts	Fish	1,16	7.38	**0.02**
	Sex	1,16	37.25	**<0.01**
	Fish × sex	1,16	2.42	0.14
	Time	6,96	2.2	0.05
	Time × fish	6,96	1.25	0.29
	Time × sex	6,96	0.7	0.65
	Time × fish × sex	6,96	2.49	**0.03**
Minimum latency to forage	Fish	1,16	3.98	0.06
	Sex	1,16	13.64	<0.01
	Fish × sex	1,16	0	0.98
	Time	6,96	3.44	**<0.01**
	Time × fish	6,96	0.46	0.84
	Time × sex	6,96	2.17	0.05
	Time × fish × sex	6,96	1.4	0.22
Group mean latency to forage	Fish	1,16	1.37	0.26
	Sex	1,16	7.95	**0.01**
	Fish × sex	1,16	0.16	0.7
	Time	6,96	1.76	0.12
	Time × fish	6,96	0.72	0.63
	Time × sex	6,96	2.87	**0.01**
	Time × fish × sex	6,96	1.52	0.18

Significant values are highlighted in bold.
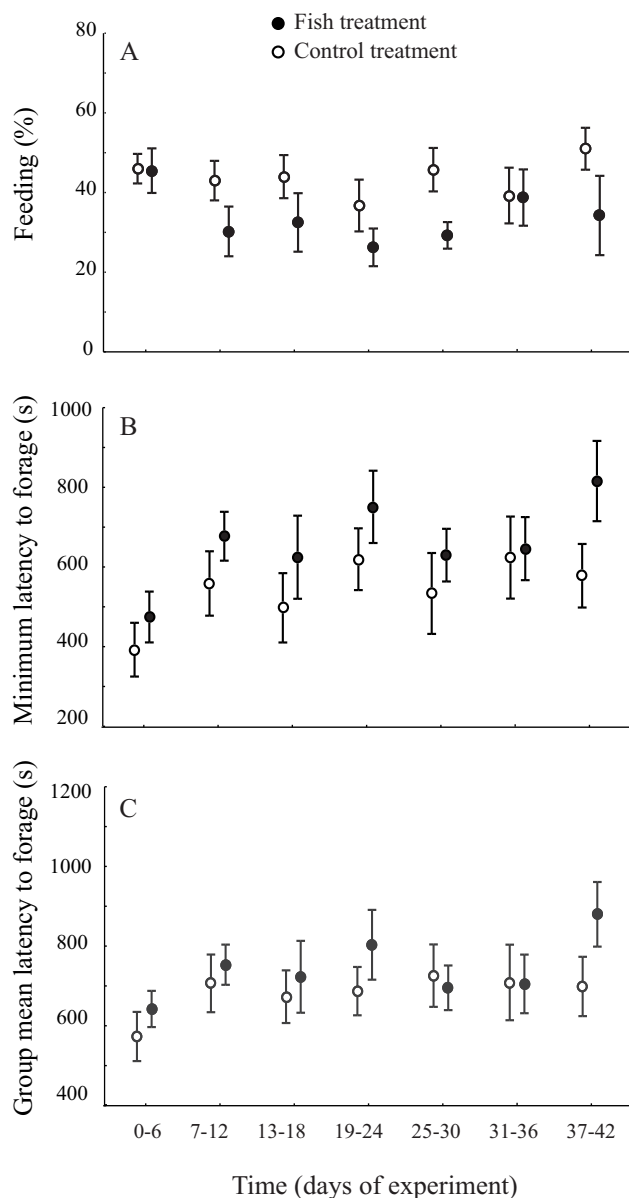
Fig. 3: Effect of fish on feeding behaviours in Alpine newts (Mean ± SE values): percentage of feeding newts (a), minimum latency to forage (b) and group mean latency to forage (c). See Table 2 for statistical results.

After 6 d of cohabitation with fish, fish presence no longer had a significant effect on newt shelter use. This suggests a possible process of habituation, which could be the result of adaptive decision making through risk assessment (Ferrari et al. 2010). As contact with fish occurred only through cues, fish could not physically disturb the newts. The lack of negative direct interactions between newts and fish could explain why differences remained low and did not persist over time. For feeding behaviour, we did not find any habituation processes as the number of feeding newts did not vary with time. Throughout the experiment, when newts came to feed, fish were particularly agitated because they detected *Chironomus* larvae. It is then possible than newts did not decrease their anti-predator response over time because of this disturbance. It is of primary importance that organisms correctly identify predator cues and make good decisions to avoid potential costs (e.g., the risk of not detecting a potential unknown predator and being eaten). This is reflected in the exclusion of long-toed salamander by trout (a carnivorous fish), where the salamander larvae are unable to recognize the introduced trout as a threat and therefore lacked any anti-predator

response (Pearson & Goater 2009). The second risk is that of not detecting or learning that an unknown organism is not dangerous, which could lead to an unnecessary decrease of important activities such as feeding (Ferrari & Chivers 2011). In this case there are no real direct survival benefits of avoidance, but only a cost. Nevertheless this anti-predatory response is not necessarily maladaptive because in this study sufficient food was available for all newts. The risk allocation hypothesis of Lima & Bednekoff (1999) suggests that the change in energy reserves determines predation risk and anti-predator decision making. In this study, the cost of exhibiting an unnecessary anti-predator behaviour was not really important because newts were not starving and could still forage later. Adjustment and readjustment of behavioural responses relative to the need and risk assessment takes time, especially if individuals lack information about the predator. The uncertainty can cause the persistence of an anti-predator behaviour despite the absence of threat (Sih 1992). This issue of uncertainty regarding the predator may be especially true with the introduction of alien species.

Conclusions

Our study aimed at understanding some mechanisms of coexistence and exclusion between species with a special focus on introduced ornamental fish and native newts. We demonstrated that interactions are not only complex at the larval stage, as shown in previous studies (e.g. with goldfish: Monello & Wright 2001; Takahara et al. 2003; Smith et al. 2008a), but also at the adult stage (see also Woody & Mathis 1998; Mathis & Unger 2012). Examining behavioural patterns can be pertinent in explaining why the same species of fish and amphibians coexist in some instances and experience exclusion in others. Specifically, fish introductions have been shown to negatively affect newt populations, sometimes as far as extirpation from ponds or lakes (Denoël et al. 2005). However, there are also numerous documented cases where such species exist in sympatry (Monello & Wright 1999; Denoël & Lehmann 2006; Denoël et al. 2009). It is also important to determine long-term effects of coexistence between adult amphibians and introduced fish. In the case of non-predatory introduced fish and adult newts, the fitness costs associated with anti-predator behaviour could become a disadvantage for newts in the long run (Gall & Mathis 2010). Adaptive response requires an increase of anti-predator behaviour in the presence of a predator (Lima 1998), but also entails not responding to a non-predator (Smith et al. 2008b). Learning mechanisms through habituation and risk assessment are essential areas of study for understanding how a complex situation such as fish introduction results in coexistence or exclusion of native amphibians with fish.

Acknowledgements

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