In: Zuffi, M.A.L. (ed.) 2006. *Atti del V Congresso Nazionale Societas Herpetologica Italica*. Firenze University Press, Firenze

Within-pond spawning site selection in *Rana dalmatina*

Gentile Francesco Ficetola, Maurizio Valota, Fiorenza De Bernardi

Dipartimento di Biologia, Università degli Studi di Milano,
V. Celoria 26, 20133 Milano Italy;
E-mail: francesco.ficetola@unimi.it

Abstract. We evaluated if *R. dalmatina* females laid their eggs randomly within a pond or if they prefer some microhabitats. We observed preferences for the areas with more submerged deadwoods and vegetation, presence of emergent ground and low water depth. Tadpoles growing in these areas could have increased growth rate and survival, thus these preferences could enhance the fitness of offspring.

Keywords. Spawning, site selection, *Rana dalmatina*

INTRODUCTION

All behaviours that could enhance the fitness of offspring can be considered "parental cares". Only a limited number of anurans provide complex parental cares such as egg and tadpole attendance, or offspring feeding (reviewed by Lehtinen & Nussbaum, 2003). However, also the selection of spawning site is a behaviour that can enhance the fitness of offspring. Females can laid eggs in sites with less predators, with more food availability or with optimal thermal features, thus increasing survival or growth rate of tadpoles. A large number of studies evaluated if the females actively select the breeding waterbodies, and if this selection could be adaptive. Almost all studies observed that females do not chose randomly the breeding wetlands: this selection is generally interpreted to have positive effects on offspring (e.g., Binckley & Resetarits, 2003). However, wetlands are not homogeneous environments: within a pond, many microhabitats can be recognised, with differences in important features such as water temperature and depth, distribution of animal and plants, and sun exposure. Thus, if eggs are laid in different locations within a pond, tadpoles can hatch in different environments. In turn, these differences can be important for survival and/or growth of embryos: data about the movements of tadpoles are scarce; however, the tadpoles that hatched close to a more suitable microhabitat could be advantaged, if compared with tadpoles that hatched far from suitable areas. Despite these considerations, only a small number of studies evaluated whether amphibians laid randomly their eggs within a pond, and the possible consequences of site selection. Here, we investigated whether , within a single pond, the agile frog *Rana dalmatina* laid eggs in microhabitats with selected features. Kecscés and Puky (1992) observed that *R. dalmatina* laid eggs in areas less than 25 cm deep and with abundant vegetation; Ancona and Capietti (1996) performed an attempt to quantify the within site selection of *R. dalmatina* and *R. temporaria*, but without clear results: to our knowledge, no complete and quantitative data on this selection exist. *Rana dalmatina* can be an excellent species to study the within pond spawning selection, since their eggs are easily identifiable and it is an explosive breeder, thus temporal differences between females are minimal, reducing the risk that differences in selection are caused by temporal variation.

METHODS

We studied a single *R. dalmatina* population, breeding in a pond within the "Ca' del Re" moor (Parco Regionale delle Groane, Lombardy, Northern Italy). In this pond, every year several tenths of *R. dalmatina* females breed. In early spring 2003, we selected 36 *R. dalmatina* clutches laid within this pond; the minimum allowed distance between two selected clutches was 1 m, to reduce spatial autocorrelation. We also selected 29 random points; minimum allowed distance between two random points or between a random points and a clutch was 1m. Random points were selected along the pond banks, since all egg masses were laid close to banks. A square frame ($1m^2$) divided by a 0.1 x 0.1 m grid was overimposed to each clutch and to each random point, to better measure environmental variables. For each frame, we measured eight environmental variables (Tab. I). If clutches were laid under the water surface, clutch depth was also measured. Since some egg masses were not fixed to substrate or vegetation, movements of masses are possible. To overcome the risk of data pseudoreplication, all egg masses were measured in two consecutive days; anyway, we did not observe substantial movements of egg masses throughout the study. We used logistic regression to analyse clutch distribution. A forward stepwise procedure was used to assess which variable should be added to the model: we used the likelihood ratio to select the variables that further reduced the log-likelihood of the model (Menard, 1995), until any new variable did not reduce it by any significant value. A variable was retained in the final model if remove P on the last step was <0.05. If necessary, variables were transformed using natural logarithms or arcsine-square root, to meet the assumptions of parametric models.

RESULTS AND DISCUSSION

Most clutches were found at the water surface, only 8 clutches were found at a depth of 5-29 cm. Clutch presence was positively associated to the number of submerged deadwoods within the frame, submerged vegetation % and emergent ground %, and negatively associated to water depth (Tab. II). The model explained 28.1% of null deviance; it is possible that low percentage of deviance explained by the model is caused by the movement of some unfixed egg masses.

This model suggest that, within a pond, *R. dalmatina* females do not laid eggs randomly (χ^2 = 25.153, d.f. = 4, P < 0.0001): females select some microhabitat features; in turn, these features could influence the offspring. Here, we present some possible explanations of the selection for the four environmental features. (1) Number of submerged deadwoods. The preference for areas with abundant submerged deadwoods is easily explainable: *R. dalmatina* and several other brown frogs frequently fix their eggs to submerged woods. Several explanations has been proposed for this phenomena: for example, fixing eggs could reduce the risk that eggs would be drifted; fixing eggs under the water surface could reduce the risk of freezing during cold night or of predation. (2) Low water depth. In areas with lower water depth, the temperature raise more quickly in sunny days: a warm temperature causes a faster growth and development rate (Ancona & Capietti 1996); in turn, fast growth and development are believed to correlate positively with larval survival (see Semlitsch 2002 for references). (3) Abundant submerged vegetation. In areas with more submerged vegetation tadpoles could find more food. The association of *R. dalmatina* clutches with abundant vegetation has been showed also by Kescés and Puky (1992). (4) Relatively abundant emergent ground. The areas with more emergent ground are those more close to the banks, and with lower water depth: a further advantage of these areas could be the difficult access by large aquatic predators, such as fish. Indeed, also in areas with abundant vegetation fish predation is lower, however, in areas with abundant vegetation invertebrate predators (such as Odonata) can be more abundant (Gunzburger & Travis 2004).

In summary, our study shows that the selection of breeding site within a pond could be important for the fitness of tadpoles. Non random breeding site choice has been demonstrated for several amphibians, including the newt *Triturus marmoratus*, and the anurans *R. dalmatina, R. temporaria* and *Physalemus pustolosus* (Ancona & Capietti 1996, Jacob et al. 1998, Tarano 1998). If the spawning site would have important consequences for the fitness of offspring, it could be actively selected, and competition among males for the most suitable breeding sites is possible. Further studies are required to evaluate if the tadpoles growing close to the spawning site have better performance in fitness related traits. Indeed, the microhabitat distribution within a pond could interact with other environmental features to determine the spatial distribution of amphibians.

References

Ancona, N., Capietti, A. (1996): Osservazioni sulla disposizione di uova e girini di *Rana temporaria* e *R.dalmatina* in un'area prealpina. Studi Tridentini di Scienze Naturali - Acta Biologica **71 (1994):** 177-181.

Binckley, C.A., Resetarits Jr, W.J. (2003): Functional equivalence of non-lethal effects: generalized fish avoidance determines distribution of gray treefrog, *Hyla chrysiscelis*, larvae. Oikos **102:** 623-629.

Gunzburger, M.S., Travis, J. (2004): Evaluating predation pressure on green treefrog larvae across a habitat gradient. Oecologia **140**: 422-429.

Jacob, C., Hoffman, O., Braun, C., Crivelli, A.J., Veith, M. (1998): Characteristics of intra-pool egg deposition sites of *Triturus marmoratus* (Latreille, 1800) and *T. helveticus* (Razoumowsk, 1789). In: Current studies in herpetology, p 215-219. Miaud, C., Guyetant, G., Eds. SHE, Le Bourget du Lac, France.

Kecscés, F., Puky, M. (1992): Spawning preference of the agile frog, *Rana dalmatina* B. In: Proc. Sixth Ord. Gen. Meet. S. E. H., Budapest 1991, p. 251-254. Korsos, Z., Kiss, I., Eds.

Lehtinen, R.M., Nussbaum, R.A. (2003): Parental care: a phylogenetic perspective. In: Reproductive biology and phylogeny of Anura, p. 343-386. Jameson, B.G.M., Ed. Science Publishers, Enfield (NH)

Menard, S. (1995): Applied Logistic Regression Analysis. Sage: Thousand Oaks, CA.

Semlitsch, R.D. (2002): Critical elements for biologically based recovery plans of aquatic-breeding amphibians. Conserv. Biol. **16**: 619-629.

Tarano, Z. (1998): Cover and ambient light influence nesting preferences in the Tungara frog *Physalaemus pustulosus*. Copeia **1998**: 250-251.

TABLES

Environmental features	Average (± SE)	
	Clutches (n = 36)	Random points (n = 29)
Slope of the nearest bank (0: < 30°; 1: < 45°; 2: > 45°)	0.69 ± 0.12	0.52 ± 0.14
Water depth	36.72 ± 2.29 cm	43.24 ± 2.65 cm
Distance from the woodland	14.58 ± 1.87 m	13.97 ± 2.07 m
Number of submerged deadwoods (within the frame)	0.25 ± 0.11	0
Submerged vegetation % (within the frame)	62.64 ± 5.60 %	50.69 ± 7.13 %
Emergent vegetation % (within the frame)	12.92 ± 2.88 %	26.21 ± 6.44 %
Emergent ground % (within the frame)	1.53 ± 0.82 %	0 %
Submerged debris % (within the frame)	12.92 ± 3.57	11.38 ± 4.08

Table I. Environmental features measured.

Variable	*B*	χ^2	d.f.	P
N of submerged deadwoods	12.816	9.860	1	0.002
Water depth	-0.062	6.318	1	0.012
Submerged vegetation %	2.131	11.043	1	0.001
Emergent ground %	24.503	5.695	1	0.017
Constant	0.348			

Table II. Logistic regression model explaining *R. dalmatina* distribution. *B*: logistic regression coefficients.