Volume 10, Number 3

July 2000

ISSN 0268-0130

THE HERPETOLOGICAL JOURNAL

Published by the
BRITISH HERPETOLOGICAL SOCIETY

Indexed in
Current Contents

HERPETOLOGICAL JOURNAL, Vol. 10, pp. 111-118 (2000)

BREEDING MIGRATION AND OVIPOSITION OF THE CHINHAI SALAMANDER, *ECHINOTRITON CHINHAIENSIS*

FENG XIE[1], LIANG FEI[1], CHANGYUAN YE[1], CHUNMO CAI[2], ZUWANG WANG[3] AND MAX SPARREBOOM[4]

[1] *Chengdu Institute of Biology, the Chinese Academy of Sciences, Chengdu 610041, China*

[2] *Zhejiang Museum of Natural History, Hangzhou 310012, China*

[3] *Institute of Zoology, the Chinese Academy of Sciences, Beijing 100080, China*

[4] *Van Neckstraat 99, 2597 SC Den Haag, the Netherlands*

Breeding migration, oviposition, egg development and larval migration to water were studied in the Chinhai salamander, *Echinotriton chinhaiensis* during three consecutive breeding seasons. During 1997, 1998 and 1999, mainly females were found around the three ponds where breeding was recorded. Females migrate to breeding sites in late March and April and deposit egg clutches on the banks of the breeding ponds. Characteristic features of these egg-laying areas are high humidity, thick cover of plant debris and location on slopes bordering the water's edge. The eggs develop on land and hatch in early May, when the hatchlings are washed into the ponds during heavy rains. Experiments show that the eggs also develop normally when placed in water. In the natural habitat neither adults nor eggs were ever found in water. Reproduction in this species is dependent on a combination of very specific requirements, which make the species particularly sensitive to the environmental changes that threaten the scarce habitat in which it has been able to survive thus far.

Key words: *Echinotriton chinhaiensis*, *Echinotriton andersoni*, breeding, conservation

INTRODUCTION

In this paper we present the first results of a three-year study of the ecology and life history of the Chinhai salamander, *Echinotriton chinhaiensis* (Chang, 1932). The genus *Echinotriton* consists of two species, *E. chinhaiensis*, occurring in Zhejiang in China, and *E. andersoni*, inhabiting the Ryukyu Islands, Japan (Nussbaum & Brodie Jr, 1982; Cai & Fei, 1984; Nussbaum, Brodie Jr & Yang, 1995). *Echinotriton* is unique among amphibian genera in having an anteriorly curved spine on the posterolateral surface of each quadrate. *Echinotriton* is most similar to *Tylototriton*, but differs in a number of significant morphological and life history features. The ribs of *Echinotriton* are free of muscular attachment distally, sharp-tipped, and often penetrate the skin through the primary warts (Nussbaum & Brodie Jr, 1982). The adults are completely terrestrial and deposit their eggs on land, whereas the larvae develop in lentic water bodies.

E. chinhaiensis is uniformly black on the dorsal and ventral surfaces, with only the underside of the tail, toes and fingers coloured orange (Fig. 1). It differs from *E. andersoni* in that it lacks the rows of secondary warts running on each side of the vertebral crest, between vertebral column and the row of primary warts supported by the ribs. Total length is approximately 12 cm in males and 14 cm in females. The body is broad and flattened; the head is broad and triangular in shape (Chang, 1932, 1936; Cai & Fei, 1984). In both sexes the cloacal opening consists of a longitudinal slit. When slightly opened, the cloaca of the female is smooth on the inside, whereas that of the male is more rugose. When carrying eggs, females have distended abdomens. *E. chinhaiensis* is known only from the type locality and two nearby valleys east of the city of Ningbo (respectively Chengwan, Ruiyansi and Qiushan, district of Beilun, province of Zhejiang, China), where it inhabits a forest area 100-200 m above sea level. Over the last 20 years, the species has been reported only incidentally from the type locality. Earlier exploratory work (Cai & Fei, 1984; Fei, 1992) signalled that the population of Ruiyansi still looked relatively healthy but was isolated and vulnerable, and had to be considered endangered. As a consequence, in 1988 *E. chinhaiensis* was listed in the grade 2 category of major state protected wildlife (Zhao, 1998), which implies that since that time the capture and handling of this salamander have been licensed by the state government.

Little is known about the reproductive biology of *Echinotriton*. Some information on occurrence and habits is available for the Japanese species, *E. andersoni* (Utsunomiya, Utsunomiya & Kawachi, 1978), and ecological work is in progress on a population in Okinawa (Satoshi Tanaka, pers. comm.). In the aforementioned preliminary work (Cai & Fei, 1984),

Correspondence: M. Sparreboom, Van Neckstraat 99, 2597 SC Den Haag, The Netherlands. *E-mail*: m.c.sparreboom@hetnet.nl

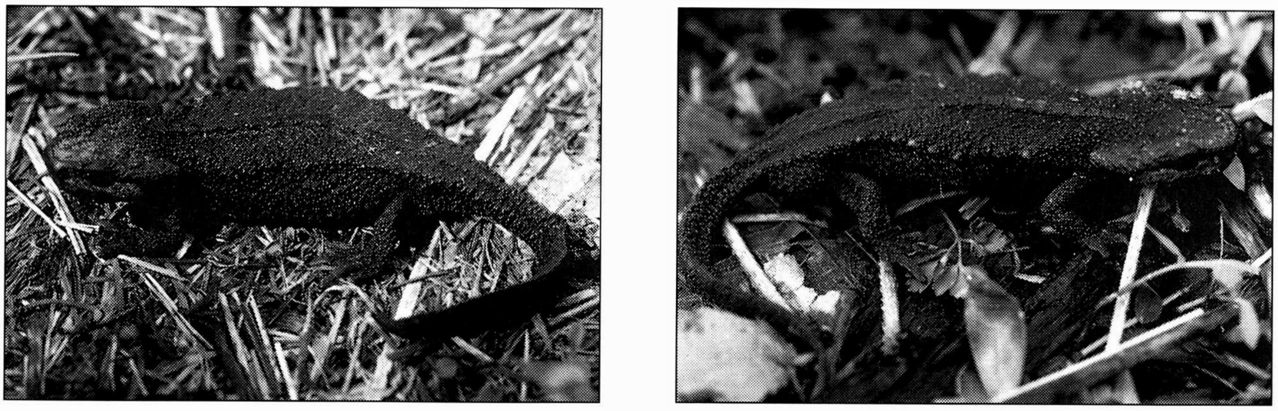

FIG. 1. *Echinotriton chinhaiensis*, female (left) and male (right). Photo by M. Sparreboom.

the authors describe a neotype of the species and report on reproductive habits, defensive posture, habitat requirements and larval development. To a considerable extent reproductive success appears to be dependent on the site of egg deposition, from where the hatchlings must reach the pond (Cai & Fei, 1984; pers. obs.). Fei (1992) reports on the endangered status of this salamander. These papers form the starting point of the present study, in which we report our observations on female breeding migration and oviposition.

STUDY SITE

The study site is situated in the district of Beilun, east of the city of Ningbo in the south-eastern Chinese province of Zhejiang, at 29°8' N, 121°8' E. The region is characterized by an oceanic, subtropical climate, where four seasons can be distinguished and humidity is high all year round. Mean annual temperature is approximately 16°C. The temperature is higher than 10°C for about 234 days per year. Annual precipitation is about 2280 mm, with about 1880 mm of rain falling in May, the wettest month of the year. The valley in which the study site is located is surrounded by hills on three sides. Vegetation consists of secondary mixed forest of broad-leaved trees and pines. Streams and water holes – some of them natural, others man-made – are bordered by agricultural land. There is small-scale farming, with mainly orange orchards, tea plantations, bamboo and flowering plants. Breeding was observed in three small ponds, two of which are situated 7 m apart, with the third located at approximately 200 m distance. During surveys of nine other water bodies in the valley – consisting of temporary and perennial streams, walled water tanks, water reservoirs for agricultural purposes and irrigation ditches – no salamanders, eggs or larvae were found.

FIG. 2. Breeding habitat (pond 1) of *Echinotriton chinhaiensis* in Ruiyansi Forest Park, Zhejiang, April 1999: dense vegetation cover on slopes of a small pond; the banks are covered with leaf litter. Photo by M. Sparreboom.

The spawning habitats around the ponds to which the females migrate (Fig. 2) typically have dense plant cover; this vegetation is composed of an upper layer of evergreen broad-leaved trees, a middle layer of shrubs, and a lower layer of grasses, creating a dark and humid habitat. The egg-laying areas consist of slopes and flat ground directly bordering the ponds; the surface consists of loose soil and stones and is invariably covered by a thick leaf litter. The ponds in which the larvae develop are small (4.2-8.75 m^2), shallow (25-36 cm at the deepest point) and semi-permanent. Their main source of water is rain and they have a pH of 6 to 7. Other amphibian species in these ponds include *Hylarana latouchii*, *Microhyla mixtura* and *Rhacophorus megacephalus* (Huang, Cai, Jin, Gu, Zhang, Guo & Wei, 1990; Fei, Ye, Xie & Cai, 1999), the tadpoles of at least the latter species forming part of the diet of the Chinhai salamander larvae.

METHODS

Our observations were made at three spawning sites between 7 April and 25 May 1997; from 25 March to 25 April 1998; and from 31 March to 24 April 1999. The surroundings of the ponds were searched by carefully raking the leaf litter and turning stones. In 1997 animals were measured, weighed and marked at the site of capture. Individual toe-clipping was applied by cutting a combination of two toes. Growth of toes in specimens marked during the first year and recaptured the second year was slow, allowing individual recognition. The toes were preserved in alcohol.

EFFECTS OF TOE-CLIPPING

In 1997 we began toe-clipping salamanders before they had spawned, but we noted that some females wandered off without spawning in the vicinity of the pond. Of the first 10 females marked in 1997, two were

TABLE 1. Annual recaptures of adult female *Echinotriton chinhaiensis* at breeding sites in the Chinese province of Zhejiang. The 34 recaptures in 1999 represent 6 from 1997 and 28 from 1998. See text for details.

Year	No. of animals	marked	recaptures
1997	50	31	/
1998	88	53	17
1999	89	54	34

found near a large, recently-laid clutch, and both left the breeding area the day after being toe-clipped. The other eight individuals were about to start egg-laying at the time they were found and marked. Two of these animals were later found depositing their eggs under stones – too far from the water for the larvae to have a chance of reaching the pond. One animal deposited a large clutch at the place where we found it; five individuals left the breeding area before laying eggs or after having laid just a few eggs, and were not seen again. Noting this apparent disruption in the breeding routine, we decided to abandon toe-clipping before spawning. Consequently, in 1998 and 1999 we followed a different procedure: each day at 08.00 hr all animals we had found were taken to the field station, weighed and measured. They were kept individually in a box furnished with a moist strip of cotton until they had completed egg-laying. This usually took one or two days. After egg-laying the eggs were counted; the animals were weighed again and marked individually by toe-clipping (Donnelly, Guyer, Juterbock & Alford, 1994). Eggs and animals were returned to the capture site. We have no indication of inflammation or infections caused by clipping, as the animals moved out of the area and were not seen again that year. However, given the recapture percentage of >50% (Table 1), we trust that toe-clipping *per se*, if applied after oviposition, did not seriously affect individual survival and capacity to reproduce. Laboratory observations support this impression.

Due to the secretive habits of this species, we missed a number of migrating adults. Occasionally, we found new clutches after the female had already left. Table 1 does not include females whose presence we have inferred from these clutches.

Each clutch was marked with a stake to which a piece of white cloth or a sticker with a code was attached. Clutch size, location and type of substrate were described. Climatological data such as precipitation and temperature of water and nearby land surface were recorded daily.

Development of the embryos was studied in the laboratory. We kept the eggs under different conditions, both on land and in water (i.e. on soil, or at depths of 1 cm, 5 cm, 10 cm, 15 cm and 20 cm), and observed their development and hatching rate under controlled conditions. The correlation coefficient was used to test for a relationship between hatching time and different water levels. Filtered spring water and running tap water were used; the water was changed every 48 hrs.

Observations of mating activity were conducted in the field station during two weeks in early April 1999. The sexes were kept separate until observation, which began at 23.00 hr and continued until 01.00 hr. Pairs of salamanders were placed together in a polyethylene container measuring 60 cm x 50 cm, furnished with soil and a hiding place. A Sony video camcorder with infrared capability was used to observe and record behaviour patterns in the dark. Room temperature varied from 10°C to 16°C and was similar to the temperature outside.

RESULTS

FEMALE MIGRATION

During the three consecutive egg-laying seasons of our study, the presence of 60 adult females was established in 1997 (50 individuals actually seen, plus 10 individuals whose presence was suggested by an egg-clutch), 88 in 1998 and 105 in 1999 (89 animals actually seen and 16 detected by their clutches). Two males were found in 1997, three in 1998, and three in 1999, which suggests a 'local' sex ratio of about 30:1. The number of males that was found at the breeding sites is too low to have a reliable estimate based upon it.

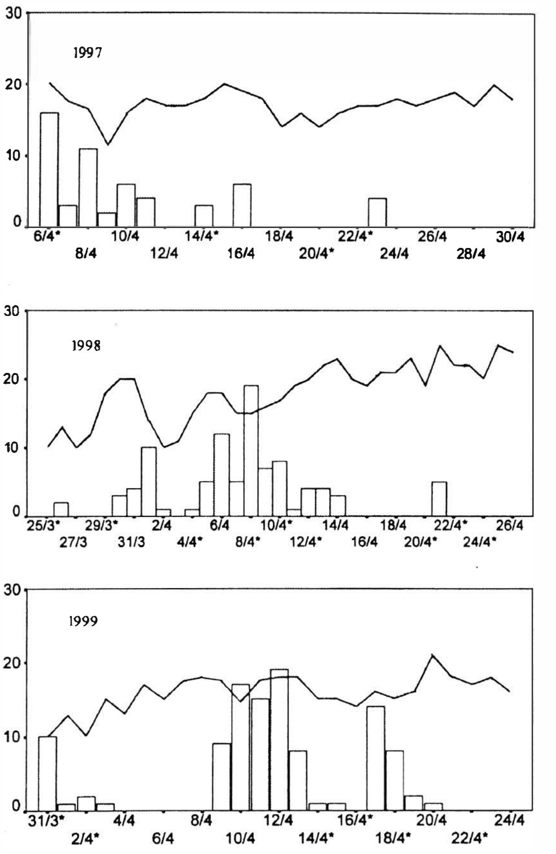

FIG. 3. Migration of female *Echinotriton chinhaiensis* to breeding site in three consecutive years (1997-99); vertical bars indicate no. migrating individuals; continuous line indicates temperature trend; *, rainy days.

In 1998 we recaptured 55% of the females marked in 1997, and in 1999 we recaptured 53% of the animals first marked in 1998 and 19% of those marked in 1997 (Table 1).

The migration data for 1997, 1998 and 1999 are shown in Fig. 3. All eggs were deposited between 26 March and 22 April. The animals do not migrate simultaneously but in waves, showing a relation to the variations in temperature and rainfall. Migration was most frequent on days with an air temperature of 17.7±3.8°C, (n= 4, data for 1997 and 1998). The surface temperature at the breeding sites was around 8°C at the end of March, gradually rising to 15°C at the end of April. Migration mostly took place on humid or rainy evenings between 19.00 hr and 24.00 hr, showing a peak around 21.00 hr. It often followed a thunderstorm after dusk. Migration was sometimes curtailed temporarily when it rained heavily. During the breeding season of 1999 very few migrating animals could be observed directly. There were no thunderstorms that year.

The females stayed in the egg-laying places for about three days and then wandered off. Females leaving the breeding site after laying were not found again the same year. Only on two occasions in 1997 was a female found who had wandered off before spawning and after being toe-clipped, but who migrated in again several days later to lay eggs.

EGG-LAYING BEHAVIOUR

Usually egg-deposition takes about two days. Having arrived at the spawning area, the female chooses a suitable spot and makes a simple and shallow 'nest'. By turning round and pressing her head and body against the muddy forest floor she creates a space on the mud floor under the leaf litter. Exactly how the female prepares the ground we have been unable to observe. The female's body and head are often covered with mud at this time, which suggests that she has engaged in some kind of digging activity. Approximately one day after arriving at the spawning site the female starts laying eggs. Her cloaca is slightly opened and fluids excreted from her cloaca indicate the beginning of egg-deposition. The female lays her eggs one by one, taking about one hour for two eggs. We found no variation in egg-laying speed. Some eggs may be deposited singly, and some grouped in a cluster, more or less sticking together by the outer jelly capsule. On five occasions we found egg clutches deposited in six or seven superimposed layers in one 'nest'. Females at a nesting site were usually found crouching more or less beside the eggs, their bodies coiled in a typical "S" shape (Fig. 4). Occasionally we found that females laid empty jelly capsules. This appeared to mark the end of spawning. When disturbed, females would temporarily stop egg-laying. Animals captured during oviposition and taken to the laboratory, would resume egg-laying after one day, on all kinds of substrate, and also in small, temporary holding boxes. Occasionally, clutches were found under stones or on a side of the slope facing away from the pond. Although the eggs in such clutches developed normally, the hatchlings most likely would not be able to find their way to the water and would die.

FIG. 4. *Echinotriton chinhaiensis*, female laying eggs, visible after removal of leaf litter. Photo by M. Sparreboom.

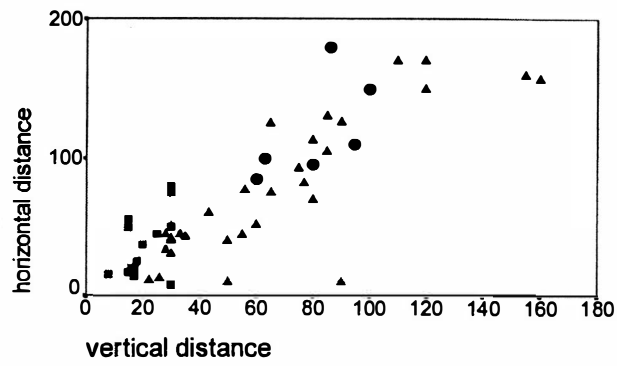

FIG. 5. Distribution of egg-masses of *Echinotriton chinhaiensis* on the banks of the three breeding sites studied, expressed in distance from the water (cm).

LOCATION OF NESTS

Egg clutches were always deposited under a thick layer of humid and rotting foliage. The substrate of a nest site included loose soil, humus and grass roots.

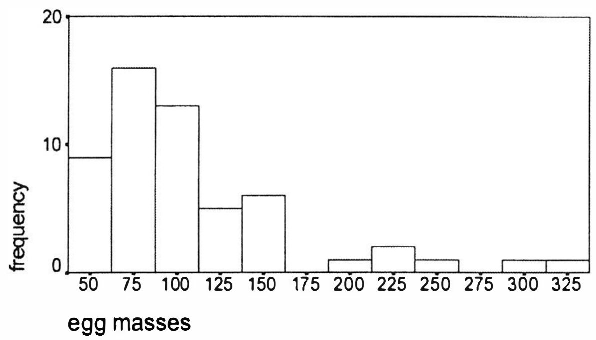

FIG. 6. Number of eggs (50 to 325) in egg masses (n = 55) found in the field, showing evidence of communal nesting. Mean± SE single clutch size measured in laboratory was 69± 21. Data gathered in 1997 and 1998.

TABLE 2. Hatching rate and duration of embryonic development in *Echinotriton chinhaiensis*, under experimental conditions. * eggs placed on earthen substrate covered with leaf litter and sprayed every day; ** measurements refer to last-hatched embryos.

	simulated natural habitat*	water depth				
		1 cm	5 cm	10 cm	15 cm	20 cm
No. hatched	105 (94 %)	10 (100%)	10(80%)	10(80%)	10(90%)	10(80%)
Embryonic period	8-15.5	9-14.5	8-20.5	9.5-11.6	11.5-25.6	9.5-21.6
No. days to hatching	8	6	13	34	46	44
Hatchling SVL (mm)	17.4±0.7 *n*=50			20.5±2.1 *n*=3**		25 *n*=1**

This environment provides the necessary temperature, humidity and protection for embryonic development. The loose soil holds water while maintaining a degree of ventilation. Where such microhabitats were found, the clutches were scattered around the banks of the pond, close to the water's edge. The location of the egg-masses on the banks of the three egg-laying areas in 1997 is shown in Fig. 5. The vertical distance of the clutches to the water varied from 8 cm to 65 cm, with an average distance of 58 cm and a highest frequency at 30 cm. The horizontal distance of the clutches from the pond varied from 7 cm to 180 cm, with an average of 73 cm and a highest frequency at 45 cm. The gradients of the slope varied from mildly sloping away from the pond to flat to steep (-20° to 90°) and 95.7 % of the nest sites were on the slopes facing the water. Distance was positively correlated with gradient: the clutches found on flat land were usually located closer to the water than those deposited on slopes. In the field we found egg masses containing 50 to 325 eggs (Fig. 6). The average±SE single clutch size of clutches laid in the laboratory was 69± 21 (n = 54), and we assume that this was approximately the same in the natural habitat. Accordingly, a substantial number of the egg-masses found in the field (around 30%) were composed of multiple clutches, laid by two to five females, and accounting for about 50% of the total number of eggs. We actually observed that several females deposited their eggs at the same nesting site at about the same time, with some females laying their eggs on top of other eggs. We did not observe eggs being eaten by conspecifics, neither did we see any other forms of intraspecific competition at the breeding site.

Development and Hatchling Migration

The embryo of *E. chinhaiensis* develops on land, but can also develop normally and hatch in water, as our laboratory experiments show (Table 2). A high proportion of eggs hatched, both in a simulated terrestrial habitat (94%), and in water of different depths (80-100%). The proportions of eggs hatching at different water depths did not differ significantly (t=0.46, P=0.14) but there was a positive correlation between water depth and time to hatching (r=0.959). Hatchling size also increased with increasing depth of water.

Chinhai salamander larvae have a limited capacity to move over land, namely by wriggling with rapid tail movements. By these tail movements, recently hatched larvae are even capable of leaping some 10 cm from the ground. Observations in the laboratory suggest that a number of hatchlings may reach the water, even if they are on level ground and are unaided by rains flushing them into the pond (Xie, unpubl.). In the natural habitat, plants and other obstacles, as well as predators, may form a fatal hindrance to the hatchlings' descent to the water.

DISCUSSION

Breeding Migration

The vast majority of individuals we caught were gravid females on their way to – or just arrived at – the breeding sites. As all eggs are usually laid as one clutch within a short time, and male salamanders were not anywhere near at that time, we conclude that these females must have been inseminated prior to arrival at the spawning sites. The sex ratio at the breeding sites was heavily skewed towards females. The few males that were found were discovered under stones near the egg-laying sites at the beginning of the observation period. Intensive searching in the wider surroundings of the breeding sites did not produce more males. Cai & Fei (1984) collected more males in early April than in other seasons, but always fewer than they did females (25 males versus 42 females, captured over three years, 1978, 1979 and 1983). These males were mostly found during excavation works, some 50 m away from breeding ponds one and two, and not at the same sites as females. Our finding is similar to observations on *E. andersoni*, of which females constitute the vast majority of individuals caught near the breeding sites (Utsunomiya *et al*., 1978; Satoshi Tanaka, pers. comm.). Apparently, only females migrate to the spawning sites. Males have a lower 'catchability' and are as hard to find as females outside the breeding season.

Mating

It remains unclear where, when and how mating takes place. We think that mating does not take place at the egg-laying sites immediately preceding oviposition.

Nor does it occur in water, as no adult has ever been found in water. Our observations in the experimental set-up at the study site in 1999 suggest that courtship and mating take place on land. Three males captured during the first days of April 1999 were used for these observations. These individuals were rather emaciated and had a slightly swollen cloacal region. They were seen pursuing females for several hours and showing various movements that could be interpreted as orientation and courtship behaviour. The females used for the experiments were captured during their migration to the spawning site and had probably already been inseminated. They were unresponsive to the males' approaches and hence no complete courtship sequence could be observed (Feng Xie & M. Sparreboom, pers. obs.). The three males showed activity during the first week of April only and from then on remained hidden, no longer showing any interest in the females. If the three males were latecomers, this observation might be considered evidence of a mating period earlier in the season, possibly in March. The earliest date we found sperm in the cloacas of females kept in captivity in Chengdu was 10 March (Feng Xie, pers. obs.). We have no evidence of insemination before the winter.

NESTING SITES

The 'nesting' sites are so specific that the presence of one or more females can almost be predicted. It appears that the female does not simply drop the eggs at the oviposition site, but makes a small clearing in the ground to receive the eggs. Females arriving later at the same place presumably do not need to make this effort, but profit from the 'pre-selected' oviposition site. Large eggs laid on land and eggs deposited in a single clutch are reproductive characteristics usually associated with parental care (Nussbaum, 1985), but in *E. chinhaiensis* we have not observed any form of parental care. The female leaves the oviposition site immediately after egg-laying. Utsunomiya *et al.* (1978) reported that female *E. andersoni* repeatedly push the eggs together with their snouts. We have not observed this phenomenon in *E. chinhaiensis*. Apart from this observation, our observations on oviposition and nesting site are largely in agreement with observations by Utsunomiya *et al.* (1978) on *E. andersoni* on Tokunoshima Island in Japan.

The habitat requirements of the animal lie within a narrow range and apparently few places are suitable for optimal egg development. Egg masses consisting of several clutches are the result. Such assemblages may have the advantage of keeping moisture inside the assemblage at a higher level. As was observed by Cai & Fei (1984) and Fei (1992), prolonged periods of drought are the greatest threat to survival and normal development of the eggs.

PREDATION

No direct evidence of predation on *Echinotriton* adults or eggs has been found. Occasionally animals were found with a forelimb or foot missing, which may be the result of attempted predation. The finding of small clutches of about 50 eggs (Fig. 6) suggests that (1) a predator may have eaten some of the eggs; (2) some disturbance may have caused the female to wander off before completing oviposition; (3) the female may have chosen more than one suitable spawning site to distribute the eggs; or (4) is an indication of a lower fecundity of some individuals.

EVOLUTION OF TERRESTRIAL OVIPOSITION IN SALAMANDRIDS

If the development of the eggs can take place equally well in water and on land, as our laboratory experiments indicate, this raises the issue of what then may be the selective advantage of terrestrial oviposition for the species. Or is terrestrial oviposition a side effect of a specialization to a terrestrial life of the adult salamanders? At present it is only possible to speculate on the answers.

Terrestriality of reproductive modes has evolved many times in amphibians (Salthe & Mecham, 1974; Duellman & Trueb, 1986). For urodele amphibians examples can be found among plethodontids and ambystomatids (Petranka & Petranka, 1981; Jackson, Scott & Estes, 1989; Petranka, 1998), and also in the Salamandridae (Veith, Steinfartz, Zardoya, Seitz & Meyer, 1998). Within the family Salamandridae the genus *Salamandra* exhibits different stages of an evolution towards further independence from water. *Mertensiella luschani* is viviparous and not dependent on water bodies (Polymeni, 1994). In the genus *Tylototriton* oviposition usually takes place in water, but in some species eggs may optionally also be laid on land (for *T. verrucosus*, see Kuzmin, Dasgupta & Smirina, 1994; for *T. kweichowensis,* see Tian, Shun & Li, 1997; Fleck, 1999; and for *T. shanjing,* see Yang, 1991, and Liu Zhijun, pers. comm.). In *T. wenxianensis* and *T. asperrimus* mating has not yet been observed, but both sexes enter water in summer whereas oviposition is on land (Deng & Yu, 1984; Fei, Ye & Yang, 1984; Ye, Fei & Hu, 1993). In the genus *Echinotriton* the tendency towards terrestrial oviposition has evolved further and the tendency to breed in water has been lost altogether.

Salthe & Mecham (1974) describe three basic reproductive patterns among salamanders: "In mode I, numerous relatively small, darkly pigmented ova are abandoned in the open in static water. In mode II, fewer but relatively larger unpigmented ova are fastened to the substratum in a limited area beneath objects in, or adjacent to, running water. The female usually remains in attendance. In mode III a very few, very large unpigmented ova are deposited in a nest hidden in, or under, objects on land and the female usually remains in attendance; development is direct (no larval stage), and this mode is restricted to plethodontids." The reproductive pattern of *Echinotriton* – large, unpigmented eggs deposited on land under leaf-litter;

adjacent to stagnant water; with the female not remaining in attendance – is exceptional among salamanders and does not fit into this classification. It shows some characteristics of Mode II of Salthe and Mecham (1974), and has probably evolved from the largely aquatic reproductive pattern as is found in *Tylototriton*, which is itself best understood as an example of the Mode I category.

CONSERVATION

As a consequence of its specialized reproductive mode, *E. chinhaiensis* has very specific requirements in its breeding environment. If one of the features that characterize its breeding habitat disappears, this salamander cannot survive (Fei, 1992). At the type locality, a pond which is surrounded by bamboo woods, we did not find a single salamander in April 1999, though we discovered a new locality in the valley of Qiushan, where we found two adult females. The suitable habitats are scarce. The known range of the species is limited to a very small area, which makes protection all the more a matter of great urgency. The immediate threats consist of, first, the use of pesticides, which are washed into the breeding ponds at the time the salamander larvae are developing. At the smallest of the three breeding ponds, eight egg-masses were found in March and April 1999, more than 100 young larvae were found in the pond at the end of June that year, but at the end of July, when the water was polluted by pesticides used in the neighbouring orange orchards, not a single larva was found. In the other two ponds, some distance from the orchards, many larvae could still be found (Chunmo Cai & Feng Xie, pers. obs.). Secondly, the terrestrial habitat is endangered by further encroachment on the forest, including activities such as felling trees, building roads and extending cultivation of the ground. During the last 15 years, more woodland in the immediate vicinity of the breeding sites has been cleared and the space used for orange and tea plantations, thus further reducing the species' habitat. At present, efforts are being made to raise public awareness about the importance of saving this species from extinction and to raise more funds for conservation. Sites have been designated where new ponds have now been dug as an insurance against catastrophes. To be effective in the long term, protective measures will have to be explained to the farmers in the valley. Such measures might include persuading farmers to limit their use of pesticides, to use other water sources for cleaning their equipment, and to limit further expansion of their cultivated land in the valley.

ACKNOWLEDGEMENTS

We thank Satoshi Tanaka for sharing with us some unpublished observations on *Echinotriton andersoni* in Japan; we thank Sheng-Xian Zhong for his help in the preparation of the manuscript and two reviewers for constructive criticisms on an earlier version of the paper. This work was supported by the Chinese Academy of Sciences (grant No. KZ951-A1-105 to Liang Fei), the Chengdu Diao Science Foundation and the Science and Technology Commission of Beilun District. Max Sparreboom was supported by a STAR grant of the United Nations Development Programme.

REFERENCES

Cai, C. M. & Fei, L. (1984). Description of neotype of *Echinotriton chinhaiensis* (Chang) and its ecology and habit. *Acta Herpetologica Sinica* **3**, 71-78 (In Chinese, with English abstract).

Chang, M. L. Y. (1932). Notes on two salamanders from Chekiang, *Tylototriton chinhaiensis* sp. nov. and *Triturus sinensis* (Gray). *Contr. Biol. Lab. Sci. Soc. China* **8**, 201-212.

Chang, M. L. Y. (1936). *Contribution a l'étude morphologique, biologique et systématique des amphibiens urodèles de la Chine*. Librairie Picart, Paris.

Deng, Q. X. & Yu, Z. W. (1984). A new subspecies of salamander from Sichuan – *Tylototriton asperrimus pingwuensis*. *Acta Herpetologica Sinica*, Chengdu, new ser. **3**, 75-77 (In Chinese with English abstract).

Donnelly, M. A., Guyer, C., Juterbock, J. E. & Alford, R. A. (1994). Techniques for marking amphibians. In *Measuring and Monitoring Biological Diversity, Standard Methods for Amphibians*, 277-284. Heyer, W. R., Donnelly, M. A., McDiarmid, R. W., Hayek, L. A. C. & Foster, M.S . (Eds). Washington, London: Smithsonian Institution Press.

Duellman, W. E. & Trueb, L. (1986). *Biology of Amphibians*. New York: McGraw-Hill.

Fei, L. (1992). *Echinotriton chinhaiensis* (Chang) and its endangered status. *Chin. J. Zool.* **27**, 39-41 (In Chinese).

Fei, L., Ye, C. Y. & Yang, R. S. (1984). A new species and new subspecies of the genus *Tylototriton* (Caudata: Salamandridae). *Acta Zool. Sinica.* **30**, 85-91 (In Chinese, with English abstract).

Fei, L., Ye, C.Y., Xie, F. & Cai, C. M. (1999). *Microhyla mixtura* Liu et Hu, a new record of Microhylidae from Zhejiang province (Amphibia: Anura). In *Zoological Studies in China*, 239-240. Chinese Soc. Zool. (Ed.) Beijing: Chinese Forest Press. (in Chinese, with English summary).

Fleck, J. (1999). Weitere Beobachtungen an den Gattungen *Echinotriton* / *Tylototriton*. *Elaphe* 7, 66-67.

Huang, M., Cai, C., Jin, Y., Gu, H., Zhang, S., Guo, H. & Wei, J. (1990). *Fauna of Zhejiang: Amphibia Reptilia*. Hangzhou: Zhejiang Science and Technology Publishing House (in Chinese).

Jackson, M. E., Scott, D. E. & Estes, R. A. (1989). Determinants of nest success in the marbled salamander (*Ambystoma opacum*). *Can. J. Zool.* **67**, 2277-2281.

Kuzmin, S. L., Dasgupta, R. & Smirina, É. M. (1994). Ecology of the Himalayan newt (*Tylototriton verrucosus*) in Darjeeling Himalayas, India. *Russ. J. Herpetol.* **1**, 69-76.

Nussbaum, R. A. (1985). The evolution of parental care in salamanders. *Misc. Publ. Mus.Zool. Univ. Michigan* **169**, 1-50.

Nussbaum, R. A. & Brodie Jr, E. D. (1982). Partitioning of the salamandrid genus *Tylototriton* Anderson (Amphibia: Caudata) with a description of a new genus. *Herpetologica* **38**, 320-332.

Nussbaum, R. A., Brodie Jr, E. D. & Yang, D. (1995). A taxonomic review of *Tylototriton verrucosus* Anderson (Amphibia: Caudata: Salamandridae). *Herpetologica* **51**, 257-268.

Petranka, J.W. (1998). *Salamanders of the United States and Canada*. Washington, London: Smithsonian Institution Press.

Petranka, J. W. & Petranka, J. G. (1981). On the evolution of nest site selection in the marbled salamander, *Ambystoma opacum*. *Copeia* **1981**, 387-391.

Polymeni, R. (1994). On the biology of *Mertensiella luschani* (Steindachner, 1891): a review. *Mertensiella* **4**, 301-314.

Salthe, S. N. & Mecham, J. S. (1974). Reproductive and courtship patterns. In *The Physiology of the Amphibia* vol. 2, 309-521. Lofts, B. (Ed.) New York: Academic Press.

Tian, Y. Z., Shun, I.Q. & Li, S. (1997). Observing the reproductive habit of *Tylototriton kweichowensis*. *Chin. J. Zool.* **32**, 20-23 (In Chinese).

Utsunomiya, Y., Utsunomiya, T. & Kawachi, S. (1978). Some ecological observations of *Tylototriton andersoni*, a terrestrial salamander occurring in the Tokunoshima Island. *Proc. Jap. Acad.* Ser.B **54**, 341-346.

Veith, M., Steinfartz, S., Zardoya, R., Seitz, A. & Meyer, A. (1998). A molecular phylogeny of 'true' salamanders (family Salamandridae) and the evolution of terrestriality of reproductive modes. *J. Zool. Syst. Evol. Research* **36**, 7-16.

Yang, D. T. (Ed.) (1991). *Amphibian Fauna of Yunnan*. Beijing: China Forestry Publishing House (In Chinese).

Ye, C. Y., Fei, L. & Hu, S.Q. (1993). *Rare and Economic Amphibians of China*. Chengdu: Sichuan Publishing House of Science and Technology (In Chinese).

Zhao, E. (1998). Amphibia and Reptilia. In Wang, S. (Ed.) *China Red Data Book of Endangered Animals*. Beijing, Hong Kong, New York: Science Press. (Chinese and English).

Accepted: 6.6.00