Temporal habitat shift of a polymorphic newt species under predation risk

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The temporal partitioning hypothesis suggests that the evolution of different diel activity rhythms in animals might facilitate the coexistence between prey and predators. However, the temporal shift of habitat use induced by predation has rarely been observed. The study of such a mechanism is particularly relevant for introduced species, because it might explain how native species can persist or decline in response to the presence of alien species. The introduction of fish into ponds inhabited by amphibians has severe consequences for their occurrence and abundance. Fish particularly affect an alternative newt phenotype, the paedomorph, which does not undergo metamorphosis and maintains larval traits such as gills at the adult stage. In a laboratory design, we assessed the diel patterns of habitat use in the two distinct morphological phenotypes of palmate newt (*Lissotriton helveticus*) in the presence or absence of goldfish (*Carassius auratus*). Both newt phenotypes avoided a risky habitat more in the presence than in the absence of fish. This habitat shift was more pronounced during the daytime (i.e., when the risk could be considered higher for the newts) than during night-time. However, in contrast to metamorphs, paedomorphs showed less adaptive changes according to temporal risk and remained in their shelter for most of the time. Temporal and habitat partitioning at the diel scale between native and alien species might promote their coexistence, but diel change can also imply a cost in the overall reduction of the time allocated to essential activities, showing that species interactions remain complex.

Key words: Alien species, amphibians, antipredator behavior, behavioral avoidance, diel pattern, paedomorphosis, risk-allocation hypothesis, risk assessment.

INTRODUCTION

Many studies have shown that prey balance potentially risky important activities such as foraging and mating, in response to predation pressure (Lima and Dill, 1990). A decrease in activity and in the use of refuges lower the risk of predation by decreasing the chance of detection and the probability of an encounter with a predator (Lima, 1998a; Skelly, 1994). However, individuals should manifest trade-offs between vigilance to potential risk and essential activities through a correct risk assessment of the level of threat (Lima, 1998a, b). Indeed the predation risk can present a high degree of spatial and temporal variability (Ferrari et al., 2009).

Spatial variability in predation pressure is a keystone of predator–prey interaction research but the temporal variability has received attention only more recently. Particularly, the risk allocation hypothesis was only proposed in the late nineties by Lima and Bednekoff (1999), and predicts that prey adjust their antipredator behavior to temporal variation in the perceived risk. Indeed, predation risk varies throughout diel, lunar and seasonal cycles (Sih et al., 2000) and therefore, prey should modify their avoidance behavior according to this variation in the level of threat (Ferrari and Chivers, 2009; Kronfeld-Schor and Dayan, 2003). It is believed that the level of predation has played a significant role in shaping the long-term evolution of diel cycle patterns in animals. Indeed, the temporal partitioning of habitats between prey and their predators at the diel scale might have evolved as a mechanism of coexistence: visual predators that are active during the day have driven prey species to a nocturnal lifestyle in a wide variety of taxa (Kronfeld-Schor and Dayan, 2003). This is the case for salamander larvae that increase foraging activity at night when the lack of light reduces the risk of being detected by predators (Manenti et al., 2013). Another well-known example is the diel periodicity of the downstream drift of stream species: a much higher drift number occurs at night than during the day, to minimize the risk of predation by visually hunting predators (Douglas et al., 1994; Oberrisser and Waringer, 2011). However, less research has been performed into whether the diel patterns of habitat use can undergo rapid change following modification in the risk of predation. Indeed, the relative rigidity of diel rhythmicity in animals might explain the rarity of temporal shifts induced by predation (Kronfeld-Schor and Dayan, 2003). Nevertheless, the question of a potential temporal shift in habitat deserves particular attention, because ecosystems are rapidly being disrupted anthropogenically (Poudel et al., 2015), especially regarding the massive introduction of alien species into the natural environment.

The introduction of alien species is one of the major causes of biodiversity loss (Gherardi, 2007). The introduction of fish into wetlands has had a particularly disastrous impact on organisms such as amphibians, which are currently declining worldwide (McGeoch et al., 2010; Stuart et al., 2004; Wake and Vredenburg, 2008). Indeed, many amphibians evolved in aquatic environments that are devoid of fish and the introduction of fish species rapidly leads to population extinctions (Denoël et al., 2005a; Salo et al., 2007). The goldfish (*Carassius auratus*) is one of the most introduced and invasive ornamental species in the world (Maceda-Veiga et al., 2013; Savini et al., 2010). It is largely introduced into ponds inhabited by amphibians, where it causes their decline (Denoël and Ficetola, 2014; Denoël et al., 2013). In laboratory experiments, goldfish caused behavioral avoidance, with a reduction in sexual and foraging behavior (Winandy and Denoël, 2013a, 2015a). This species can also predate salamander eggs and larvae (Monello and Wright, 2001), but usually not adult newts, due to gape-size limitations. However, it can peck newts – a behavior that causes newts to avoid fish contacts (Winandy and Denoël, 2015a).

A specific feature of newts and salamanders is the occurrence of two alternative developmental phenotypes in some populations: a fraction of the larvae metamorphose and become metamorphs, and the others mature into paedomorphs and retain aquatic life traits, such as gills. The expression of each phenotype depends on environmental variables and has been shown to be linked to a quantitative trait locus in ambystomatids (Denoël et al., 2005b; Voss et al., 2012). This polyphenism is adaptive in some species and allows resource partitioning and early reproduction in paedomorphs (Denoël et al., 2005b), but is now endangered by fish introductions. Reports have shown massive declines in paedomorphs following fish introduction, whereas metamorphs either decline or are maintained in populations (Denoël et al., 2005a; Denoël and Ficetola, 2014). In comparison with metamorphs, paedomorphs have a more larval structure and size that might make them more sensitive to fish encounters (Andreone et al., 1993; Denoël et al., 2009; Winandy and Denoël, 2015b) and might explain

the lower foraging and activity of paedomorphs in the presence of fish (Winandy and Denoël, 2015a).

The aim of this study was to assess the diel variation in the habitat use of both phenotypes (metamorphs and paedomorphs) of the palmate newt (*Lissotriton helveticus*) in response to the presence of goldfish. The goldfish is considered to be a mostly diurnal species with a rhythm that is synchronized by the photoperiod (Iigo and Tabata, 1996; Sánchez-Vázquez et al., 1997). In newts, the light–dark cycle during the aquatic phase entrains a rhythmic pattern of activity, with a morning and dusk activity often associated with courtship behavior and a nocturnal activity more related to foraging behavior (Dolmen, 1983a; Griffiths, 1985; Kesler and Munns Jr., 1991; Martin et al., 1989). It is unknown whether newts can modify their diel patterns of habitat use in response to fish introduction. However, it has been shown that species that can exhibit both nocturnal and diurnal activity are good models to test whether diel activity patterns are responsive to variations in predation pressure (Metcalfe et al., 1999). Therefore, in a laboratory design, we assessed the presence of newts in a risky habitat as a function of fish presence and the diel period (day versus night). Our primary hypothesis was that in the presence of goldfish, newts avoid risky habitats, i.e., those occupied by fish, and consequently, hide more in shelters during the day than during the night. We also expected a stronger avoidance response from paedomorphs than from metamorphs.

MATERIAL AND METHODS

Study organisms and laboratory maintenance

At the start of the reproduction period (29 March 2013), we caught 96 adult palmate newts (48 metamorphs and 48 paedomorphs, with 24 individuals of each sex) by dip-netting in a pond devoid of fish ("Le Coulet North-East", Larzac Plateau, France located at 43.820°N, 3.540°E). Paedomorphs had a smaller snout-vent length (i.e., from the tip of the snout to the end of the cloaca) than metamorphs in the study population (mean $\pm$ SE: 33.96 ± 0.26 mm and 42.82 ± 0.48 mm, respectively, $t_{94} = 16.19$, $P < 0.001$). We stored newts (keeping morphs and sex separated) for one day in four large tanks (80×40 cm, 20 cm water depth) before bringing them to the laboratory in six, 3-L tanks ($30 \times 20 \times 18$ cm) placed in a refrigerated box (230 L). At the end of the experiment, all newts were released into their capture habitat following the recommendations of the capture permit. All materials used for capture were well washed and disinfected before and after use following the recommendations of the study permit. The goldfish came from a fish retailer (Blue Coral, Herstal, provider of the Aquarium of the University of Liège). Before the beginning of experiment, goldfish were maintained in a large tank (180×80 cm, 60 cm water depth) at a mean temperature of 18°C and at a photoperiod of 11 h light/13 h dark. Goldfish had a mean ($\pm$ SE) total length of 11.8 ± 0.2 cm ($n = 12$), which is a typical size found in the wild (Winandy and Denoël, 2015a, b). This size was also chosen to test only nonconsumptive effects of goldfish on newt. Consequently, care was taken to plan the experiment in order to avoid any risk of wounds to 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 Animal Ethics Commission (Protocol No.1246).
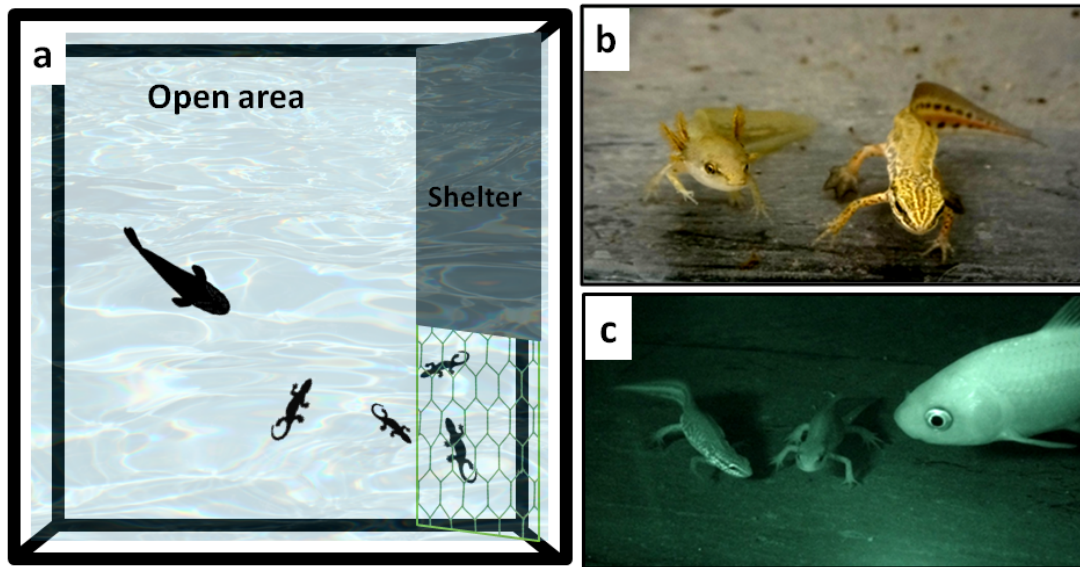
Figure 1

(a) Tank configuration ($n = 24$) with an individual goldfish ($n = 12$) and four palmate newts (one of each phenotype and each sex), (b) the two newt phenotypes: the paedomorph (on the left) and the metamorph, and (c) infrared image showing the presence of palmate newts in the risky habitat (i.e., the open area) at night.

On 31 March 2013, we distributed the newts among 24 identical tanks (60 × 60 cm, 40 cm water level; 135 L), with four individuals (one of each sex and each phenotype) per tank. Goldfish were simultaneously placed in 12 of the 24 experimental newt tanks. Goldfish were placed in direct contact with newts because previous research showed the importance of interactions rather than only cues to elicit newt avoidance (Winandy & Denoël, 2013b, 2015a). All tanks were independent (not connected) and were oxygenated with an air diffuser. The bottom of the tanks was covered with pieces of slate to come up to a more natural environment (Larzac ponds often have substrate covered by stones or concrete). In each of the 24 tanks, newts were provided with one large shelter (20 × 60 cm). The shelter was closed by a grid to prevent the fish from entering inside. The part of the aquarium outside the shelter is hereafter referred to as the "open area", where newts were in direct contact with the goldfish (Figure 1a). The ambient air temperature was regulated and controlled daily to maintain the water temperature at a mean of 14.69°C (SE = 0.05°C). Newts were fed with 50 mg thawed *Chironomus* larvae per newt every day in the afternoon (at 15:00). This is a typical newt prey and the quantity given corresponds to the newts' needs as shown by stomach content analyses of palmate newts in the wild (Demars, 2004). We chose to feed newts at a different time to the experimental observations (morning and night), to avoid the effect of food presence on their habitat use. Goldfish began to receive the same food as newts one month before the beginning of the study. During the study period, 200 mg thawed *Chironomus* larvae was provided to the goldfish at the surface of the water so that the newts did not consume them (the goldfish consumed food rapidly). The goldfish were fed just after newts. We established a photoperiod (with one Lumilux de lux 2350-lm daylight tube, L36W/12-950, and one Sylvania Professional tube, 36W DECOR183) that reflected the natural cycle at the capture location: 12 h light/12 h dark (8:00 to 20:00). The illumination intensity was measured using a lux meter (L202 PMS Photometer, Macam Photometrics Ltd, Livingstone, UK) and the mean intensity was 186.3 lux for daylight and 0.0104 lux for light at night. The laboratory was devoid of windows so there

was no effect of natural day light. However in the double door entry of the laboratory, a LED lamp (2W, Jansjö, Ikea) was switched on the all time, thus providing the minimum light intensity needed at night and reflecting then better night conditions than full darkness. Newts and goldfish can see in the night (Himstedt, 1967; Tavolga, 1977), but their visual acuity is better in higher light conditions (Himstedt, 1973; Neumeyer et al., 1991).

Experimental procedure

To assess the impact of fish presence on diel patterns of habitat use in newts, we compared the behavioral patterns in 24 tanks: 12 as a control treatment (without fish) and 12 as the fish treatment (with a goldfish). We used a scan-sampling method, which consists of instantaneous sampling of the individual position at set time intervals (Martin and Bateson, 2007), to assess the presence of each newt ($n = 96$) in the open area, which is more risky than the shelter as it is the only area inhabited by fish. The tank only contained two habitats, so that if newts were not in the open area, they were necessarily in the shelter. We could easily distinguish each individual from each other because each aquarium only contained one individual of each sex and phenotype. The sexual dimorphism is pronounced in palmate newts (tail filament, large cloaca, hind-foot web only present in males) and the two phenotypes possess strong morphological differences, such as the presence of gills only in paedomorphs (Figure 1b) (Winandy and Denoël, 2015b). There was therefore no observer bias because all individuals can be attributed to be either under or outside shelter without error and because all individuals are perfectly identifiable in each tank. Three series of observations (i.e., scans) were performed 45 min apart, both during the day (between 9:30 and 11:30) and the night (between 20:30 and 22:30). We chose these time periods because newts are particularly active in the morning and early at night during the breeding period (Martin, 1982). The scan-sampling procedure was repeated every week for 12 weeks (i.e., a total of 6,912 individual scans). This duration is representative of the long aquatic period of palmate newts in Larzac (Gabrion et al., 1977; Denoël, 2005b). We avoided disturbance of the newts by keeping distance to the tanks (approximatively 1m) and by moving very slowly between each tank observation. We did not notice any effects on newt behavior. To be able to move around the laboratory and to observe newts in the dark (Figure 1c), we used infrared video cameras (Sony HDR-CX730E and HDR-HC3E, Sony Corporation, Japan). Infrared wavelength cannot be discriminated by newts (Grüsser-Cornehls and Himstedt, 1976) or by goldfish (Neumeyer, 1986).

Statistical analyses

We used an information-theoretic approach based on the Akaike information criterion (AIC) to evaluate the support for hypotheses that explain variation in the habitat use in the palmate newt. The AIC is a numerical value used to rank competing models. Parsimonious models that explain more variation have the lowest AIC value and are considered to be the best models (Burnham and Anderson, 2002). We built a generalized linear mixed model (GLMM), assuming a binomial distribution to test the effect of fish (fish versus control treatment), phenotype (paedomorph versus metamorph), diel period (day versus night) and sex on the extent of use of the open area by newts ($n = 96$). We also included the different interactions between fish, phenotype and diel period in the model. Time (12 weeks of repeated measure), individual identity and tanks were included as random factors (individuals nested within tanks) to take into account the dependence between the data (Pinheiro & Bates, 2000). We built GLMMs representing combinations of these hypotheses and then calculated the AIC corrected for a small

sample size (AICc) for each model and ranked the models on the basis of their AICc values (Burnham & Anderson, 2002). For each candidate model, we calculated the Akaike weight w, which represents the probability that a given model is the best approximating model, given the data and the set of candidate models. Afterwards, from the AICc analyses, we used the model-averaging function to made inferences based on the set of candidate models (with a delta AICc < 4), instead of basing the conclusions only on the single best model (Mazerolle, 2006). Indeed, when several models rank highly (i.e., delta AICc < 4), there may be not only one single best model. It is therefore best to employ a model-averaging approach to obtain robust parameter estimates or predictions. This procedure computes a weighted average of parameter estimates to be calculated, such that little weight is given to parameter estimates from models that contribute little information about the variance in the response variable (Grueber et al., 2011). Once the model-averaged estimates are calculated, the 95% confidence intervals (CIs) are used to assess the magnitude of the effect: there is a strong effect when the CI excludes 0 (Mazerolle 2006). Finally, to assess the possible difference in phenotype response according to the diel period and the fish presence, we used a Tukey contrast test to bring out significant pairwise comparisons.

For all analyses, we chose an *a priori* level of significance of 0.05. All analyses were performed in R using lme4, MuMIn and multcomp packages (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The AICc analyses showed that all parameters and their interactions were included in a least one of the best models (i.e., with a $\Delta AIC \leq 4$; Table 1). The model averaging procedure confirmed the effect of fish on the proportion of newts present in the open area. In the fish treatment, fewer newts used the open area than in the control treatment (mean ± SE: 29.89 ± 0.80 % and 65.10 ± 0.63 % respectively; Table 2). There was an effect of phenotype, with paedomophs using the open area less than metamorphs (mean ± SE: 43.13 ± 0.91 % and 51.86 ± 0.85 %, respectively, Table 2). There was a slight effect of diel period on the use of open area showing that newts used a little more the open area during day than at night (mean ± SE: 48.35 ± 0.91 % and 46.64 ± 0.86 %, respectively, Table 2). There was also an effect of sex, with females using the open area slightly more than males (mean ± SE: 49.75 ± 0.87 % and 45.25 ± 0.90 %, respectively, Table 2).

There was a strong interaction effect between fish presence and the diel period on the proportion of the open area used by newts, showing that newts in the fish treatment used the open area more at night, whereas newts in the control treatment used the open area more during the day (Table 2, Figure 2a). There was also a strong interaction effect between the diel period and phenotype: metamorphs showed a higher use and paedomophs a lower use of the open area at night (Table 2, Figure 2b). There was no interaction between fish and phenotype, suggesting that the presence of fish affected both phenotypes similarly (Table 2). The interaction between fish, phenotype and the diel period was also not significant. However, Tukey contrast tests revealed different mechanisms in phenotypes according to the diel period (Figure 3): metamorphs in the control treatment used the open area equally during night and day (Z = -2.34, P = 0.36) but in the fish treatment, they used the open area more at night than during the day (Z = 3.39, P = 0.02; Figure 3a). In contrast, paedomophs in the control treatment used the open area more during the day than at night (Z = -8.47, P < 0.001), but in the fish treatment, they used the open area equally during the night and daytime (Z = -1.77, P = 0.72; Figure 3b).

Table 1

Comparison of models explaining the presence of palmate newts in the open area, that is, outside shelters of the experimental tanks.

Fish	Pheno	Period	Sex	Fish x Period	Fish x Pheno	Pheno x Period	Fish x Pheno x Period	AIC$_c$	Delta AIC$_c$	Weight
-2.12	-1.28	0.29	-0.48	-0.98		1.26		2364.0	0.00	0.38
-1.89	-1.08	0.49	-0.45	-1.18	-0.27	1.14		2364.1	0.09	0.36
-1.93	-1.13	0.45	-0.45	-1.10	-0.16	1.22	-0.18	2365.8	1.83	0.15
-2.03	-1.22	0.49		-1.18		1.12		2367.7	3.73	0.06
-1.90	-1.10	0.47		-1.16	-0.28	1.15		2369.1	5.10	0.03
-1.93	-1.13	0.43		-1.07	-0.19	1.24	-0.21	2371.0	6.96	0.01

The models are ranked from best to worst (with support ≥ 0.01), according to AICc scores (small-sample corrected Akaike Information Criterion) and Akaike weights. Coefficients of the included parameters with the sign of the relationship (+ or -) are reported. The considered parameters were fish (fish versus control treatment, with fish as a baseline in the table); phenotype (Pheno: paedomorph versus metamorph, with paedomorph as a baseline); D.diel period (period: day versus night, with day as a baseline); sex (male versus female, with male as a baseline). The interactions between fish, phenotype and diel period were also assessed.

Table 2

Summary results of average GLMM models (based on the best model with ΔAIC ≤ 4) relating the effect of parameters on the presence of palmate newts in the open area, that is, outside the shelters of the experimental tanks.

Parameters	Importance	Average coefficients	SE	95% CI
Fish	>0.99	-2.00	0.24	-2.47, -1.52
Phenotype	>0.99	-1.18	0.23	-1.64, -0.72
Period	>0.99	0.40	0.20	0.02, 0.79
Sex	0.94	-0.46	0.16	-0.77, -0.15
Fish*Period	>0.99	-1.08	0.23	-1.53, -0.63
Fish*Phenotype	0.54	-0.24	0.34	-0.90, 0.43
Phenotype*Period	>0.99	1.20	0.22	0.77, 1.63
Fish*Phenotype*Period	0.16	-1.18	0.39	-0.95, 0.59

The importance of all parameters (i.e., the sum of Akaike weights) and the mean coefficients of included variables with the sign of the relationship (+ or -) are reported. The parameters considered in the best models were: Fish (fish versus control treatment, with fish as a baseline in the table); Phenotype (paedomorph versus metamorph, with paedomorph as a baseline); Diel period (Period: day versus night, with day as a baseline); Sex (male versus female, with male as a baseline). The interactions between fish, phenotype and diel period were also assessed.
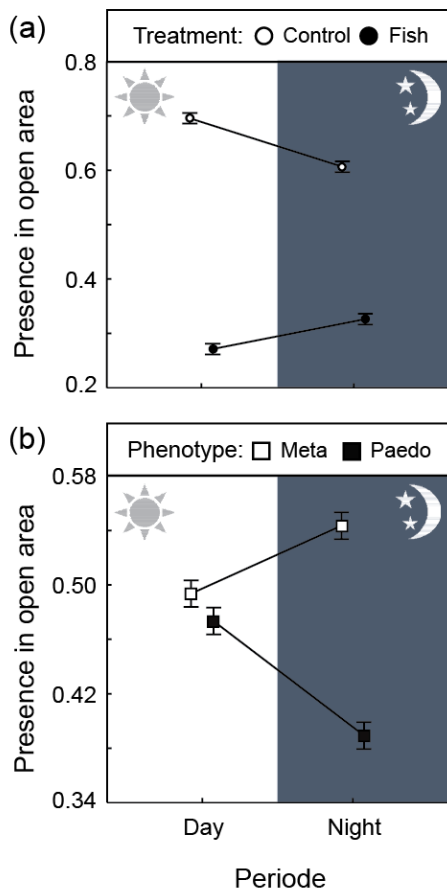
Figure 2

Interactions between (a) diel period and fish, and (b) between diel period and phenotype on the use of the risky habitat (i.e., the open area of the aquaria) by palmate newts (mean ± SE). Newts not present in the open area were hidden under a shelter. See Table 2 for results of the statistical analyses. The left white side and the right grey side represent observations during the day and night-time, respectively. Meta: metamorphs and Paedo: paedomorphs, the two newt phenotypes.
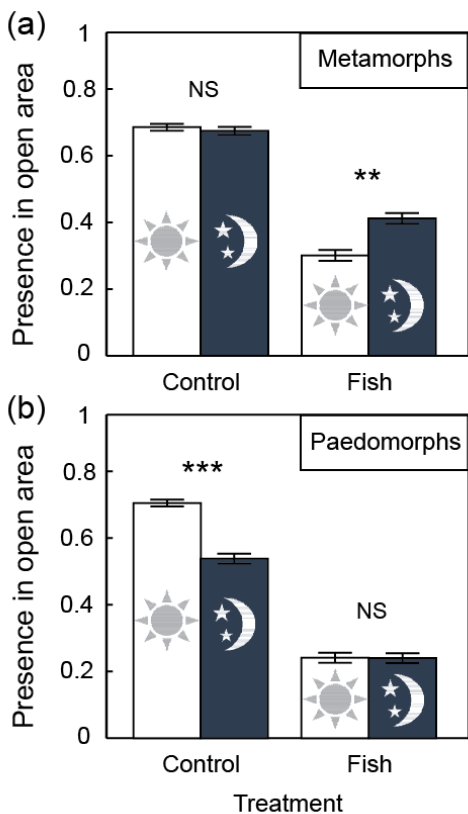

Figure 3

Effect of fish and diel period on the use of the risky habitat (i.e., the open area of the aquaria) in (a) metamorphs and (b) paedomorphs (mean ± SE). Newts not present in the open area were hidden under a shelter. NS: not significant, ** p < 0.01, *** p < 0.001 (see results for statistical analyses). White bar: day; dark bar: night.

DISCUSSION

Our results support the risk-allocation hypothesis (Kronfeld-Schor and Dayan, 2003; Lima and Bednekoff, 1999), suggesting that in the presence of a potential predator, newts exhibited a temporal shift of habitat use. In the presence of fish, newts avoided the risky habitat more during the daytime than at night, whereas in the absence of fish, the inverse pattern was observed. This adaptation to temporal risk of predation might favor coexistence between the introduced fish and native amphibians. However, the results also showed phenotypic variation in the response to fish presence: paedomorphs exhibited less adaptive flexibility than metamorphs in their temporal use of risky habitats. This might contribute to explain why the coexistence between fish and paedomorphs is much rarer in the natural environment than with metamorphs (Denoël and Ficetola, 2014).

Temporal and habitat shift

The impact of fish on the behavior of newts includes habitat shifts towards a safer place. In the presence of fish, newts avoided the risky habitat and consequently, remained in the shelter. This is not surprising, because the most common response to predation threat is the avoidance of risky habitats and the use of shelters (Hartman and Lawler, 2014; Lima, 1998a; Stuart-Smith et al., 2008). Field studies have shown that newts are most likely to be present in ponds with plentiful vegetation, which provides shelters for both adults and larvae (Hartel et al., 2007; Landi et al., 2014). Moreover, aquatic vegetation is important for egg oxygenation (Wimpenny, 1951, but see Miaud, 1995) and protection against predators (Miaud, 1993).The availability of safe habitats can then favor the persistence of newts in habitats invaded by fish (Winandy et al., 2015). Although refuge habitats allow risky encounters to be avoided and, thus, provide escape from potential predation, they often provide fewer foraging and mating opportunities (Bridges, 2002; Lima and Dill, 1990; Winandy and Denoël, 2013a). Therefore, preys are expected to make trade-offs by adapting their habitat use according to the level of predation. That means that prey may balance the cost of shelter use against the risk of encounter with a potential predator to keep opportunity to breed and feed (Lima, 1998a, b). We also found an effect of sex on habitat use showing that males used more the shelter than females. The behavior of females may be explained by their larger size making them less threatened by the risk of predation. Moreover, females may also use more the open area to escape courtship pressures from males in the shelter (see also Verrell, 1984) or to search for substrate for their eggs as females can use different plants to lay their eggs (Miaud, 1995).

As well as habitat preference, the time allocation is important for the variation in predation pressure (Ferrari and Chivers, 2009; Ferrari et al., 2008). By using less risky areas when it is expected to be more dangerous, i.e., during the day, palmate newts adopt an adaptive avoidance strategy. Goldfish are more diurnal than nocturnal, which thereby further decreases the risk of encounters with newts at night rather than during the daytime. A similar diel variation was also observed in juvenile perches under predation risk: they went from open water at night into shelters in daylight (Jacobsen and Berg, 1998; Nunn et al., 2012). It is expected that during the night, the darkness allows prey to be less detected by visually hunting predators (Oberrisser and Waringer, 2011). However, avoidance behavior can also be influenced by the diel rhythm of predator activity rather than by the lighting condition (Madison et al., 1999). Although goldfish can see in dark conditions (Tavolga, 1977), they exhibit a diurnal activity pattern (Iigo and Tabata, 1996; López-Olmeda et al., 2006), which might explain the decrease in newt avoidance behavior at night. This suggests adaptive decision-making through risk assessment,

in which individuals display an effective anti-predator response, depending on the temporal variation in predation risk (Bosiger et al., 2012; Ferrari and Chivers, 2009; Ferrari et al., 2010).

The results also showed a slight effect of the diel period on the behavioral pattern of newts, but the difference between day and night was very low (48 and 47% respectively) and the lower limit of the 95% interval of confidence close to zero (but see next section for interactions with phenotype). This suggests that newts did not use their aquatic habitats quite differentially during the day and night-time. This is in agreement with previous research: in the terrestrial phase, newts are almost only crepuscular and nocturnal, but in the aquatic phase, such as during breeding, they exhibit two peaks of activity that might cause them to leave a refuge place to forage especially during early night and mating during daylight, especially in the morning and in the dusk (Dolmen, 1983a; Griffiths, 1985; Martin et al., 1989). However, during the aquatic phase, palmate newts can also actively forage during the daytime (M. Denoël, pers. obs.). Therefore, species such as newts that can be either nocturnal or diurnal according to several factors such as season, light intensity and temperature (Dolmen, 1983b), are suitable models to test the effect of predation pressure on diel activity patterns, because of their great flexibility in diel phasing (Kronfeld-Schor and Dayan, 2003; Metcalfe et al., 1999). This flexibility can therefore be adaptive in response to the risk of predation and suggests a mechanism of coexistence between species that share the same environment (Kronfeld-Schor and Dayan, 2003). Many animals are able to shift temporal activity into nocturnal or diurnal patterns to avoid predation (Esslinger et al., 2014; Ross et al., 2013; Sönnichsen et al., 2013). Such temporal partitioning generally implies a cost in the overall reduction of activity patterns (Kronfeld-Schor and Dayan, 2003). For instance, the shift from aperiodic to nocturnal activity of salamander larvae in the presence of trout allows them to decrease the risk of predation, but with the consequence of a lower food intake (Barr and Babbitt, 2007). Our results showed that even when newts exhibited a diel change in the use of habitat, they nevertheless still hid more often in the shelter in the presence than in the absence of fish. Moreover, some activity patterns cannot shift into a nocturnal or diurnal profile: for example, some resources are only available at a precise time of the day, making a temporal shift of foraging difficult for the feeders on these resources (Schoener, 1974). Furthermore, even if newts can also mate in the dark (Denoël and Doellen, 2010; Treer, 2015), newt courtship is associated with visual displays and colorful ornamentation, which explains the peak in mating behavior in light conditions (Himstedt, 1971; Martin et al., 1989; Wambreuse and Bels, 1984). These factors might explain why a diel shift in activity remains relatively rare (Monello and Wright, 2001; Schoener, 1974) or is not sufficient regarding the cost of such antipredator strategies.

Differential response between metamorphs and paedomorphs

Globally, paedomophs are less abundant in open areas than metamorphs. In natural ponds inhabited by both phenotypes, spatial differences between the two morphs of palmate newts has already been observed, showing that paedomorphs are mostly found in deep waters, whereas metamorphs are more frequent in shallow area (Denoël, 2005a). This higher abundance of paedomorphs in the deepest habitats can be explained by their gilled mode of respiration, whereas metamorphs rely more on water-surface breathing (Seliskar and Pehani, 1935). Phenotypic variation in habitat use in shallow ponds was also observed in the alpine newt; paedomorphs prefer microhabitats with aquatic vegetation and metamorphs inhabit more open areas without vegetation (Denoël and Andreone, 2003). This habitat partitioning can be only partially explained by diet segregation, because in ponds, a large overlap in food resources between the two morphs is found (Denoël and Andreone, 2003). Another explanation for the

use of vegetation areas by palmate newt paedomorphs might be their greater vulnerability towards predators. Paedomorphs are smaller and their skin is thinner than in metamorphs (Andreone et al., 1993; Winandy and Denoël, 2015b), making encounters with large potential predators more risky. The results here are also congruent with those found in previous studies, where paedomorphs were shown to exhibit avoidance behavior towards direct contact with fish more strongly than metamorphs (Winandy and Denoël, 2015a). However, in the natural environment, microhabitat availability can become a limited resource after fish introduction, as fish such as goldfish can reduce the amount of vegetation in which newts can hide (Richardson et al., 1995). This might partially explain the lower prevalence of paedomorphs than metamorphs in ponds invaded by fish (Denoël and Ficetola, 2014).

Regardless of the presence of fish, the two phenotypes exhibited different diel patterns of habitat use. Although metamorphs were equally present outside their shelter (i.e., in the open area) during both day and night, paedomophs were less frequent in the open areas at night; therefore, palmate newt paedomorphs might be more diurnal than metamorphs. Such temporal partitioning could be considered to be adaptive in allowing a broader use of resources, with each phenotype specializing in micro-habitats and food at a different time in the absence of predation risk (Schoener 1974). Indeed, prey availability varies across space and time and both phenotypes have been shown to use differently these resources in the natural environment (Denoël et al., 2004). In contrast, in the presence of fish, the presence of both phenotypes decreased in the open area, regardless of the time period. Although the response of paedomorphs was as important during day as at night, metamorphs showed a greater use of the open area at night than during the day. Therefore, only metamorphs adapted their time allocation of habitat use in the presence of fish. Because metamorphs showed no diel preference in the presence of fish in the open area, this might make them more flexible in adapting to ecological change (Kronfeld-Schor and Dayan, 2003; Metcalfe et al., 1999). Moreover, the diel alteration of habitat use in other species such as larval tiger salamanders appears to be a short-term predator avoidance behavior that is employed only when a risk of predation exists, showing that flexibility in microhabitat use might depend on the intensity of predation risk (Holomuzki, 1986). However, diel modifications in activity patterns are usually not common in species that show a preferred activity phase, because animal species can be evolutionarily constrained in their activity pattern and therefore, the plasticity in adapting to ecological change can be limited (Kronfeld-Schor and Dayan, 2003). In comparison with metamorphs, the potential lack of behavioral plasticity of paedomorphic newts might explain why they are more threatened by alien fish introduction (Denoël et al., 2005a; Denoël and Ficetola, 2014). Palmate newts therefore persist in some fish-invaded environments, but at the cost of their intraspecific diversity.

CONCLUSIONS

The exhibition of phenotypic plasticity in response to environmental change is an essential adaptive trait in an increasingly disrupted world (Hendry et al., 2008; Sih et al., 2011). The study of anti-predator strategies through risk assessment is essential to understand the interactions between native and introduced species (Ferrari et al., 2015; Winandy and Denoël, 2013b). As shown in the present study, temporal and habitat partitioning at the diel scale between native and introduced species can promote their coexistence through the avoidance of direct encounters. However, diel change can imply a cost in the overall reduction of time allocated in essential activities that might then affect individual fitness (Barr and Babbitt, 2007; Kronfeld-Schor and Dayan, 2003). Therefore, the study of non-consumptive effects of alien

species through the anti-predator decision-making (i.e., avoidance behavior) of native species, brings new understanding concerning the complexity of species interactions and the potential ecological consequences of such introductions (Lima, 1998b; Winandy and Denoël, 2015a).

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