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## Reproduction of the pig-nosed turtle *Carettochelys insculpta* (RAMSAY, 1886) at the Rotterdam Zoo

### Abstract

Rotterdam Zoo has had pig-nosed turtles in its animal collection since the large exhibition NOTOGAEA (on Notogaeian flora and fauna) in 1978, and added several specimens since. Over the years the animals have been kept in two different enclosures, and in several combinations, due to size differences, availability of space and aggressive behaviour. In 2004 three of the animals were kept together in a 15.000 l aqua-terrarium, in the company of other large freshwater turtles and fishes. The animals were separated again as one of them, later determined to be a male, had damaged one of the others, as occurred in the years before on numerous occasions. In January 2005 one of the remaining turtles, obviously a female, was found to have left the exhibit during the night, and to have produced three eggs of which two were broken. She had done this in the public area of the building where the aqua-terrarium is situated, the Rivière-Hall. After the female had been placed in an off-exhibit enclosure, she produced another 21 eggs after injection of *Calcium-Sandoz*® and Oxytocine. Of these eggs, 15 successfully hatched, after 84–112 days, and most after submersion in water. This is believed to be the fourth captive breeding ever, and the first one that documents the complete reproduction process in captivity.

### Introduction

The pig-nosed turtle *Carettochelys insculpta* is the only surviving member of its genus and the family Carettochelyidae. It is found in northern Australia and southern New Guinea. The species is also known as Fly River turtle, after the New Guinean river tributary where it was first collected.

From this single specimen, the species was described by Dr. E. P. RAMSAY in 1886.

Epidermal scutes are absent. The shell is covered with a continuous skin, similar to soft-shelled turtles. The limbs are paddle-shaped, as in marine turtles, and each bears two claws. The fleshy proboscis, with the nostrils placed anteriorly, gives the species its common name: pig-nosed (or pig-nose) turtle.

Fully adult, they may grow to 55 to 60 cm (CANN 1978) and weigh more than 20 kg. Although primarily herbivores, animal matter is also taken.

DOODY et al. (2002) sexed the animals in north Australia by inspecting the tail length. In our case, with animals of different sizes, this did not prove to be simple. Generally speaking, the tail of the males is larger.

The animals are largely aquatic, and are found in larger and smaller rivers, ponds, and lagoons, with a soft sand or mud bottoms; sometimes also in brackish coastal waters.

*Carettochelys* is now included in the suborder Cryptodira, superfamily Trionychoidea (GEORGES & WOMBEY 1993).

The IUCN has classified the species as vulnerable (IUCN, 2004). CITES: Appendix 2.

### The history of pig-nose turtles at Rotterdam Zoo

The first three specimens, probably two females and one specimen of unknown sex that died shortly after arrival, originating from New Guinea, were obtained com-

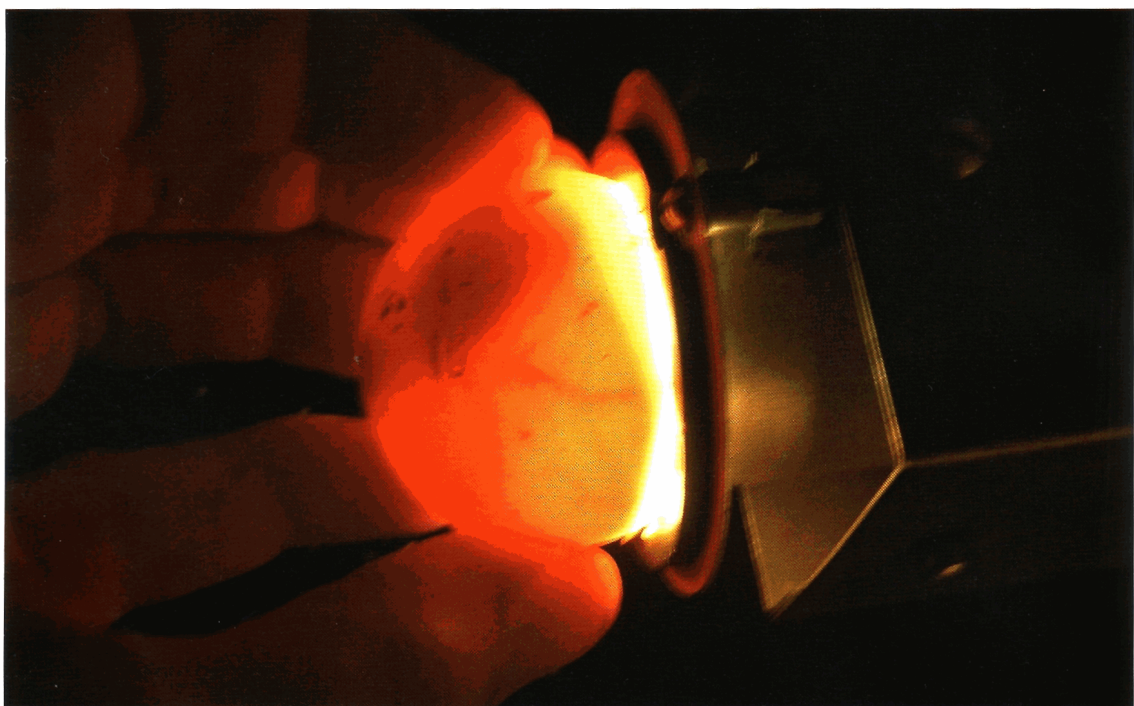


Fig. 1. Embryo at an age of five weeks.

mercially from a local reptile dealer in May 1978, in an era that was not yet against the policy of Rotterdam Zoo to obtain reptiles from pet shops.

At that time very little was known about the species (it had not been recorded from Australia until 1969 (CANN 1978)! The fact that around that time PRITCHARD (1979) reports on only four known captive specimens worldwide (two in Taronga Zoo, Sydney, one in the Bronx Zoo, New York, and one in the lab of Dr. ROBERT MERTENS) illustrates the level of knowledge on husbandry and captive reproduction. We saw a specimen when we visited the Aquarium of the Berlin Zoo in 1976. Whether or not this concerned the specimen from Dr. MERTENS is not known to us. Dr. MERTENS had died a year earlier, in 1975.

Except for intra-specific aggression, the animals are not difficult to keep in captivity.

As we were unsuccessful in getting our animals to breed, or in obtaining extra specimens, we sent our presumed female out on breeding loan to a well-known German turtle breeder in 1988. He had a male specimen, but as this project didn't result in successful matings either, the female was returned to the Zoo in 1990.

Later, two more sub-adult specimens (just very recently determined as males) did become available on loan from a private person in 1995; a fully grown female, purchased, from a private German keeper and one of (still) unknown sex, donated, 1998. One of the specimens acquired in





Fig. 2. *Carettochelys insculpta* two hours after hatching. Note that the margins of the scutes are already very flat.

1978. Contrary to our original assumption it turned out to probably be a male. It was loaned for breeding purposes to Frankfurt Zoo (Germany) in 1999. In Rotterdam this resulted in a group of five specimens, of which three are usually kept together in the 15,000 l aquarium.

In 2004 the two females shared the large aqua-terrarium with one of the males that had arrived in 1995.

#### Housing and husbandry

In 1978 the original first group of 3 specimens was housed in a small  $1.25 \times 1.25$  m aquarium for a very short quarantine period. Shortly after that they were housed in the 15,000 l aquarium.

This aquarium measures  $15 \times 1.5 \times 1.0$  m and has steep edges with overhang-

ing tropical plants (e.g. *Monstera*, *Spatyphyllum* and *Scindapsus* spp.). The aquarium itself does not have any plant decoration. A 15 cm layer of sand covers the bottom. For egg-laying, a 2 m<sup>2</sup> heated area with 30 cm deep sandy bottom is situated on one side of the aqua-terrarium. A sloping haul-out is provided on the edge for easy access to the nesting area. The situation with respect to decoration and shape of the aqua-terrarium did not change over the years.

However, the fish population the turtles shared the aquarium with changed several times. During the 1980s they shared the aquarium successively with three Australian lungfishes *Neoceratodus forsteri*; during the 1990s followed by arowanas *Osteoglossum bicirrhosum* and arapaimas

*Arapaima gigas*, pacu's *Colossoma macropomum* and red-tailed catfish *Phractocephalus hemioliopterus*. Since 2000 also four adult Arrau terrapins *Podocnemis expansa* are housed in the same aquarium. Very recently the fish population was reduced to a large group of twin-bar characins *Tetragonopterus argenteus*.

Due to temporarily changes in behaviour of individual specimens, the combination of the group of *Carettochelys* changed several times. Sometimes, injured specimens were separately housed in off-exhibit tanks to recover.

The aquarium is filtered by an open biological filter, consisting of two 150 × 75 × 50 cm tanks filled with a sand and gravel. Crude waste-material is filtered out by a cotton-wool filled section. One third of the water contents is changed twice a week. The water temperature is 28 °C and this is maintained by the central heating system, controlled via a thermostat, situated in one of the two filter compartments.

### Diet

Pig-nosed turtles are opportunistic feeders, largely omnivorous, but they consume a higher ratio of herbivore food, such as fruits (such as *Pandanus*-fruits), flowers and leaves, either direct from the river bank vegetation, or after it has fallen into the water (ROEMPP 2003). According to GEORGES & WOMBEY (1993) their herbivore diet includes *Ficus racemosa*, *Syzygium forte* and *Pandanus aquaticus*. Aquatic plants such as algae, eelweed *Vallisneria* spp., and water nymph *Najas tenuifolia* are also eaten when available. They also eat molluscs, crustaceans, fishes and mammals (as carrion).

CANN (1978) observed his captive specimens to accept most food offered: crabs; freshwater crayfish, worms, water hyacinth bulbs *Eichhornia crassipes* and fruit.

In Rotterdam Zoo it at first did not prove easy to get the animals to feed, probably due to the conditions they had experienced during holding and transport while in transit from New Guinea to the dealer's shop in Rotterdam. We learned from the keeping staff in Berlin Zoo that boiled potatoes could prove to be feeding stimulus. This turned out to be successful and from then on further feeding problems in this species were not experienced.

During the first decade in Rotterdam they were mainly fed on minced meat mixed with vitamin and calcium/mineral supplements twice a week and vegetable matter as lettuce, endive, tomatoes, apples and bananas three times a week.

Since 1990 all fresh water turtles at Rotterdam Zoo are fed with a gel-food developed in Germany by both Prof. Dr. WALTER SACHSSE and INGO PAULER. This gel-food is made in house in the Zoo and consists of freshwater fish, commercially manufactured dog-food, ox heart, beef, spirulina algae, shrimps, milk, water, vitamin- and calcium/mineral supplements and high quality gelatine.

### Reproduction biology

In northern Australia the nesting season is in the dry season, from mid-July to October or early November, depending on the geographic location (DOODY et al. 2003a). The animals nest on sandy, loamy or muddy banks and beaches, adjacent to the river (GEORGES & WOMBEY 1993). Females in Australia reproduce every second year, but produce two clutches per year (DOODY et al. 2003a). Clutch size ranges from 7 to 19, but averages around 10 eggs. The females show gregarious behaviour when nesting and during oviposition (up to 16 females have been observed on one beach), but this does not seem to reduce the risk of predation (DOODY et al. 2003b). Nests are susceptible to

predation by monitor lizards (*Varanus* spp.) and, in Northern Australia, by being trampled upon by feral water buffalo (*Bubalus bubalis*).

TDS (Temperature Dependent Sex determination) is known in this species; the range of temperatures within which both sexes are produced was found to be very narrow (around 1 °C) and was found centred around 32 °C. This range separates the range of temperatures within which only females are produced (> 32.5 °C), from the range within which males are produced (< 31.5 °C) (YOUNG et al. 2004).

At 30 °C the embryos enter a diapause (aestivation) within the egg after 64-74 days (WEBB et al. 1986). Yolk is then used at such a rate that the yolk supply can last up to about 60 more days. Data from natural nests in New Guinea show that incubation periods in the wild range from 86-102 days at 31.6 °C. This would confirm that this diapause occurs within this 59 day limit (GEORGES & WOMBEY 1993). Hatching was stimulated experimentally by reducing the availability of oxygen. This indicates that in the field the stimulus is either the first rains of the season, or flooding (WEBB et al. 1986). The aestivation would ensure that hatching would coincide with favourable conditions in the field.

### Captive breeding

In several zoos mating attempts and oviposition has been observed, although much of the time, eggs have been deposited in the water. On May 25<sup>th</sup> 2001, eggs were found at the bottom of a tank in the *Wilhelma Zoo* (Stuttgart, Germany). Most eggs were eaten or destroyed by the fish in the tank (*Pangasius* spp.) or by the turtles themselves – only two could be saved for incubation. This was done at 30 °C and 80 % humidity in Perlite. The humidity was increased after 136 days, and one

juvenile hatched on 4 October 2001, after assisted hatching. The other embryo had died, and it was assumed that assisted hatching could be necessary. The breeding group consisted of seven animals, many of which had been living in the Zoo for 27 years (KOCH pers. comm., ROEMPP 2003).

Also in 2001, a hatchling was found swimming in the main pool of an exhibit at the *Bronx Zoo* (New York, U.S.A.), among fishes, turtles and crocodiles. It must have emerged from a clutch that had been deposited on the nesting beach in the exhibit. After intensive search of the enclosure, more eggs were found but these did not develop during incubation (ROEMPP 2003).

In September 2003, ten hatchlings were found in a large, off-exhibit enclosure in the *Miami Metrozoo* (Florida, U.S.A.). It is believed that these hatchlings were from two separate, undetected nests. The parents had been maintained in that pool for several years (ANONYMUS 2004).

### Breeding at Rotterdam Zoo

As stated above, the breeding trio shared the large aqua-terrarium during parts of 2004. There was a lot of aggression between them and nothing indicated that copulations had taken place. Nevertheless, this must have been the case, as fertile eggs were produced in 2005. Mating behaviour was probably interpreted as aggressive behaviour. Courtship and mating in this species sometimes looks very aggressive.

This means that the female, which arrived as an adult animal in 1978, had produced her first clutch of fertile eggs almost 27 years after her arrival at the Zoo.

After we noticed increasing damage on the carapace of the female, the male was taken out of the public tank in November 2004.





Figs. 3-5. Hatching under water.

The female laid 24 eggs on January 10, 2005. In search of suitable nesting site, the female had ignored the beach that had been specially made for her by the keeping staff, and had left the exhibit through the planter that separates the aquatic part of the tank from the public area. She was found by the night watchman very early in the morning at the bottom of the stairs leading to the public lavatory. Of the three eggs that she laid there, two were broken. After the female was recovered from the public area she was put in an off-exhibit container with a layer of peat, where she produced the rest of the eggs, twenty one in number, approximately one hour after the injection of Calcium-Sandoz and Oxytocine

### Incubation

As hatchling sex is influenced by the incubation temperature, the eggs were incubated in four human incubators at different temperatures: 28, 30, 31.5, and 32 °C, following WEBB et al. (1986). The majority of the embryos incubated at 28-30 °C become males, and at 32 °C become females. Only after the hatchlings emerged did we become familiar with the recent paper by YOUNG et al. (2004) on the pivotal range, as cited above. If we would had had that information before, we would probably have chosen slightly different incubation temperatures.

Vermiculite was used as a substrate, with two exceptions: two eggs were incubated on a peat- vermiculate mix, one at 30 °C and one at 32 °C.

On the second day a small white dot became visible on the eggs and by the fourth day, the eggs turned completely white. When the eggs were candled on day 18, three eggs showed signs of fertilization. On day 27, 15 eggs turned out to be fertile, with an embryo of about 20 mm in length visible (see fig. 1).





**Fig. 6.** Immediately after hatching the egg membrane and a few blood vessels are still visible in this specimen.

On March 15 (day 61), egg number 10 (32 °C) was submerged in water for five minutes. The egg floated and there was apparently no effect. This was repeated on day 66 (for 10 minutes) and day 75 (30 minutes), still without any result. On April 7 (day 84) the egg sank immediately and hatched after 20 minutes. Eggs nos. 6, 7 and 8 also sunk, and these subsequently hatched after 15 minutes.

The next day (day 85) egg nos. 4, 5 and 6 (also from 32 °C) hatched after 15, 20

and 23 minutes, and on day 90, egg no 3 hatched after being submerged for 32 minutes. This egg was specially selected and kept to hatch in front of the press and the audience as a special event when the Rotterdam Zoo and EAZA launched the 2005 EAZA ShellShock Campaign in the Netherlands. The complete data on the hatching can be found in Table 1.

Only one egg (number 15) hatched in the incubator, thus without being submerged in water. This was an egg that was incubated at 28 °C.

Prior to submersion all eggs were checked for viability with help of a so called Buddy-Egg, a commercially developed apparatus for registration of the heartbeat of bird embryos. At Rotterdam Zoo, we already had very positive experiences using this device for checking eggs of crocodiles *Paleosuchus palpebrosus* and Gila monster *Heloderma suspectum*. All viable eggs proved to have a rate of 78 to 85 heartbeats per minute.

### Hatching

Just a few minutes elapsed between the first hole in the egg shell and the complete hatching. One hatchling needed five minutes to complete the process, but took a

breath at the surface whilst hatching. All embryos are situated in the upper part of the egg during incubation, and as soon as the egg sinks to the bottom of the tank they turn upside down to the bottom, such that the heavy embryo is then at the lowest point.

At hatching the carapace has visible ribs and is shaped just as round as the egg. The weaker parts, including the spiny edges, spread out after 30 to 45 minutes and become tighter during the following days.

On a number of occasions the hatchlings are completely covered with the egg membrane attached to the umbilical chord, which falls off after several minutes. On two occasions the keepers had to free the

Egg No.	Incubation temperature (°C)	Incubation time (days)	Hatching method	Hatch date
1	31	97	submerged	04/17/2005
2	31	97	submerged	04/17/2005
3	32	93	submerged	04/13/2005
4	32	89	submerged	04/08/2005
5	32	89	submerged	04/08/2005
6	32	89	submerged	04/08/2005
7	32	88	submerged	04/07/2005
8	32	88	submerged	04/07/2005
9	32	88	submerged	04/07/2005
10	32	88	submerged	04/07/2005
*11	30	-	-	dead embryo
*12	30	-	-	dead embryo
13	30	-	-	infertile
*14	30	-	-	dead embryo
15	28	109	incubator	04/28/2005
16	28	110	submerged	04/29/2005
17	28	110	submerged	04/29/2005
18	28	still incubating	-	-
x19	30	140	opened	05/29/2005
20	30	still incubating	-	-
21	30	116	submerged	05/05/2005
22	30	116	submerged	05/05/2005

\* On May 5, this egg was opened after it did not show any pulse on the Buddy-Egg. It contained a full grown, dead embryo.

x On May 29 this egg did not show any pulse on the Buddy-Egg. It was opened and it contained an almost fully grown, living embryo. After removal of the egg-membrane the "hatchling" was housed in a small plastic aquarium with shallow water. Although looking very lively it died after two days.

**Table 1. Data on the hatching.**



hatchling from this membrane as it was also attached to the fore limbs and neck of the hatchling. On some occasions the membrane and umbilical chord stayed attached to the plastron for two or three days.

#### Hatchling husbandry

All hatchlings were initially housed separately in small plastic containers measuring  $30 \times 17 \times 15$  cm, in 7 cm of water. Some floating plastic plants were provided for cover. Water temperature was kept at 26 to 28 °C, air temperature at 28 °C.

After three days the hatchlings were fed with mosquito larvae, aquatic plants, zoo-made gel-food, Tetramin®, floating plants. Most food items were taken readily, but the fact that the mosquito larvae were

layer of sand. After moving into this aquarium the hatchlings directly buried themselves into the sand with just the top parts of the carapace and their heads above the sand. The water temperature is kept at a constant 28 °C.

#### Conclusions

It is indeed possible to breed *Carettochelys insculpta* in captivity. All three known breeding events happened with animals that had spent a long time in captivity. Aggression between the animals and consequent female carapace damage is related to reproductive behaviour. As was to be expected, the eggs hatched after being submerged in warm water, but also eggs

**Table 2. Morphometrics of eight hatchlings after 1 or 2 days after hatching.**

Hatchling No.	Weight (g)	Carapace length (mm)	Date
1	30.0	58.0	04/13/2005
2	31.3	55.5	04/13/2005
3	27.1	54.5	04/13/2005
4	24.7	55.7	04/13/2005
5	29.0	58.8	04/13/2005
6	26.7	53.3	04/13/2005
7	19.1	50.5	04/13/2005
8	24.0	54.4	04/17/2005

refused may indicate that the hatchlings are herbivorous initially. At the age of one month, some hatchlings started to feed on smaller earthworms.

Eight hatchlings were measured and weighed after one to two days (see Table 2).

Three weeks after birth, the first eight hatchlings were housed together in a  $150 \times 75$  cm aquarium with a water level of approximately 12 cm. The decoration consisted of some stones, aquatic plants and artificial plants for shelter. The bottom was covered with a few centimetres thick

that were not submersed hatched in the incubator.

The problem seems to be how to offer suitable nesting beaches that the females will indeed use and this may now be the single-most important factor to concentrate on for improving subsequent breeding conditions. These beaches should probably resemble the specifics of the natural situation better, the details of which may not be sufficiently known to animal managers outside of the natural range of the species.

In Rotterdam, this will be the focus of attention for the next breeding seasons.

### Additional data

During preparation of this manuscript, one egg that we had been able to salvage from the bottom of the aquarium and that was produced by our second female, hatched on May 27, 2005.

This egg hatched in the incubator and the incubation period at 31 °C was 106 days. This is the fifth case of breeding that is known to the authors.

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