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Reproductive Behavior in the Long-tailed Salamander (*Onychodactylus fischeri* Boulenger).

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Abstract. - We studied reproductive behavior in wild and captive *Onychodactylus fischeri*. Reproductive behavior and physiology is triggered by warming water temperatures in the spring. Pair formation begins at 7° C and spawning at 9° C water temperature. Fertilization is external. The development of distinctive femoral musculature and skin on the posterior surface of the hindlimbs in the male is for grasping the eggs during fertilization. Pairs of salamander experimentally injected with a synthetic analogue of gonadotropin releasing hormone exhibited typical mating behavior and produced fertile clutches of eggs.

Key words: Onychodactylus fischeri , salamander, reproduction, external fertilization, courtship, gonadotropin releasing hormone.

Introduction

The long-tailed salamander, *Onychodactylus fischeri* , is one of the most poorly known amphibians in Russia. Emeliyanov (1947) noted that spawning takes place from the time salamanders appear in the spring until the middle of July. Regel and Epshtein (1975) concluded that reproduction in this species is not restricted to a specific season. The discovery of a clutch with some hatched larvae indicated that oviposition probably occurred in the spring (Kozik, 1991). It is difficult to establish the precise time of oviposition since the length of embryonic development for this species is unknown. The length of embryonic development for the related species *O. japonicus* is 120 days (Hayase and Oseki, 1983) and it breeds in the winter (Akita, 1989). The absence of precise information on the timing and method of reproduction of the long-tailed salamander prompted us to undertake this study.

Methods and Materials

This study was conducted from May through July, 1991 on the Primorsky krai a tributary of the Mineral'naya River. Daylight observations of the location and reproductive state of salamanders in nature were made along a 1000m transect along Kitaisky Spring. The search effort was directed to the time of maximum diurnal activity. All captured salamanders were individually numbered by toe-clipping using standard techniques. A total of 601 salamanders were captured of which 102 were recaptured. All salamanders were measured and weighed on a triple beam balance. We noted the degree of development of such male secondary sexual characters as the distinctive femoral musculature and skin development on the posterior surface of the hind limbs. We recorded such female characteristics as the relative size of oocytes observed through the abdomen, their passage into the oviducts and the condition of the ventral opening to the cloaca.

The long-tailed salamander has secretive habits and we determined that observation of natural oviposition was unlikely. Therefore, we stimulated reproductive behavior hormonally in captive individuals. Salamanders were housed outdoors in the shade of an awning in 8 liter aquaria with 3-5 cm of running water and shelters. Aquarium water was changed daily. Each of seven males and seven females were injected with 1-5 µg/individual/day of a synthetic analogue of gonadotropin releasing hormone for 7-12 days. All individuals were determined to be ready for breeding before injection. Males and females were kept separate until ovulation began; then they were paired.

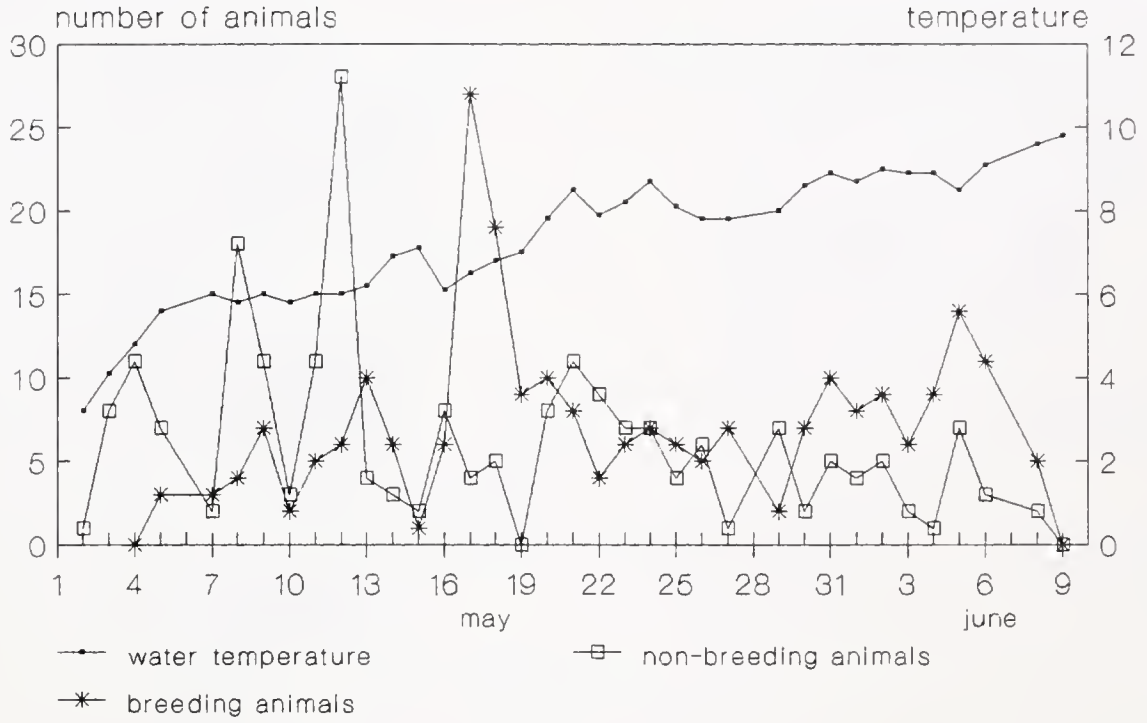

FIG. 1. Pattern of *Onychodactylus fischeri* activity and water temperature during the spring..

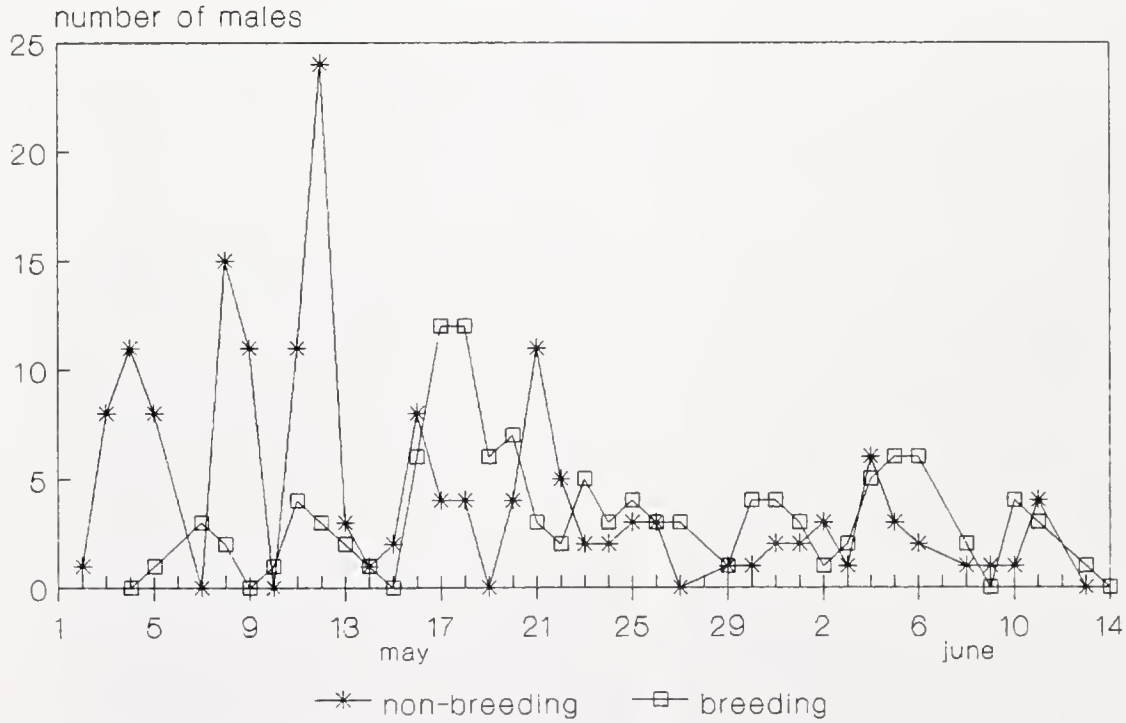

FIG. 2. Frequency occurrence of breeding and non-breeding male *Onychodactylus fischeri*.

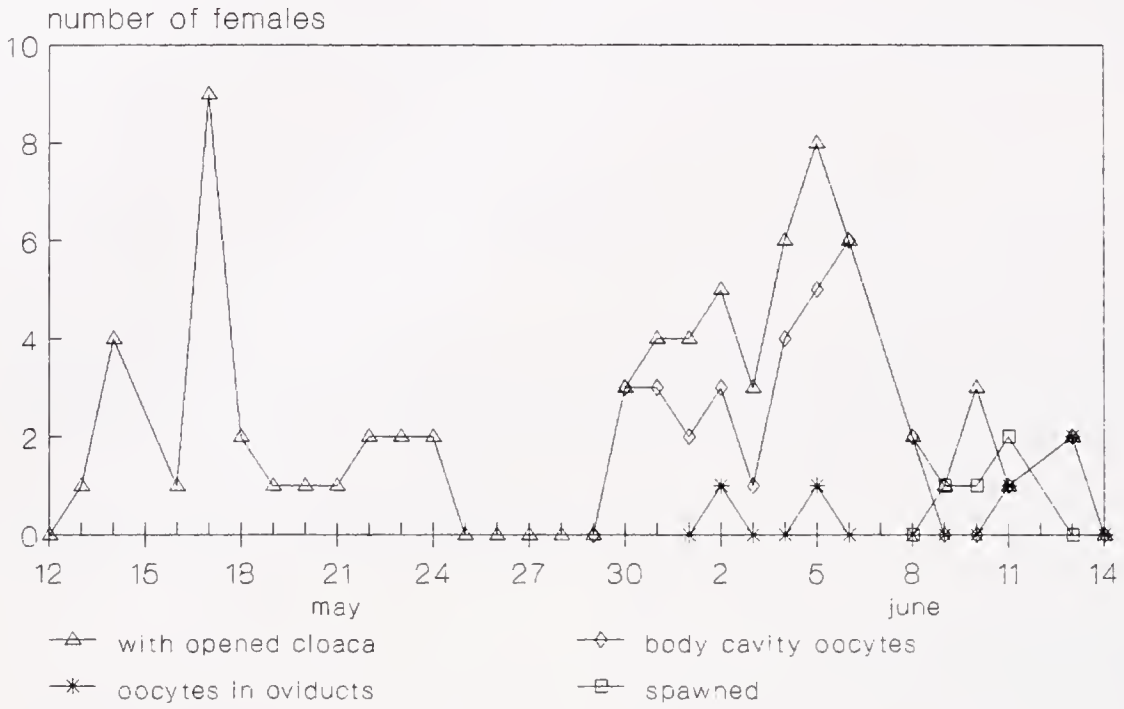

FIG. 3. Frequency occurrence and reproductive state of female *Onychodactylus fischeri*.

Results and Discussion

The first observations of active salamanders were made in late April to early May at a water temperature of approximately 3° C and while sections of the stream were still covered with ice. The number of animals active each day increased as temperatures warmed during the spring (Fig. 1). The first salamanders observed did not exhibit readiness for breeding. Oocytes were not visible through the abdomen wall of females and males did not exhibit hindlimb muscle and skin development. All animals were in a poor nutritional state. The peak number of non-reproductively ready salamanders occurred on 12 May when water temperature reached 6° C. Not all salamanders leave their winter shelters simultaneously due to differential warming rates along the slopes and this is reflected in Fig. 1. After 12 May the number of non-reproductive salamanders decreased to a constant level by 20 May.

The peak number of reproductively ready salamanders was encountered on 17 May when the last stretches of the stream were ice free. These animals appeared to be well nourished. From 15 May to 15 June a majority of the males encountered were in breeding condition (Fig. 2).

By 20 May post-hibernation aggregations of salamanders began to disintegrate and the individuals dispersed along the stream. This marked the beginning of the breeding season.

By 10 May we began encountering females with opened cloacas (Fig. 3). By the end of May females with ovulated oocytes in the body cavity were observed and by 10 June we found salamanders with oocytes in the oviducts. We first observed a female that had spawned or oviposited her eggs on 9 June. In early June we observed 3 male-female pairs of salamanders with at least one member in reproductive readiness. These observations agree with previous accounts (Regel and Epshtein, 1975).

In the hormonally treated females the caudad displacement of oocytes usually took

FIG. 4. Courtship behavior of *Onychodactylus fischeri* with the male (light salamander) approaching the female's (dark salamander) vent, rubbing against the female's abdomen and straddling the female.

two to four days after injection. In another day oocytes appeared in the body cavity and three days later they filled the oviducts. From hormone injection to readiness for spawning usually took from six to 10 days. We observed 6 cases of spawning, two of which were followed by spermatophore deposition.

In the hormonally treated animals approximately one to two days before spawning the female salamander ceased most activity and assumed a typical pre-spawning posture on a stone with the rear third of the body in the water. At this time the male moves about the aquarium, periodically approaches the female touching his snout to her vent, rubbing his body on hers and resting beside her (Fig. 4). The

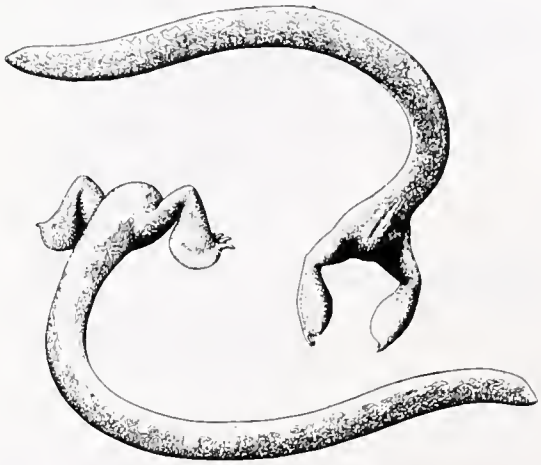

FIG. 5. Comparison of the male *Onychodactylus fischeri* hindlimbs and tail in normal posture and in the pre-fertilization posture with the legs extended. Note the distinctive heavy femoral musculature and skin development on the posterior of the hindlimbs.

male then extends his rear legs and holds this position (Fig. 5).

When spawning begins the female attaches a mucous cord to the stone with a pair of egg sacs each containing one to eight eggs. The eggs are five to six mm in diameter. The mucous capsule is thick and strong unlike that of *Salamandrella keyserlingii* and *Ranodon sibiricus*. During egg deposition (30-40 min.) the male remains sitting beside and in contact with the female. When the egg sacs appear the male enters the water and approaches the female's vent with his snout. In this position the male's body begins to undulate. When the male nudges the egg sac with his snout this causes a burst of excited thrashing from side to side by his snout. This dislodges the egg sac from the female. The male moves immediately over the egg sac with his legs extended and grasps the egg sac with his forelimbs, positioning it between his hindlimbs. The male then grasps the egg sac with the hindlimbs and pulls the base of the sac against his vent and deposits the spermatophore (Fig. 6).

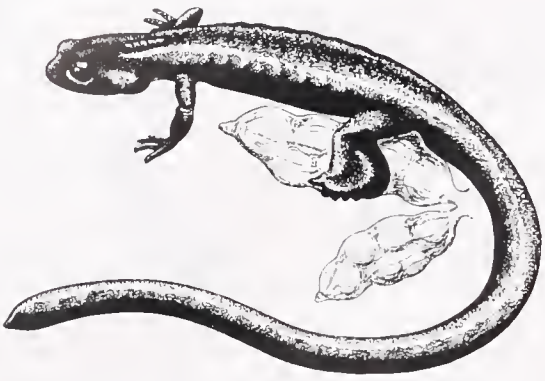

FIG. 6. Male *Onychodactylus fischeri* grasping the egg sac with the hindlimbs in apposition with the vent during spermatophore deposition. Note the distinctive heavy femoral musculature and skin development on the posterior of the hindlimbs.

Those individuals that are ready for breeding emerge from hibernation later than those that are not reproductively ready. We believe that the triggering mechanism for breeding is the elevation of the stream temperature as can be seen in the increased activity of reproductively ready salamanders when the water temperature reaches 6 to 7° C (Fig. 1). At this time females with open cloacas begin to appear. When water temperatures have reached 8° C most females have opened cloacas and many have oocytes in the body cavity. At 9° C water temperature some females have oocytes in the oviducts. By the time water temperature reaches 10° C most females have oocytes in the oviducts and some are beginning to spawn (Fig. 3). We believe increasing temperature is the primary triggering mechanism for reproductive readiness, courtship behavior and spawning.

We found very few post reproductive females on the surface (n=3 in 1990; n=5 in 1991). Very few males were observed on the surface with their legs extended in the reproductive posture. Spawning activity coincided with the period of optimum temperature for activity and low water levels. Salamanders generally remain near the area of spawning until the next reproductive season.

These salamanders are sparsely distributed and do not form breeding aggregations. Male salamanders seem to seek out females well before spawning (i.e. 15-20 days), perhaps by olfaction. The males initiate courtship behavior at this time and it may continue for up to 10 days thus assuring that the male is likely to be in attendance to fertilize the eggs when the female spawns. This lengthy courtship period is the longest known of our native amphibians. This pattern contrasts with that of *Mertensiella caucasica*, the Caucasian salamander, a species with a similar ecology but different reproductive pattern from that of *O. fischeri*. The Caucasian salamander has internal fertilization that induces ovulation. Courtship and copulation take much less time and the pair is together for a much shorter time than are long-tailed salamanders. These represent two very different reproductive strategies for two distantly related species living in similar habitats.

Summary

Our studies have demonstrated that reproduction in *O. fischeri* is triggered by warming water temperatures in the spring. Pair formation begins at 7° C and spawning at 9° C water temperature. Fertilization is external. The development of distinctive femoral musculature and skin is for grasping and holding the eggs during fertilization. Pairs of salamander experimentally injected with a synthetic analogue of gonadotropin releasing hormone exhibited typical mating behavior and produced fertile clutches of eggs.

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