

## Habitat selection and reproduction of newts in networks of fish and fishless aquatic patches

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The spatial distribution of organisms in patchy environments can be determined by the relationship between habitat quality and animal behaviour. In species with complex life cycles, such as pond-breeding amphibians, the selection of a suitable wetland is crucial. While the traditional view of amphibian ecology suggests strong site fidelity and low vagility, more recent research highlights mechanisms involving active site choice through avoidance behaviour and bet-hedging strategies in heterogeneous environments. The introduction of fish to the aquatic environment is one of the factors that may alter site selection and lead to local dispersal. In this context, we investigated the habitat choice of Alpine newts, *Ichthyosaura alpestris*, in networks of fish (*Carassius auratus*) and fishless aquatic patches. Using a laboratory design consisting of aquaria divided into two water tanks connected by a terrestrial platform, we assessed newt distribution and movement between patches. During the breeding period, we compared the reproductive success of individuals in two types of networks. We found that newts escaped fish by rapidly changing aquatic patches and then aggregating in safe aquatic patches that were free of fish. In the fish network, newts maintained reproduction, but the high local abundance resulted in decreased sexual activity and egg production and increased use of the terrestrial habitat. However, in the fishless network, newts moved between aquatic patches several times, exhibited more courtship behaviour and laid more eggs than they did in the fish networks. Our results showed both adaptive habitat switching due to environmental risks in the fish network and habitat supplementation (i.e. use of alternative resources) in the fishless network. Such studies on movement behaviour and habitat selection have conservation implications in showing that the persistence of native species in invaded networks depends on the rescue effect, with immigration to fish-free habitats potentially preventing local extinction.

*Keywords:* alien species, behavioural avoidance, fish introduction, habitat selection, movement ecology, spatial connectivity

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Understanding patterns in the distribution and abundance of organisms is one of the main challenges in ecology. The existence of a relationship between habitat structure or quality and animal behaviour helps to determine the spatial distribution of organisms and improves our understanding of habitat selection (Boyce et al. 2016; McLoughlin, Morris, Fortin, Vander Wal & Contasti 2010). Studying habitat choice is particularly relevant in species with complex life cycles requiring both terrestrial and aquatic habitats that provide nonsubstitutable resources (Denoël, Perez, Cornet & Ficetola 2013; Dunning, Danielson & Pulliam 1992). Indeed, such habitat complementation is present in many stream- or pond-breeding insects and amphibians that radically shift from major habitats (i.e. water to land) at metamorphosis. Adults often only use water for reproduction, whereas aestivating, wintering and dispersal usually occur in the terrestrial habitat (Wilbur 1980). In insects and anurans, it is well known that another process, habitat supplementation, can be involved, where individuals supplement their resource levels by moving to patches that provide similar resources within an accessible part of the local landscape (Dunning et al. 1992). For instance, pond-breeding species can use several aquatic habitats and lay eggs in various sites (Khatchikian, Dennehy, Vitek & Livdahl 2010; Refsnider & Janzen 2010). This can result from an active choice or from a bet-hedging strategy, if assessment of habitat quality is not possible (Kaplan & Cooper 1984; Schulte et al. 2011). Indeed, the highly unpredictable variability of environmental conditions can make it almost impossible for parents to predict the future quality of a pond for their offspring development. Therefore, depositing eggs in several selected habitats could minimize the risk of total offspring loss in uncertain environments (Erich, Ringler, Hödl & Ringler 2015).

However, the traditional view of the community ecology of a variety of amphibian species, particularly newts and salamanders, is that they show strong breeding site fidelity and low vagility, which are characteristics of a metapopulation structure (Smith & Green 2005). This pattern has been found at a regional scale where amphibian communities live in wetlands that are isolated by distances beyond the normal range of terrestrial movement (Gill 1978; Joly & Miaud 1989). In newts, the usual distance of migration is within a few hundred metres (Denoël et al. 2013). However, at a more local scale, when the interpond distances are short, some research suggests a 'patchy population' model of organization, showing more active habitat selection (Petranka & Holbrook 2006; Sinsch 2014). In particular, studies have shown that many amphibians have evolved behavioural avoidance mechanisms in response to unfavourable aquatic patches (Resetarits 2005; Resetarits & Wilbur 1989).

Movement between habitats and levels of aggregation can vary with numerous ecological factors, but resource distribution and predation risk are often two of the most important factors influencing habitat selection (Amburgey, Bailey, Murphy, Muths & Funk 2014; Heithaus et al. 2007; Indermaur, Schaub, Jokela, Tockner & Schmidt 2010). The best example is breeding site selection, where females may assess habitat quality for their future offspring and avoid giving birth or laying eggs in ponds containing predators or with high conspecific densities, which may increase competition for resources (Resetarits & Wilbur 1989; Rieger, Binckley & Resetarits 2004). Therefore, within the limits of their movement capacities, field studies have shown that amphibians can colonize alternative ponds where they can breed (Kopecký, Vojar & Denoël 2010; Perret, Pradel, Miaud, Grolet & Joly 2003). It is yet unknown

how species such as newts and salamanders use alternative breeding habitats during a single period of reproduction.

One of the factors that may affect population structure in wetlands, and lead to dispersion, is the introduction of alien species (Consentino, Schooley & Phillips 2011; Unglaub, Steinfartz, Drechsler & Schmidt 2015). Freshwater ecosystems are among the most invaded in the world and many levels of ecological organization can be affected simultaneously, including individuals, populations, communities and ecosystems (Ricciardi & MacIsaac 2011). Among invasive species, fish are one of the main causes of amphibian decline (Bucciarelli, Blaustein, Garcia & Kats 2014), as amphibians did not usually coevolve with fish (Cox & Lima 2006). Many longitudinal environmental studies have confirmed exclusion patterns between fish and amphibians by showing amphibian extirpation after the introduction of fish and resilience after their removal (Knapp, Boiano & Vredenburg 2007). Decreases in native amphibian populations can be caused by consumptive effects, i.e. the direct predation of eggs, larvae or adults (Leu, Lüscher, Zumbach & Schmidt 2009), and indirectly through resource competition (Joseph, Piovia-Scott, Lawler & Pope 2011). However, a nonconsumptive effect that is often overlooked, but can also have ecological consequences, is behavioural avoidance in response to fish (Binckley & Resetarits 2003; Petranka & Holbrook 2006). This could range from microhabitat shifts within a pond (Orizaola & Braña 2003b; Teplitsky, Plenet & Joly 2003) to shifts between aquatic and terrestrial habitats (Winandy, Darnet & Denoël 2015). The consequences are usually a decrease in both foraging and mating opportunities (Winandy & Denoël 2013b, 2015). Moreover, in response to the presence of fish, newts can leave the water and remain on land during the entire mating period, thereby forgoing reproduction (Winandy et al. 2015). Indeed, in a landscape structure in which ponds were isolated, even when high emigration of newts was observed, only a very small number of movements between distant fish and fishless ponds were observed (Unglaub et al. 2015). Therefore, a high connectivity between ponds could offer amphibians more choices in site selection (Joly, Miaud, Lehmann & Grolet 2001; Marsh, Fegraus & Harrison 1999). Wholesale shifts of breeding populations between ponds were observed after the introduction of fish into some ponds of a network (Petranka & Holbrook 2006). It is of great importance to determine how organisms use such networks during the breeding period, i.e. whether they show habitat supplementation and habitat selection based on behavioural avoidance, and what the consequences are in terms of reproductive output.

In this study, we aimed to assess the habitat selection and movement patterns of an amphibian species, the Alpine newt, *Ichthyosaura alpestris*, at a local scale in two connected aquatic habitats with and without fish. We used goldfish, *Carassius auratus*, as a model species because this is the most introduced ornamental species in the world (Maceda-Veiga, Escribano-Alacid, de Sostoa & García-Berthou 2013) and it is causing declines in newts (Denoël & Ficetola 2014; Denoël et al. 2013). Goldfish are not thought to predate on adult newts, but they can forage on the eggs and larvae of salamanders (Monello & Wright 2001). We took an experimental approach, using a laboratory-replicated design throughout the period of newt reproduction. Using a PIT-tag marking method, we recorded individual movements and newt abundance in networks with or without fish. We also assessed the impact of habitat

selection on sexual activity and egg production. We predicted that newts would be capable of active habitat choice for breeding. Therefore, in fish pond networks, newts should avoid fish patches and reach higher densities in fishless patches, resulting in reduced reproduction in comparison to fishless networks.

## METHODS

### *Species*

We caught 64 adult Alpine newts using dip netting (32 individuals of each sex) at the beginning of the reproductive period (March 2014) in a fishless pool (Romerée, Belgium, 50°08'N, 4°40'E). There were no fish present in the ponds within the usual dispersal distance of newts around the capture site, so the newts were completely naïve to fish. After capture, we kept the sexes separated in six 9-litre tanks, which were filled with water, stuffed with towels, and then placed in two large 230-litre refrigerated boxes. We then brought them directly to the laboratory. The Alpine newts had a mean  $\pm$  SE snout–vent length of  $5.27 \pm 0.53$  cm ( $N = 64$ ).

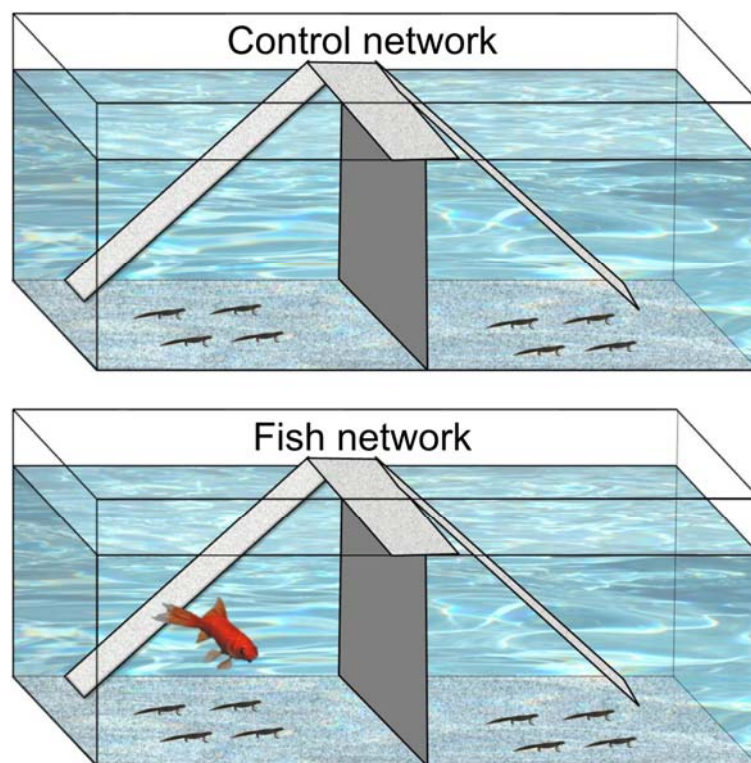
We used four goldfish from the Aquarium of Liège and stored them in a large tank (180×80 cm and 60 cm water depth) in our laboratory. They had a typical orange colour and a mean  $\pm$  SE standard length (i.e. from the tip of the snout to the posterior end of the last vertebra) of  $14.2 \pm 0.39$  cm.

### *Laboratory maintenance*

We distributed the newts between eight identical and independent aquaria networks (116 × 60 cm and 40 cm water depth, 278 litres). Each network consisted of two identical tanks, separated by waterproof, opaque glass. A terrestrial platform (60 × 17.5 cm) allowed the newts to move between the two tanks. The platform was made of a slab of slate and devoid of shelter; therefore, it functioned only as a connection between aquatic patches. An access ramp (60 × 16 cm with angle of 40°) was placed in the tanks to facilitate the transition between aquatic and terrestrial habitats. Four individual newts (two of each sex) were distributed into each tank, giving a total of eight newts in each of the eight networks at the start of the experiment. In four networks, the control networks, both tanks were fishless, while in the other four networks, the fish networks, one goldfish was present in one of the two tanks (Fig. 1). An oxygen diffuser was placed in each tank to maintain a high oxygenation of the water (mean  $\pm$  SE =  $9.91 \pm 0.02$  mg/litre). The ambient air temperature was regulated to maintain an average water temperature of 14.75 °C (SE = 0.05 °C). We established a photoperiod of 13:11 h light:dark (daylight tube, OSRAM L36W/965 Biolux and lumilux warm white tube, OSRAM L36W/830). We placed a support for egg laying in every tank. The newts were fed with 75 mg of thawed *Chironomus* larvae per newt every day in the afternoon. The food was provided in the water under a grid to avoid consumption by the fish (the grid was removed at the end of the day when all the food was consumed). The fish were fed at the same time as the newts on 800 mg of *Chironomus* larvae. They ate their food quickly, leaving the newts no time to forage on it.

### *Marking method*

Individual identification was essential to the experiment; therefore, 5 days before the newts were put into the tanks, they were all marked with a PIT-tag (RFID Mark, 134.2 kHz, Réseumatique, Bernay, France,  $8 \times 1.4$  mm, 30 mg). Before tagging, the newts were anaesthetized using phenoxyethanol (0.8 ml/litre) in tanks filled with 2 litres of water (four newts per tank). Marking began as soon as the newts were asleep, usually after around 15 min in the anaesthetic bath. The PIT-tag was injected under the skin near the hindlimbs and pushed in the direction of the forelimbs. After marking, all the newts were placed in fresh oxygenated water tanks (30 litres, four newts per box) for 5 days. They all recovered well. This marking method has been shown to have no detrimental effects on Alpine newts, including on their movement and behaviour (Perret et al., 2003; Winandy & Denoël 2011). We could easily identify each newt using a PIT-tag reader with an external waterproof stick antenna (AB10625 Loligo System, Copenhagen, Denmark). However, to avoid the presence of the stick reader disturbing the newts during behavioural observations, we also used sketches of unique spots and special features on the newts to differentiate them from each other. Therefore, the stick reader was only used after observation sessions to confirm the visual identifications.



**Figure 1.** Configuration of the experimental set-up: eight networks consisting of two tanks linked by a terrestrial platform. In each network, there were eight newts (four males and four females) initially placed with four (two males and two females) in each tank. There were four control networks, consisting of two tanks free of fish (above) and four fish networks, one of the two tanks being inhabited by a goldfish (below).

## *Experimental procedure*

Newt behaviours were compared at various levels (see Fig. 1). (1) At the network type level there were four control networks ( $N = 32$  newts) and four fish networks ( $N = 32$  newts). (2) At the treatment level, there were eight tanks in the control treatment (each tank within the four control networks), four tanks in the no-fish treatment (the tanks without fish within the fish networks) and four tanks in the fish treatment (the tanks containing a goldfish within the fish networks).

For 8 weeks, we recorded in which tank (or terrestrial platform) each newt in the network was found. We conducted 12 observation sessions per week, in the morning (at 0900 hours) and in the afternoon (at 1400 hours), from Mondays to Fridays and one session per day during weekends. We could therefore assess the number of movements, as well as differences in newt abundance, between the two tanks of a network and the use of the terrestrial habitat.

To assess sexual activity, we used a focal sampling method (Martin & Bateson 2007) to observe the newts in each tank for 3 min. We repeated the focal sampling twice a day (morning and afternoon), 5 days a week (for 8 weeks), to obtain the number of courtship events that occurred in each tank of a network. To assess reproductive output, we counted the eggs in each tank every week. We changed the egg supports every day, placing them in additional tanks to avoid predation risk.

## *Statistical analyses*

First, we assessed whether the newts moved from their release tank (i.e. the initial tank) to the other tank in each network during the experimental period (1 = moved, 0 = remained in the release tank the entire time). We used generalized linear mixed models (GLMMs assuming a binomial distribution) to test the effects of initial tank treatment (control,  $N = 8$  tanks; no-fish,  $N = 4$  tanks; fish,  $N = 4$  tanks) and sex on newt movement ( $N = 64$  newts). The network identity was included as a random factor. We also used a GLMM to test the effect of initial tank treatment and sex on the latency (i.e. the number of days, using a square root transformation) before moving to the other tank. We completed these analyses using a Tukey contrast test to assess significant pairwise differences between the three treatments.

Second, we used GLMMs (using a Poisson error distribution) to test the effects of network type (control network,  $N = 4$  and fish network,  $N = 4$ ), sex, time (8 weeks, included as an ordinal factor), and the interaction between network and time on: (1) the number of movements between tanks made by each newt, and (2) the number of times that each newt was observed in the terrestrial habitat ( $N = 64$  newts). The network and individual identities were included as random factors (individuals were nested within networks).

Finally, we used GLMMs to assess the effect of network, time and their interaction on (1) the absolute difference in newt abundance between the two tanks of a network (using a Gaussian distribution), (2) the number of courtship events and (3) the number of eggs per week (both using a Poisson error distribution;  $N = 8$  networks). The network identity was included as a random factor. We completed these analyses by assessing the potential effect of newt density

on reproduction. We used a GLMM to assess the effect of treatment (control,  $N = 8$  tanks; no-fish,  $N = 4$  tanks; fish,  $N = 4$  tanks) on the number of courtship events per male (for each tank we calculated the total number of courtship events per week and then divided that by the mean number of males present during that week) and on the number of eggs laid per female (for each tank, we calculated the total number of eggs laid each week and then divided that by the mean number of females present during that week). The network identity was included as a random factor.

For all analyses, we assessed significance using likelihood ratio tests with an a priori level of significance of 0.05. Analyses were performed in R 3.0.2 ([www.r-project.org](http://www.r-project.org)) using the lme4 and multcomp packages.

### *Ethical note*

In this study, we aimed to determine the potential nonconsumptive effects of goldfish on adult newt behaviour. Indeed, given their gape size limitation, the fish could not forage on the newts or wound them, but may still have frightened them. During all experiments, newts could avoid fish by changing their aquatic habitat. Direct interactions between newts and fish are essential in this type of experiment if we are to understand newt avoidance, as this is how they interact in the wild (Winandy et al. 2015; Winandy & Denoël 2013a). Moreover, this study mimics natural conditions in ponds where goldfish are introduced and coexist with newts (Denoël & Ficetola 2014). The study was carried out in an accredited laboratory of the University of Liège (LA1610429), and the research project was approved 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 Supérieur Wallon de la Conservation de la Nature. In the laboratory, all individuals were checked and fed every day. At the end of the experiment, all newts were released alive and unharmed into their capture habitat following the recommendations of the capture permit and the fish were kept in the laboratory. All materials used for maintenance and capture were well washed and disinfected before and after use following the recommendations of the study permit.

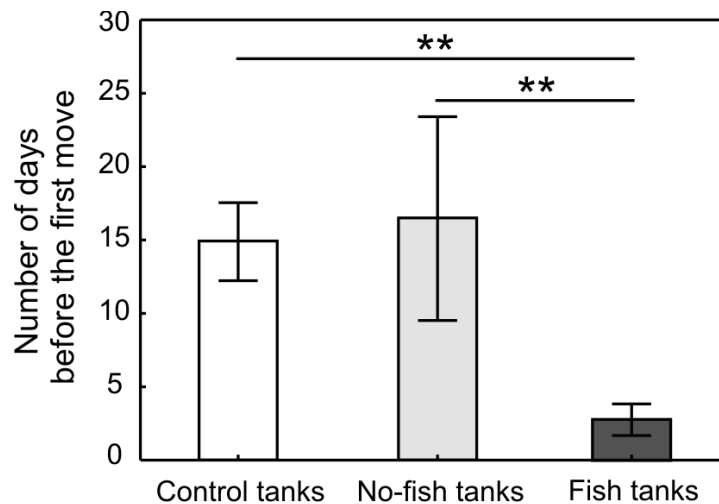
## **RESULTS**

### *Movement between aquatic patches*

During the experimental period, 50 of the 64 newts (i.e. 78%) moved from their initial tank to the other available tank. There was a significant effect of tank treatment (control, no-fish, fish) on the propensity of newts to move from their initial tank (GLMM, estimate = -1.71, SE = 0.73,  $\chi^2_2 = 20.49$ ,  $P < 0.001$ ,  $N = 64$  newts). In control tanks, a mean  $\pm$  SE of  $91 \pm 5\%$  of newts changed tank during the experiment. In fish and no-fish tanks,  $94 \pm 6\%$  and  $38 \pm 13\%$  of newts changed tanks, respectively. Tukey contrast tests showed significant differences between the control and no-fish treatments ( $Z = -3.43$ ,  $P = 0.002$ ) and between fish and no-fish treatments ( $Z = -2.90$ ,  $P = 0.01$ ), but not between the fish and control treatments ( $Z = 0.38$ ,  $P = 0.92$ ) showing that more newts moved in the control and fish treatments than in the no-fish treatment. More specifically, we observed only 18 moves from no-fish tanks to fish tanks, and newts stayed in the fish tanks for only  $1.28 \pm 0.14$  days. There was also a significant effect of

sex on movement (GLMM: estimate = -1.86, SE = 0.91,  $\chi^2_1 = 5.19$ ,  $P = 0.02$ ,  $N = 64$  newts) indicating that more females than males moved ( $88 \pm 6\%$  and  $69 \pm 8\%$ , respectively). The network identity included as a random factor did not explain any of the variance.

There was a significant effect of tank treatment (control, no-fish, fish) on latency before the first tank change (GLMM: estimate = -1.10, SE = 0.51,  $\chi^2_2 = 15.14$ ,  $P = 0.001$ ,  $N = 64$  newts; Fig. 2). Tukey contrast tests showed significant differences between the control and fish treatments ( $Z = -3.54$ ,  $P = 0.001$ ) and the fish and no-fish treatments ( $Z = 3.27$ ,  $P = 0.003$ ), indicating that newts in the fish treatment changed tank faster than newts in the control and no-fish treatments. There was no significant difference between the no-fish and control treatments ( $Z = 1.04$ ,  $P = 0.54$ ) on the number of days before the first tank change. There was a significant effect of sex (GLMM: estimate = 1.83, SE = 0.47,  $\chi^2_1 = 14.46$ ,  $P < 0.001$ ,  $N = 64$  newts), indicating that females moved sooner than males (mean  $\pm$  SE:  $6.61 \pm 2.09$  days and  $17.68 \pm 3.06$  days, respectively).



**Figure 2.** Effect of initial tank treatment (control tanks inside the control networks, no-fish and fish tanks inside the fish networks) on the number of days before the first move by newts (mean  $\pm$  SE,  $N = 64$ ) from the initial tank to the other tank in the network. \*\*  $P < 0.01$  (Tukey contrast test, see Results for statistical analysis).

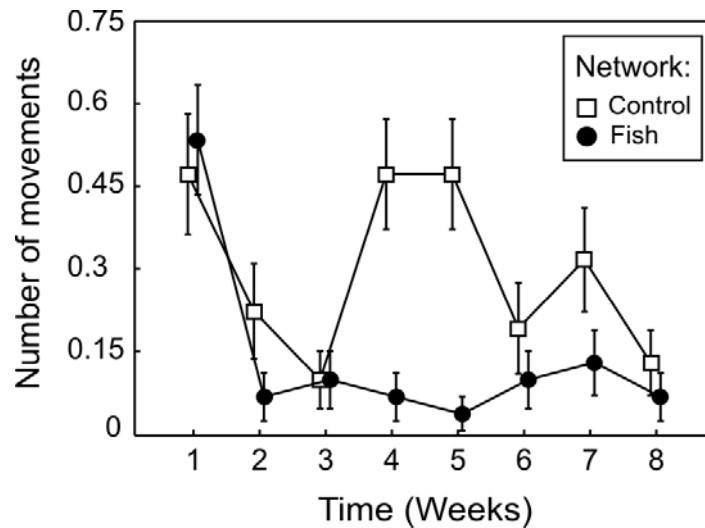
There was a significant effect of network type (control network versus fish network) on the number of movements between tanks made by newts ( $N = 64$  newts) during the experiment (Table 1), indicating that newts in the control networks changed tanks twice as often as newts in the fish networks (mean  $\pm$  SE:  $0.29 \pm 0.03$  and  $0.13 \pm 0.02$  changes per newt and per week for control and fish networks, respectively). There was also a significant effect of time on the number of movements made by newts (Table 1), indicating a decrease in movement between tanks across the 8 weeks. There was also a significant interaction between the network type and time (Table 1), indicating that after 3 weeks there was a peak in newt movement between tanks in the control networks (Fig. 3). There was no effect of sex on the number of movements made by newts (GLMM: estimate = -0.23, SE = 0.23,  $\chi^2_1 = 1.11$ ,  $P = 0.29$ ,  $N = 64$  newts), indicating that males changed tanks as often as females.



**Table 1.** Results of the generalized linear mixed-effects models (GLMMs) testing the effect of network type (control versus fish) and time (8 weeks of experiment) and their interaction on newt movement, presence in terrestrial habitat, newt abundance, number of courtships and egg production in each tank of the networks

Variables	Parameters	Estimates	SE	$\chi^2_1$	<i>P</i>
Movement between tanks	Network	0.79	0.22	13.21	<b>&lt;0.001</b>
	Time	-0.13	0.04	9.79	<b>0.002</b>
	Network * Time	0.23	0.010	5.46	<b>0.02</b>
Terrestrial habitat use	Network	-2.63	0.89	9.75	<b>0.002</b>
	Time	0.10	0.04	8.69	<b>0.003</b>
	Network * Time	0.12	0.10	1.63	0.2
Difference in newt abundance between tanks	Network	-4.84	0.72	13.07	<b>&lt;0.001</b>
	Time	0.16	0.04	103.88	<b>&lt;0.001</b>
	Network * Time	0.33	0.06	29.67	<b>&lt;0.001</b>
Courtship	Network	0.48	0.12	6.32	<b>0.01</b>
	Time	-0.22	0.03	82.90	<b>&lt;0.001</b>
	Network * Time	0.10	0.05	3.36	0.07
Eggs	Network	0.58	0.24	4.48	<b>0.03</b>
	Time	-0.02	0.01	11.20	<b>0.001</b>
	Network * Time	0.05	0.01	10.82	<b>0.001</b>

The variables ‘movement between tanks’ and ‘terrestrial habitat use’ were scored at the individual level ( $N = 64$  newts) each week counting the movements between tanks and the proportion of time spent in the terrestrial habitat. The variables abundance, courtship and eggs were scored at the network level ( $N = 8$  networks) each week counting the difference in newt abundance between tanks of a network and the number of courtships displayed and eggs laid in each network. Significant values are highlighted in bold.



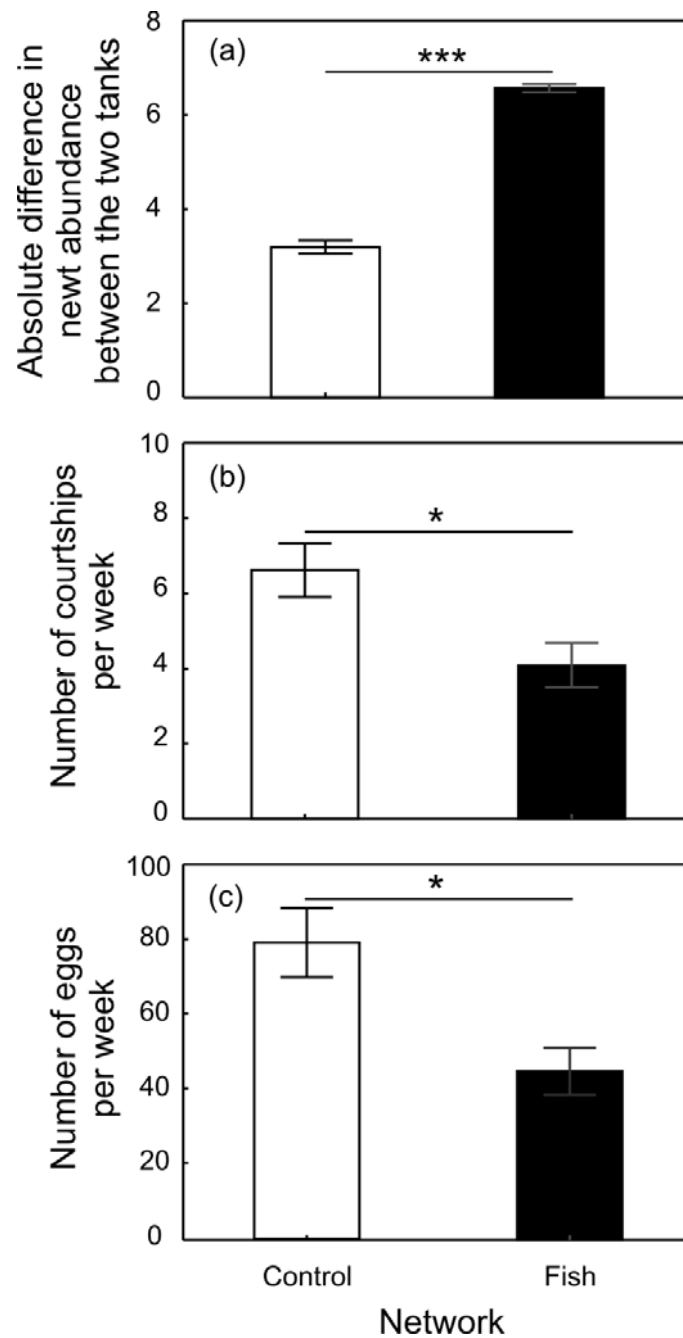
**Figure 3.** Effect of network type (control versus fish network) on the number of newt movements between tanks across time (mean  $\pm$  SE,  $N = 64$ ). See Table 1 for the statistical analysis. Each fish network contained an aquatic tank with a goldfish and an aquatic tank free of fish.

#### *Presence in terrestrial habitat*

There was a significant effect of network type on the number of times newts ( $N = 64$  newts) were observed in the terrestrial habitat (Table 1), indicating that newts in the control networks used the terrestrial habitat four times less often than newts in the fish networks (mean  $\pm$  SE:  $0.11 \pm 0.04$  and  $0.45 \pm 0.08$  numbers of terrestrial habitat uses per newt and per week, respectively). There was a significant effect of time on the use of the terrestrial habitat (Table 1), indicating an increase in terrestrial habitat use across the 8 weeks. There was no significant interaction between network type and time (Table 1). There was a significant effect of sex on the number of times that newts were observed in the terrestrial habitat (GLMM: estimate =  $-1.59$ , SE =  $0.67$ ,  $\chi^2_1 = 6.10$ ,  $P = 0.01$ ,  $N = 64$  newts), indicating that males used the terrestrial habitat seven times less often than females (mean  $\pm$  SE:  $0.07 \pm 0.02$  and  $0.49 \pm 0.09$  number of terrestrial habitat uses per newt and per week, respectively).

#### *Abundance in aquatic patches*

There was a significant effect of network type on the absolute difference in newt abundance between the two connected tanks of a network (Table 1,  $N = 8$  networks), indicating that the absolute difference was much higher in the fish networks than in the control networks (Fig. 4a). There was also a significant effect of time, indicating an increase in the absolute difference in newt abundance across weeks (Table 1). The significant interaction between time and network type shows that the absolute difference in newt abundance varied across time only in the control networks, while remaining stable in the fish networks (Table 1).



**Figure 4.** Effect of network type (control versus fish network) on (a) the absolute difference in newt abundance between the two tanks of a network, (b) the number of courtships displayed each week per tank, and (c) the number of eggs laid each week per tank. Means  $\pm$  SE are shown ( $N = 8$ ). \*  $P < 0.05$ ; \*\*\*  $P < 0.001$  (see Table 1 for statistical analysis). Each fish network contained an aquatic patch with a goldfish and an aquatic patch free from fish.

## Reproduction

Throughout the experiment, newts in the control networks displayed courtship 1.6 times more than did those in the fish networks (i.e. 212 versus 131 courtship events, respectively). There was a significant effect of network type on the number of courtships displayed in networks (Table 1,  $N = 8$  networks), indicating that there was less sexual activity in the fish networks than in the control networks (Fig. 4b). There was a significant decrease in the number of courtships across time, but no significant interaction between time and network type (Table 1). When we looked at the effect of tank treatment (control,  $N = 8$  tanks; no-fish,  $N = 4$  tanks; fish,  $N = 4$  tanks), we observed only six courtships in the fish treatment (from a total of 343 courtships). We therefore assessed the effect of newt density on the control and no-fish treatments. The mean density  $\pm$  SE of newts in the control treatment tanks and the no-fish treatment tanks was  $3.98 \pm 1.9$  and  $7.23 \pm 0.6$  newts, respectively. We found a significant effect of tank treatment (control,  $N = 8$  tanks and no-fish,  $N = 4$  tanks) on the number of courtships displayed per male present in a tank (GLMM: estimate = -0.51, SE = 0.25,  $\chi^2_1 = 3.17$ ,  $P = 0.04$ ), indicating that males courted less often in the no-fish than in the control treatment (mean  $\pm$  SE:  $1.1 \pm 1.0$  and  $1.7 \pm 1.4$  courtships per male and per week, respectively).

During the experiment, newts in the control networks laid 1.8 times more eggs than those in the fish networks (i.e. 2530 and 1426 eggs, respectively). There was a significant effect of network type on the number of eggs laid in the networks (Table 1,  $N = 8$  networks) (Fig. 4c). There was an effect of time on egg production (Table 1), indicating an increase in eggs laid up to the fifth week and then a decrease in egg production until the end of the experiment. There was also a significant interaction between time and network type (Table 1), indicating that newts in the fish networks decreased their deposition of eggs sooner. When we looked at the effect of tank treatment (control,  $N = 8$  tanks; no-fish,  $N = 4$  tanks; fish,  $N = 4$  tanks) we observed only three eggs laid in the fish treatment (from a total of 3956 eggs) and only during the first week. Therefore, we assessed the effect of newt density only on the control and no-fish treatments. We found a significant effect of tank treatment (control,  $N = 8$  tanks and no-fish,  $N = 4$  tanks) on the number of eggs laid per female present in the tank (GLMM: estimate = -8.48, SE = 4.31,  $\chi^2_1 = 3.97$ ,  $P = 0.04$ ), indicating that females laid fewer eggs in the no-fish than in the control treatment (mean  $\pm$  SE:  $12.3 \pm 9.8$  eggs and  $20.8 \pm 15.3$  eggs, respectively).

## DISCUSSION

The results of this study show the effect of fish introduction on habitat selection and distribution of newts in networks of aquatic patches at a local scale. In particular, newts rapidly adapted their spatial distribution to the environmental risk, thereby avoiding fish-invaded habitats. This habitat shift allowed the persistence of reproductive activities in mosaics of adequate and unsuitable habitats, but reduced individual fitness (i.e. sexual activity and egg production). These results can provide an additional explanation of the distribution patterns of organisms within fish and fishless habitats. Indeed, this distribution is not only caused by local extirpation but also by an active avoidance of risky habitats, with increasing densities in the

most favourable reproductive patches. These experimental results on habitat selection have implications for conservation and species management (Petranka & Holbrook 2006).

### *Spatial distribution in fish and fishless networks*

The presence of fish in a pond network strongly influenced the movement of newts. After only a few days, almost all the newts from the fish patches immigrated to the fishless patches. This avoidance of fish-invaded habitats partly explains the rarity of coexistence between amphibians and fish in the wild (Unglaub et al. 2015). During their period of reproduction, newts avoided fish habitats and made very few movements between tanks. Most newts from fishless patches did not move to fish patches, and if they did they stayed there for a very short time. This rapid shift can be explained as a strong antipredator response leading to complete fish avoidance (Petranka & Holbrook 2006). This is particularly effective, as by doing so newts avoid all detrimental contact, such as harassment, with fish (Winandy & Denoël 2015). Newts from a fishless network initiated their first move much later and made several changes in aquatic patches during the breeding period, indicating a more explorative tendency. In their natural environment, newts can show nomadic tendencies, moving between local ponds from one year to another (Perret et al. 2003), and, when ponds are very close to each other, making multiple movements between ponds even during the breeding period (Kopecký et al. 2010; Weddelling, Hachtel, Sander & Tarkhnishvili 2004). High connectivity between local ponds greatly facilitates movement because terrestrial dispersal could be risky for amphibians. Indeed, travel in the terrestrial habitat is associated with desiccation and predatory risk (Rothermel & Semlitsch 2002). In our experiment, the proximity of the two aquatic habitats meant that movement between them involved little or no risk. These observations agree with field data showing numerous movements between pools in connected patches such as forest ruts and low emigration between distant ponds (Kopecký et al. 2010; Unglaub et al. 2015).

We also found a strong difference in the use of the terrestrial habitat depending on the presence of fish in the network. In fish networks, newts were observed on the terrestrial platform four times more often than newts in the fishless networks. A terrestrial habitat provides a safe place when the aquatic habitat becomes less hospitable. Therefore, newts could avoid contact with fish by remaining on land. In our experimental design, however, the lack of suitable terrestrial shelter could force newts to remain in the water. Therefore, the availability of a more suitable terrestrial habitat could increase the effect of fish by allowing newts to move between contrasting habitats (water to land). While habitat complementation is common in amphibians, such a shift during the breeding period can lead to newts forgoing aquatic reproduction (Winandy et al. 2015). Newts could also avoid the high densities in the fishless tank by leaving the water sooner (Grayson & Wilbur 2009). Indeed, spatial distribution and movement between habitats had a strong effect on local newt abundance. In fish networks, all newts aggregated into a single aquatic patch, doubling the initial abundance, while in the control networks, the abundance of newts between aquatic patches was more balanced. Consequently, at a local scale, fish presence causes habitat compression, where individuals are forced to stay together in the remaining suitable fishless patches (Resetarits 2005). Therefore, the habitat selection depends on the interaction between density effects and factors that determine habitat suitability. In this study, the risk of predation applied stronger pressure than conspecific density on newt spatial

distribution. It has been shown that juvenile frogs choose to remain at high densities in high-quality habitats rather than in low-quality habitat even if the density is lower (Patrick, Harper, Hunter, Jr & Calhoun 2008). Therefore, breeding site selection appears to be determined more by predation risk than by competition avoidance (Heithaus et al. 2007; Indermaur et al. 2010). However, in our study, we assessed the density effect independently of food competition, as food was distributed in line with the number of newts present in each patch. In natural settings, higher densities may drain food resources more rapidly, which may be more detrimental and force newts to opt for terrestrial life.

#### *Effect of habitat selection on newt reproduction*

Antipredator strategies are particularly costly when they reduce essential activities such as reproduction (Lima 1998; Winandy & Denoël 2013b). Unfavourable environmental conditions can even lead amphibians to skip reproduction (Cayuela et al. 2014; Winandy et al. 2015). In our study, almost no newts bred while coexisting with fish but they still had the opportunity to breed by finding a more suitable habitat free of fish. However, less courtship was observed in the fish than in the fishless networks, even when the same number of males and females were present in both network types. One explanation for this could be an indirect effect of the presence of the fish through a detrimental density effect in the fishless patches. By aggregating in a single breeding patch, newts can reach high densities that can negatively affect sexual activity. Indeed, sexual competition between male newts could increase mating harassment towards females, which show aversion to such harassment and alter their behaviour to avoid mating (Grayson, De Lisle, Jackson, Black & Crespi 2012). Our results are in line with these observations, as we showed that females used terrestrial habitats seven times more often than males, which could be their response to the high densities. In networks without fish, newt density was more balanced between the two aquatic habitats. Moreover, after a few weeks, newts displayed exploratory behaviour by moving between aquatic patches. These movement patterns explained the variation in local newt abundance over time. Male relocations can be explained by the need to find other females to breed with (Kopecký et al. 2010). Indeed, as the breeding season progresses, females become less willing to mate and increasingly choosy (Gabor & Halliday 1997). Our results showed that females began to move earlier in the breeding period than males, which could be explained by their search for oviposition sites. In pond-breeding species, nest site selection strongly affects offspring survival and female choices are based on a range of environmental cues such as hydroperiod, as well as the presence of conspecifics, predators and competitors, which can serve as negative cues of reproductive success (Egan & Paton 2004; Schulte et al. 2011).

A major maternal strategy is risk spreading, which consists of splitting egg production across several water bodies (Crean & Marshall 2009). Indeed, in highly unpredictable environments, organisms can select multiple oviposition sites to minimize the risk of offspring loss (Erich et al. 2015; Refsnider & Janzen 2010). Such bet hedging is particularly adaptive for avoiding full or major reproductive failure when only one site is chosen (Andersson & Åhlund 2012; Refsnider & Janzen 2010). In our study, newts in the control networks showed habitat supplementation by using more than one aquatic patch in which to reproduce. This can minimize the risk of competition and the risk of cannibalism by other adult newts, as Alpine

newts are known to eat the eggs of their species, although females can wrap their eggs which limits the risk of predation (Miaud 1993; Orizaola & Braña 2003a). Moreover, in our study eggs were removed every day so that the data were not biased by potential egg predation. The number of eggs laid by females in the fishless network (158 eggs per female) matches the average found in a previous study of around 100 - 200 eggs during a breeding period (Miaud 1990). In the fish networks, females showed active habitat selection by laying all their eggs in the safe patches free of fish. However, these females laid fewer eggs (89 eggs per female) than females in the control networks. This suggests an avoidance of high-density habitats for egg laying. However, the number of laid eggs remained near the range of the previous study (Miaud, 1990) indicating that the safe patches could maintain an acceptable reproductive output. However, if the eggs had not been removed every day the high density of newts may have increased the risk of cannibalism from adults and decreased reproductive output further. Moreover, limiting egg production also allows females to save energy, possibly increasing the reproductive value of subsequent years (Reyer, Frei & Som 1999). Indeed, amphibians may decide to attempt reproduction in years that present more suitable environmental conditions (Cayuela et al. 2014).

### *Conservation implications*

Plasticity in movement is demonstrated by an organism's ability to switch from one patch to another (Sinsch 2014). The nomadic behaviour of adult newts suggests high rates of breeding migration and dispersal (Perret et al. 2003). Such movements are dependent on complex interactions between density and environmental factors (Sinsch 2014). Because amphibians are extremely sensitive to environmental disturbances, understanding the factors that influence their breeding site selection is essential for their conservation. Movement ecology is therefore a critical component for explaining species persistence (Pittman, Osbourn & Semlitsch 2014). Amphibians respond to habitat disturbance and fish invasions with high rates of local interpond movement, indicating that avoidance behaviour is a key mechanism in breeding site selection (Petranka & Holbrook 2006). In a well-connected landscape, adaptive habitat switching in response to environmental risks supports the 'patchy population' model of organization (Petranka & Holbrook 2006; Smith & Green 2005). A good example comes from amphibian populations living in clusters of temporary and permanent pools. The proximity between reproductive patches favours individual movement and provides alternative choices for counterbalancing the local risk of drying (Cayuela, Cheylan & Joly 2011; Kopecký et al. 2010). However, site selection based on habitat suitability and more particularly on fish presence can also occur at a broader scale, affecting regional dynamics of metapopulations (Resetarits 2005; Werner, Yurewicz, Skelly & Relyea 2007).

Wetland restoration programmes should therefore consider movement ecology and antipredator behaviour when configuring the distribution of aquatic patches in the landscape. They should also account for the permeability of the matrix between alternative patches to facilitate movement (Decout, Manel, Miaud & Luque 2012; Joly et al. 2001). Indeed, the quality of the terrestrial habitat is essential and affects individuals' willingness to move (Popescu & Hunter, Jr 2011; Scott, Komoroski, Croshaw & Dixon 2013). Consequently, the rescue effect of multiple nearby ponds with high terrestrial connectivity can allow adult amphibians to seek out alternative habitats, which may prevent a local extinction and enhance the long-term

persistence of patchy populations (Consentino et al. 2011; Denoël, Scimè & Zambelli 2016; Petranka & Holbrook 2006).

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