

Predator complement determines the relative success of tadpoles of the *Rana esculenta* complex

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ABSTRACT

Question: Does the identity of the apex predator in a system predict the relative success of closely related amphibian larvae?

Organisms: Larvae of the hybridogenetic European frog, *Rana* sp. *esculenta*, and its sexual host, *R. lessonae*.

Site: Three ponds supporting predatory fish and four ponds without fish but containing large invertebrate and amphibian predators in northern Switzerland.

Background: *Rana esculenta* is a better competitor than *R. lessonae* in a wide range of conditions and is also a larger, more fecund frog than *R. lessonae*. Under most conditions, models predict competitive exclusion of *R. lessonae* followed by extinction of *R. lessonae*.

Methods: In the field, we measured the change in frequency of the two taxa from the larval stage to metamorphosis. In the laboratory, we measured the activity of the two taxa and measured their vulnerability to odonate predators.

Conclusions: In the presence of fish, the frequency of *R. lessonae* declined relative to *R. esculenta* from the larval stage to metamorphosis. In the absence of fish and presence of other predators, the opposite was true. *Rana esculenta* was more active than *R. lessonae* and more vulnerable to predation. The two taxa are adapted to different predator complexes and the hybridogenetic system is maintained by occasional dispersal between dissimilar water bodies.

Keywords: activity, co-existence, distribution, habitat segregation, hybridogenesis, predation, *Rana esculenta* complex, tadpoles.

INTRODUCTION

Species are non-randomly distributed in space. At the global scale, this is largely explained by their evolutionary origin. At the local scale, distribution is determined by the physiological

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tolerances of species as well as their ability to co-exist with competitors, predators and disease organisms. In small lakes or ponds, the presence or absence of large predatory fish can dramatically alter which species are present (reviewed in Wellborn *et al.*, 1996). For example, some species of damselfly are restricted to lakes and ponds without fish, while others cannot co-exist with the large invertebrate predators that are common in the absence of fish (Stoks and McPeck, 2003). Amphibian distributions can be similarly structured by predator complement (Richardson, 2001).

European hybridogenetic frogs provide a particular challenge to our understanding of amphibian distributions. *Rana* kl. *esculenta* Linnaeus 1758 is a hybrid between *R. ridibunda* Pallas 1771 and *R. lessonae* Camerano 1882. However, throughout much of *R. esculenta*'s range in central Europe, *R. ridibunda* is absent. *Rana esculenta* reproduces as a sexual parasite of the parental species, *R. lessonae*, by producing gametes that are exclusively *R. ridibunda* in origin (Uzzell and Berger, 1975; Uzzell *et al.*, 1980), a process known as hybridogenesis (Schultz, 1969). When the hybrid, *R. esculenta*, mates with the parental species, *R. lessonae*, the offspring are exclusively *R. esculenta*. Natural matings between two *R. esculenta* only very rarely produce metamorphs (Hotz *et al.*, 1992; Vorburger, 2001). Thus, *R. esculenta* can only persist in the presence of *R. lessonae*.

However, *R. esculenta* is a better competitor than *R. lessonae* in a wide range of conditions (Semlitsch and Reyer, 1992; Semlitsch, 1993a) and is also a larger, more fecund frog (Reyer *et al.*, 1999) than *R. lessonae*. In the absence of some conditions that favour *R. lessonae*, we expect that *R. esculenta* should competitively exclude *R. lessonae* and then go extinct for lack of mates (Hellriegel and Reyer, 2000; Som *et al.*, 2000). Co-existence could also be enhanced if *R. lessonae* had some advantage in the adult phase of the life-cycle, but the evidence is inconsistent (Holenweg Peter, 2001; Anholt *et al.*, 2003).

If each taxon does best in a unique habitat, this could also explain the long-term persistence of this system (Moore, 1977) provided that there is sufficient dispersal to ensure that matings between *R. lessonae* and *R. esculenta* still occur (Hellriegel and Reyer, 2000).

Rana esculenta is in many ways intermediate to its two parental species, *R. ridibunda* and *R. lessonae*. *Rana ridibunda* commonly co-occurs with fish (Pagano *et al.*, 2001) and has lower predation risk than larvae of frogs that are less commonly found with fish (Teplitsky *et al.*, 2003). *Rana lessonae* is more often found in habitats that are smaller (Holenweg Peter *et al.*, 2002), warmer (Negovetic *et al.*, 2001), often oxygen depleted (Plénet *et al.*, 2000a,b), and consequently do not contain fish (Abt Tietje and Reyer, 2004). *Rana esculenta* is more often found in habitats that are larger, cooler, more permanent (Pagano *et al.*, 2001), and consequently contain large fish predators.

Here we investigate whether predator complement can shift the relative frequency of *R. lessonae/esculenta* larvae in seven small water bodies of northern Switzerland. We also conducted laboratory experiments to determine whether the risk to predation of *R. lessonae* and *R. esculenta* tadpoles differs consistently with their relative performance in the field.

METHODS

Field study

Collections

We made four to six collections at each of seven water bodies in Kantons Zürich, Schaffhausen and Thurgau of northern Switzerland (Table 1). The first collection was

Table 1. Sample sizes of amphibian larvae and metamorphs collected from the seven ponds in northern Switzerland

Name	Location UTM	Pond type	Larvae sampled	Metamorphs sampled	Sampling occasions
Dicki	691.3/290.9	Dragonfly	213	113	6
Enteler	692.4/274.7	Dragonfly	496	92	4
Hellberg	703.9/239.2	Dragonfly	455	89	5
Hundsruggen	701.2/240.1	Dragonfly	230	51	6
Äpelhusenhof	701.6/279.5	Fish	92	80	5
Redlikon	698.4/234.8	Fish	394	107	4
Werdhof	695.0/272.7	Fish	116	91	5

shortly after breeding, between 13 June and 15 July 1996. On each visit, two or three people collected tadpoles for 90–120 min using 50 × 20 cm long-handled dip nets. Unbaited minnow traps were left in place for 1–2 h. The tadpoles collected were a tiny fraction of those present and did not appreciably affect population density. At the same time as we collected tadpoles, we captured predators in both the dipnets and the minnow traps.

Larvae were taken to the laboratory where they were anaesthetized using buffered tricaine methane sulphonate (MS-222), rinsed and then individually frozen at –80°C in Eppendorf tubes until their genotype could be determined by protein electrophoresis using the methods of Uzzell and Berger (1975) and Hotz (1983). We collected metamorphs in late summer of 1996, between 20 August and 20 September. We used hand capture in addition to the previous methods for capturing metamorphs. Metamorphs were taken to the laboratory and given toe clips, which were stored and frozen in Eppendorf tubes for later identification by electrophoresis. Metamorphs were returned to the natal pond.

Data analysis

For each pond we had a measure of the frequency of the two taxa, *R. esculenta* and *R. lessonae*, as larvae and as metamorphs. We tested whether the frequency of the two genotypes changed over the course of the season as a function of the predator complement in a pond using a generalized linear model with binomial errors in S-Plus 2000 (Venables and Ripley, 1999). Because the frequency of the two genotypes varies among ponds, we fit a term for pond first and then tested whether there was a significant interaction term between the presence of fish and the stage (larva or metamorph) at which the genotypes were sampled.

Activity study

Recording

As part of a larger set of experiments, we artificially crossed (Berger *et al.*, 1994) five *R. lessonae* and seven *R. esculenta* females with four *R. lessonae* males (each used three times) to create 12 clutches of tadpoles of the same taxon as the mother. After hatching, we raised clutches separately in 1000-litre outdoor tanks containing bacterial and algal flora growing on natural *Typha* sp. detritus.

We measured the activity of 131 individual tadpoles (Gosner stage 26–32, snout–vent length 4–9 mm) that had been randomly assigned to 10 × 30 cm containers filled to a depth of 5 cm with aged tap water. We placed a single large odonate, *Anax imperator* Leach 1815, larva inside a bottomless cylindrical cage (5 cm wide × 10 cm high with 2 mm mesh size) in each experimental container. Larvae were starved for 48 h before the experiments. Two pairs of containers were videotaped using two PAL-VHS video cameras on a switching timer. Each video camera was mounted 1 m directly above a pair of experimental containers. The timer switched the signal from the two video cameras every 5 min over the course of 75 min (Anholt *et al.*, 2000).

At the end of 60 min, the cages were removed and the *Anax* larva allowed to hunt. When recorded, the exact time of capture was noted. If capture occurred while the videotape was recording the alternate pair of containers, the midpoint of the observation period was assigned as the time of capture.

From the 30 min recorded (out of 60 min) before release of the predator, we measured the size of the tadpole, and the frequency and duration of each tadpole's active and inactive periods. For those periods when a tadpole was active, we measured movement speed five times per second using a custom macro written in AIL, the macro control language of Optimas. The macro located the tadpole, advanced the video five frames (0.2 s), relocated the tadpole and calculated speed in the interval. This was iterated until the end of the active period. From all such measurements, we calculated an average movement speed while active for each tadpole. Tadpoles swam on or near the bottom of the water column. Distances travelled along the bottom were much greater than the 5 cm depth of the water column. The distance of the camera above the experimental tanks reduced distortions due to parallax to near zero (Anholt *et al.*, 2000).

Analysis

We tested whether taxon, body size or activity predicted survival time using a Cox proportional hazards model implemented in S-Plus 2000. On two occasions, the video-recorder stopped recording before 75 min. Animals that had not been captured by the end of recording were included in the data and treated as being right-censored in the analysis.

RESULTS

Field study

The ponds either contained fish or large invertebrate predators but not both. Three ponds (Werdhof, Äpelhusenhof, Redlikon) contained the large fish *Tinca tinca* (Linnaeus 1758) or *Rutilus rutilus* (Linnaeus 1758). We collected no large aeshnid dragonfly or salamander (*Triturus* spp.) larvae from these ponds. Four ponds (Hundsrudden, Hellberg, Dickie, Enteler) contained invertebrate predators such as aeshnid dragonfly larvae, dytiscid larvae and salamander larvae, but no predatory fish (Table 1).

In the presence of fish, the proportion of *R. lessonae* larvae in the ponds varied from 19 to 37%, while in the absence of fish, the proportion of *R. lessonae* larvae varied from 29 to 87% (Fig. 1). In the presence of fish, the proportion of *R. lessonae* declined in the metamorphs by 5–7%, while the proportion increased in the absence of fish by 9–36%. The proportion of *R. lessonae* in the metamorphs in fishless ponds reached between 50.4 and 98.9%. The change in frequency between larvae and metamorphs was highly dependent on the status of

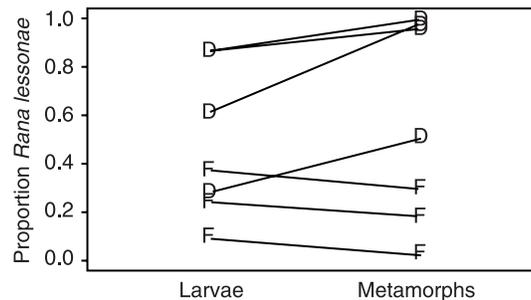


Fig. 1. Proportion of the sample made up of *Rana lessonae* for ponds containing dragonflies (D) or fish (F). Larvae were collected after breeding was completed and egg masses had hatched. Metamorphs were collected at the end of the summer.

the pond (stage \times status interaction term $\chi^2 = 60.0$; d.f. = 1; $P = 10^{-14}$; Fig. 1). A much more conservative test that ignores the magnitude of the changes is a binomial test. The one-tailed probability of seven correct predictions in seven attempts is $1/128 = 0.008$.

Activity study

Rana esculenta was active for a larger proportion of time than *R. lessonae* (mean \pm standard error: 47.6 ± 2.7 and $38.2 \pm 3.7\%$ respectively; angular transformed data $t = 2.13$; d.f. = 129; $P = 0.035$). When active, *R. esculenta* and *R. lessonae* moved at similar speeds (1.89 ± 0.11 and $1.79 \pm 0.15 \text{ cm} \cdot \text{s}^{-1}$ respectively; $t = 0.52$; d.f. = 129; $P = 0.61$). There was no detectable difference in the mean size of the taxa used in the activity study ($t = 0.45$; d.f. = 129; $P = 0.65$).

Almost one-third (28 of 87) of the *R. esculenta* larvae were captured by the dragonfly predator, compared with less than one-tenth (4 of 44) of the *R. lessonae* larvae (Fig. 2). Despite the difference in activity between the two taxa, there was no detectable effect of time active or movement speed measured before release of the predator on the probability an individual would be captured. Similarly, tadpole size had no detectable effects on mortality (Table 2).

DISCUSSION

The results of this study support niche separation based on shared enemies (Holt and Lawton, 1994) as an explanation for the continued co-existence of the hemiclinal hybrid *Rana esculenta* and its sexual host *R. lessonae*. Each taxon does best in a slightly different habitat. While *R. esculenta* larvae were more vulnerable to dragonfly predation than *R. lessonae* larvae, they survived better in dragonfly-free ponds containing fish. In addition, *R. esculenta* adults tend to be associated with larger water bodies than *R. lessonae* (Pagano *et al.*, 2001; Holenweg Peter *et al.*, 2002; but see Van Buskirk, 2003), which differ in other respects than their ability to support fish. *Rana lessonae* is also more tolerant of low oxygen conditions (Plénet *et al.*, 2000a,b) and warmer temperatures (Negovetic *et al.*, 2001).

Although the relative frequency of *R. lessonae* increased in the absence of fish and that of *R. esculenta* increased in their presence, some dispersal between dissimilar ponds would be required for *R. esculenta* to continue to persist (Hellriegel and Reyer, 2000). This does not exclude

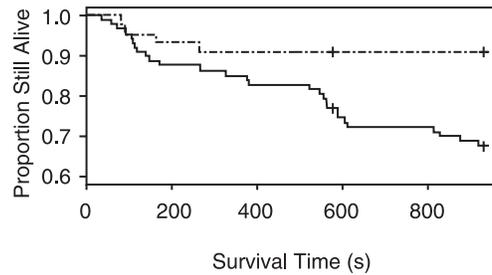


Fig. 2. Survival curve for *R. esculenta* (solid line) and *R. lessonae* (dashed line) tadpoles exposed to a dragonfly predator. Crosses mark right-censored data.

Table 2. Cox proportional hazards analysis of survival data

Effect	z-score	P-value
Taxon	2.4730	0.013
Movement speed	-0.5673	0.57
Proportion of time active	1.1138	0.26
Body size	0.0593	0.95

other mechanisms that have been put forward to explain the persistence of this hybridogenetic system. Higher survival of adult *R. lessonae* (Holenweg Peter, 2001; but see Anholt *et al.*, 2003), preference of females of both taxa to mate with *R. lessonae* males (Reyer *et al.*, 1999; Som *et al.*, 2000), and niche partitioning within ponds based on temperature (Negovetic *et al.*, 2001) could all contribute to reducing the rate at which the hybridogen, *R. esculenta*, would be expected to replace the parental species, *R. lessonae*.

The differential success of two taxa on different parts of the temporary to permanent water body continuum has been well documented in other amphibians. Green frog larvae are eliminated from permanent waters by centrarchid fishes, while bullfrog larvae are eliminated by the large dragonfly larvae that are common in the absence of fish (Werner and McPeck, 1994). There has been repeated evolution of morphological and behavioural traits that allow the co-existence of amphibian larvae with either fish or invertebrate predators (Richardson, 2001). Similar collections of traits exist for damselflies (Stoks and McPeck, 2003), which includes higher sensitivity to the predator prevalent in the most commonly occupied habitat.

Increased activity is associated with higher rates of food acquisition in amphibians (Rist *et al.*, 1997) as well as higher encounter rates with predators (Skelly, 1994). This trade-off is particularly important in the presence of ambush predators such as odonates (Anholt and Werner, 1995, 1998).

We found that larval *R. esculenta* are more active than larval *R. lessonae* in the presence of dragonflies and Semlitsch (1993b) found they were also more active in the presence of newt predators. In both cases, the mortality rate was higher for *R. esculenta*. This is consistent with the observed decline in frequency of *R. esculenta* relative to *R. lessonae* in fishless ponds where odonate and newt predators were present. Conversely, *R. esculenta* larvae are less active than *R. lessonae* when exposed to fish odours (Horat and Semlitsch, 1994). This would make *R. lessonae* relatively more vulnerable to fish predators and would increase the relative frequency of *R. esculenta* in ponds containing fish, as we observed.

We were unable to predict which individual tadpoles were more likely to survive using the proportion of time active, or average swimming speed prior to release of the predator. However, relative predation risk among amphibian larvae is consistently associated with level of activity (Semlitsch, 1993b; Skelly, 1994; Anholt and Werner, 1995; Richardson, 2001).

We hesitate to extend this result to explain all occurrences of this hybridogenetic system because considerable variation exists among hemiclinal lineages for anti-predator traits (Frei, 1997) and life-history traits in the face of competitive pressures (Semlitsch *et al.*, 1997). There is also variation in life-history traits at the LDH-B locus in both the sexual host species, *R. lessonae* (Schmidt *et al.*, 1998), and in the hybridogen, *R. esculenta* (Hotz and Semlitsch, 2000). There may also be local adaptation within taxa to physical and biotic regimes, but this remains to be investigated.

Despite the unusual nature of this system where the hybridogen *R. esculenta* is dependent on the parental host *R. lessonae* for reproduction while at the same time being strong competitors, co-existence appears to be mediated in the same way as it is in other larval anurans. Although their distributions overlap widely, *R. esculenta* is more successful in larger, more permanent water bodies that harbour fish but exclude invertebrate predators, while *R. lessonae* is more successful in smaller, warmer water bodies that do not support fish but consequently have abundant invertebrate predators.

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