Amphibians forgo aquatic life in response to alien fish introduction

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Species with complex life cycles are good models to understand trade-offs between life in hostile and favourable habitats. Newts remain in breeding wetlands for a long period and are strongly affected by fish introduction; however, mechanisms of the exclusion observed in the field are still not well known. In particular, whether newts skip breeding and leave water for land in response to fish introduction and how aquatic shelter may influence their choice remain open questions. To investigate these questions, we experimentally studied the use of aquatic and terrestrial habitats during the breeding season of palmate newts, *Lissotriton helveticus*, in the presence and absence of goldfish, *Carassius auratus*. We determined the consequences of habitat choice on newt fitness by assessing sexual activity and number of eggs. There was a strong, significant avoidance of the aquatic environment in the presence of fish, particularly when no aquatic shelter was available. This escape from the water had an impact on reproduction: newts decreased their sexual activity and laid fewer eggs. The availability of shelters favoured coexistence but did not prevent a large proportion of the newts from leaving water and skipping reproduction. This study shows how the presence of fish and the absence of aquatic shelters can lead to newts forgoing aquatic life, thus improving our understanding of the mechanisms behind the coexistence and exclusion patterns found in the wild. More broadly, these data contribute to explaining aquatic versus terrestrial life in favourable and unfavourable environments.

Keywords: amphibian decline, behavioural avoidance, complex life cycles, habitat selection, species exclusion

Many species have a complex life cycle that requires both terrestrial and aquatic habitats; while wintering and aestivating often take place on land, reproduction is exclusively aquatic (Pittman, Osbourn & Semlitsch, 2014; Wells, 2007; Wilbur, 1980). Theoretical models have proposed that the selection of terrestrial and aquatic habitats depends on their respective costs and benefits (Werner, 1986; Wilbur & Collins, 1973). In particular, habitat choice has functional relationships with reproduction and survival, and, consequently, fitness covaries directly with habitat use (Morris, 2011). Both abiotic and biotic habitat characteristics have been shown to affect habitat selection (Albeny-Simões et al., 2014; Amburgey, Bailey, Murphy, Muths & Funk, 2014; Indermaur, Schaub, Jokela, Tockner & Schmidt, 2010). For instance, adult amphibians can shorten the time spent in water when the density is too high (Grayson & Wilbur, 2009), while they can remain aquatic during winter to avoid colder conditions on land (Pilliod, Peterson & Ritson, 2002). During reproduction, a pond's hydroperiod strongly regulates the habitat suitability for aquatic-breeding species (McCaffery, Eby, Maxell & Corn, 2014; Williams, Heeg & Magnusson, 2007), but habitat quality is also determined by resource competition and predation risk (Indermaur et al., 2010; Rieger, Binckley & Resetarits, 2004).

The presence of potential predators, such as fish, is a critical determinant of the occurrence and abundance of prey, including organisms as varied as amphibians and insects (Binckley & Resetarits, 2008; Blaustein, Kiflawi, Eitam, Mangel & Cohen, 2004). It is believed that the evolution of pond-breeding species, such as newts, was made possible because such habitats are usually devoid of fish or because predatory pressure is limited. Indeed, in contrast to many anurans, newts are long-term breeders that remain in the water for weeks or even months to actively court and lay eggs one by one (Wells, 2007). The selection of a suitable breeding habitat is, therefore, of primary importance both for the adult newts' offspring and for themselves. The introduction of alien species, such as fish in naturally fish-free environments, thus constitutes a major threat by risking amphibians' use of the aquatic environment (McGeoch et al., 2010). Among other factors, this contributes to the current amphibian decline, which has been identified as part of the sixth world mass extinction (Stuart et al., 2004; Wake & Vredenburg, 2008). A large number of environmental studies support these conclusions by showing exclusion patterns between fish and pond-breeding amphibians (Kats & Ferrer, 2003), amphibian extirpations after fish introduction (Pilliod et al., 2010) and resilience after fish removal (Knapp, Boiano & Vredenburg, 2007; Vredenburg, 2004).

The disappearance of many amphibian populations after fish introductions can have different causes. The two primary reported detrimental effects are direct predation on adults, larvae and/or eggs (Leu, Lüscher, Zumbach & Schmidt, 2009; Monello & Wright, 2001; Orizaola & Braña, 2006) and competition through the consumption of important resources such as food (Joseph, Piovia-Scott, Lawler & Pope, 2011). An additional mechanism that can lead to exclusion patterns is avoidance behaviour. On the one hand, the adults can check breeding sites and avoid those occupied by potential predators such as fish (Binckley & Resetarits, 2003; Hopey & Petranka, 1994; Orizaola & Braña, 2003a; Rieger et al., 2004). On the other hand, behavioural avoidance can be expressed in the aquatic habitat where amphibians can increase the use of microhabitats as shelter in the presence of fish (Orizaola & Braña, 2003b; Teplitsky, Plenet & Joly, 2003). This is nevertheless a costly antipredator response, as it can impair fitness

through a reduction of important activities, such as foraging and reproduction (Lima, 1998; Winandy & Denoël, 2013a, b).

An important nonconsumptive effect that has not been studied so far is whether the absence of newts from fish habitats is due to their transient or permanent escape from the aquatic environment to the terrestrial habitat and whether the availability of aquatic shelter could affect this behavioural choice (for instance, by allowing coexistence between newts and fish). Understanding these patterns is essential to explaining the use of aquatic versus terrestrial environments by amphibians in fish and fishless habitats (Langerhans, 2007).

To test these hypotheses, we monitored the aquatic versus terrestrial use of habitats and the associated fitness consequences in the palmate newt, *Lissotriton helveticus*, in response to the presence of goldfish, *Carassius auratus* (Fig. 1). We used goldfish as a model species because this is the most introduced and invasive ornamental fish in the world (Maceda-Veiga, Escribano-Alacid, de Sostoa & García-Berthou, 2013) and because it has caused amphibian decline (Meyer, Schmidt & Grossenbacher, 1998). Both exclusion and coexistence patterns between newts and goldfish have been found, but the mechanisms that may explain these observations are still not well known (Denoël, Džukić & Kalezić, 2005; Denoël & Ficetola, 2014). Goldfish can forage on eggs and larvae of salamanders (Monello & Wright, 2001), but are not thought to predate on adult newts. This suggests that exclusion patterns observed in the field can be particularly explained by nonconsumptive processes such as behavioural avoidance (Winandy & Denoël, 2013b). We chose palmate newts because they are in decline and are affected by goldfish in the wild (Denoël, 2012; Denoël & Ficetola, 2014). Our approach was experimental, using a laboratory replicated design during the entire breeding period of newts. We assessed a gradient of behavioural responses considering four different treatments mimicking high- to poor-quality aquatic habitats using both biotic and abiotic factors: no fish with aquatic shelter, no fish without aquatic shelter, fish with aquatic shelter and fish without aquatic shelter. Along this environmental gradient, we expected an increase in the use of the terrestrial habitat and a decrease in sexual activity and reproductive success of the newts.

Figure 1. Palmate newt leaving water for the terrestrial habitat in response to goldfish presence.

METHODS

Study organism and laboratory maintenance

We caught 96 palmate newts by dip netting (48 individuals of each sex) at the beginning of the reproductive period (11 March 2014) in a fishless pool (Romerée, Belgium: 50°08'N, 4°40'E). Newts had no prior experience with fish because no fish were found in the varied waterbodies within the usual dispersal distances of newts (i.e. up to 400 m) around the capture site. In their natural habitat, they coexisted with alpine newts, *Ichthyosaura alpestris*. After capture, we directly brought newts to the laboratory, keeping sexes separated, in several tanks (2 h transport); six 9-litre tanks filled with water and stuffed with towels were placed in two large refrigerated boxes of 230 litres. At the end of the experiment, all newts were released in good health into their capture pond (26 May 2014) in accordance with capture permits.

In the laboratory, we distributed the newts between 24 identical and independent aquaria (60 × 60 cm, 40 cm water level; 135 litres) with four individuals (two males and two females) per aquarium. Before their release in the aquaria, we carefully measured them using a ruler. They had a mean ± SE snout–vent length (i.e. from the tip of the snout to the end of the cloaca) of 4.24 ± 0.03 cm (*N* = 96). An oxygen diffuser was placed in each aquarium to maintain a high oxygenation of water (mean ± SE = 9.70 ± 0.05 mg/litre). The ambient air temperature was regulated to maintain water temperature at an average of 14.7 °C (SE = 0.05 °C). We established a photoperiod of 13:11 h light:dark (Lumilux de lux 2350 lm daylight tube, L36W/12-950 and Sylvania Professional tube, 36 W DECOR183). In all aquaria, a terrestrial habitat was composed of a 23 x 46 cm floating 'Turtle Dock' (Zoo Med Laboratories Inc., San Luis Obispo, CA, U.S.A.) covered by natural moss taken at the capture location (watered daily to maintain the humidity at an average of 84.75%, SE = 0.05%) and containing a shelter (14 x 11 cm and 6 cm high). In 12 of the 24 aquaria, we placed one large aquatic shelter (20 × 60 cm) closed by a grid to avoid fish entering. In every aquarium, we also put two supports for egg laying (strips of filter papers). In the aquarium providing an aquatic shelter, one support was placed under the shelter. Newts were fed with 50 mg of *Chironomus* larvae per newt every day in the afternoon. This food is a typical newt prey and the quantity given was ad libitum, corresponding to the newts' needs. Food was provided on the dock and in the water under a grid to avoid consumption by fish (the grid was removed at the end of the day when all the food was consumed).

We obtained the goldfish from the Aquarium of Liège and stored them in a large tank (180 × 80 cm, 60 cm water depth) in our laboratory. They had never been in contact with amphibians prior to the experiment and they were all healthy. They had a mean ± SE standard length (i.e. from the tip of the snout to the posterior end of the last vertebra) of 11.4 ± 0.18 cm (*N* = 12). We placed the fish at the same time as the newts in 12 of the 24 experimental newt tanks as described above; the conditions applied to the fish were similar to those applied to the newts. Goldfish and newts were in direct contact, but the fish could not get into the aquatic shelter. Similar to the newts, goldfish were fed with *Chironomus* larvae (500 mg per fish every day).

Experimental procedure

We used four experimental treatments ($N = 6$ aquaria and $N = 24$ newts for each treatment): control (no fish) with an aquatic shelter, control without aquatic shelter, fish with aquatic shelter and fish without aquatic shelter (Fig. 2). The experiment lasted 8 weeks (17 March–25 May 2014), i.e. the duration of the aquatic and reproductive period.

Figure 2. Experimental aquaria ($N = 24$) with four treatments: (a) control (i.e. without fish) with aquatic shelter, (b) control without aquatic shelter, (c) fish with aquatic shelter and (d) fish without aquatic shelter. In all aquaria, a terrestrial habitat with a shelter was available. Four newts were present in each aquarium.
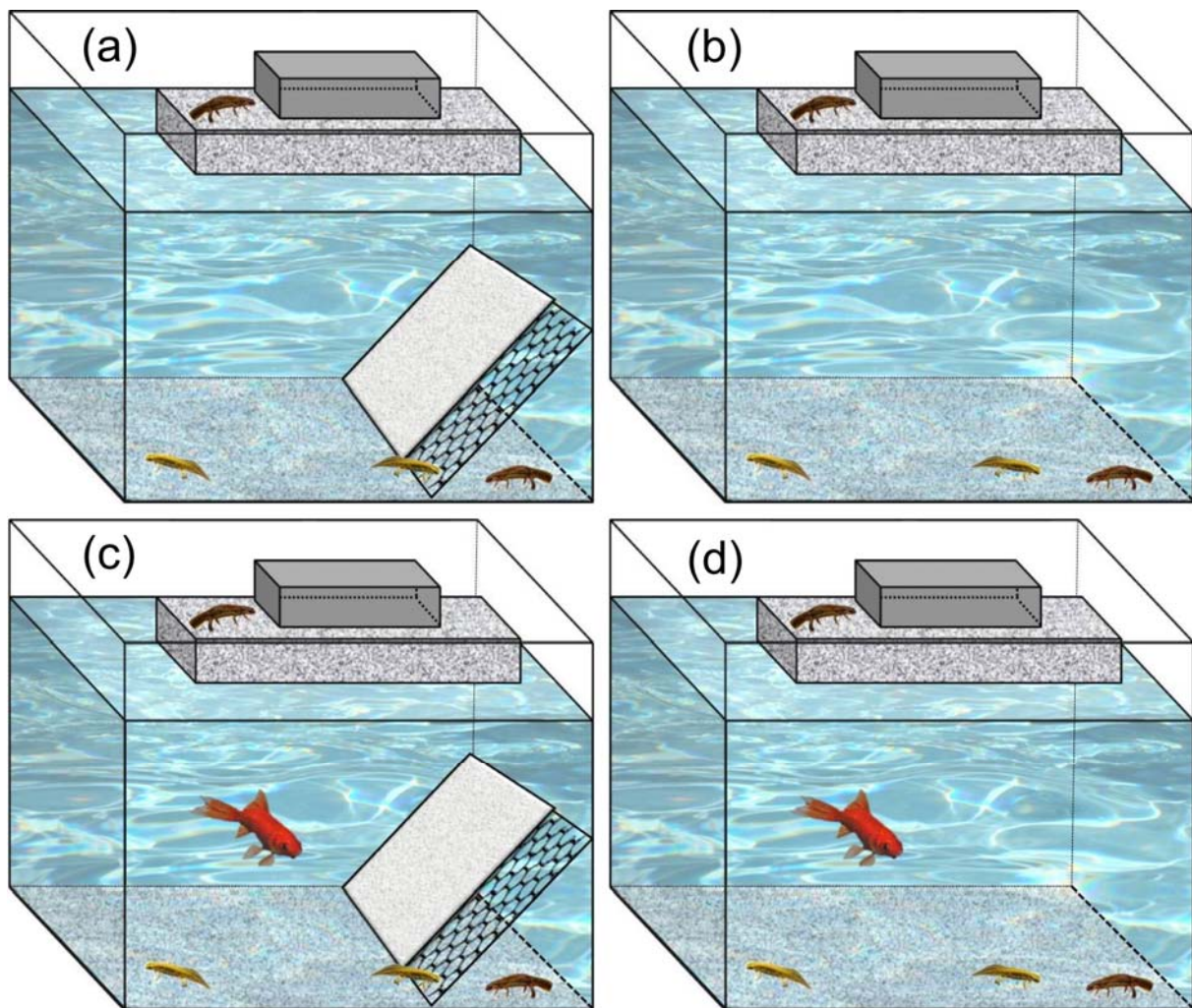
By using sketches of unique features of the newts, we were able to visually recognize each of the four individuals from each aquarium (Winandy & Denoël, 2011). To assess the use of the terrestrial habitat by newts, we used a scan sampling (Martin & Bateson, 2007) method twice a day (morning and afternoon), 5 days a week. Therefore, we obtained the proportion of terrestrial habitat use of each newt based on 10 repeated measures every week during the 8 weeks (i.e. 7680 observations of presence/absence in each habitat). To assess sexual activity, we used a focal sampling (Martin & Bateson, 2007) method on the four newts of each aquarium for 3 min. We repeated the focal sampling measure twice a day (morning and afternoon), 5 days a week (during the 8 weeks) to obtain the number of courtship events for each newt per week (i.e. 7680 observations of occurrence of sexual activity). To assess reproductive success, we counted the eggs in each aquarium every week (i.e. 192 records). We changed the egg supports every day, placing them in independent tanks to avoid predation risk.

Statistical analyses

We used a generalized linear mixed model (GLMM) to test the effects of three variables as fixed effects: fish (presence versus absence), aquatic shelter (presence versus absence) and week (ordinal variable; 1–8 weeks), and interactions between these variables, on (1) the proportion of terrestrial habitat use (using binomial error distribution), (2) the number of sexual activity events (using a Poisson error distribution) and (3) the number of eggs (using a Poisson error distribution). We assessed significance using likelihood-ratio tests (ANOVA of the model with a particular explanatory variable versus the model without that variable). In analyses of terrestrial habitat use and sexual activity, aquaria and individuals were included as random factors (individuals nested within aquaria). In the analyses of eggs, we included aquaria as a random factor. We also included, as a covariate, the number of newts present in the water for the analyses on sexual activity and the number of females present in water for the analyses on eggs. Finally, to assess the gradient of responses between the four treatments on terrestrial habitat use and reproduction, we used a Tukey contrast test. We chose an a priori level of significance of 0.05. Analyses were performed in R 3.0.2 (www.r-project.org) using lme4 and multcomp packages.

Ethical note

The aim of this study was to determine only the potential nonconsumptive effects of goldfish on adult newts by observing their behaviour. Consequently, care was taken to plan the experiment accordingly and, thus, to avoid any newts being harmed. Moreover, newts had the opportunity to hide in protected areas from fish; a large terrestrial shelter was available all the time so newts could avoid any direct contact with fish. This study mimics natural conditions in wild and garden ponds where goldfish are introduced and coexist with newts (Denoël & Ficetola, 2014). No alternative design would have been suitable since a direct interaction between fish and newts is the key to understanding newt avoidance and the only situation present in the wild (Winandy & Denoël, 2013b). Therefore, goldfish were able to touch newts

and could try to 'peck' them. However, given gape size limitations of fish in comparison with newt size, fish could not catch or wound them. However, the direct contact may frighten the newts. The study was carried out in an accredited laboratory of the University of Liège (LA1610429), and the research project was accepted by the university's ethics commission (Protocol No. 1246). The collecting permit was issued by the Service Public de Wallonie (SPW), following approval by the Conseil Superieur Wallon de la Conservation de la Nature. In the laboratory, all individuals were checked and fed every day. At the end of the experiment, newts were released alive and unharmed into their capture habitat following the recommendations of the capture permit. All materials used for capture and maintenance were well washed and disinfected before and after use following the recommendations of the study permit.

RESULTS

Terrestrial habitat use

The GLMM analysis of the proportion of terrestrial habitat use (Table 1) indicated a significant effect of fish: in the presence of fish, more newts left the water than in the control treatment (mean ± SE of 0.16 ± 0.01 and 0.67 ± 0.02 newts on land per aquarium for control and fish treatments, respectively). There was also a significant effect of the presence of an aquatic shelter: when a shelter was available in the water, fewer newts left the water than when it was absent (0.28 ± 0.02 and 0.55 ± 0.02 newts on land per aquarium for the treatments with and without aquatic shelter, respectively). Finally, there was a significant effect of week indicating an increase in terrestrial habitat use with time. Tukey contrast tests showed a significant gradient of response between the four experimental treatments (Fig. 3a). There was also a significant interaction between fish, aquatic habitat and week (Fig. 4).

Sexual activity (courtship)

The GLMM analysis on sexual activity (Table 1) indicated a significant effect of fish, i.e. less sexual activity in the presence than in the absence of fish (mean ± SE of 0.91 ±0.06 and 0.24 ±0.04 courtship events per newt and per week for control and fish treatments, respectively). There was also a significant effect of the presence of the aquatic shelter: there were more courtship displays exhibited when an aquatic shelter was available than when it was not (0.86 ± 0.06 and 0.29 ± 0.04 courtship events per newt and per week for the treatments with and without aquatic shelter, respectively). Finally, there was a significant effect of week, indicating a decrease in the number of courtship events with time. Tukey contrast tests showed a significant gradient of response between the four experimental treatments (Fig. 3b). The presence of newts in water (included as a covariate in the model) had a significant effect on sexual activity, as it affected positively the number of courtship events ($\chi^2_1 = 76.38$, $P < 0.001$).

Figure 3. Effects of the four treatments (mean ± SE) on (a) the proportion of terrestrial habitat use, (b) sexual activity (number of courtship events per week) and (c) egg production (number of eggs laid per week) in palmate newts ($N = 96$ newts, $N = 24$ aquaria). The treatments involve the absence and presence of goldfish and aquatic shelter. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (Tukey contrast test).
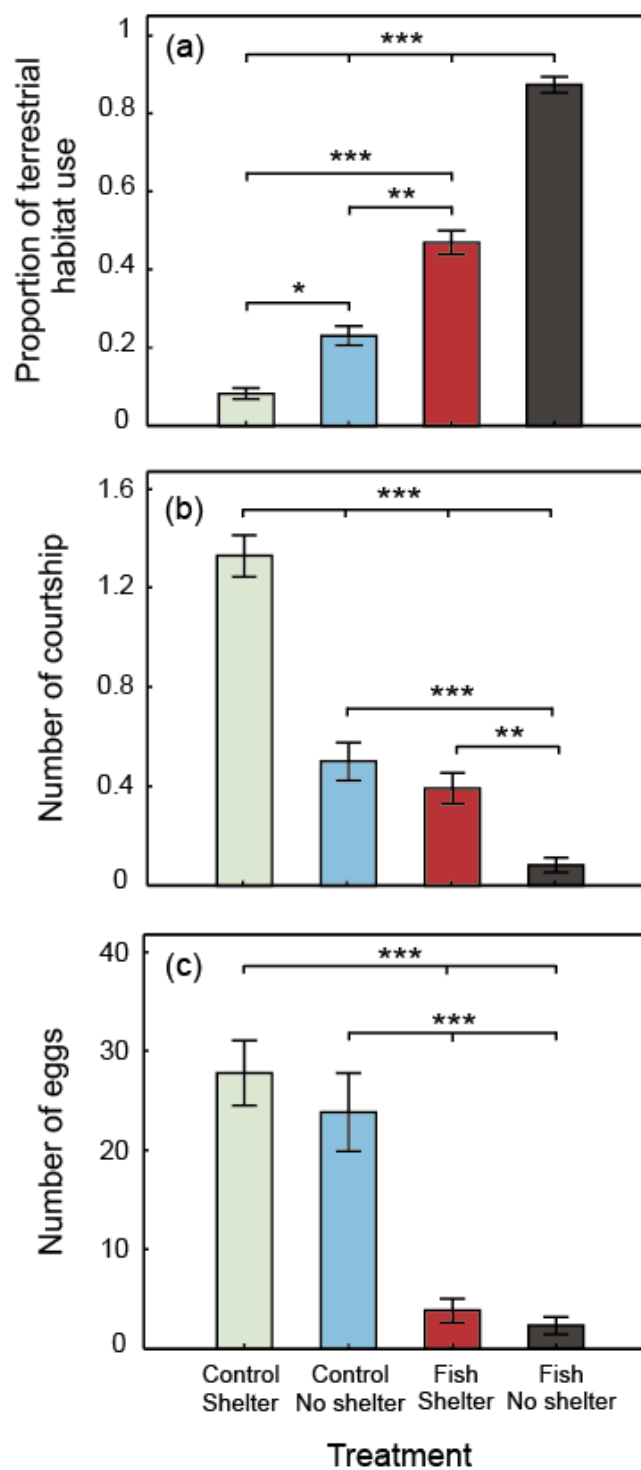
Table 1 Generalized linear mixed models (GLMMs) evaluating the effect of fish, aquatic shelter, week and their interactions on newt behaviour

| Variables	Factors	Estimate	SE	95% CI	χ^2_1	*P*
Terrestrial habitat use	Fish	5.79	0.60	4.52, 7.28	80.28	**< 0.001**
	Aquatic shelter	-3.53	0.59	-4.90, -2.31	32.65	**< 0.001**
	Week	0.31	0.06	0.18, 0.44	24.78	**< 0.001**
	Fish*Aquatic shelter	-1.77	1.17	-4.30, 0.69	1.79	0.180
	Fish*Week	-0.09	0.12	-0.37, 0.17	0.63	0.430
	Aquatic shelter*Week	0.13	0.11	-0.12, 0.38	1.09	0.297
	Fish*Aquatic shelter*Week	0.09	0.01	0.06, 0.11	17.05	**< 0.001**
Sexual activity (courtship)	Fish	-0.69	0.23	-1.17, -0.25	9.28	**0.002**
	Aquatic shelter	0.81	0.21	0.39, 1.26	14.25	**< 0.001**
	Week	-0.10	0.02	-0.14, -0.05	20.60	**< 0.001**
	Fish*Aquatic shelter	-0.70	0.49	-1.65, 0.28	2.00	0.156
	Fish*Week	0.05	0.06	-0.06, 0.15	0.71	0.400
	Aquatic shelter*Week	0.02	0.05	-0.07, 0.12	0.24	0.626
	Fish*Aquatic shelter*Week	0.03	0.05	-0.06, 0.12	1.60	0.205
Production of eggs	Fish	-2.75	0.66	-4.37, -1.46	14.81	**< 0.001**
	Aquatic shelter	0.60	0.65	-0.71,2.04	0.84	0.359
	Week	-0.20	0.01	-0.22, -0.18	429.01	**< 0.001**
	Fish*Aquatic shelter	1.14	1.31	-1.65, 4.07	0.75	0.386
	Fish*Week	-0.08	0.03	-0.14, -0.02	6.03	**0.014**
	Aquatic shelter*Week	0.04	0.02	0.003, 0.08	4.52	**0.033**
	Fish*Aquatic shelter*Week	-0.31	0.07	-0.44,-0.18	37.29	**< 0.001**

The behavioural response variables were the proportion of terrestrial habitat use, the sexual activity (number of courtship events) and the production of eggs (number of eggs laid). $N = 96$ newts, $N = 24$ aquaria; newts nested in aquaria as random effects. Significant values are highlighted in bold.

Production of eggs

The GLMM analysis on egg production (Table 1) showed a significant effect of fish, with a total of 2475 eggs in control aquaria and 297 eggs in aquaria with fish (mean ± SE of 25.8 ± 2.6 and 3.1 ± 0.7 eggs per week and per aquarium for the control and fish treatments, respectively). There was also a significant effect of week, showing a decrease in the number of eggs with time, and a significant interaction between week and fish, showing a very low production of eggs over time for the fish treatment, and a decrease in egg production with time for the control treatment. There were also significant interactions between aquatic habitat and week, and between fish, aquatic habitat and week (i.e. a more pronounced decrease in the number of eggs in the control without aquatic shelter than in the control with aquatic shelter). Tukey contrast tests showed a significant difference between both the fish groups and the control groups (Fig. 3c). The presence of females in the water (included as a covariate in the model) was significant and affected positively the production of eggs ($\chi^2_1 = 73.28$, $P < 0.001$).

Indeed, females were seen in water 2.5 times more often in the absence than in the presence of fish (mean ± SE of 84 ± 1.6% and 33 ± 3.4% of water residency, respectively). Taking into account the presence of females in the water, there were still 3.3 times fewer eggs laid by aquatic females in the presence of fish than in the absence of fish.

Figure 4. Effects of the four treatments (mean ± SE) on the proportion of terrestrial habitat use across time in palmate newts (*N* = 96 newts, *N* = 24 aquaria). The treatments involve the absence and presence of goldfish and aquatic shelter.
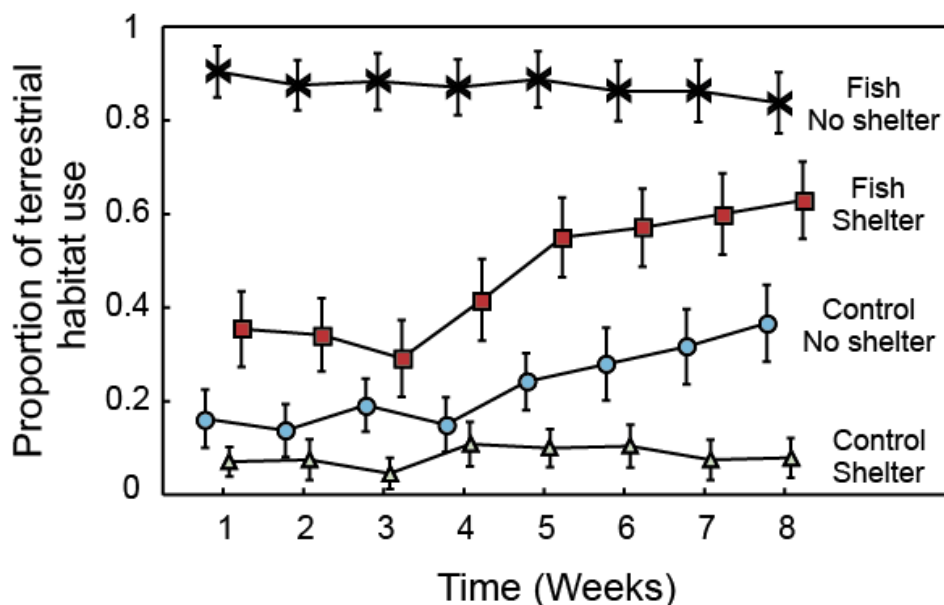
DISCUSSION

The results presented in this behavioural study highlight an important cause of the rarity of coexistence patterns observed between fish and pond-breeding amphibians in the wild (Bucciarelli, Blaustein, Garcia & Kats, 2014; Denoël & Ficetola, 2014). In unfavourable aquatic habitats, i.e. in the presence of fish in our design, newts chose to leave their aquatic habitat for terrestrial life (Fig. 1) and, therefore, give up reproduction. When an aquatic shelter was available, more newts stayed in the water and displayed courtship; however, despite this choice, their reproductive success was very low. By exploring these nonconsumptive behavioural effects, this study contributes to the understanding of the complex patterns of newt distribution in fish and fishless habitats, but also of the consequences of fish introduction on amphibian diversity.

Impact of fish presence on habitat use in newts

Both biotic and abiotic conditions influence the occurrence of amphibians in their breeding habitat (Indermaur et al., 2010; Van Buskirk, 2005). Indeed, newt distribution can be explained by the quality of their habitat such as deep and fishless ponds (Denoël & Ficetola, 2008). When these conditions deteriorate, newts are no longer found or are present in lower population densities (Denoël et al., 2005; Denoël & Ficetola, 2014). By showing that newts can avoid contact with fish by leaving the aquatic habitat, our results provide a new empirical explanation in support of such field observations (Langerhans, 2007). This habitat shift is in agreement with models on complex life cycles that predict life on land when the aquatic habitat provides poor aquatic conditions (Werner, 1986; Whiteman, 1994; Wilbur & Collins, 1973). When not leaving for the entirety of the breeding season, this allows for a reduction in the contact time with fish.

The escape of newts to terrestrial shelters highlights that these habitats are essential to newts, not only for aestivation or overwintering (Denoël & Ficetola, 2008), but also for the whole year in providing a safe place when aquatic habitats become less hospitable. Indeed, newt survival has been shown to be higher on land than in the water in another newt species (Unglaub, Steinfartz, Drechsler & Schmidt, 2015). Previous studies showed the importance of the forest in the core zone around the breeding ponds of amphibians including newts (Denoël & Ficetola, 2008; Gustafson, Malmgren & Mikusiński, 2011; Indermaur & Schmidt, 2011). Indeed, forests can contain moist and shaded ground, which is favourable for feeding, but also for migration, aestivating and overwintering (Jehle & Arntzen, 2000; Marty, Angélibert, Giani & Joly, 2005; Schabetsberger, Jehle, Maletzky, Pesta & Sztatecsny, 2004). The consideration of the effects of fish should, thus, focus not only on the aquatic environment, but also on its terrestrial surroundings (Ficetola, 2015). The presence of a favourable terrestrial habitat near a breeding pond invaded by goldfish could allow multiple movements of newts between aquatic and terrestrial habitats, therefore limiting the contact with fish while keeping the opportunity to reproduce.

Along with the terrestrial habitat, aquatic microhabitats are essential environmental features to sustain newt populations. Field ecological studies have shown that amphibians, such as newts, are present in ponds with plentiful vegetation, which provides shelter for both adults and larvae, and substrate for spawning (Hartel et al., 2007; Joly, Miaud, Lehmann & Grolet, 2001; Landi, Piazzini & Saveri, 2014). In this study, we found that the availability of aquatic shelter allows half of the newt population (53%) to coexist with fish, whereas almost all newts (87%) left the water when they had no aquatic shelter. The use of aquatic shelters is the most frequently described avoidance strategy in response to fish introduction in the natural environment (Hartman & Lawler, 2014; Petranka, Kats & Sih, 1987). However, the presence of the aquatic microhabitat is also important regardless of fish presence: in an aquatic habitat that provided shelter and was devoid of fish, most newts (92%) stayed in the water for the entire experiment (8 weeks), while, in the absence of an aquatic shelter, they started to leave the water after only 4 weeks. This concurs with a previous study showing that, when the aquatic environmental conditions were unfavourable (too high a density), newts began the migration to the terrestrial habitat sooner (Grayson & Wilbur, 2009). Our results support the hypothesis of

newt selectivity based on the quality of their aquatic habitat (Denoël & Lehmann, 2006). They show that microhabitat availability can allow the persistence of populations in both fish and fishless habitats (see also Winandy & Denoël, 2013b). Indeed, the increase in aquatic vegetation could reduce the impact of introduced predators and competitors on native species (Kiesecker, Blaustein & Miller, 2001; Pearl, Adams, Leuthold & Bury, 2005). However, in the natural environment, goldfish introduction often leads to a reduction in vegetation that could provide shelter (Richardson, Whoriskey & Roy, 1995). Safe habitats in the water can, therefore, be a limited resource, making the coexistence between fish and amphibians difficult.

Impact of fish presence and habitat on newt reproduction

Antipredator behaviour has been shown to imply a reduction in the time spent on essential activities such as reproduction (Lima & Dill, 1990; Strauss, Lau & Carroll, 2006). Although such a trade-off between breeding and predation risk can be adaptive (Rohr & Madison, 2001), skipping reproduction entirely is certainly not. In the presence of fish, newts maintained some reproduction (see also Winandy & Denoël, 2013b), but the production of eggs was very limited and not compensated for density effects (i.e. by aquatic newts that could have deposited more eggs in the absence of competitors). Indeed, there were eight times fewer eggs deposited in fish environments than in favourable fishless habitats. Therefore, the presence of aquatic shelters made the persistence of sexual activity possible, but poorly improved the egg deposition rate. Although goldfish can be predators of eggs and salamander larvae (Monello & Wright, 2001), in this study, females were able to wrap their eggs, and therefore, protect them against predation (Miaud 1993; Orizaola & Braña, 2003a). In our study, eggs were removed from the aquaria every day; thus consumption by fish was unlikely and indeed not observed. Moreover, in aquaria with aquatic shelter, newts could lay eggs under the shelter. Therefore, the difference in the number of eggs between the control and fish treatments was not due to predation, but was partly because females left the water. By fleeing the aquatic habitat in response to the presence of fish, newts forgo reproductive opportunities.

When an aquatic shelter was available and newts stayed in the water, this low reproductive success could be explained by both a reduction in sexual activity and females deciding not to lay eggs. This confirms that, in the natural environment, the absence of eggs and larvae may indicate not only a high predatory pressure, but also a decrease in reproductive effort (Ficetola et al., 2011). Therefore, in the wild, the measure of reproductive success may allow a more accurate assessment of the impact of introduced predators than the mere presence of adults (Ficetola et al., 2011). Oviposition habitat selection has been largely studied in pond-breeding insects such as mosquitos, beetles and midges (Binckley & Resetarits, 2005; Blaustein et al., 2004; Brodin, Johansson & Bergsten, 2006) and in amphibians, particularly in anurans (Egan & Paton, 2004; Schulte et al., 2011). In these taxa, females choose to breed more in sites without predators than in ponds with a high risk of predation for their offspring. Therefore, the escape from water in the presence of fish could be interpreted as dispersal away from fish to potentially more suitable ponds. However, in our study, females did not have a choice to breed in a habitat devoid of fish; nevertheless, they decided not to lay eggs in the presence of fish. An

explanation for this forgoing of reproduction could be that the behavioural response is not based on an assessment of the different breeding possibilities in order to maximize reproductive success. Indeed, it has been shown in mosquitos that females do not adjust their decisions to the heterogeneity in habitat quality. In contrast, they responded to the absolute value of a potential oviposition site, and not to the relative value in comparison with other sites (Kiflawi, Blaustein & Mangel, 2003). Therefore, the ability of females to assess habitat quality for offspring can strongly influence reproductive success (Rieger et al., 2004): in a positive way when multiple breeding sites are available at a low interpond distance, but in a negative way when the breeding site assessed as unsuitable by females is isolated from other ponds. Unfortunately, current anthropogenic pressure, such as the destruction and fragmentation of habitats, leads more to the isolation of breeding and terrestrial habitats (Da Silva & Rossa-Feres, 2011; Denoël & Ficetola, 2008; Greenwald, Gibbs & Waite, 2009). Indeed, the lack of landscape connectivity between suitable aquatic and terrestrial habitat constrains many amphibian species with aquatic reproduction to undertake risky migration in a more disturbed environment, which could probably contribute to population decline (Becker, Fonseca, Haddad, Batista & Prado, 2007). Moreover, compared with flying pond-breeding insects, semiaquatic amphibians, such as newts, have more limited dispersal capability (Ficetola & De Bernardi, 2004), making migration to alternative aquatic habitats possibly more problematic. In landscapes in which ponds are isolated, although a high emigration of newts was found in fish ponds, few movements were observed between distant fish and fishless ponds (Unglaub et al., 2015). However, as long as newts still occur in fish ponds (even without reproductive success) or in nearby habitats, as it is often the case (see e.g., Denoël, Scime, & Zambelli, 2016), there is potential for their conservation. In particular, if management provides new adequate aquatic sites near the invaded ponds (Manenti, Bonelli, Scaccini, Binda, & Zugnoni, 2014) or if fish disappear naturally (e.g., after pond drying) or by removal (Vredenburg, 2004), there is hope for the persistence of newts and for an increase in their population.

The long-term consequences of skipping reproduction may be difficult to assess because amphibians can make strategic decisions over their lifetime about whether to breed or not to breed. For instance, amphibians may benefit from skipping reproduction by reducing the number of eggs laid in a breeding season, then saving energy, allowing for a higher reproductive value the subsequent year (Reyer, Frei, & Som, 1999). Indeed, reproduction in amphibians may incur a high energy cost that can lead to the decision to skip a breeding opportunity and attempt reproduction in years that present suitable environmental conditions for both adults and offspring (Cayuela et al., 2014; Church, Bailey, Wilbur, Kendall, & Hines, 2007). Therefore, the decision of an organism to reproduce depends on environmental conditions that directly affect individual fitness and the probability of offspring survival (McNamara & Houston, 1996). However, in the case of fish introduction, the poor environmental quality repeatedly affects a population over more than one year; both the decision to skip reproduction and the impossibility to find another breeding habitat may lead to the extinction of the population.

In conclusion, escape from fish habitats by pond-breeding amphibians is a mechanism that contributes to explaining the negative spatial association observed in the field between introduced fish and amphibians (Denoël & Ficetola, 2014; Orizaola & Braña, 2006; Pilliod et

al., 2010). Fish species, such as goldfish, that have been shown to be problematic for the larval stage of amphibians through consumptive effects (Monello & Wright, 2001) are, thus, also detrimental for the adult stage of amphibians through nonconsumptive effects, i.e. by behavioural avoidance. Habitat selection has a direct functional relationship with survival and reproduction and, therefore, strongly influences fitness (Morris, 2011). In this study, the consequences were particularly acute, as the escape response implied a complete habitat shift for a large part of a population, with the fitness consequence of forgoing reproduction. More broadly, these results also show the importance of integrating behavioural patterns and habitat heterogeneity in conservation biology studies (Caro, 2007).

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