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WORLD WIDE FUND FOR NATURE AUSTRALIA FINAL REPORT

Project P125

RESCUING THE WESTERN SWAMP TORTOISE *PSEUDEMYDURA UMBRINA* FROM EXTINCTION: AN ECOPHYSIOLOGICAL APPROACH TO CAPTIVE BREEDING

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1.0.0. BACKGROUND TO THE PROJECT

The Western Swamp Tortoise *Pseudemydura umbrina* is the most endangered vertebrate in Australia. The first Western Swamp Tortoise known to science was sent to the Vienna Museum of Natural History in 1839. In 1901 it was scientifically descibed and named by Siebenrock. No further specimens were collected until 1953 when two were found near Warbrook, only 30 km north-east of the centre of the city of Perth.

Following the interest generated by the rediscovery of a presumed extinct species so close to Perth, the Government of the day, aided by a public appeal for funds, created two Class A nature reserves that protected much of its remaining habitat. These are Ellen Brook Nature Reserve of 65 ha and Twin Swamps Nature Reserve of 155 ha.

Biological studies of the Western Swamp Tortoise commenced shortly after it was rediscovered. Initially, Dr. David Ride, then Director of the Western Australian Museum, coordinated field searches and kept a captive colony in the back yard of his Nedlands home. Then, in 1963 Professor A.R. Main of the University of Western Australia initiated and supervised a project by several Zoology Honours Degree students (Lucas 1963) and subsequently a Ph.D. thesis (Burbidge 1967). The captive colony of Dr David Ride (13 tortoises) was transferred to Perth Zoo in 1963.

The studies in the 1960s, especially the Ph.D. study of Dr A.A. Burbidge, revealed the very special biology of the Western Swamp Tortoise and the fact that only about 200 animals were surviving in the two nature reserves (Burbidge 1967). In 1968 Dr A.A. Burbidge commenced work as a Research Officer with the Department of Fisheries and Fauna (Wildlife component now part of the Department of Conservation and Land Management) and continued studies on the Western Swamp Tortoise during the 1970s and 1980s (Burbidge 1981, 1984, 1987). These studies demonstrated that the numbers of *Pseudemydura umbrina* in the wild dropped to about 30-90 animals in the late 1970s and to 20-30 animals in 1987.

The records of the 13 captive animals which were transfered from Dr Ride's colony to Perth Zoo in 1963 are not comprehensive. It seems likely that most, if not all, of these were taken from the wild as adults. Information on the origin and on the identity of individual animals had not been recorded before the animals were transferred and no system for identifying individuals was instigated by Perth Zoo.

The Western Swamp Tortoises in Perth Zoo were kept as a single group in concrete display tanks with a planted land area. The exhibit was entirely enclosed with heavy wiremesh. The first captive breeding of the species was

recorded in 1966 and the subsequent 11 years saw the production of 26 hatchlings, 14 of which survived at April 1978 (Spence *et al.* 1979). However, only four or five of these captive offspring survived by 1987.

During the late 1970s reproduction of the species in Perth Zoo became erratic. In 1979, in order to provide a more intensive and scientifically based approach to solving the problems associated with reproduction in captivity, all three remaining females of breeding age were transferred to the Western Australian Wildlife Research Centre (then part of the Department of Fisheries and Wildlife, now part of the Department of Conservation and Land Management). During the breeding season they were examined by X-ray and when oviducal eggs have been confirmed egg laying was induced by oxytocin injection. During the first year three eggs were produced and incubated artificially at 30° C. Two hatchlings were obtained, but both died in their first year. In 1980 19 eggs were produced (at this time two more females from the Twin Swamps population were taken into the captive group at the Wildlife Research Centre) and artificially incubated; six of these hatched, but again the hatchlings did not survive for more than a few months.

No eggs were produced by the captive animals between 1981 and 1987.

In March 1987 the author arrived at the University of Western Australia to work with Professor S.D. Bradshaw on the reproductive biology and endocrinology of the long-necked tortoise *Chelodina oblonga* and, in collaboration with Dr Bruno Colombe, developed a non-invasive technique using ultra-sound scanning to assess the reproductive condition of female tortoises and turtles (Kuchling 1989). In May 1987 the author started to examine all captive Western Swamp Tortoises in Perth Zoo and later those in the Western Australian Wildlife Research Centre.

At that time the conservation status of the Western Swamp Tortoise was critical. The wild population was reduced to 20-30 animals and on a continuous downward trend. Seventeen animals remained in captivity of which only three were adult females. Captive breeding had reached a standstill, with no eggs having been laid for six years. The females had been caught about 30 years earlier - presumably as adults. The concern was that they were too old to reproduce.

The ultra-sound method was a breakthrough for the assessment of the reproductive condition of the captive female *P. umbrina*, enabling counts and measurements to be made of developing ovarian follicles as well as oviducal eggs. Previously it was not known whether eggs were being developed until ovulation had taken place and the shell had been laid down. Even then, palpation of eggs in Western Swamp Tortoises is difficult because of the small leg openings between the carapace and plastron, and the

only certain method of assessing oviducal eggs was via the use of radiography. Because ovarian activity in the old *P. umbrina* females, as shown by ultra-sound scanning, was known to be continuing, the author proposed immediately changes of husbandry and captive management to improve the captive breeding situation.

These improvements - mainly changes of the feeding regime - probably induced two of the three adult females then in captivity to ovulate and to lay a total of seven eggs during the spring of 1987. These eggs were incubated artificially at 24° C, but none hatched, the embryos dying at an early stage of development. The reasons for this are not clear: recent results suggests that the most likely explanation is poor quality eggs caused by long-term inadequate nutrition of the females that produced them.

2.0.0. ORIGINATION OF THE PROJECT

The success of obtaining some eggs from captive females in 1987 and their subsequent failure to develop and hatch demonstrated on one hand that the situation was not hopeless, but on the other hand that a more concerted and sophisticated approach to captive breeding was necessary in order to rescue the Western Swamp Tortoise from extinction.

In late 1987, the Department of Zoology of the University of Western Australia (Professor S.D. Bradshaw and Dr G. Kuchling) and the Department of Conservation and Land Management (Dr A.A. Burbidge) developed a budget for a two and a half year project; **"Rescuing the Western Swamp Tortoise from Extinction: an Ecophysiological Approach to Captive Breeding"**. WWF Australia agreed to contribute slightly less than half the necessary funds (Project P125). Additional funds were sought and obtained from the Australian National Parks and Wildlife Service, the Western Australian Nature Conservation and National Parks Trust Account and the Department of Conservation and Land Management.

The project has been supervised and coordinated by the **Western Swamp Tortoise Captive Breeding Project Management Committee** of Dr Andrew Burbidge (chair), Mr John DeJose (Perth Zoo) and Professor Don Bradshaw and Dr Gerald Kuchling (Department of Zoology, The University of Western Australia). The project utilised tortoises kept at Perth Zoo and at the Department of Conservation and Land Management's Wildlife Research Centre. Perth Zoo and the Department of Conservation and Land Management have supported the project with staff and with additional funds and the University of Western Australia has provided facilities and financial administration. Perth Zoo has constructed new facilities for the captive tortoises and hatchlings and for the incubation of the

eggs. Construction of these facilities has been part funded by external sponsorship.

2.1.0. COLLABORATORS

I thank the following persons for direct collaboration in the breeding project:

in CALMs Wildlife Research Centre:

Dr Andrew Burbidge, Mr Phil Fuller, Mr Mike Churches, Dr David Coates, Mr Graham Hall.

in Perth Zoo:

Mr John DeJose, Mr Darryl Miller, Dr Bill Gaynor, Ms Georgina Thiele, Mr Dean Burford, Mr David Knowles, Mr Ron Dencio, Mr Klaas Gaikhorst, Mr Ray Dixon, Mr Ivan Loach, Mr Rick Dunlop, Mr Greg Burford, Mr Toni Hodge, Mr Peter Brindley.

at the Department od Zoology of the University of Western Australia:

Prof. Don Bradshaw, Ms Nancy Scade, Mr Stan Hopwood, Mr Tom Stewart, Dr Bruno Colombe.

and further:

Dr Marie Mulcahy, Mr David Groth and my wife Guundie.

3.0.0. OBJECTIVES OF THE PROJECT

- To overcome the stand-still in captive breeding of the Western Swamp Tortoise
- To get the last captive P. umbrina to produce as many offspring as possible
- To investigate and understand the ecophysiological requirements for successful captive breeding
- To develop new rearing techniques for hatchlings; one of the main problems in the past was mortality of young animals
- To train the technical staff of Perth Zoo and CALM in the breeding techniques which should become routine
- To stimulate further conservation activities for the Western Swamp Tortoise, especially improvements of the two reserves where the last wild P. umbrina occur

- To restock the population in the wild with captive bred animals

4.0.0. RESEARCH METHODOLOGY

The complex problems and objectives this project had to tackle resulted in an array of different methods and approaches. The methods included:

- experiments with new designs of enclosures, ponds, aestivation pens, hatchling nurseries
- ultra-sound examination of the reproductive organs of females to assess the reproductive state
- recording of food intake, body weight, growth and behaviour
- hormonal induction of egg laying
- artificial incubation of eggs under different conditions
- candling of eggs to monitor development
- improved diets and feeding regimes
- sampling of blood for plasma enzyme electrophoresis, karyology and DNA-fingerprinting to assess the genetic variability of the breeding stock
- mating management

A main goal of the project was to improve the captive breeding techniques for this species. Many methods and techniques used in the project have been newly developed or adapted and modified from existing techniques and are as such results of the project. A description of these techniques is given in the results-section.

5.0.0. THE ANIMALS USED IN THE PROJECT

The captive breeding project is mainly based on animals of the old captive stock of Perth Zoo and some of its offspring from the 1960s and 1970s and on animals from the former Twin Swamps population which have been taken into captivity in the early 1980s. During the course of the project, four Western Swamp Tortoises have been found on private land outside the Nature Reserves and included in the captive breeding programme. In addition, some animals from the Ellen Brook population have been taken into captivity temporarily in order to increase the gene pool of the captive breeding population and to secure wild animals against fox predation.

The following account of animals does not include wild tortoises which were only caught, examined and released and not used for captive breeding and does not include the hatchlings produced during the project.

Table 1: At the start of the project in 1988 the captive population consisted of the following animals:

	males	females	juven.
animals of the former captive colony of Dr Ride	7	2	-
captive offspring	4	1	-
Twin Swamps population	1	1	1
total	12	4	1

Table 2: The following animals died during the project (only very old wild caught animals of the old captive stock died):

	males	females	juven.
1988	2	-	-
1989	2	-	-
total	4	0	0

Table 3: The following animals have been caught on private land and included into the captive population:

	males	females	juven.
12.09.1988		1	
15.08.1990			1
26.08.1990	1	1	
total	1	2	1

Table 4: The following animals of the Ellen Brook population have been taken in captivity temporarily .In parentheses: animals already released after some weeks in captivity. All the others will be returned into the newly fox proof fenced area in Ellen Brook Nature Reserve during winter 1991:

	males	females	juven.
1988	-	2	-
1989	(2)	4	-
1990	1	2	1
total	3 (-2)	8	1

Table 5: By 31.12.1990 the permanent captive stock (excluding the hatchlings from 1989 and 1990 and the Ellen Brook animals) consisted of the following animals:

	males	females	juven.
old wild caught Zoo stock	3	2	-
former captive bred stock	4	1	-
Twin Swamps population	2	1	1
animals from private land	*)	2	1
total	9	6	2

*) One male caught on private land in 1990 is a marked animal from the Twin Swamps population and shown here as such.

6.0.0. RESULTS

6.1.0. REPRODUCTION

At the start of the project very little was known about reproduction of *P. umbrina*. It was known that mating occurs in winter and early spring and that ovulation must occur some weeks before the three to five eggs are laid in November or early December. A female dissected by Dr. David Ride on 7 September 1959 had five follicles (ova) of about 20 mm diameter in the ovaries. The smallest female known to have produced eggs was of 120 mm carapace length (Burbidge 1981).

6.1.1. Ultra-sound scanning

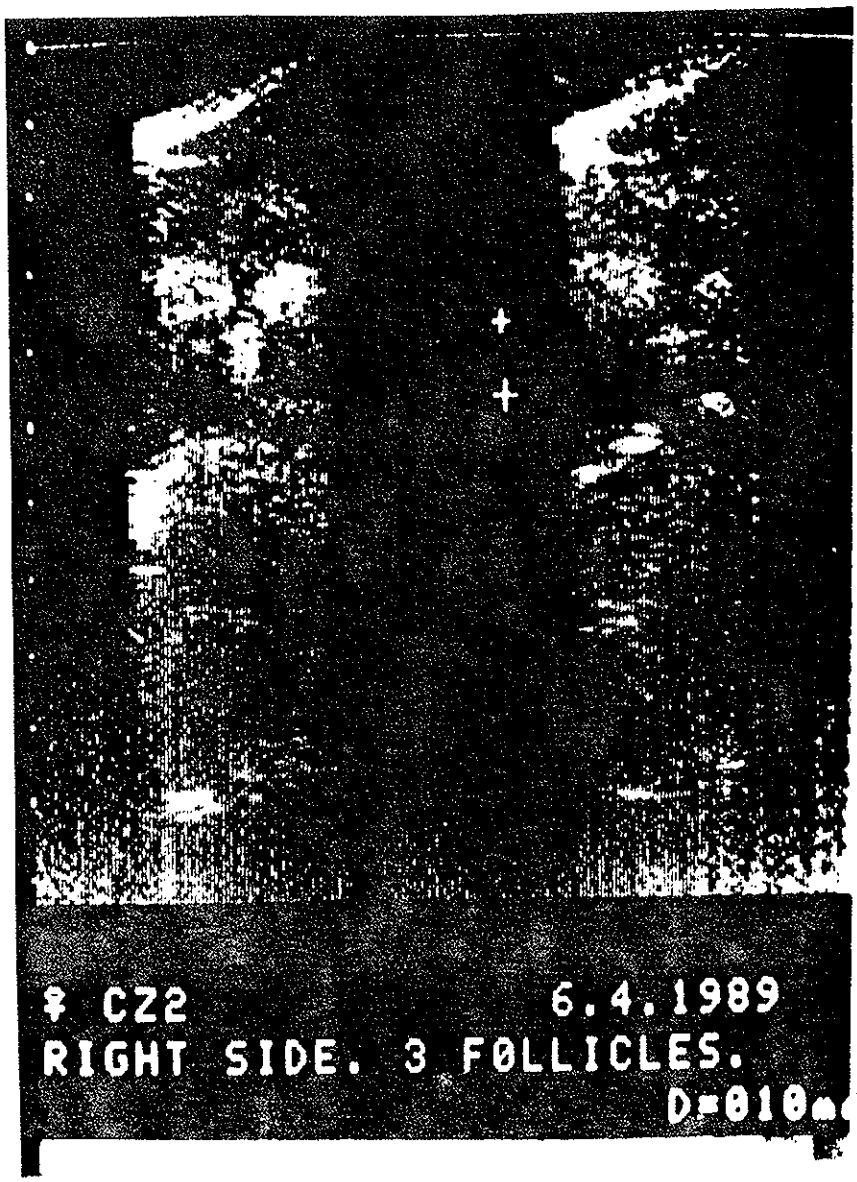
The failure of the captive females to produce eggs was a main reason for the initiation of this project, the understanding of the ovarian cycle therefore an important aspect. The method used to monitor the ovarian activity in individual females was ultra-sound scanning, a technique described for *Chelodina oblonga* (Kuchling 1989). A Toshiba Sonolayer-L SAL 32B with a linear array

probe IOB-502H has been purchased and used in the project. Due to the small size and shell opening of *P. umbrina* this small intra-operative ultrasonic probe gave more accurate results than the larger probe used with *Chelodina oblonga*. The longterm monitoring of individual females over several years improved the accuracy of interpretation of ovarian structures observed by ultra-sound scanning over that described previously (Kuchling, 1989). Generally all captive females are examined by ultra-sound scanning at monthly intervals, but during critical times such as ovulation and egg laying examinations were often made daily. Aestivating females are only examined in one and a half to two month intervals.

The results of the ultra-sound assessments are summarized in Figures 1-19. Ovarian follicles under the size of 4-5 mm diameter are difficult to identify and generally not included in the assessment. They are previtellogenic, have less echogenicity then larger follicles and are present all year round. Therefore they provide little useful information concerning current reproductive activity.

The typical ovarian cycle starts in summer, January to February, when follicles commence to become vitellogenic and increase in size to over 5-6 mm diameter. In ultra-sound examinations, developing follicles are echogenic in nature and spheroid in conformation (Fig. 1). Vitellogenesis, the accumulation of yolk material and therefore energy input into the eggs, begins during aestivation. Typically, reproductively active females have follicles of 14-15 mm diameter when aestivation terminates in May or June, in the captive animals as well as in animals caught in the wild at this time of the year. During winter, the largest follicles further increase until they reach the preovulatory size of 16-18 mm in early spring (Fig. 2). Ovulation occurs between late September and end of October. Ultra-sound imaging permits the recognition of freshly ovulated ova in the oviducts even before the egg shell membranes are laid down: they are of an elongate conformation (*versus* spherical prior to ovulation) and surrounded by a thin non-echogenic layer - the beginning of the albumin secretion (Fig. 3).

The eggs remain four to six weeks in the oviducts before being laid. In ultra-sound scanning, soft-shelled eggs are elongate, with an echogenic spherical to slightly elongated yolk surrounded by a nonechogenic albumin layer and an echogenic shell (Fig. 4). The shell becomes increasingly echogenic as progressive mineralisation of the shell occurs. Eggs are ready to be laid when the shell reflects most of the ultra-sound and its acoustic shadowing obscures the internal yolk and albumin layers (Fig. 5). Nesting activity and egg laying occurs from the end of October to the middle of December.



Firure 1: Ultra-sound image of vitellogenic ovarian follicles. The scale shows centimeters.

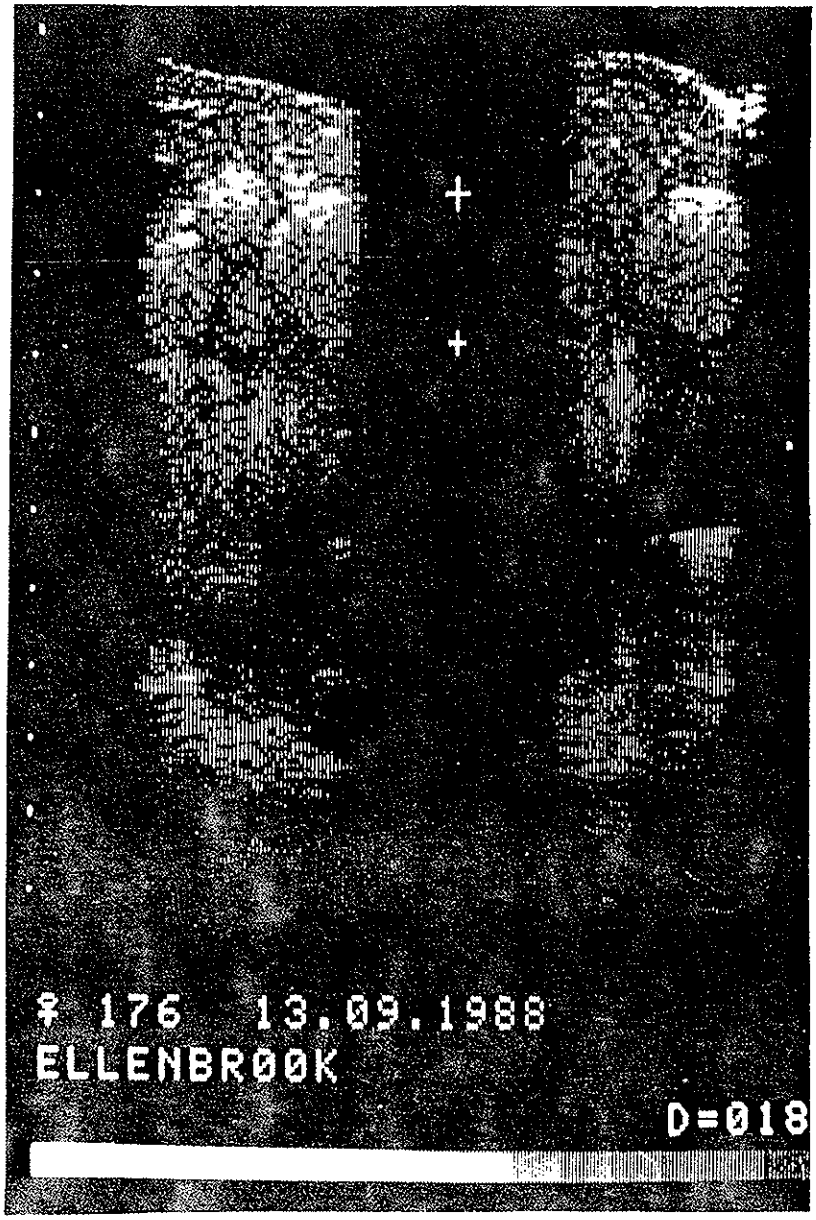


Figure 2: Ultra-sound image of preovulatory ovarian follicles.

Figure 3: Ultra-sound image of freshly ovulated ova.

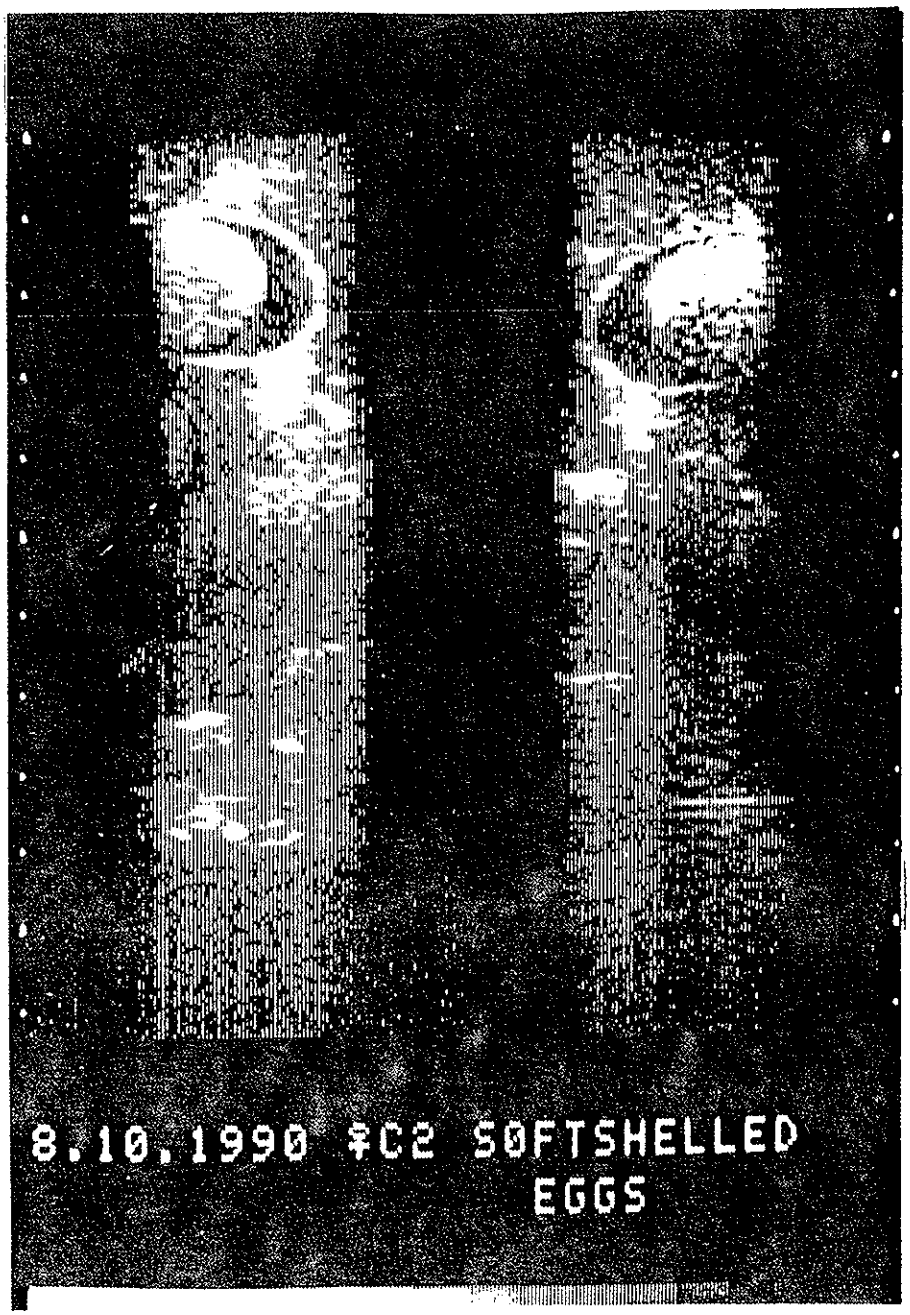


Figure 4: Ultra-sound image of soft-shelled oviducal eggs.

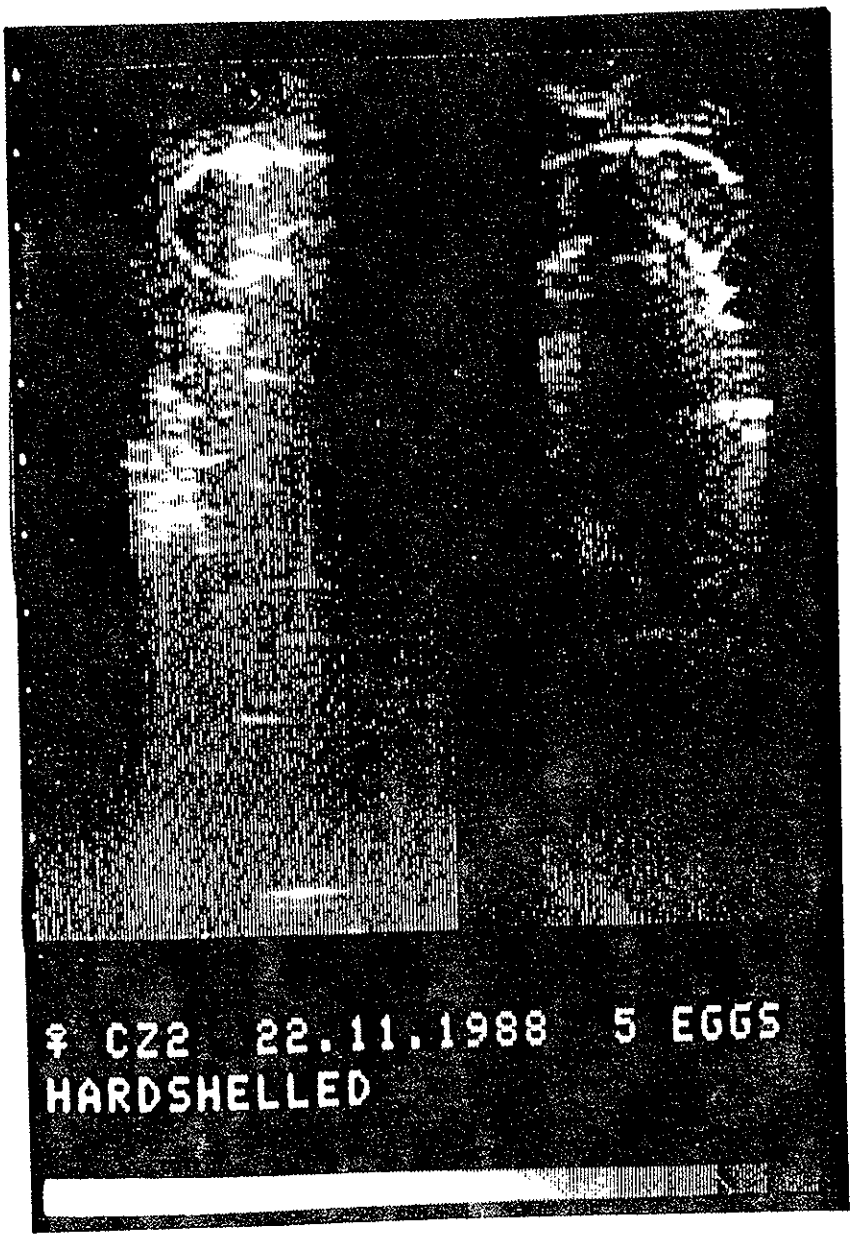


Figure 5: Ultra-sound image of hard-shelled oviducal eggs which are ready to be laid.

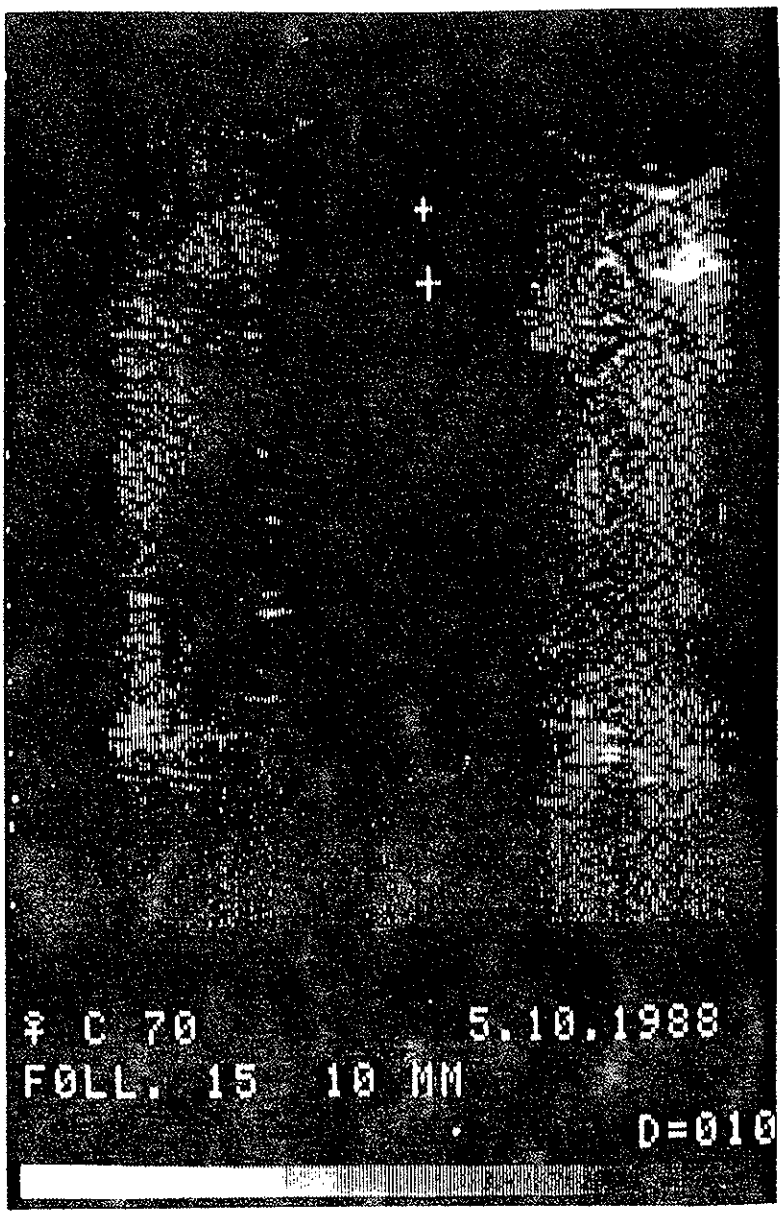


Figure 6: Ultra-sound image of atretic ovarian follicles.

In most animals studied some follicles never reach preovulatory size and in some cases follicles of preovulatory size may not ovulate. These follicles become atretic, they start to decrease in size and ultimately disappear. They appear as only roughly spherical structures on the ultra-sound image and have varying degrees of echogenic materials and areas, from very high echodensity to anechoic. The first sign of a follicle becoming atretic is often a slight deviation from a clearly spherical shape and increased echodensity. In later stages of atresia dense structures seem to have anechoic holes and caverns (Fig. 6). This may reflect the degeneration of yolk globules and the progressive removal of yolk constituents during atresia. When atresia of a follicle starts it may take from a few weeks to up to about ten months for individual follicles to completely disappear from the ultra-sound image.

As in *Chelodina oblonga*, it is not possible in *Pseudemydura umbrina* to identify corpora lutea or ovulation scars by ultra-sound scanning. They seem to have nonechogenic characteristics and are indistinguishable from other structures in the body cavity. Their identification, however, is of no direct relevance for the captive breeding programme where the reproductive condition of individual females is regularly monitored over many years.

6.1.2 The ovarian cycle

Under optimal conditions females breed every year (Fig. 7, 8). Vitellogenesis starts in January or February and the follicles grow continiously until late September to early November when ovulation occurs. Frequently a few preovulatory follicles do not ovulate and a few follicles do not reach preovulatory size. These become atretic and are reabsorbed

A young female in Perth Zoo became sexually mature during this project and laid eggs for the first time in 1989 with a carapace length of 117 mm. Her ovaries were already cyclic in the two previous years, since monitoring started, with follicles growing to near preovulatory size (Fig. 9). A young female from the Ellen Brook population has yet to ovulate, despite having follicles growing every year (Fig. 12); she is smaller (109 mm carapace length) than all females known to have laid eggs. Another young female (111 mm carapace length) caught in Ellen Brook Nature Reserve in November 1989 had follicles of 14-15 mm in the ovaries (Fig. 16) but obviously had not reached the stage of egg production. Therefore, the ovaries become active and cycle several years before a female starts to lay eggs for the first time.

The smallest females found to produce eggs were two wild animals from Ellen Brook Nature Reserve. One was caught on 23.10.1989 with four preovulatory follicles. One

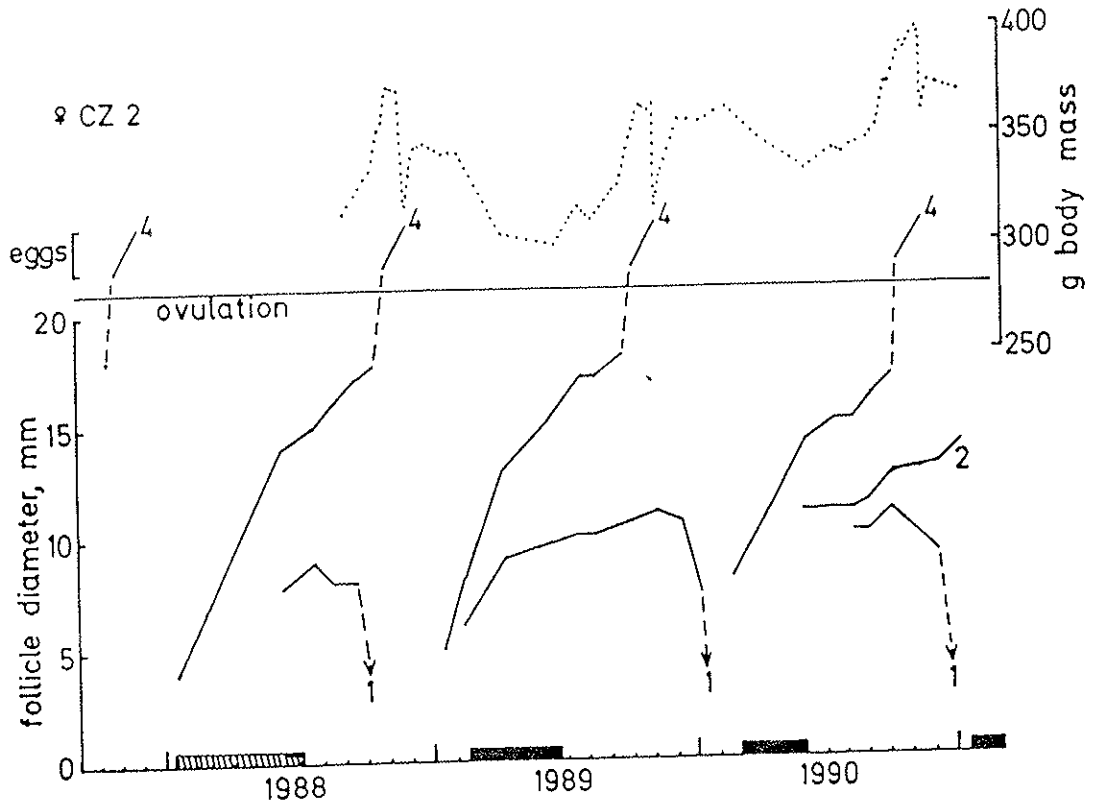


Figure 7: Female of the old Zoo stock; diagram of follicular growth, ovulation, oviposition, atresia (solid and dashed lines) and female body mass (dotted line). Black bars: animal in aestivation pen; hatched bar: presumed aestivation. Numbers: number of eggs laid or numbers of atretic follicles which disappeared.

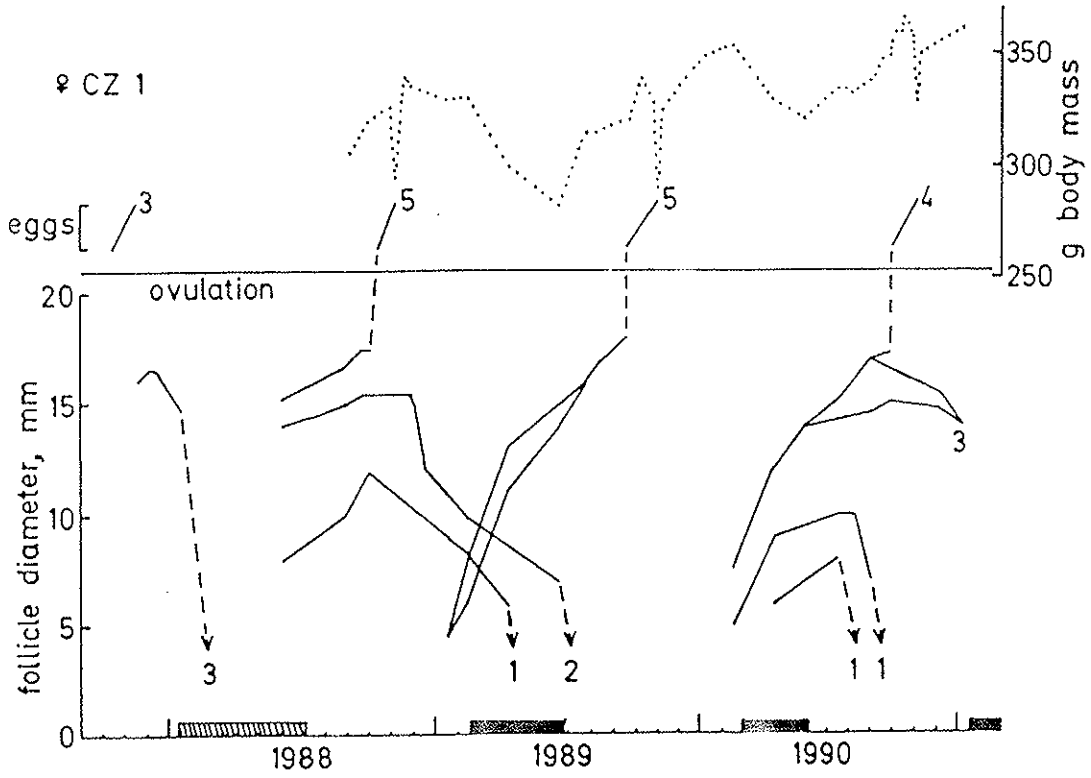


Figure 8: Female of the old Zoo stock; symbols as in Figure 7.

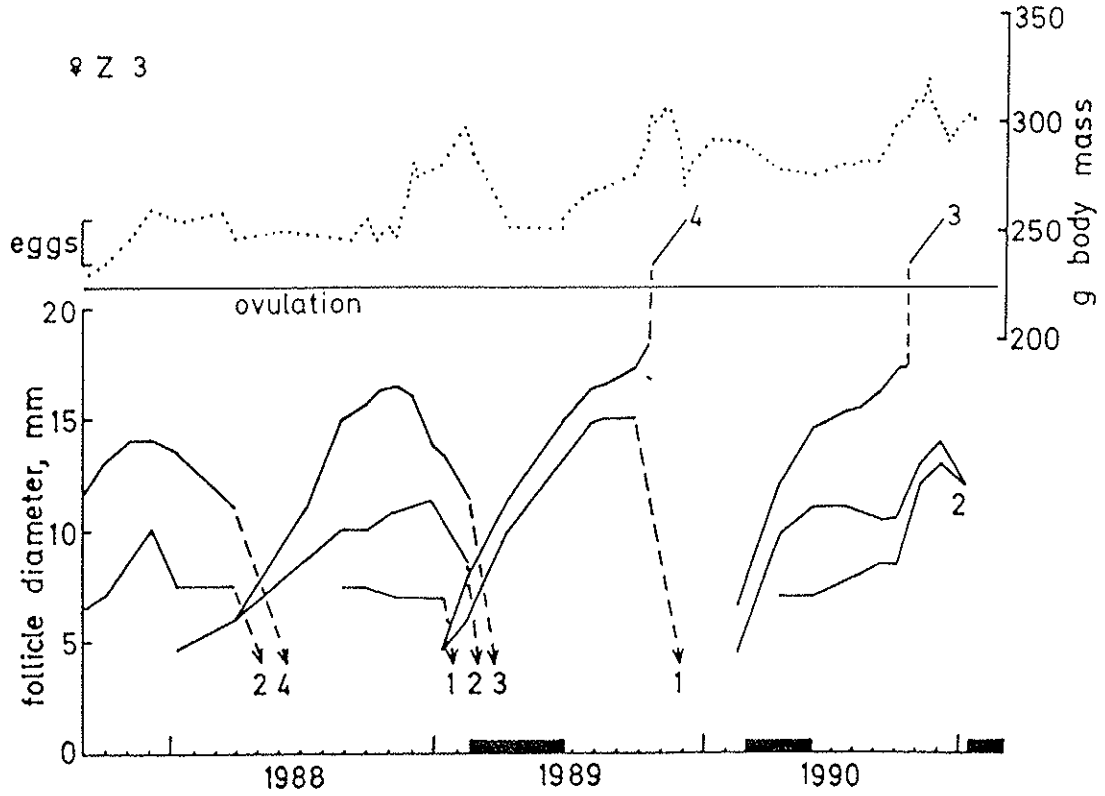


Figure 9: Young female of Perth Zoo, captive bred in the late 1960s or early 1970s; produced eggs for the first time in 1989; symbols as in Figure 7.

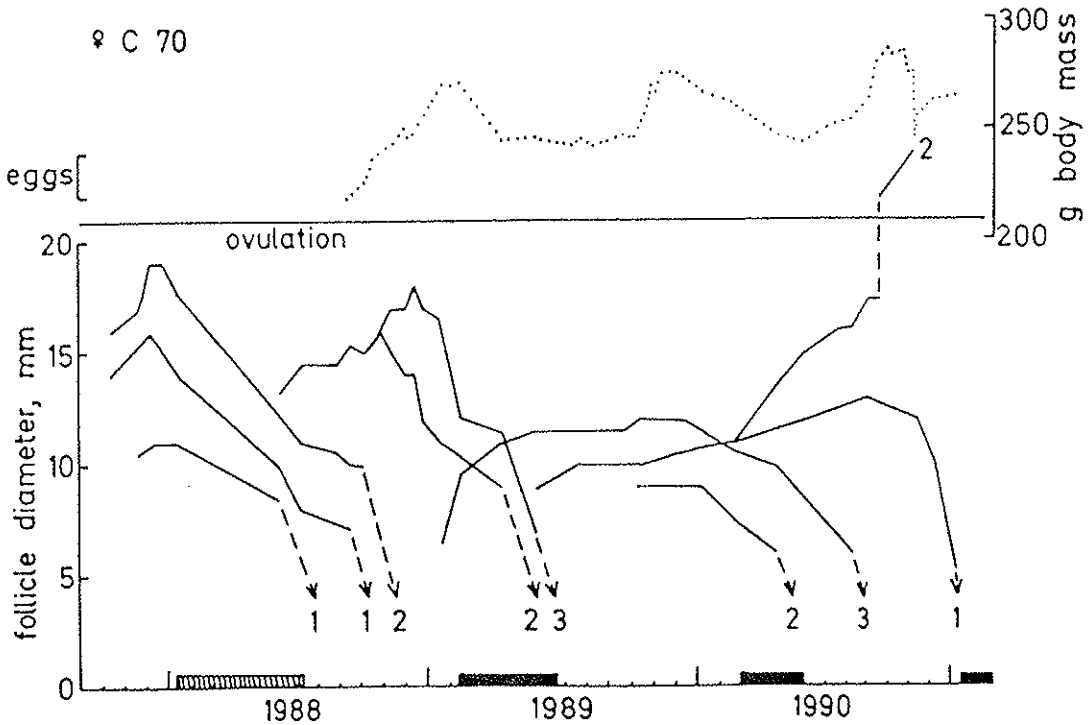


Figure 10: Adult female of the former Twin Swamps population, since 1980 in captivity; symbols as in Figure 7.

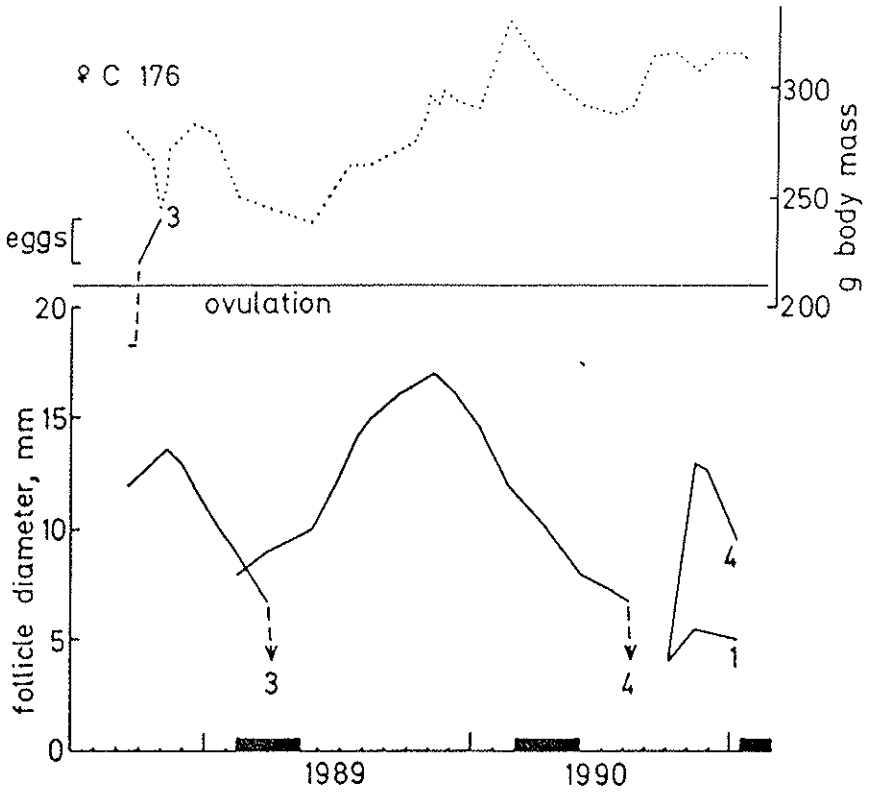


Figure 11: Adult female, caught south of Ellen Brook Nature Reserve, since September 1988 in captivity; symbols as in Figure 7.

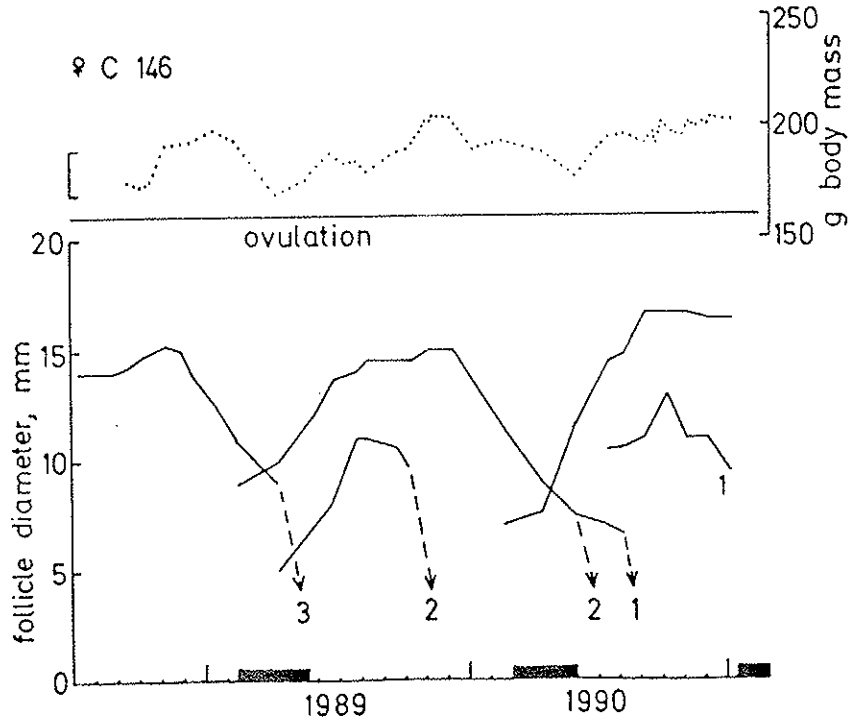


Figure 12: Subadult female of the Ellen Brook population, temporarily in captivity since July 1988; symbols as in Figure 7.

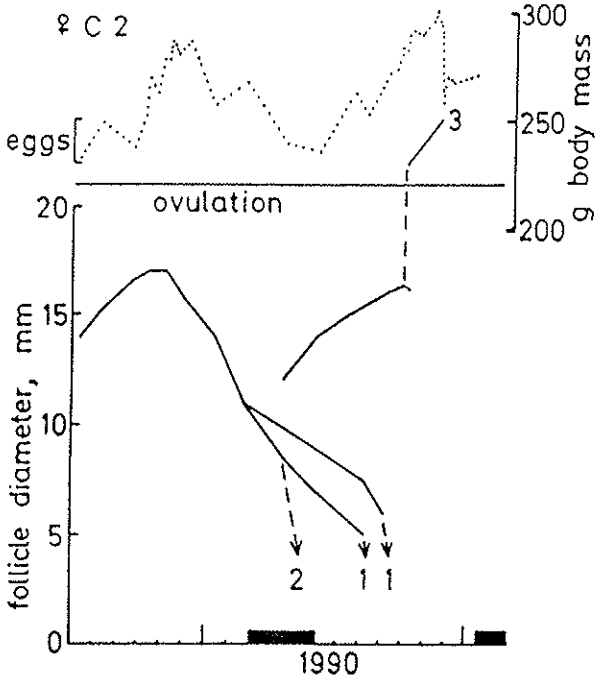


Figure 13: Adult female of the Ellen Brook population, temporarily in captivity since July 1989; symbols as in Figure 7.

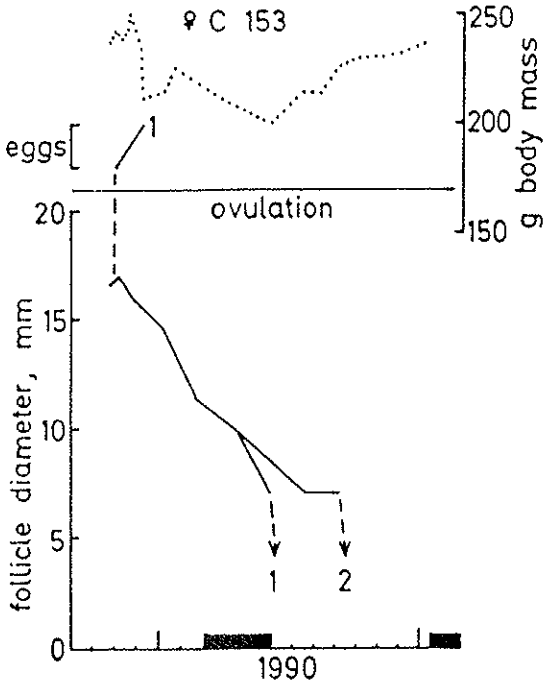


Figure 14: Young female of the Ellen Brook population, temporarily in captivity since October 1989; symbols as in Figure 7.

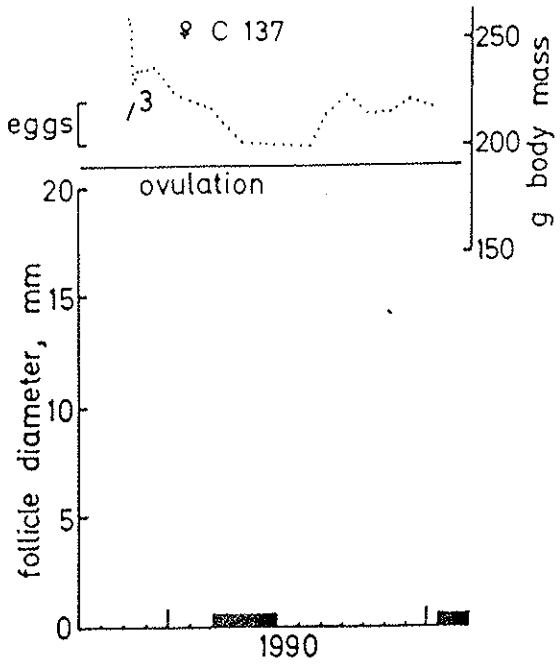


Figure 15: Young female of the Ellen Brook population, temporarily in captivity since November 1989; symbols as in Figure 7.

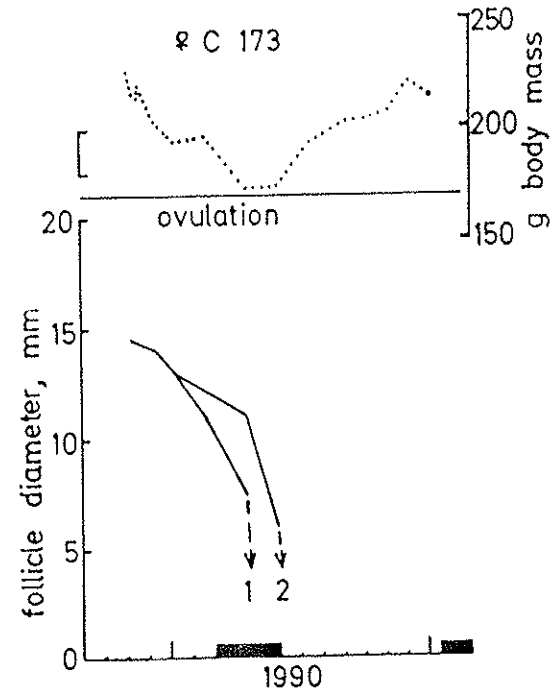


Figure 16: Subadult female of the Ellen Brook population, temporarily in captivity since November 1989; symbols as in Figure 7.

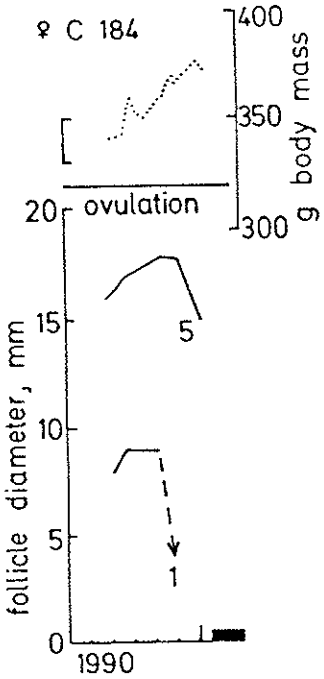


Figure 17: Adult female from a private property, taken in captivity in August 1990; symbols as in Figure 7.

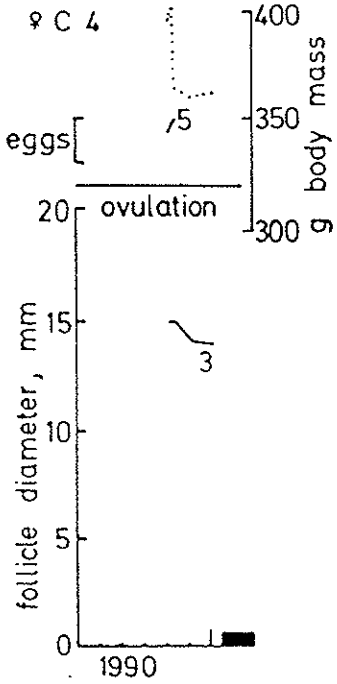


Figure 18: Adult female of the Ellen Brook population, temporarily in captivity since November 1990; symbols as in Figure 7.

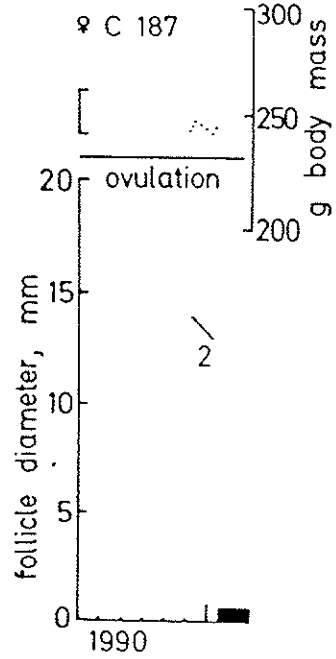


Figure 19: Adult female of the Ellen Brook population, temporarily in captivity since December 1990; symbols as in Figure 7.

follicle ovulated on 30.10.1989 when the female had a carapace length of 116 mm (Fig. 14). Another female caught on 07.11.1989 with three eggs in the oviducts in Ellen Brook Nature Reserve had a carapace length of 113 mm (Fig. 15).

Several females did not lay eggs regularly despite being sexually mature. A female from Twin Swamps laid eggs when taken into captivity in 1980, but did not lay again until November 1990. During 1987 and 1988 she had follicles growing to preovulatory size but ovulation did not occur and they became atretic (Fig. 10). Generally it seems that ovulation does not occur after early November. Follicles which did not reach more than 16 mm by early November did not ovulate. In 1987 and 1988 the follicles of this female may have been growing too slowly to be ready in time for ovulation. This phenomenon will be discussed in more detail in section 6.4.0.

In 1989 the same female had no follicles growing to preovulatory size, despite some follicles becoming vitellogenic during summer (Fig. 10). It is not clear why this happened, but this female was kept under artificially short photoperiods from 03.12.1988 to 19.01.1989 and received several gonadotropin injections (see chapter 6.4.0.). This treatment may have negatively influenced the growth of follicles in the next season. However, a young female in Perth Zoo had been subjected to the same photoperiodic conditions from 08.12.1988 to 06.01.1989 without hormone treatment and laid eggs in 1989 (Fig. 9).

Surprisingly, some follicles which had a diameter of 10-11 mm in winter and spring 1989 continued to grow during 1990. Two of these ovulated on 18.09.1990, the earliest date of ovulation ever observed in *P. umbrina* (Fig. 10). These follicles grew for about 15 months.

The female which was caught on a sheep paddock south of Ellen Brook Nature Reserve on 12.09.1988 ovulated about two weeks later and laid eggs in 1988, but has not produced eggs since (Fig. 11). She had follicles grown to preovulatory size by early November 1989, but they did not ovulate. The reason for this is not clear. In 1990, follicles started to grow only in September and could not reach preovulatory size.

A female which was caught on 06.07.1989 in Ellen Brook Nature Reserve had follicles growing to preovulatory size in 1989 but did not ovulate this year. In 1990 she grew follicles and ovulated (Fig. 13). Another female found on private property on 26.08.1990 had follicles growing to preovulatory size in spring 1990 but did not ovulate (Fig. 17).

In summary, from nine females with active ovaries taken into captivity from the wild during this project: two were obviously too small or young to produce eggs (Fig.

12, 16); two had eggs in the oviducts when caught (Fig. 15, 18); two ovulated about two weeks after being caught (Fig. 11, 14); two had follicles growing in the season they were caught but did not ovulate (Fig. 13, 17); and one was caught after having laid eggs (Fig. 19).

6.1.3. Ovulation

The history shows that, under some circumstances, captivity can effectively suppress egg production (see chapter 1.0.0.): none of the captive females produced eggs between 1980 and 1987. When I started the ultrasound examinations of the captive females in 1987 it became obvious that the stand-still in captive breeding had been caused by the failure of follicles to ovulate. My assumption was that this failure had to do with the nutritional status of the females and the feeding regime. After changing the annual feeding pattern as well as the food quality (see chapter 6.2.3.), two of the four long term captive females began a regular egg laying pattern which has now lasted over four breeding seasons (Fig. 7, 8). The young female which was captive bred in Perth Zoo in the late 1960s or early 1970s laid eggs in 1989 and 1990 (Fig. 9). One long term captive female which laid eggs in 1980, however, took three years of the same food and feeding conditions to start to ovulate again, which was not until 1990 (Fig. 10).

The weight curves of the females in figures 7-19 indicate that ovulation is typically preceeded by a sharp increase in body weight. It is likely that females have to experience a heavy feeding bout and weight gain in spring in order to ovulate. This may be an adaptation to the ephemeral habitat of *Pseudemydura umbrina*: in the case of reduced food supply in spring (e.g. because of low water levels after a dry winter) the coming dry season may be particularly long and it may be necessary to save and reabsorb the energy of the yolk material in order to survive the next summer.

Two females ovulated between seven and fourteen days after being taken into captivity (Fig. 11, 14), despite the fact that freshlycaught animals are generally disturbed by the new environment and reluctant to take food, especially artificial food. Ovulation seems to be triggered by a physiological preparation that cannot easily be switched off one or two weeks prior to the actual event.

On the other hand, in two females which were taken into captivity in Winter (July and August), the follicles continued to grow to preovulatory size but did not ovulate in spring, despite the fact that the females received live food and that at least one was feeding and gaining weight (Fig. 13, 17). It is clear that in *Pseudemydura umbrina* ovulation is the most sensitive and critical event in the female reproductive cycle and the

first one to be negatively affected by disturbance or suboptimal captive conditions.

This is in marked contrast to the situation in the long-necked tortoise *Chelodina oblonga*. I planned to use this species as a model to study the endogenous and exogenous control of ovulation, but I was unable to find any reasonable means to prevent ovulation in this species once follicles reached preovulatory size. Neither food deprivation for several months (up to five months) nor low or high temperatures (in between its ecological boundaries: 16° and 26°C) nor short or long photoperiods were able to supress ovulation or even the production of two consecutive egg clutches. *Chelodina oblonga* is a highly aquatic species which normally lays two or three clutches of eggs between late September and early February, with ovulations occuring from August to January.

The sensitivity of ovulation in *Pseudemydura umbrina* to food intake and conditions of stress may reflect its unpredictable, ephemeral habitat and the need to balance energy input into reproduction against the survival chances of adult females in long dry periods.

6.1.4. Egg production

The mean egg mass of all clutches is summarized in Table 6 together with the female body mass prior to laying. Egg mass is positively correlated with female body mass (Fig. 20). There is some variability of the egg mass of individual females from year to year and no clear correlation between egg mass and clutch size. It may, however, well be that a more extensive data set of clutches from individual females would reveal a negative correlation between clutch size and egg mass. The clutch mass as percent of the female body mass (Table 7) varies in individual females from year to year. Three females which laid eggs in 1989 and 1990 had a higher relative clutch mass in 1989 than in 1990. The clutch mass does not differ between wild-caught animals which laid eggs shortly after capture (mean = 8.60 +- 4.16, n = 4) and animals which are more than twelve months in captivity (mean = 8.75 +- 1.16, n = 5).

A young female from the Ellen Brook population and the young captive bred female in Perth Zoo both had one egg in their clutches from 1989 which obviously had thinner shells than the other eggs. One egg cracked during laying and one cracked when the eggs were removed from the nest. The female of the Twin Swamps population which had not produced eggs since 1980 laid two eggs in 1990, but one may have been destroyed during nesting, only its shell remains were recovered from the nest. The ultra-sound data of the oviducal eggs before laying suggested that one may have had a thinner shell than the other one.

Table 6: Mean egg mass per clutch +- SD; number of eggs per clutch (n=.),[.] = number of eggs destroyed during.laying, not included in mean; body mass of females {...g} before laying eggs.

	CZ1	CZ2	C176	Z3	C137	C153	C4	C2	C70
1987	8.46+- 0.22g (n=3) {319g}	?							
1988	6.93+- 0.49g (n=5) {327g}	7.80+- 0.19g (n=4) {372g}	8.86+- 0.65g (n=3) {268g}						
1989	8.01+- 0.22g (n=5) {327g}	9.67+- 0.32g (n=4) {365g}		7.19+- 0.15g (n=4) {308g}	7.75+- 0.45g (n=3-[1]) {253g}	6.20g (n=1) {226g}			
1990	7.28+- 0.26g (n=4) {353g}	9.15+- 0.26g (n=4) {390g}		7.60+- 0.10g (n=3) {306g}			10.08+- 0.59g (n=5) {402g}	8.97+- 0.15g (n=3) {292g}	8.50g (n=2-[1]) {274g}

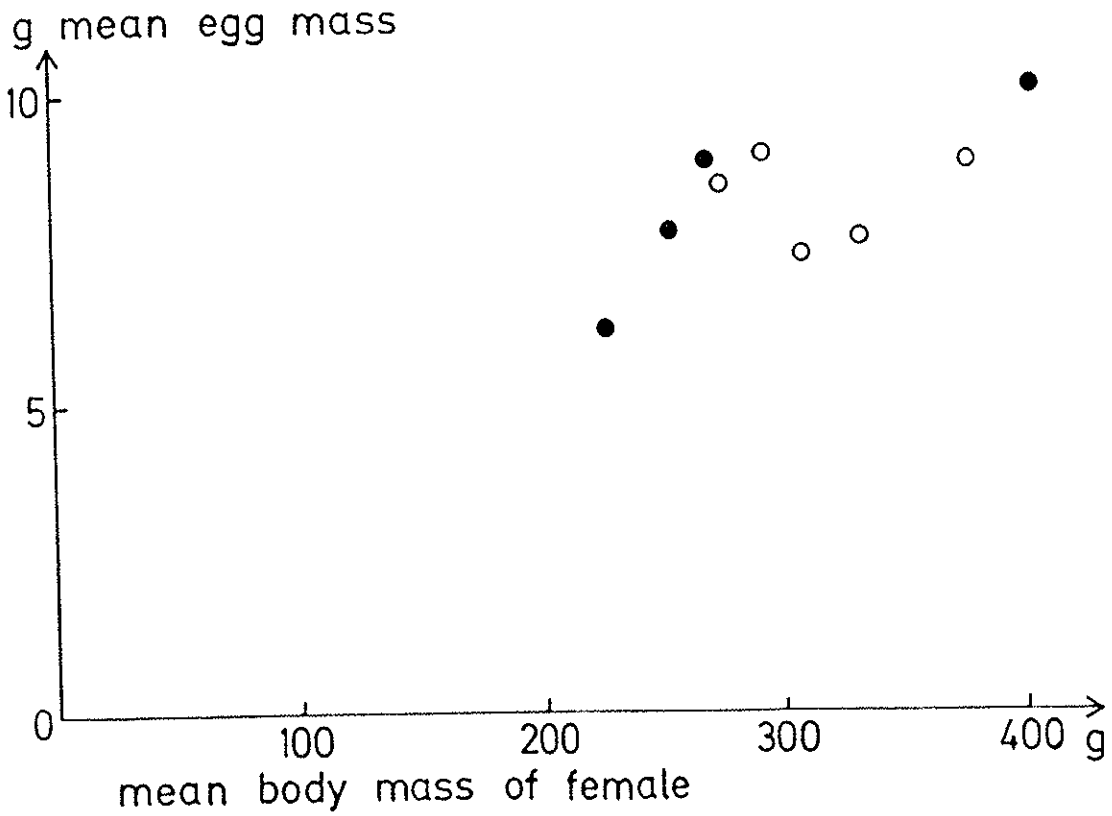


Figure 20: Relation of egg mass to female body mass; mean of all eggs laid per female in captivity from 1987 to 1990. Circles: females > 12 months in captivity before oviposition; dots: females < 2 months in captivity before oviposition.

Table 7: Total clutch mass in percent of female body mass

	CZ1	CZ2	C176	Z3	C137	C153	C4	C2	C70
1987	7.96 %	?							
1988	10.60 %	8.39 %	9.92 %						
1989	12.25 %	10.60 %		9.34 %	9.19 %	2.74 %			
1990	8.25 %	9.38 %		7.45 %			12.54 %	9.22 %	6.88 %

6.1.5. Nesting

A total of 16 egg clutches of *P. umbrina* have been laid in captivity between 1987 and 1990 by nine females. Twice egg laying was induced by oxytocin (see chapter 6.4.0.), 14 clutches (from 8 females) were laid in nests constructed by the females between 2 November and 8 December (compare Fig. 7-18). One female which laid eggs on 26 October 1988 after oxytocin inducement "nested" on 31 October 1988: she dug a nest hole and closed and covered it without laying any eggs.

The nest sites chosen by females have been slightly different in different years. In 1987 and 1988 all nests (n=4) were located at the dryest and hottest places in the enclosures, on sandy hills or slopes with scarce vegetation (some grass tussocks). In 1989 three nests were built on similar sites, but two were in dense grass and vegetation in rather moister soil. In 1989, only one nest was on top of a dry sandy hill and five nests were in dense grass and vegetation. Therefore, from 1987 to 1990, eight nests have been constructed at the driest and hottest place available and seven nests in dense grass and vegetation at moister places.

Two to ten days before nesting the females start to leave the water frequently and walk around the whole enclosure. They may climb up on wire mesh to more than one metre above ground level and often climb over their enclosure fence. Normally they start to dig holes at different places before building a nest. These trial diggings may start 8 days before the actual nest is built or only some hours beforehand. The trial holes are not filled in with earth material like finished nests but simply abandoned. It may well be that individual females prefer certain nesting environments which the enclosures do not offer and that their searching behaviour is prolonged in captivity.

The search for a nest site often starts under cloudy low pressure conditions and nesting itself sometimes takes place during light rain. From 1987 to 1989 this association between weather conditions and nesting seemed very obvious, but during 1990 several nestings occured on sunny and hot days. Nesting on hot days (often after many days of trials and searching) and the choice of nest sites in dense grass and vegetation on moist soil may be artefacts of captivity, but since nesting has never been observed in the wild, no conclusions can be drawn.

The nesting behaviour of *P. umbrina* is unique for chelonians: The nest cavity is dug and constructed with the front feet, the animal digging head first a nest hole in the soil to a depth of about 10 cm. Then the female turns around in the nest, lays the eggs and closes the nest hole with its hind feet. In all other chelonians in which nesting behaviour of has been observed and described, the females dig the nest and egg cavity with

their hind feet. Only during the preparation of the nesting area, which can include digging a "body pit", are the front feet normally involved.

The eggs, embryos and developed hatchlings of *P. umbrina* have to survive the hot and dry summer in the nests until the winter rains soften the soil, which may be six to eight months after laying. Obviously the nest has to be at a certain depth underground in order to smooth environmental extremes. It may be impossible for the relatively small P. umbrina to dig with their hind feet a hole deep enough to safeguard their eggs. The unique nesting behaviour of *P. umbrina* may be an adaptation to its peculiar habitat and life style as well as its small size.

6.1.6. Male reproductive activity

The project paid less attention to male than to female reproduction, firstly because at the start of the project no problems were obvious with the male reproductive performance as opposed to the female one and secondly because there are no non-invasive techniques available to investigate the male reproductive physiology of chelonians. The information on male reproduction is restricted to behavioural observations and to the assessment of the fertility rate of eggs.

Burbidge (1981) reports that mating occurs in water in winter and early spring. Observations during this project confirmed this time to be the main mating time. Sexually active males seem to try to mount any Swamp Tortoise they encounter. In 1987 and early 1988 ten males and one young female were kept together in an enclosure with two ponds in Perth Zoo. Frequently males mounted each other trying to copulate, sometimes three males would be on top of each other and grasp the shell of the underlying one with all four feet so firmly that the whole animal pile could be lifted out of the water without disintegrating. The males of *P. umbrina* are bigger than the females, the mating strategy is a relatively simple one of "forced insemination": during the mating season males try to overpower any female (or even an other male) they meet.

Since winter 1988 males and females have been generally kept separate and only put together at various times in winter and spring to copulate. Copulations or males mounting females have only been observed from May to September. From October to December no indications have been seen of males mating with females. During the last three years a male was only once observed mounting another male in the middle of October and once in early November. In contrast, males frequently mount other males (and females, when available) between February (if kept in water and not aestivating) and September. Most activity seems to occur in late autumn and early winter, the time after aestivation. On 26.08.1990 a copulating

pair of *P. umbrina* was found in shallow water on private property between the Twin Swamps Nature Reserve and the Ellen Brook, the only recent observation of mating activity in the wild.

The cessation of mating activity in spring may be of biological significance. During winter in the confined space of captivity, males tend to exclude the females from the ponds by chasing them continiously. In the wild this is the time when vast areas of the swamps are flooded and the animals have the opportunity to disperse and may not meet too frequently. In spring, before the swamps dry out, the water retreats into small pools with high food availability and temperatures are optimal for feeding. The decline of mating activity at this time presumably allows the animals to concentrate in the last highly productive water bodies. The importance of a high food intake in spring for ovulation and egg production has been discussed in chapter 6.1.3.

Sperm normally remain viable in the oviducts of turtles and tortoises for long periods after insemination. The dissociation between peak mating activity and ovulation in this species may be an adaptation to its peculiar life style of an activity season confined to winter and spring.

6.2.0. CAPTIVE MANAGEMENT

6.2.1. Housing of captive stock

In 1987 three adult female and two adult male *Pseudemydura umbrina* were kept together in one enclosure with one pond in CALMs Wildlife Research Centre in Woodvale. One juvenile was kept there in an Aquarium. Perth Zoo maintained ten males and one subadult female together in an enclosure with two ponds.

During the course of this project the captive colony has been kept divided between the Wildlife Research Centre and Perth Zoo. Several new ponds and enclosures of different designs have been constructed and tested. Figure 21 shows a standardised pond design which has been found to be the most useful. Generally every female has her own pond and two or three males share one pond. However, because of the ten animals from the Ellen Brook population which are temporarily in captivity, the situation at present is slightly more crowded: by 31.12.1990 seven enclosures and ponds in the Wildlife Research Centre and seven in Perth Zoo harboured a total of 27 animals.

Experience has shown that the new tortoise enclosure in Perth Zoo has a very suitable location because of its full sun exposure all day and year round. In the Wildlife Research Centre the enclosure is located under big trees. Especially in winter and early spring water temperature

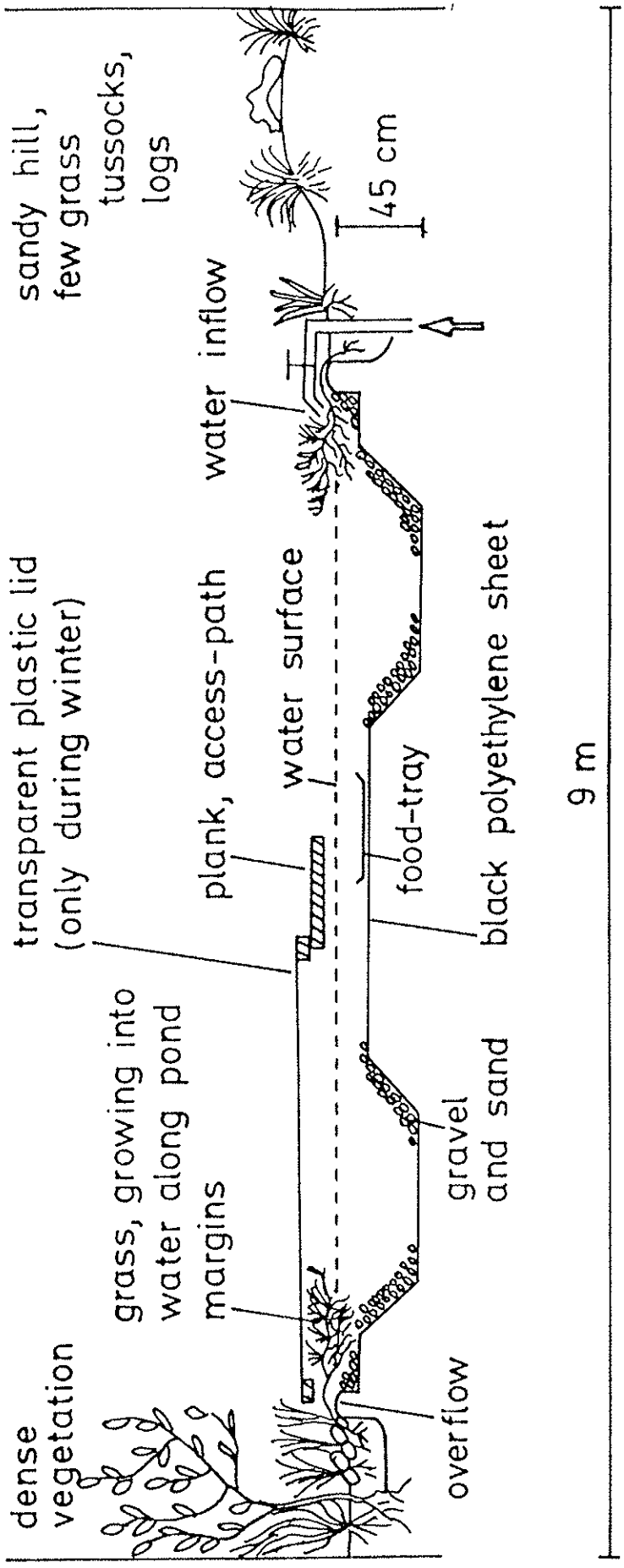


Figure 21: Longitudinal section through a standardised pond and enclosure for the maintainance of the breeding stock.

and sun insolation is a critical factor for food intake. In the Wildlife Research Centre every pond has been equipped with a 100 W Aquarium heater in order to offer a possibility for thermoregulation. In both Perth Zoo and the Wildlife Research Centre, half the pond surface is covered by a transparent plastic lid during winter to offer the choice of a protected and warmer microclimate. Generally the emphasis is to keep the Swamp Tortoises under climatic conditions similar to that of their wild habitat. The swamps have a very high sun insolation and the situation in Perth Zoo seems to come close to that condition.

At the start of the project it was planned to get the tortoises to aestivate in their standard enclosures, but for two reasons this did not proove useful. Firstly, most animals were reluctant to leave the water and start to aestivate (under the more crowded conditions in the past some of the animals normally left the water in summer). Secondly, the zoo enclosures were not rodent proof. In December 1988 a male started to aestivate in a tunnel in the ground which was provided for this purpose; during a routine check in early February 1990 only his skeletal remains were found. There is a possibility that mice or rats caused his death.

Because of this problem six rodent proof aestivation pens of about two square metres each have been constructed in Perth Zoo in early 1990. In the Wildlife Research Centre there are two aestivation pens of about six and eight square metres in a rodent proof enclosure. Basically the aestivation sites are heaps of leaf litter (about 80 cm high) which cover most of the ground area. Each pen has a small water puddle of 6-10 cm depth to which the animals always have access. During the hot summer months the whole area is shaded by shade-cloth which is about two metres above the ground.

6.2.2. Mating management

Since July 1988 captive male and female *P. umbrina* are generally kept in separate ponds and enclosures and only brought together for mating purposes. As discussed in chapter 6.1.6. mating activity occurs mainly between February and September. The captive animals now have to aestivate during summer and autumn as have their wild counterparts. For this reasons mating management has to take place between the end of aestivation and the end of September.

Females of *Pseudemydura umbrina*, as those of many other tortoises and turtles, may be able to store sperm over long periods, possibly up to several years. (In the Widlife Research Centre there was one female that had not been with a male for two years and she laid a clutch of three eggs, one of which appeared to be infertile; Burbidge in lit. 31.08.1987). On one hand this makes mating management easy, because there is no need to time

insemination of females with ovulation. On the other hand it is an obstacle, because potentially males may father offspring even years after copulation with the mother. Therefore the pairing of a female with a particular male does not necessarily mean that he will be the father of all her next young.

Management of mating pairs is important for the genetic management of the captive population which will be discussed in chapter 6.3.0. Here I consider the technical aspects of mating management.

During winter 1988 males have been introduced to the females from 07-18 September and from 19-26 October but copulated only during September (until July 1988 males and females have been kept together and may well have already copulated). In 1989 males were put to the females from 15 July to 10 August. In these years chasing of females by males and mounting started normally between some minutes to several days after putting the animals together.

Insemination of a female is considered as confirmed when either several mountings are observed which last longer than 30 minutes (often several hours) or, after some days of company with a male, a female is always found out of the water. In this case it is a good test to put the female slowly back in the water near the male. If the male starts immediately to chase or mount the female and the female struggles to escape it is a sign that copulation already took place.

At this stage I did not try to confirm insemination directly e.g. by flushing the cloaca of females to look for sperm, because the emphasis was to disturb the animals as little as possible.

During winter 1990 most males showed less interest in mating and copulating and it was difficult to confirm copulations. One reason for this could be that the long term captive males were moved to aestivation pens for the first time in summer 1990. This new change for them may have affected their readyness to mate that season. On the other hand, two males from the Ellen Brook population mated without problems when taken in captivity temporarily in 1989 for this purpose, but two other wild males taken in during winter 1990 refused to mate. Therefore it is not clear why there was less mating activity in 1990 than in 1989 and 1988.

In the years 1988 and 1989 I used primarily the oldest and most senile males for matings (which belonged to the old Zoo stock), in order to get them to reproduce before it may be too late and their genes may be lost. For some of the old males this was obviously too much and three of them died during the mating exercise. Two were found dead, laying on the bottom of the ponds with water in the

lungs and one was recovered dying and was dead 30 minutes later.

Although these deaths seem to be related to the old age of the males, it is important that the ponds in which animals are brought together to mate have shallow margins all around and that all slopes to deeper areas are not smooth (e.g. the black plastic used for building ponds) but easy to climb up (e.g. stone gravel or sand). *P. umbrina* is a rather clumsy swimmer and the mating excitement together with the cool winter temperatures increase the possibility of drownings.

6.2.3. Nutrition, food and feeding regime

Pseudemydura umbrina is carnivorous. In the wild animals eat only living food such as insect larvae, crustaceans, tadpoles, and earth worms (Burbidge, 1981).

Before the project started the captive Swamp Tortoises were fed mainly on small marine fish, such as (previously frozen) whitebait *Hyperlophus vittatus*, and beef heart which was sometimes sprinkled with calcium and mineral powders. In the Wildlife Research Centre beef heart was usually mixed with a mineral supplement for pets and congealed with agar agar. The animals were kept in the same ponds and enclosures all year round and got food routinely twice or three times a week. Although they were fed over the whole year, anecdotal information suggests that the food quantity was increased during spring when more food was taken.

The diet in captivity is very different from the natural diet but it would be difficult and expensive to provide enough natural living food for the captive adult Swamp Tortoise colony. A comparison of the captive diet mentioned above and the natural diet suggests that there may have been a lack of some dietary components in captivity. The first step at the start of the project was to develop an artificial food which was based on more diverse components. Since then the recipes changed several times. Basically the mixture is developed by trial and error and based on common wisdom rather than on scientific analyses - an improvement was too urgent in order to wait for such results.

The food mixture now consists of minced prawns, fish, mice, rats, beef heart, algae powder ("Kelp"), a multi-vitamin supplement ("Supradyn") and calcium carbonate in gelatine. Other components used at some stage are squid, marron, carrots, *Daphnia*, dolomit powder, and agar agar. It is important to use all components as fresh as possible, frozen material should be thawed and processed as quickly as possible. After the gelatine congelat the food is portioned and frozen until use. Presently the standard recipe used by Perth Zoo is as follows: 560 g beef heart, 320 g whitebait, 80 g prawn, 480 g rat or

mouse, 8 g kelp powder, 10 g CaCO3 powder, 2 egg yolks, 1 capsule Supradyn, 750 ml H2O, 240 g gelatine.

Some of the captive animals and nearly all of the tortoises taken in captivity from the wild were at first reluctant to accept this food mixture. The best way to get them used to the artificial food was to offer them first tadpoles, then for some time live mosquito fish (*Gambusia affinis*) from forceps, then dead mosquito fish, then whitebait, then a food mixture based entirely on whitebait and marron, and then the standard mixture. Animals switch over to the food mixture more readily in spring, females especially after laying eggs. Some animals took only some weeks to accept the food, others took six to twelve months. Animals were not starved in order to accept this food more quickly, because starving animals do not breed and the breeding performance was, and is, the main emphasis of the project.

Good nutritional balance of the food is particularly important for the successful development of eggs (see chapter 6.2.5.). Since 1987, the developmental success rate of eggs produced by wild-caught animals was 100%, that of captive females which refused to eat the food mixture was 0% and that of captive females which ate the food mixture was 90% (see table 8). This indicates that the food mixture now used is much better than the former food for captive animals but still does not reach the quality of the food of wild animals. Generally wild caught individuals look leaner than the captive ones; the problem with the artificial food is certainly not lack of nutritional richness in terms of energy, rather a deficiency of components like minerals and vitamins.

Pseudemydura umbrina feeds only under water which, in its habitat in the wild, is normally available only for five to six months per year during winter and spring. The natural life style of the Western Swamp Tortoise is to fast six to seven months per year during aestivation, to feed in late autumn and winter when water is available but low temperatures limit digestion and to have a feeding bout in spring when it gets warmer and before the swamps dry out. This rather extreme feeding pattern seems to determine to some extent ovulation and egg production (see chapter 6.1.3.).

The management of aestivation in the captive population which was introduced during this project simulates the normal annual feeding pattern. Generally the captive animals start aestivation later then the wild ones (see next chapter) and have therefore a prolonged feeding period in late spring and early summer. This higher food intake may increase their breeding performance in the next season, but it seems equally important for the breeding performance to offer them their natural period of fasting.

The food and feeding regime of hatchlings is discussed in chapter 6.2.6.

6.2.4. Aestivation

When the swamps in the habitat of *Pseudemydura umbrina* are nearly dry, usually in November, the tortoises leave the last remaining water puddles to aestivate during the summer and autumn. Aestivation refuges vary with the soil type: at Ellen Brook Nature Reserve they are naturally-occuring holes in the gilgai clay, while at Twin Swamps Nature Reserve most aestivate under *Banksia* leaf litter or fallen branches, but few a few find holes in the ground dug by other animals or left by a rotting tree root (Burbidge 1981, Burbidge *et al.* 1990).

Under past captive conditions the animals had clear and cool water available in their ponds over the whole year. Anecdotal information and my observations during summer and autumn 1987/88 indicate that, despite the availability of water, some animals (and all females) spent much of the time in summer and autumn out of the water and hidden under grass-tussocks or leaf litter. It was however never recorded which animals chose to stay out of the water and aestivate and for how much time. Spence *et al.* (1979) reported that males generally remained in the water and continued to feed throughout the summer but that females tend to spend several weeks on land resting in the shade in the terrestrial compartment of the enclosure.

At the start of the project in 1988 I planned to get the animals to leave the water and to aestivate on there own choice. Holes in the ground of about 80 cm length and down to a depth of about 50 cm were provided as well as vegetation and leaf litter. By 1 February 1989, however, only one male of the whole captive population had chosen to start aestivation, and this male was found dead and as a skeleton in one of the holes in the ground. Because of the possibility that mice may have caused the death it was decided to use only rodent proof enclosures for aestivation in future.

The reason for the reluctance of the tortoises to stay out of their ponds in summer 1989 seems to be the spreading of the animals in several ponds and enclosures and the separation of males and females. As discussed in chapter 6.1.6. the mating activity of males sharply declines in spring. Mating activity is taken up again in summer and by February the males frequently mount other animals. If all animals are kept together the larger males chase other animals and particularly the females out of the water and those animals may start to aestivate. But without this occuring in 1989 nearly all animals prefered not to leave the water by February.

There are no indications that aestivation is important for the reproductive performance of males (e.g.

spermatogenesis). Aestivation may however improve the female breeding performance through the facilitation of a natural annual feeding (and metabolic) pattern which is important for ovulation (see chapter 6.1.3.). In this respect it is interesting to note that in the 1960s and 1970s, when captive breeding occured in Perth Zoo, the females usually aestivated (Spence *et al.* 1979) - presumably they have been driven out of the water by the males.

In summer 1989 it was decided to move only the females in special aestivation pens and to oversummer all males in their old enclosures. In Perth Zoo a plastic container (2.5 x 1.2 x 1.0 m) was filled with 30 cm of sand and half the area with 40 cm of leaf litter (and some planks and branches to create holes under it). A small basin (25 x 40 x 14 cm) filled with water was always available to them. The container was located in a roofed and shady outdoor enclosure of the animal hospital. Three females aestivated there from 18.02.-22.06.1989. Four females aestivated in the Wildlife Research Centre from 14.02.-22.05.1989 in an area of 3 x 3 m which was fenced off in a shady rodent-proof outdoor enclosure. Half the area was covered with 50 cm of leaf litter and a basin of 40 x 60 x 12 cm filled with water was always available. On 09.04.1989 all animals were weighed and examined by ultra-sound scanning, after which they continued to aestivate.

The aestivation times for individual females for all years are summerised in Figures 7-19. From summer 1990 onwards, all adult swamp tortoises, males and females, aestivated in the aestivation pens described in chapter 6.2.1. All animals are checked once at about half-time of aestivation.

The aestivation management of hatchlings and young animals is discussed in chapter 6.2.6.

6.2.5. Egg incubation

When all captive Western Swamp Tortoises were maintained in Perth Zoo in the 1960s and 1970s egg laying was never observed. The nests were constructed in the land area of their concrete display enclosure which had a 40 cm deep sand and clay soil and some vegetation growing in it. Because of the zoo's policy of non-intervention, it was not searched for eggs, no egg or clutch numbers were recorded and only the emergence of hatchlings was noted. Hatchlings emerged in eight out of fifteen years between 1963 and 1978 (Spence *et al.* 1979).

In 1979 the three adult females from Perth Zoo were transfered to the Wildlife Research Centre. During the first year three eggs were obtained by oxytocin inducement, incubated artificially at 30°C and two of them hatched. One hatchling died in its first year, the other one died in its second year. In 1980 12 eggs were

induced from the captive and some freshly caught wild tortoises. The eggs were placed on slightly moist paper tissue in sealed plastic containers and incubated at 30°C. Four of them hatched, two were opened and contained live hatchlings and six opened eggs contained dead hatchlings. All hatchlings died in their first year (Burbidge in lit. 31.08.1987, Burbidge *et al.* 1990).

After changes to the feeding regime in late winter 1987 seven eggs were obtained from the two females of the old captive stock. These eggs were incubated artificially on slightly moist paper tissue in sealed plastic containers at 24°C (temperatures recorded over the summer of 1981/82 at possible nesting sites in Ellen Brook Nature Reserve by CALM staff suggested nest temperatures in the wild are around 22 to 24°C). None of the seven eggs from 1987 hatched, with two eggs never starting development and the embryos of five eggs dying at an early stage of development.

Although both strategies of egg incubation in the past - to leave eggs in the nests in an outside enclosure and to incubate them artificially at constant temperatures on slightly moist paper tissue - worked to some extent, it was clear that an incubation method should be developed during this project which improved the hatching rates and which is simple to manage. Because of my past experiences with vermiculite as a safe incubation substrate for tortoise and turtle eggs I set up containers with vermiculite for egg incubation as shown in Figure 22. These have been used since 1988.

During recovery of eggs from a nest a number code is written on their top with a soft pencil (the eggs are brittle-shelled). Care is taken that eggs remain in the same orientation during their whole incubation time; about twelve hours after laying the embryo attaches at the egg menbrane and from then on turning an egg can suffocate the embryo. The eggs are weighed, half-buried in the vermiculite and left with as little disturbance as possible in the incubators ("Memmert" laboratory ovens).

Freshly-laid eggs are opaque in appearance. Eggs which start development normally form a white patch at the top area of the shell in 12-48 hrs. After 24 hrs this is typically a circular white dot of 3-6 mm diameter clearly distinguishable from the surrounding opaque appearance of the egg shell. This white dot continues to grow and remaines circular until it reaches 10-11 mm diameter; than it starts to form a band around the equator of the egg which then gradually expands towards both ends of the egg. This process generally occurs faster at higher temperatures, but with individual variations between eggs. In some eggs one or both ends may remain opaque, others gradually turn completely white. In a few cases no such development can be seen at all, but an egg may get gradually sprinkled with white on the whole upper surface and still develop normally. The thickness of the calcium

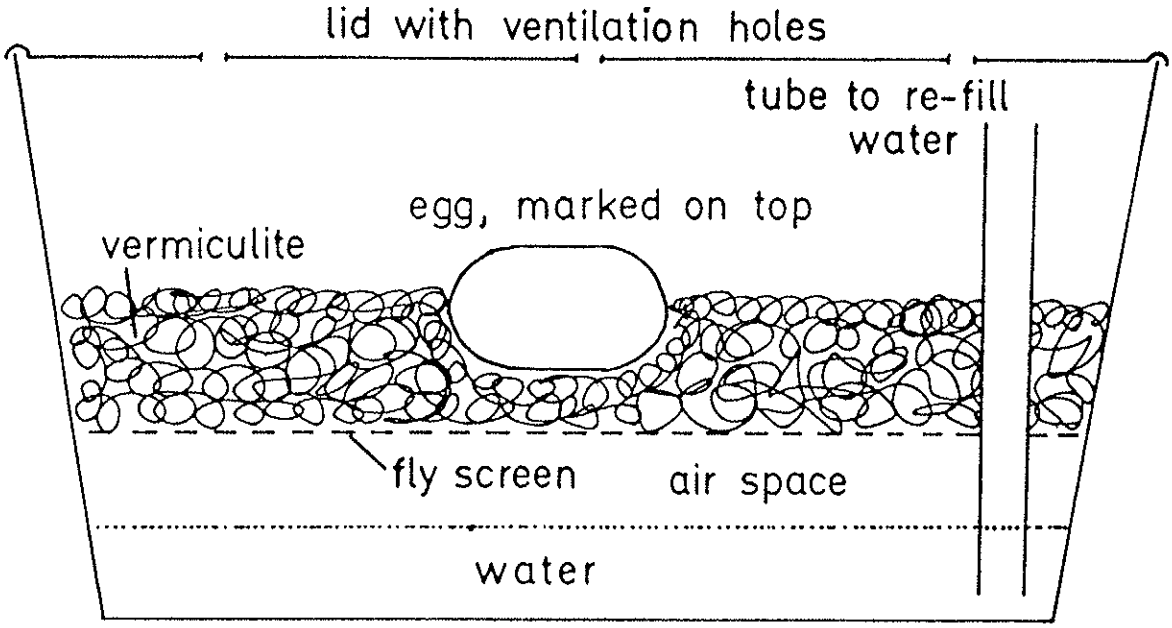


Figure 22: Section through an egg incubation container.

layer of egg shells may differ and this may influence the appearance and the clarity of this phenomenon. Infertile eggs and non-developing eggs usually remain totally opaque for several months.

Incubating eggs are routinely candled about once a month to assess their condition and progress. Candling is done with a "cold"-light source, the lamp and fibre optic cable of an endoscope. Generally an egg is only candled for five to ten seconds in order to reduce its exposure to the infrared radiation (heat) of the light bundle. After about two weeks of incubation the blood vessels of the allantois which are close to the inner surface of the shell of developing eggs can be clearly detected. (It is possible to detect the vitelline circulation earlier during development, but in order to handle eggs as little as possible this is not routinely done.) In older eggs size, orientation and movements of the embryo can be assessed in addition to the blood vessels.

In spring 1988 12 eggs were laid by three females. Three of them were left in two nests which the females constructed in the outside enclosures (one at the Wildlife Research Centre and two at Perth Zoo), one was incubated at 29°C, four at 28°C, three at 27°C and one at 25°C. The eggs were divided in four different incubators and temperatures firstly to spread the risk of equipment failure and secondly because it is not known wether *Pseudemydura umbrina* has temperature-dependent sex determination (as do most tortoises and turtles studied) or genetic sex determination (as do the three Australian chelid species studied). All twelve eggs were fertile and started to develop.

The shell of some of the artificially incubated eggs from 1988 cracked after about two and a half months of incubation. The relatively wide cracks of two eggs were sprayed with a spray bandage ("Healex) on 09.01.1989 and on 12.01.1989 but they developed fungus growing in the cracks. From 13.01.1989 on the cracks were swabbed with a fungicide ("Acriflavine" 10 mg/l H2O) and sprayed with the bandage every four to five days. On 01.02.1989 the fluid near the crack of one egg was cloudy and fungus was spreading on the allantoic membranes inside the shell. At this stage the egg was opened and the premature hatchling recovered as described in chapter 6.2.6. The second problem egg was opened on 06.02.1989.

One egg which had been left in a nest in the outside enclosure in Perth Zoo was found dead and decomposed on 19.02.1989; presumably it had been buried too shallow and got overheated and desiccated. The two other eggs in outside nests were moved to incubators of 25° and 28°C on 19.02.1989. Eleven hatchlings were produced in 1989.

In spring 1989 a total of 17 eggs was produced, but two of them had a thin shell and cracked during laying or when removed from the nest (see chapter 6.1.4.) and four

were infertile or non-developing. Six of the fertile eggs were incubated at 24-25°C and five at 29°C (spread over four incubators). Care was taken that the vermiculite was rather dry and none of these eggs cracked prematurely. Nine to eleven weeks after laying the eggs at 29°C were, over some days, cooled down to 24-25°C and maintained at this temperature until hatching. At this stage of development temperature should have no more effect on the sex of the hatchlings. The lower temperature may help to preserve "fuel" during the later part of incubation when development is finished and the hatchlings lay dormant until hatching.

In spring 1990 a total of 21 eggs were laid but one was destroyed during nesting. The 20 remaining eggs were split between four incubators, nine at 24-24.5°C and eleven at 29°C. 18 started development, but one of these died about three weeks after laying and one in its second month of development. Again the eggs at 29°C were only kept at this temperature for nine to eleven weeks and incubation was completed at 24-24.5°C

A summary of the development of all eggs laid since 1987 is given in Table 8. From the 54 intact eggs laid between 1987 and 1990, no sign of development was observed in eight eggs and eight eggs died during development (the eggs of 1990 are still incubating, the final hatching success is therefore still unknown). One of the eggs which died may have been buried too shallow in a nest, but seven of these eggs died spontaniously without obvious external reasons. All intact eggs which were produced by females less than six weeks after capture in the wild developed without problems. This indicates that during this project developmental problems of eggs are generally caused by long-term captive conditions of their mothers (15 cases) rather than by incubation problems (one case: one egg left in a nest outside).

The entire first egg clutch of the young female in Perth Zoo from 1989 did not develop, but this female refused to eat the new food mixture before then. One egg of an old captive female in 1989 and two eggs from captive females in 1987 never started to develop, but it is not clear which of these eggs were "non-fertilised" and which were fertilised and "non-developing". In 1989 the one non-developing egg from the old captive female seems to have been infertile, because all her other eggs from 1988-1990 developed without problems and she was readily eating the food mixture. With the exception of this egg and the egg which died during development in the shallow nest in 1988, 13 of the 14 eggs which were non-developing or which died have one thing in common: the females which laid them had been in captivity for more than 16 months but did not eat the new food mixture before laying or at least until three months before laying. The main reason for the failure of eggs to develop normally seems to be that they were of poor quality caused by inadequate nutrition of the females that produced them.

Table 8: Egg development, all eggs between 1987 and 1990

	Number of eggs laid	No. of eggs damaged during laying	No. of non-develop. eggs	No. of eggs dying during developm.	No of eggs hatched	No. of eggs develop. > 10 weeks	% of un-damaged eggs hatched or developing
females caught in the wild < 6 weeks before laying eggs	12	1	0	0	6	5	100 %
females > 1 year in captivity, ate no food mixture until < 3 months before laying eggs	14	1	7	6	0	0	0 %
females > 1 year in captivity, eating food mixture > 10 months before laying eggs	31	1	1	2	16	11	90 %
total	57	3	8	8	22	16	70 %

6.2.6. Hatching

From 1966 to 1978 hatchlings emerged between 24 April and 22 July in the outdoor enclosure of Perth Zoo (Spence *et al.* 1979). This may well be the normal time of hatchling emergence in the wild; at this time the soil becomes softened by the first heavy autumn rains and the swamps fill with water. Hatchlings may emerge from the nest as long as seven months after the eggs have been laid.

The captive breeding work in the Wildlife Research Centre from 1979 to 1981 demonstrated that the hatching of *P. umbrina* eggs is triggered by a sudden drop in incubation temperatures; if the incubation temperature is maintained constant the embryos develop to hatchling size but do not hatch and eventually die. This adaptation presumably prevents hatchlings emerging before the swamps fill with water in late autumn or winter (Burbidge *et al.* 1990).

A drop of the incubation temperature is now routinely used to trigger hatching of eggs. Towards the end of egg incubation all eggs are now routinely maintained at 24°C. During April a temperature drop down to 18-20°C normally induces hatching at between some hours to several days (seventeen days in two cases in 1989). Routinely eggs are induced to hatch during April, because under the artificial incubation conditions the energy reserves of eggs may run out more quickly then in natural nests (which possibly have lower mean temperatures over the whole incubation period). In one case, on 09.04.1990, an egg hatched spontaneously in the incubator at 24°C, indicating an endogenous readiness to hatch at this time of the year.

Two complete hatching events have been video-taped in April 1990 and one partly. An animal first pips the egg with its egg tubercle from the inside which causes little cracks and a short protrusion of the egg shell which smooths again. Then, after about one minute, the head is forced through the shell at this point, breaking parts of the shell off and ripping the egg membranes. The head is pulled in again and for the next 2-3 minutes only the nostrils protrude from time to time through the opening, sometimes rhythmically - obviously the animal starts breathing. Then, in one single bout of activity, head and front limbs lift off the front part of the shell; the animal then rests for two to three minutes with extended neck and front feet and breathes regularly. Then it leaves the egg, ripping off the allantois which remains either inside the egg shell or directly besides it. No yolk or yolk sac is visible externally; in naturally hatched animals its inclusion into the body cavity has already occured. The whole hatching event from the first pipping of the shell to the animal walking off takes from five to eight minutes.

In some cases developing eggs needed more sophisticated attention to produce living hatchlings. Two eggs laid on 06.11.1988 and incubated at 28°C cracked during January 1989 and had fungus invading them (see chapter 6.2.5.). In the late afternoon on 01.02.1989 (after 87 days of incubation) the shell of one of these eggs was carefully removed in the area of the head of the hatchling (as assessed by candling). Then the head and especially the nostrils were freed from all membranes to allow the animal to breath, but the hatchling was not removed from the egg. The opened egg was left overnight without disturbance and the hatchling was found outside the egg with totally internalised yolk the next morning. The allantoic stalk and the allantoic sack were thick, dense, whitish tissue structures which were strongly attached to the animal (contrary to the situation of normal hatchlings were both are thin, transparent structures which collapse and separate from the animal through the slight mechanical impacts during its leaving the egg). The allantoic stalk was cut off as close to the plastron as possible and the slightly bleeding wound was sterilized with betadine solution. The animal was kept then on dry sterile gauze for 24 hrs. This premature hatchling weighted only 3.2 g but was viable and ate its first live food after four days.

The same procedure was performed with the second egg with fungus on the cracks on the evening of 06.02.1989 (after 93 days of incubation) and the result was the same. It weighed 4.2 g.

In 1988/89 all incubation temperatures were maintained constant until the end of March - they were not lowered to 24°C after nine to eleven weeks as in the following years. The egg incubated at 29°C was transfered to 20-21°C on 19.02.1989 and transported from the Wildlife Research Centre to Perth Zoo on 20.02.1989. Fifty minutes after the transport it hatched spontaniously (117 days after being laid). It weighted 5.1 g and - from the appearance of its allantoic stalk and sac which remained inside the egg shell - had been ready to hatch. Another small egg (6.2 g when laid) which had been left in a nest outside was opened on 28.02.1989 (114 days after being laid) as described above but had a strong and thick allantoic stalk and sack and 3.9 g body mass. The speed of development certainly varies with the incubation temperature. The other eggs of 1988/89 hatched during April after the incubation temperature was dropped. The only one which had not hatched by 26.04.1989 was opened (after 145 days of incubation) but accidentaly slipped out of the egg during the opening of the shell. Some yolk (a ball of about 3 mm diameter) was still outside the body in the yolk sac and after 24 hrs this situation was still unchanged. Obviously the inclusion of yolk and yolk sac occurs during the hatching process. If animals are artificially removed from an egg they may not be able to internalise it once they are outside. The yolk sac and the allantois of this animal had to be cut off despite

the fact that both were thin and translucent. The animal was kept dry for 24 hrs and its umbilicus closed and healed without problems.

From the eggs of 1989/90 nine hatched on their own between 05.04. and 23.04.1990. One was opened on 09.04.1990 and its allantoic stalk ripped off when it left the egg. The other egg was the smallest to be laid during this project (6.2 g, only 16.3 mm width against 18-21 mm normally) and it was from the smallest female ever recorded laying an egg (and it laid only a single one); it was opened on 15.04.1990 (after 128 days of incubation) and the hatchling (3.8 g) left the egg overnight but was not very active and did not rip off its allantois by itself.

6.2.7. Rearing of hatchlings

The method of rearing hatchlings of *P. umbrina* in Perth Zoo in the 1960s and 1970s was as follows: newly-hatched babies were transferred to a nursery section as soon as they were found. The nursery was an outdoor concrete display tank in which the water was kept thick with *Daphnia*. As the babies progressed, they were offered finely-chopped fish (whitebait) and sheep's heart. With this method, 14 of the 26 hatchlings found between 1966 and 1977 were alive by April 1978 (Spence *et al.* 1979). No system for identifying individuals and their progress was instigated. Five of the captive offspring were alive by 1987.

The hatchlings obtained in 1980 and 1981 in the Wildlife Research Centre were kept indoors in a laboratory in aquaria on a bench adjacent to a window where they were exposed to (glass-filtered) sunlight. During winter the water was heated to 24°C. They were fed on live food (mainly *Daphnia* and *Notonectes*) which was caught in Star Swamp and other swamps and on chopped fresh fish. All hatchlings except one from 1980 died in between 6 and 204 days without having increased their hatching weight. One animal which hatched on 23.06.1980 did not gain weight during its fist winter and spring, but started to grow later and died on 04.04.1982 at 20 g body mass.

The limited success of rearing hatchlings of *P. umbrina* in the past shows that the methods were unsatisfactory if the goal is to rear as many captive bred animals as possible for reintroduction to the wild. Therefore this project had to develop better methods to rear hatchlings.

Nursery

The Western Swamp Tortoise Captive Breeding Management Committee decided in late 1988 that all hatchlings should be reared in Perth Zoo. Because of unforeseen delays, Perth Zoo was not able to provide the planned outdoor hatchling nursery at the time when the first batch of *P. umbrina* hatched in summer and early autumn 1989. The

hatchlings had to be kept at the animal hospital until 21.06.1989. They were kept individually in transparent plastic containers of 26 x 19 x 10 cm with 4-5 cm of water and a dry clay area with clay from the tortoise habitat. During the day a lamp heated one dry corner to 30-35°C, the night temperature in the airconditioned room was 21°C. On sunny days the animals were moved to an aquarium outdoors in the sun where they basked extensively.

A prototype nursery-unit was built in June 1989 and the actual nursery during summer 1990. It consists of six outdoor ponds in six separate units of 1.2 x 2.2 m. The ponds can easily be drained, refilled, heated with aquarium heaters, and covered with wire mesh and fibre glass lids (e.g. overnight). The ponds themselves have been built out of black polyethylene sheets. The whole bottom area and the slopes to the shallow and dry parts of the enclosures are covered with clay from a clay pan in the tortoise habitat to provide the microorganisms and plants (which germinate out of seeds in the soil) which occur there. A deep part of 30-40 cm water depth is surrounded by shallow areas of 3-8 cm water depth. All units are 60 cm above ground level and provide access from three sides.

In 1990 hatchlings were moved to the nursery a few hours to two days after hatching. The eleven hatchlings are spread over three different ponds. The hatchlings from 1989, since moving to the nursery, were always kept together in one pond.

Food

During their first year the hatchlings from 1989 were mainly fed with live food (*Daphnia*, *Notonectes*, Culicidae and Chironomidae-larvae) from the duck-ponds in Perth Zoo and with a food-mixture more or less similar to that which the adults get (the recipe changed several times, in some batches *Daphnia* made up about 30%).

Because of the possibility of microbial contamination of the live food from the duck-ponds the hatchlings from 1990 received only live food which was caught in relatively unpolluted farm dams near Ellen Brook and Twin Swamps Nature Reserves (mainly *Notonectes*, sometimes *Daphnia*), or which is bred in live food breeding ponds in Perth Zoo (mosquito-larvae), or which is purchased from commercial breeders (brine-shrimps). Before being offered to the hatchlings, the brine-shrimps are kept in saltwater and fed with yeast. Since winter 1990 all youngsters from 1989 and 1990 have beeb given only live food and no food mixture.

During their first autumn, winter and spring food is offered twice a day to the hatchlings *ad libidum*

Growth

The growth of all hatchlings is summarised in figure 23. Due to health problems in their first months of life, which still has not been overcome, the hatchlings from 1989 grew slower than those from 1990, which have already caught up with them.

In the wild, in order to survive the first summer, hatchlings have to grow during their first growing season to a minimum of 25 g in Twin Swamps Nature Reserve and to at least 17 g in Ellen Brook Nature Reserve (Burbidge 1981, 1984). The tortoises feed only under water, which is normally available in their habitat for five to six months between end of May and November. Captive hatchlings have a relatively longer first growing season. The growth rate of the captive hatchlings from 1990 seems to approximate that recorded in wild ones.

This is a significant success of the project. For example, a hatchling from the Twin Swamps Nature Reserve raised in captivity in the Wildlife Research Centre between 1982 and 1987 had a growth rate of only about 10% of that recorded by wild animals (Burbidge in lit. 31.08.1987). No records exist of the growth of hatchlings in Perth Zoo in the 1960s and 1970s.

Health

All eleven hatchlings from April 1990 are in good health and have had no serious problems so far. In late September and October five animals had, over variable times, some white material building up in eye corners which they normally lost after some days. Sometimes the left and the right eyes were affected intermittently. In all three hatchling ponds some animals showed this condition which disappeared without treatment and which did not seem to have a negative impact on the animals. Despite investigation of the white material by the zoo veterinarian and by the Microbiology Laboratory of the W.A. Department of Agriculture its nature or agent could not be established.

The eleven hatchlings from 1989 developed health problems during their stay in the temporary accomodation in the animal hospital of Perth Zoo between February and June 1989: their skin shed in clumps, some had swollen eyes and eye lids, some had respiratory problems, some had swollen toes and feet and lost nails or entire toes. The delayed completion of the outdoor nursery also meant that the already sick hatchlings had to be shifted outside at the beginning of winter, during the coldest time of the year.

Treatment of sick animals was done in close collaboration with the zoo veterinarian (Dr Bill Gaynor), the Zoo hospital staff, animal keepers, and with the Animal Health Laboratory of the Western Australian Department of

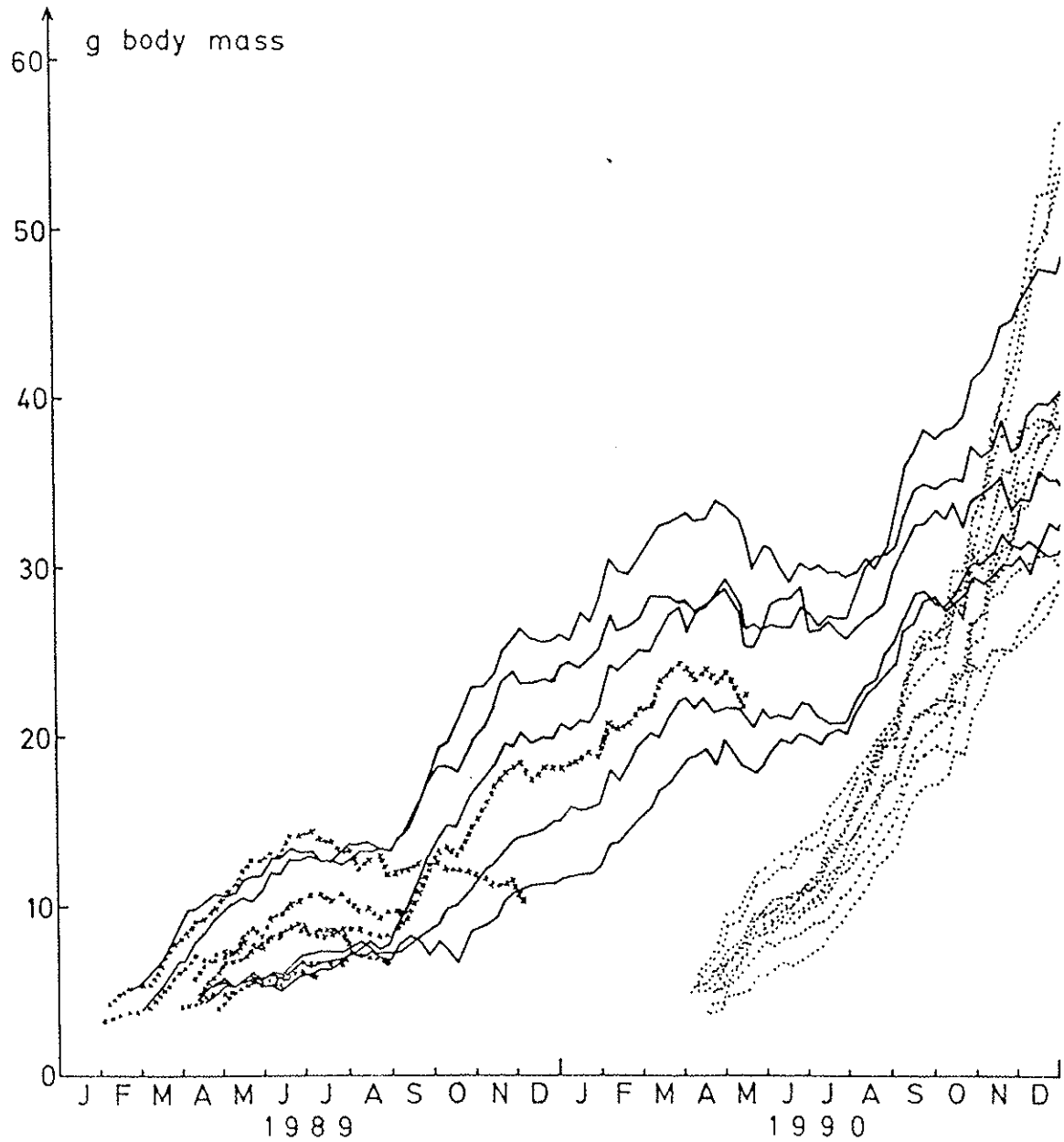


Figure 23: Growth curves of the hatchlings from 1989 (solid lines: survivors; xx-lines: animals which died) and of the hatchlings from 1990 (dotted lines).

Agriculture. It seems likely that the problems were (and are) caused by bacterial infections, since we found by way of trial that chloramphenicol (chloromycetin) treatment improved their condition, either applied as an ointment or injected intraperitoneally. Other treatments included disinfectant baths, keeping animals dry and warm overnight, increasing the acidity of the pond water to pH 6 and heating the pond with aquarium heaters. Microbiological examination of swab samples and of dead hatchlings revealed such a number of bacteria and fungi that it was not possible to identify the pathogen which caused the problems.

Five of the eleven hatchlings from 1989 died during their first winter and spring. A further animal developed problems after maintainance in an aestivation pen from 24.04.1990 to 12.05.1990: It became disorientated and did not react to obstacles at its path in the normal way. It became weak, its condition worsened and despite chloramphenicol injections it was found dead on 20.05.1990. The post-mortem (done by the Agricultur Department of W.A.) revealed that it had died because of a *Pseudomonas* infection of the lung and other organs. This infection was not necessarily the same as the one which caused the swollen feet and the other deaths.

The five remaining hatchlings from 1989 developed again problems with swelling of their digits and feet and the breaking off of claws during winter 1990. Build ups of dead skin on their feet had to be removed once a week. It became obvious that the infection still persists in all these animals and that their condition worsens during winter, despite the fact that their ponds were heated overnight. In spring their condition improved and stabilised by summer without treatment, but their reduced growth when compared with the hatchlings from 1990 indicates that they still have not totally overcome their health problems.

Aestivation

During February and March 1990 some of the then six surviving hatchlings from 1989 often spent several days in a row under leaf litter in the dry compartment of their enclosure. At this stage it was decided to move the three heaviest juveniles to an aestivation pen which resembled those which are used for the adults (see chapter 6.2.4.). On 10.04.1990 they were moved and all three disappeared under the leaf litter within hours. Over the next three weeks all three gained weight - apparently they filled up their cloacal bladders with water as a storage for aestivation. On 24.04.1990 the remaining three juveniles were shifted to this aestivation pen and two of them behaved in the same way and settled down under the leaf litter. One however was reluctant to hide under the leaves and moved constantly around in the pen (this animal died four weeks later

because of a *Pseudomonas* infection). On 12.05.1990 all animals were moved back in a nursery pool.

This experience with aestivation of juveniles in 1990 was not very encouraging. It was decided not to shift hatchlings and juveniles to aestivation pens in 1991 but to provide enough leaf litter in their standard nursery enclosures in order to let them choose between aestivation and remaining in their ponds.

6.3.0. GENETIC MANAGEMENT

Pseudemydura umbrina is in a severe genetic bottle-neck with only around 30 animals of breeding age for the whole species. In this situation loss of genetic variability can be a serious constraint for long-term survival and the future evolutionary potential of a species. Conservation actions have to take this into account and genetic management of the captive population is necessary in order not to lose the genetic diversity of the founder stock. Another potential problem for small populations is inbreeding and its possible consequence of depression of fertility and vitality in the population.

In the 1960s and 1970s in Perth Zoo the whole adult breeding group was kept in a random mating situation in one enclosure. Records indicate that the 13 founders included four females of breeding age (Spence et al. 1979). It is not known whether the captive-bred animals from this time were produced by one or by several females; the rate of reproduction as recorded could have been the result of only one or two breeding females (Kuchling and DeJose 1989).

In 1987 the captive population included 14 animals of breeding age (including 3 captive bred ones), three females and eleven males. These were split into two groups: one in the Wildlife Research Centre which included all three females and two males; the second in Perth Zoo consisted of nine adult males, one subadult male and one subadult female. Apart from the fact that the animals did not reproduce between 1980 and 1987, this management excluded 9 of the 14 potential breeders from any chance to contribute their genes to future generations; it reduced the potential captive breeding population size of 14 breeders to an effective one of maximally 5.

At the start of the project the first step of genetic management of the captive population was to change the composition of the two subgroups in order to widen the potential genetic input into offspring. The second step was to abolish the random mating situation of the captive population which occured in the past 25 years in order to enable the management of the contribution of individual animals.

Several aspects have to be considered when choosing management options for preserving genetic diversity of the small population of *P. umbrina*. Conservation biological evaluations in other species show that when animals are bred in captivity to re-establish a wild population, the methods of randomly selecting breeding pairs, choosing the best breeders, or managing mating pairs by allozyme data result in substantially reduced genetic diversity of the reintroduced population. Genetic management based on pedigree analysis (Equalizing founder contribution, or maximizing founder genome equivalents, or maximizing allelic diversity) produces more genetically diverse release populations (Haig *et al.* 1990).

But the most important rule is: the less time (in generations) a species spends in captivity and the sooner reintroductions start the better for its genetic situation. The severe genetic bottle-neck of the species is relatively recent (in generation times). Because of the longevity of *P. umbrina* (> 50 years) many individuals of the critically small world population (less than 50 individuals without the captive bred hatchlings of the last two years) are still survivors from a time when the population was larger (e.g. 200-300 animals 25 years ago). The captive population still consists overwelmingly of wild-caught individuals. A continuation of the present captive breeding success should leed to a rapid population expansion. The use of F1 offspring to re-establish populations in the wild will effectively prevent any potentially detrimental genetic drift during the time the species has to be bred in captivity. Of course the long-term maintainance of the captive population will be essential - but in years, not necessarily in generations.

The breeding strategy (presumably facultative mate selection, no pair bonds) and the - for an animal of this size - unusually long reproductive span of individuals (in any case > 30 years for females) means that it will be possible in the long term to use more or less all genetic management strategies during one breeding generation. The extensive generation overlap in this species will even allow options which are not generally available in other captive breeding programmes. This will include the pairing of unrelated individuals of different generations of animals - a possibility not even considered by the available programmes of PVA-analysis (U.S. Seal, personal communication Nov. 1990).

The third step of genetic management of the captive breeding programme of *P. umbrina* was to preferentially use the oldest males (and of course any female available) to engender offspring in order to save their genetic heritage before their demise. During 1988 and 1989 the old and senile males of Perth Zoo were introduced to the females to copulate, but unfortunately three of them died during this exercise (see chapter 6.2.2.).

At this stage the fourth step of genetic management of the population was to introduce a rotation scheme so that every female mates with a different male in consecutive years and that all individuals contribute their genes. Due to some problems with mating activity in 1989 and 1990 this scheme could not be rigidly followed. During 1989 two wild males and during 1990 one wild male of the Ellen Brook population were used for matings with captive females to expand the captive gene pool. A problem to consider is the ability of females to store sperm over several years. In future the contribution of individual males should be assessed by DNA-fingerprinting - in the case a suitable method can be found.

The importance of single individuals to such a small breeding population cannot be overstated. In this respect the recovery of four individuals from marginal, disturbed habitats on private properties outside the nature reserves and their inclusion in the captive breeding programme (they would have been lost for the wild population) is genetically very significant.

It would be helpful for the genetic management of the species to assess the genetic variability which exists in the last wild population as well as in the captive founder stock. In the long term, the reconstruction of patrilineages for captive bred animals (e.g. by DNA-fingerprinting) could be a further helpful tool for genetic management. The actual sire of an offspring cannot be assessed without doubt by knowing the mate of the mother in the last mating season - females can store sperm for several years.

During the project several techniques have been assessed which could help in the genetic management of the species. These were chromosomal studies, plasma enzyme gel electrophoresis and DNA-fingerprinting. Two constraints had to be taken into account: The necessity to sample living tissues from the tortoises to perform these tests and financial aspects.

The main constraint was and is the sampling of animals. Since the main goal of the project was and is to improve the breeding performance of the captive tortoises no chances could and can be taken that would reduce it. In critical times any invasive procedure could easily interfere with ovulation and therefore egg production. The problem is that nearly any time during the year has some significance for reproduction of *P. umbrina*. Females were only sampled after the egg laying time in the first half of December, some time before aestivation and resumption of vitellogenesis. Males were sampled between October and December. This timing minimized interference with either aestivation and the onset of ovarian activity, mating in winter or egg production in spring.

During 1988 and 1989 blood samples (50-150 l) were collected from all adult captive animals in heparinized capillary tubes, centrifuged and plasma and blood cells stored in liquid nitrogen. Some animals from the Ellen Brook population were also sampled. In addition, muscle biopsies have been taken from the tails of two males for tissue culture experiments at the Department of Clinical Cytogenetics of the State Health Laboratories (Dr Marie Mulcahy). Theoretically, from such a biopsy, cell material with all the genetic properties of the animal could be grown in any quantity for any genetic or chromosomal test. These two samples were however contaminated and did not grow, although this method (and Chromosome banding) worked well with muscle biopsy samples from three species of *Chelodina*.

During 1990 plasma enzyme electrophoresis (by Dr David Coates, CALM) and DNA-fingerprinting (by Dr David Groth Curtin University of Technology) could detect only a very little genetic variability and a low level of heterozygosity in 17 *P. umbrina*. These results are still inconclusive and the question remains open if the genetic resources of the species are limited or wether the techniques used to detect genetic variability are appropriate. Samples of more animals and additional techniques should be used in future.

6.4.0. ARTIFICIAL REPRODUCTIVE TECHNOLOGIES

Few artificial reproductive technologies which are helpful for captive breeding of reptiles have been developed and successfully applied. The most significant of these is the induction of egg laying of gravid females by oxytocin (a mammalian hormone which has not been found in reptiles; Ewert and Legler 1978). Artificial insemination has been used in several reptile species with no (Samour 1986) or limited success (Quinn et al. 1989). For example, in the U.S. captive breeding programme for the critically endangered madagascan tortoise *Geochelone yniphora*, electro-ejaculation and artificial insemination gradually incapacitated or killed all males - during 15 years this programme produced one single hatchling and a conservation prize was awarded for this "success".

A healthy captive breeding success was and is imperative for the persistence of *Pseudemydura umbrina* and should not be jeopardized by unnecessary technological gadgets. This consideration restricted the use of artificial reproductive techniques - see the instructive example above (although we did not get any conservation prize). Induction of egg laying in females with oviducal eggs by oxytocin injection was successfully used in *P. umbrina* in 1979 and 1980 by A. Burbidge and was used on three occasions during this project (see chapter 6.1.4.).

Some experiments were done to attempt to induce ovulation in females which did not ovulate. In spring 1988 the one

female from the Twin Swamps population and the young female in Perth Zoo both had preovulatory follicles in the ovaries which had not ovulated by late November (see chapter 6.1.3.). An experiment was started to see if photoperiodic and temperature manipulation would induce ovulation.

The young Zoo female was moved to a controlled temperature and photoperiod room at the Department of Zoology of the University of Western Australia on 08.12.1988 and kept under L:D 11:13 (L:D = light : dark cycle) and a day:night temperatures of 22°:16°C in an Aquarium of 80 x 50 cm ground area and 12 cm of water depth. In this way early spring conditions were simulated in the hope that they might facilitate ovulation. However, the follicles started to decrease in size and on 06.01.1989 the experiment was terminated and the animal was moved back to Perth Zoo.

The Twin Swamps female was kept under the same light dark and temperature conditions from 03.12.1988 to 19.01.1989. Her largest follicles remained at a preovulatory size of 17-18 mm diameter but ovulation did not occur. On 28.12.1988 an experiment was conducted to induce ovulation by gonadotropin treatment. The female recieved 1200 IU PMSG ("Folligon") intraperitoneally at 1210 hrs and at 1955 hrs. This treatment produced no observable effect and it was repeated on 10.01.1989 with 2000 IU PMSG at 1420 hrs and at 2135 hrs. Neither ovulation nor any other effect of the treatment could be observed and the animal was shifted back in her outdoor enclosure on 19.01.1989.

Comparable hormonal treatments have been successful in inducing ovulation of preovulatory follicles in *Anolis* lizards (Jones et al. 1988) and are routinely used in husbandry of domestic animals. So far, they never worked in turtles (Paul Licht, pers. comm. Sept. 1989). There is however the possibility that the follicles of the female *P. umbrina* which was treated with PMSG did not ovulate because the disintegration process of atresia may already have started. Despite the fact that several investigators stated that follicles can be "rescued" from atresia by PMSG treatment, it has now been shown that once a follicle begins to degenerate in vivo, it cannot be recruited for ovulation by PMSG (Hirshfield 1989).

The Twin Swamps female did not have any follicles growing to preovulatory size in the 1989 season (Fig. 10). Because of the possibility that the hormonal treatment interfered with her next ovarian cycle no further experiments to induce ovulation artificially have been conducted.

Because of these unsuccessful results it seems generally more sensible for the captive breeding of *P. umbrina* to interfere as little as possible with artificial reproductive techniques. The only exception is the

inducement of egg laying by oxytocin, which can be very useful in certain cases (e.g. when a laying female retains one egg of her clutch in the oviducts; when natural nesting is not possible or cannot be observed). This method has proved to be secure and successful in P. umbrina and many other tortoises and turtles.

7.0.0. TRAINING OF TECHNICAL STAFF

During the first part of the project, before winter 1989, there was no continuity of technical staff allocated to the project and therefore no real training of specific technical staff for captive breeding.

During winter 1989 the Western Swamp Tortoise Captive Breeding Management Committee decided that at the end of the project, by July 1991, Perth Zoo will take over the responsibility for captive breeding of *P. umbrina*. From this stage on, in the captive colony at Perth Zoo, all day to day work, the routine of which was established, was primarily done by Zoo keepers under the direction of the principal investigator. By April 1990 one of the keepers (Mr. Dean Burford) has been assigned full time to the project in order to be trained as principal keeper for the Western Swamp Tortoise breeding programme. Since then he has been trained by the principal investigator to do all practical project work in Perth Zoo, including monitoring of oviducal eggs in females, egg incubation (recovery of eggs, monitoring of egg development), all handling of hatchlings and managing and monitoring of mating and aestivation. The results of the training are satisfactory and the routine work should not result in problems in future.

From the beginning all treatments of sick Western Swamp Tortoises have been done in close collaboration with the animal hospital staff of Perth Zoo. This expertise is there and the aspects of the nutritional requirements for captive breeding have been frequently discussed with the zoo veterinarian (Dr Bill Gaynor). Ultra-sound scanning of females has been regularly done in collaboration with keepers or animal hospital staff.

After June 1991 captive breeding of the Western Swamp Tortoise will be discontinued at the Wildlife Research Centre; the captive stock which is kept there will be moved to Perth Zoo and the animals from the Ellen Brook population will be released into the now fox-proof reserve. No CALM-staff were trained in captive breeding techniques.

8.0.0. SIGNIFICANCE OF RESULTS

* The Western Swamp Tortoise did not breed successfully in captivity for many years. The project got the captive population to reproduce again.

* The research into female reproduction has resulted in an understanding of ecophysiological mechanisms which control their reproductive performance.

* Survival and growth rate of captive hatchlings was very poor in the past. The project developed successful techniques to rear them.

* Staff of Perth Zoo have been trained in all captive breeding techniques which were developed during the project. Perth Zoo will continue the breeding programme.

* The Western Swamp Tortoise appeared to be heading towards extinction. It is the most endangered vertebrate animal in Australia and the most endangered chelonian species in the world. After its continuous decline over the last 25 years the world population of *P. umbrina* has now increased by more than 30% as a result of this project.

* The most important objective of the captive breeding project is to produce enough animals for reintroduction into the wild and the reestablishment of a second wild population. This project provides the logistic basis for this work.

* The success of the project initiated new conservation actions for the Western Swamp Tortoise which have been written down in Wildlife Management Program No 6. This is an official document of the Western Australian Department of Conservation and Land Management which prescribes management actions and strategies for the next ten years to ensure long term survival of this species.

* The results of this project clearly generate long-term commitments of the conservation authorities to rescue the Western Swamp Tortoise and its habitat.

9.0.0. RECOMMENDATIONS

The captive breeding programme will be continued by Perth Zoo. The main recommendation is that all improvements and changes of husbandry management introduced by this project should be maintained in order not to erode the breeding performance of the captive population. Particularly critical aspects are:

- allocation of sufficient staff time to the breeding programme
- use of experienced, trained staff who has an understanding of the biology of *P. umbrina* (e.g. field experience)
- an optimal food mixture for adults, especially in regard to the freshness of ingredients and to minerals and vitamins
- *ad libidum* feeding of breeding stock in winter and spring until aestivation starts
- pairing of males and females only for some weeks in winter (from the end of aestivation until September). Males and females should be kept separate during the rest of the year
- adults should be encouraged to aestivate from early January to late May
- eggs should be moved to incubators within 12 hrs of laying, marked on the top and never turned during incubation
- incubation temperatures above 25°C should be reduced to about 24°C after 9-10 weeks of incubation
- in April, hatching of eggs should be induced by a drop of the incubation temperature to 19-21°C
- hatchlings should be kept in outdoor nursery pools from their first day onwards
- During their first year hatchlings should be reared with live food, the change to the artificial food mixture should be gradual over one or two years

Recommendations concerning the genetic management are:

- matings of males and females should be continued on a rotation basis in the next few years
- all captive adults have to be involved in matings (not necessarily all in one year)

- new techniques of DNA-fingerprinting should be used to assess the genetic variability of the captive and the wild population

- the temporary use of wild males as mates for captive females should be continued

- new DNA-techniques should be applied for the reconstruction of patrilineages of offspring

- as many pedigree data as possible have to be recorded and pedigree analyses should be performed for all hatchlings

- in the long-term, mating plans should be drafted and supervised by a conservation biologist according to increased knowledge of the genetic situation of the founders

- the selection of captive offspring for reintroductions has to be done under population genetic considerations

The recommendations above are limited to the captive breeding programme itself. Further aspects of conservation management and protection of the Western Swamp Tortoise are prescribed in Wildlife Management Program No 6 of CALM and not repeated here. The full implementation of this program over the next ten years is absolutely essential for the long-term persistence of P. umbrina.

10.0.0. EXECUTIVE SUMMARY

The following tasks have been executed:

* Breeding ponds and enclosures have been designed and constructed in Perth Zoo and in the Wildlife Research Centre

* a system to keep males and females separate over most of the time per year has been introduced to maximize reproductive output

* the reproductive conditions of females have been monitored by ultra-sound scanning

* two breeding groups have been established in order to maximize the number of animals involved in breeding

* a rotation system for matings has been introduced to preserve the genetic diversity of the founder stock

* a new food mixture has been developed which greatly enhances the viability of the eggs laid by captive animals

* all captive adult females have been induced to produce eggs

* aestivation pens have been designed and constructed

* aestivation management has been established for the breeding stock

* blood samples have been taken from the adults in captivity and analyses of genetic variability have been started

* a new method of egg incubation was developed

* a hatchling nursery has been designed and built in Perth Zoo

* the rearing management for hatchlings has been sucessfully improved during the project

* Experience has been gained in the treatment of sick hatchlings

* keepers in Perth Zoo have been trained in captive breeding techniques

* The world population of the species has been increased by more than 30% (excluding the 16 developing eggs existing on 31.12.1990)

11.0.0. REPORTS AND PUBLICATIONS ARISING

11.1.0. PUBLISHED PAPERS

Kuchling, G. and DeJose, J.P. (1989): A captive breeding operation to rescue the critically endangered Western Swamp Turtle *Pseudemydura umbrina* from extinction. Int. Zoo Ybk 28, 103-109.

Burbidge, A.A., Kuchling, G., Fuller, P.J., Graham, G. and Miller, D. (1990): The Western Swamp Tortoise. Western Australian Wildlife Management Program No. 6. Department of Conservation and Land Management, Perth.

11.2.0. PUBLICATIONS IN PREPARATION

Kuchling, G. Reproduction of the critically endangered Western Swamp Tortoise *Pseudemydura umbrina*.

Kuchling, G. Nesting of the Western Swamp Tortoise *Pseudemydura umbrina*: the other way round.

Kuchling, G. Western Swamp Tortoise captive breeding manual.

Kuchling, G. et al. Captive breeding of the Western Swamp Tortoise *Pseudemydura umbrina*: Husbandry management and rearing of hatchlings.

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