6. EXPERIMENTAL STUDY OF THE HORMONAL CONTROL OF EGG DEPOSITION IN *TRITURUS VULGARIS*

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Abstract. We studied the impact of additional hormones on egg deposition parameters by injecting human-chorionic gonadotropin (HCG) to *Triturus vulgaris* females at different moments of the deposition period. The females had a higher susceptibility towards HCG in the early period of the egg deposition. The parameters describing egg deposition differed between newts originating from highly unpredictable environments (flooded areas, temporary ponds) and more stable environments (permanent ponds). In temporary ponds with a high risk of desiccation the end of oviposition is probably triggered by a decrease in hormones induced by environmental factors.

Key words: egg deposition, hormonal stimulation, newt, *Triturus vulgaris,* reproduction

INTRODUCTION

The smooth newt (*Triturus vulgaris*) has a wide distribution throughout Europe [5]. The reproduction of this species takes place in water, sperm transfer occurring after a complex courtship parade without amplex. The anatomy of the spermatheca allows for multiple matings, and also influences the male paternity [11]. The mating dynamic of the *Triturus* species was most studied in the smooth newt (see [8], [12]). However, the studies of the reproduction of *T. vulgaris* [8] were mostly made on populations from Great Britain that reproduce in relatively constant, favourable conditions (i.e. permanent ponds). In these conditions the females spend a period of up to six months in the water, with oviposition lasting on average 4–6 weeks [1], [13], [14]. In other European countries shorter oviposition periods were reported due to fluctuating environmental factors (e.g. [6] for Italy, [10] for Poland and [3] for Romania). Hormones play an important role in modulating behaviour patterns in amphibians [7], but their role in controlling egg deposition is not well understood.

We investigated the role of additional hormones on egg deposition parameters in smooth newt females, by injecting human-chorionic gonadotropin (HCG) to females at different moments of the deposition period.

MATERIAL AND METHODS

We collected 15 smooth newt females in early spring in 2003 immediately after the ice began to melt and before courtship started, from a temporary pond in a mixed deciduous forest near Sighişoara (N: 46º13'44.1''; E: 24º47'32.7''), Mureş Department, Romania.

Each female was measured and weighed (0.01 g precision) at the beginning and at the end of the experiment, and individually held in a four litre plastic jar. Water was changed once every week and the females were fed daily with *Tubifex* and tadpoles of *Rana dalmatina* and *R. temporaria.* A male was added to each jar for two-three days/week to ensure that females were inseminated. Plastic strips were provided for egg deposition, and the eggs were collected and counted daily [3].

We separated the animals in three groups, each consisting of five females. The wet weight of the females before the start of the experiment did not differ between the three groups (ANOVA, F=0.67, p=0.52). The group “before-after” was injected with 200 u.i. of HCG Organon freshly dissolved in a saline solution twice, before and after the start of regular oviposition, the group “after”, received a dose of HCG at the end of the regular egg deposition, and the control group was not injected.

We chose HCG because (i), it can be considered a gonadotropin agonist in amphibians due to structural similarities, (ii) is a key hormone in inducing ovulation in amphibians, and (iii) since the gonadotropin secretion depends on the central nervous stimulation, an adverse environment may compromise ovarian function through the nervous system via gonadotropin [9].

RESULTS AND DISCUSSION

1. Hormonal effect on egg deposition parameters

The HCG has a significant influence on the egg deposition rate, measured as the number of eggs deposited daily (Fig. 1). The HCG injected at the end of the breeding period triggers a second brief egg deposition period. The “before-after” group deposited fewer eggs after the second injection than the females from the “after” group, injected only at the end of egg deposition period. The “after” group before the hormonal injection has a similar egg deposition rate as the “control” group (see Fig. 1), both groups depositing most of eggs in the first part of the egg deposition period.

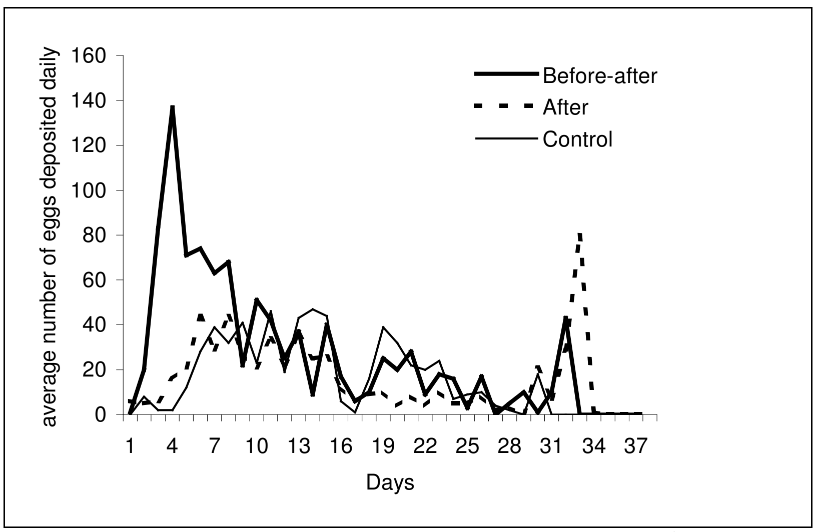

Fig. 1. Egg deposition rate in *Triturus vulgaris* females.

The “before-after” group deposited on average a larger number of eggs than the “control” group and the “after” group (t-test, $p<0.05$ for both cases) (Table 1). The differences between the average number of eggs deposited by the “control” group and the “after” group are not significant (t-test, $p>0.05$).

The wet weight of the females at the end of the experiment did not differ between the experimental groups (ANOVA, F=0.70, p=0.51). During the experiment the wet weight of the females decreased from an initial average of 1.53 ± 0.21 g to an average final weight of 1.15 ± 0.142 g. The decrease was on average of 24%, indicating that despite the intensive feeding during the experiment oviposition implies a high energetic cost. The decrease in wet weight measured as the percentage between the initial/final wet weight ratio was negatively correlated to the total number of eggs deposited (R=-0.51, p=0.05, n=14) (Fig. 2), but was not correlated to the period of oviposition (R=-0.04, p=0.88, n=14).

The number of eggs deposited were highly correlated to the length of the oviposition period (R=0.80, p<0.001, n=15), independent of the hormonal treatment (Fig. 3).

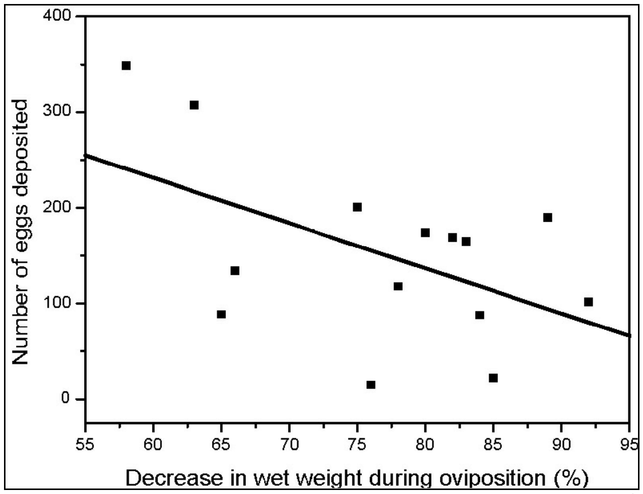

Fig. 2. The correlation between the wet weight (measured as the percentage of the initial/final wet weight ratio) and the total number of eggs deposited.

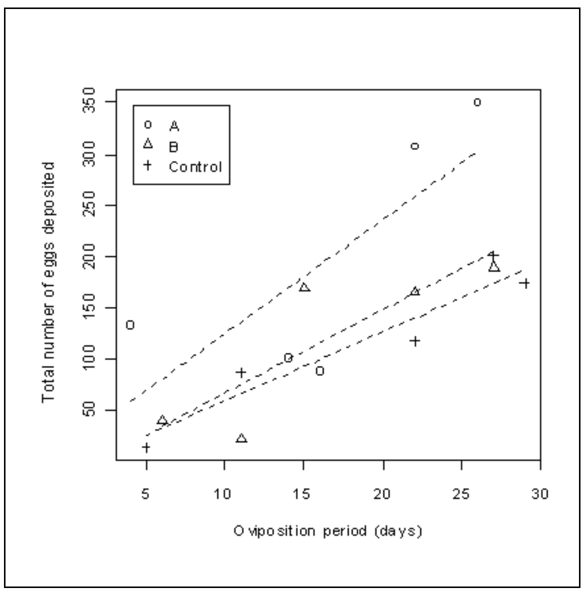

Fig. 3. The correlation between the number of eggs deposited and the length of the oviposition period.

Our results suggest a higher susceptibility of females toward gonadotropin in the early stages of the egg deposition period. Oocyte maturation, as well as ovulation, are under the control of the steroids secreted by the follicle cells, which are themselves the target of other circulating hormones on the hypothalamo-pituitary axis [2].This might be explained as an adaptation to local environmental conditions since the smooth newt population from which the females were collected reproduce in a temporary pond with a high risk of desiccation. In this case, a short and intensive egg deposition period is more advantageous than a prolonged and late breeding period. Cogălniceanu [3] found that the females originating from highly unpredictable environments did not deposit all the eggs, suggesting that the end of the oviposition is not a consequence of the depletion of mature oocytes but is probably triggered by environmental factors. Our present experiment suggests that gonadotropin has an important role in such adaptations.

Table 1

Parameters describing egg deposition in three Romanian smooth newt populations, compared with the population studied by Baker [1] in England and Cogălniceanu [3] in Romania.

Experiment	Locality	Number of eggs deposited	Oviposition period (days)	Female initial weight (g)	Average rate of oviposition (eggs/day)	Maximum no. eggs deposited per day
Present experiment	Sighişoara C	Mean=119.2 SD=73.3 (15-204)	Mean=18.6 SD=10.5 (5-29)	Mean=1.48 SD=0.15 (1.32-1.58)	Mean=4.08 (0-9.4)	31
	Sighişoara B	Mean=117.2 SD=79.5 (22-190)	Mean=20 SD=11.2 (6-31)	Mean=1.48 SD=0.24 (1.35-1.85)	Mean=3.46 (0-16)	20
	Sighişoara A	Mean=196.2 SD=122.33 (89-341)	Mean=23.4 SD=7.98 (12-32)	Mean=1.63 SD=0.26 (1.3-1.9)	Mean=8.16 (0-27.4)	70
Cogălniceanu 1999 [3]	Danube floodplain	Mean=74.6 SD=60.4 (13-195)	Mean=12.3 SD=6.4 (1-21)	Mean=1.09 SD=0-24 (0.76-1.64)	Mean=5.85 (1.6-10.6)	37
	Bucharest	Mean=51.2 SD=30.8 (15-105)	Mean=11.9 SD=8.9 (2.33)	Mean=0.96 SD=0.1 (0.7-1.4)	Mean=5.51 (1.7-17)	24
Baker 1992 [1]	England	Mean=300 SD=189 (88-637)	Mean=36.9 SD=19.7 (11-74)	Mean=2.7 SD=0.8 (1.6-4.2)	Mean=8.7 (3.9-17.3)	54

A, B, C refers to the three experimental groups of females A=”before-after” group; B=”after” group, and the “control” C group. The range for each of the measured parameters are in brackets.

2. Reproductive strategies in the smooth newt in unpredictable environments

Although the main parameters describing egg deposition as well as the initial body weight, differs in populations originating from seasonally fluctuating aquatic environments (temporary ponds and flooded areas), the differences became more obvious when compared with populations reproducing in a more stable environment (Table 1). The mean number of eggs deposited by females from populations inhabiting highly unpredictable environments ranged from 51.2 eggs [3] to 119.2 eggs (present study, control group), while females from a permanent pond deposited 300 eggs on average [1]. The differences regarding the minimum and maximum number of eggs deposited, body weight, average daily deposition rate were also higher in more stable environments (Table 1).

Unpredictable environments do not only affect the size, but also the longevity and mean age, inducing a lower annual survival rate [4].

Under unpredictable and fluctuating conditions a longer breeding period represents a disadvantage mainly due to the risks associated with desiccation, flooding or other factors that might cause high mortality among larvae and perhaps even adults.

Egg deposition in newts appears thus to be under a double control: internal (hormonal) and external (environmental factors).

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