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INSEMINATION AND EGG LAYING DYNAMICS IN THE SMOOTH NEWT, *TRITURUS VULGARIS*, IN THE LABORATORY.

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ABSTRACT

Female smooth newts begin egg deposition in spring after hibernation even if they have not been inseminated. All these eggs are unfertilized. This indicates that the sperm from the previous years are either not retained in the seminal receptacles or are incapable of fertilization. A large proportion of females do not lose receptivity after the first insemination and may collect several spermatophores during the egg-deposition period. There seems to be a positive correlation between the number of eggs deposited and the number of spermatophores transferred.

INTRODUCTION

In the genus *Triturus*, as in most other urodele amphibians, eggs are fertilized in the female's body the sperm being released from a specialized recess of her cloaca, the spermatheca. At the start of the breeding season a female newt is inseminated by a spermatophore, deposited by the male on the bottom of the water body, having been transferred to her cloaca. This happens during an elaborate and relatively long courtship. My own observations (Pecio, unpublished) indicate that the number of sperm in one spermatophore of the smooth newt, *Triturus vulgaris*, is so high that one insemination should provide the female with enough sperm to fertilize all the eggs laid in one season. One spermatophore on average contains several hundred thousand sperms, as I have found by counting the concentration of sperm isolated from single spermatophores.

One might expect then that a female should lose her receptivity after the first insemination in a season, since engaging in further courtship may be costly in terms of time and energy invested, and result in a higher risk of predation. Indeed, Verrell (1984) reported that *T. vulgaris* females lose receptivity after insemination.

The genetic analysis of the progeny collected from individual females of *T. alpestris* and *T. montandoni* has shown, however, that the progeny of a large proportion of females is sired by more than one male (Rafiński, 1981; and unpublished). This means that either a female is inseminated by sperm from several males during one breeding season or that sperm capable of fertilization survive in the female's spermatheca for more than one breeding season. Retention of sperm capable of fertilization for more than one breeding was described for *Salamandra salamandra* (Joly, 1966).

Here I consider two questions: (1) Does the female newt use sperm from the previous season for fertilization? (2) What is the influence of the number of inseminations on the receptivity of the female and the number of eggs she lays?

MATERIALS AND METHODS

On 5 April 1986, 37 female smooth newts were collected in the garden of the Cracow Institute of Zoology on their way to the pond. Each female was placed in a 1.5 l glass bowl with 0.5 l water and fragments of aquatic plants (*Elodea sp., Fontinalis sp.*) for deposition of eggs. The animals were fed with tubifex and plankton three times a week.

Two or three days before each experiment, courting males were netted from the pond adjacent to the area where the females had been collected. The males were placed in an aquarium isolated from the females, with plants, and fed with tubifex.

Observations were carried out every 2-3 days between 08.00hr and 14.00hr. Each female was placed with a male in an aquarium (80 x 30 x 15 cm) with a sand substrate. If no sexual activity was noted after 15 minutes the observation was terminated. Negative or positive response of females to males and the number of spermatophores transferred were noted. After each encounter with a male the female was returned to her glass bowl.

Every second day all the eggs laid were collected, and the number of fertilized and unfertilized eggs from each female recorded using a microscope.

The experiment was finished on 3 June, when all the females had stopped laying eggs and the males no longer exhibited breeding activity. All females were anaesthetised with MS 222, fixed in formalin, for further analysis of the spermatozoa content of the seminal receptacle.

RESULTS

Of the 37 females used in the experiment, 12 produced eggs before they were inseminated. All eggs produced by these females were not fertilized. Only one non-inseminated female had not been courted by a male. All other non-inseminated females exhibited a positive or negative response to a male, or both sexes did not show any reciprocal interest. The number of eggs laid by non-inseminated females varied between 2 and 35 (Table 1). Four of those females picked up spermatophores later, at the end of the experiment.

No. of spermatophores	No. of eggs laid max.	min.	mean	% of unfertilized eggs	No. of females
0	35	2	12.7	100	8
1	180	14	53.2	31.8	8
>1	216	47	136.7	11.9	6

TABLE 1. Number of eggs laid in relation to the number of spermatophores transferred.

	No. of spermatophores/no. of males				
	1/1	2/1	3/1	>2/>2	Total
No. of females inseminated	10	1	1	6	18
% of females inseminated	55.5	5.5	5.5	33	100
No. of sperm. picked up	10	2	3	17	32

TABLE 2. Number of females inseminated with one or several spermatophores from one or more males.

During 144 (35 successful) encounters observed, 18 females (48.6%) became inseminated with at least one spermatophore (Table 2). During the first encounter 12 females transferred one spermatophore to their cloaca, one transferred two spermatophores, and another female three. Some inseminated females remained receptive during further exposure to courting males some time after the first insemination: four females transferred spermatophores from two different males (in 6, 14, 19 and 26 days after the initial insemination), and two females transferred spermatophores from three different males (2 + 11 and 17 + 8 days after initial insemination, Table 2). One of the females inseminated three times exhibited a further positive response to male courtship within 16 days after the third insemination, which induced spermatophore deposition but not transfer to the cloaca.

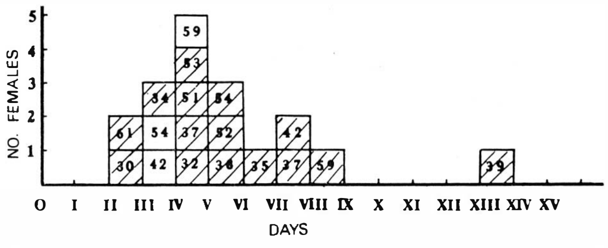

Fig. 1. Relationship between spermatophore transfer (day 0) and the start of egg laying (days 3-14). Each square represents one female. Open squares are females which initially laid unfertilized eggs.

The first eggs (6, 18, 24 in a clutch) laid by three of the inseminated females were not fertilized. Without transfer of additional spermatophores those females later laid only fertilized eggs. The remaining females produced fertilized eggs three days after initial insemination at the earliest, most of them started egg deposition between days 4 and 6 (Fig. 1). Females inseminated with one spermatophore laid on average 52.2 eggs per season, the number of eggs ranging from 14 to 180. On average females inseminated more than once laid 136.7 eggs (Table 1).

There was no significant difference (Mann-Whitney U-test) between numbers of eggs laid by females which had collected one spermatophore and numbers of eggs laid by females which had collected several spermatophores. The data indicates, however, that there might be a causative relationship between the number of eggs deposited and the number of spermatophores collected by a female. Small sample size might be the cause of the non-significant result of the statistical test.

Five to thirty-nine days after insemination females started to produce non-fertilized eggs, the total number of eggs laid per female diminished during the course of the experiment, with an increase in the number of non-fertilized eggs. After subsequent insemination these females increased egg production: most of these eggs were fertilized.

DISCUSSION

All females that were not inseminated failed to produce fertilized eggs. This suggests that the spermatozoa are not stored from season to season in female *T. vulgaris*. *Salamandra salamandra* eggs can be fertilized by spermatozoa which have survived for two years in the spermatheca (Baylis, 1939; Joly, 1966). In *T. vulgaris* even if the spermatozoa survived in the spermatheca, they apparently are not capable of fertilization or they undergo resorption after the end of the breeding season.

My data indicate that the beginning of egg-laying in a season does not depend on insemination since both the inseminated and non-inseminated females laid eggs. The process may depend on external factors, e.g. temperature, and/or the physiological state of the female. Bell (1977) showed that egg deposition is determined by the length of time spent by the female in water: a female has to spend about 40 days in water before she begins laying eggs. My data indicates, however, that this is not true in the population I studied. In my experiment, 12 females began laying eggs 10 days after being placed in bowls of water; one female inseminated a day after being placed in water laid seven eggs three days after picking up the first spermatophore. Verrell and Halliday (1985) also found that females may oviposit about 10 days after entry into water. The discrepancy between Bell's (1977) and my data and observations by Verrell and Halliday (1985) might be explained by the local differences in trophic conditions or either environmental factors.

The beginning of egg deposition after initial insemination confirms earlier data of Verrell (1984), who observed egg deposition between two and six days after initial insemination. In *T. alpestris* spermatozoa travel for at least 24 hours through the main duct of the spermatheca and during this time the spermatozoa must free themselves from the mucopolysaccharide substrate, subsequently travelling towards dorsal tubules in the seminal receptacle (Pecio, unpublished observation).

Smith (1954) suggested that "a female once inseminated will not take up any more spermatophores until those eggs ready to be fertilized have been laid", and states that a second mating would seem unnecessary since "the number of spermatozoa contained in the spermatophore is far in excess of the number produced by the female". Verrell (1984) also noted that the majority of females remain unresponsive to male courtship for 20 days after the first insemination. This study shows that 44% of non-inseminated females eagerly followed a courting male until the end of sexual sequences.

The prolonged receptiveness of females and the successive taking up of spermatophores might have both proximate, physiological and ultimate, adaptive reasons. When the tubules are filled with a great number of spermatozoa from several spermatophores (or from one large spermatophore) the functioning of the sperm distribution might be more efficient.

It seems that the amount of sperm reduces quickly in females which lay eggs each day, as some time after insemination the number of unfertilized eggs increases. This might explain the phenomenon, that the multiple inseminated females produce on average 11.9% unfertilized eggs, while once inseminated as much as 31.8%.

In this experiment some of the females after insemination with one spermatophore only produced a large number of eggs which is close to a measured value of the complete clutch size for a season (Hagström, 1980). A similar phenomenon was observed in *Ambystoma tigrinum* and *Desmognathus orhrophaeus* (Halliday and Verrell, 1984). The size of a spermatophore is highly variable and most probably depends on the level of male sexual activity. It might be that a large spermatophore may contain as may sperms as several smaller ones.

Multiple insemination can significantly increase genetic variation of the progeny produced by a female. This might be especially significant for a species which colonises transient environments and experiences sharp reductions of a population size as is the case of many *Triturus* species.

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EGG, CLUTCH AND MATERNAL SIZES IN LIZARDS: INTRA- AND INTERSPECIFIC RELATIONS IN NEAR-EASTERN AGAMIDAE AND LACERTIDAE

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ABSTRACT

We provide data on the fecundity of locally common Israeli reptiles, and use these data to examine current ideas on the reproductive ecology of lizards. Our methodology was selected in consideration of the acute problems of nature conservation in Israel. In the museum collections of the Hebrew University of Jerusalem and Tel Aviv University we used radiography to locate the shelled oviductal eggs of 164 female lizards, belonging to eleven species (Agamidae and Lacertidae). Each sample sums the species' variation over its range and over different years. Female body size, egg number and egg volume were determined. Specific clutch volumes, relative to maternal body lengths, resembled those reported in iguanid lizards from tropical America. Clutch size varied intraspecifically and, in most species, correlated to maternal size. In others, egg size was more influenced by maternal size. We argue that the latter species oviposit in more stable environments than do the majority.