

REPRODUCTION AND REPRODUCTIVE ORGANS IN *NEOPHOCAENA ASIAE-ORIENTALIS* FROM THE YANGTSE RIVER

Chen pei-xun, Liu renjun and R.J. Harrison, Institute of Hydrobiology, Academia Sinica, Wuhan, China, and Anatomy School, Cambridge University, England.

Introduction

Two species of freshwater dolphin are known to have existed in the Yangtse River, China, for over two thousand years. One is a white form, the Baiji (*Lipotes vexillifer*), the other is black and called the river pig (jiang zhu) or black finless porpoise (*Neophocaena phocaenoides*). PILLERI and GIHR (1972), on the basis of certain features, have divided the genus *Neophocaena* into three species: *N.phocaenoides* with a distribution in warmer waters, *N.sunameri* from colder Japanese waters and *N.asiaeorientalis* from the Yangtse river and its estuary. VAN BREE (1973) has, however, criticized the significance of the alleged differences and considers that the single species *N.phocaenoides* occurs in all warmer waters along coasts of southern and eastern Asia with a "ribbon-like" distribution. PILLERI and GIHR (1975) have subsequently given details of body and skull measurements of the three species to support their claims. PILLERI and CHEN PEI-XUN (1979) have described differences in the disposition of the small tubercles on the dorsal region of the body which distinguish *N.phocaenoides* from *N.asiaeorientalis*.

Nothing is known about the reproductive organs of the Yangtse finless porpoise, CHEN *et al.* (1979) considered that birth occurred between February and April and that from mid April until May calves are carried on the mothers' backs. The scanty information on reproduction in *N.phocaenoides* from warmer waters has been reviewed by HARRISON and MCBREARTY (1973-74). These authors described the appearances of the gonads from three males (130-150 cm) and three females (98-155 cm), none of which was pregnant.

Materials and Methods

Ten male and ten female *Neophocaena* were accidentally caught by fishermen or were stranded in the middle reaches of the Yangtse River over the period 1978-1981 and became available for dissection. The size of the population is not known but it has been estimated by us to be between several hundred and one thousand. Local opinion is that the population is decreasing and that the animal was more abundant below the three gorges of the river in the past. *Neophocaena* is not taken for food and has been a protected species in China for the past two years. The animals were measured and weighed. The reproductive organs were removed, weighed and fixed in 10% formalin. Histological sections were made of the gonads, especial care being taken first to identify and measure all corpora albicantia in the ovaries. Sections were stained with haematoxylin and eosin.

Foetal and newborn specimens

The foetus was in the left horn of the uterus in the five pregnant animals examined. Foetuses of 40 cm (female) and 40 cm (male) were recovered from females 79-6, (16 Jan) and 81-1 (21 Jan) respectively. Another foetus, 47 cm (male) was obtained from female 79-3, also on 16 Jan. Female 79-11 contained a large male foetus of 64 cm on 7 March.

The near-term male foetus of female 80-1 (18 March) was 73.5 cm in length. The left testis measured 3.9 x 0.9 x 1.1 cm and the right 4.0 x 0.9 x 1.0 cm; the left together with the epididymis weighed 3.4 g, the right 3.4 g. The seminiferous tubules measured 5 μ in average diameter;

they lacked a lumen and contained only spermatocytes and supporting cells. The tubules were surrounded by extensive masses of interstitial (Leydig) cells which were large, 15 - 20 μ in diameter, and were obviously being stimulated.

A newborn female 80-2, length 68.3 cm, weight 5.5 kg, abandoned by its mother, was found on 8 May 1980 by a workman and was fed on milk for two days until it died. Remnants of the umbilical cord were still present. The right ovary (1.9 x 0.4 x 0.3 mm) weighed 0.14 and the left ovary 0.17g (1.9 x 0.4 x 0.35). There was no indication of hypertrophy of interstitial cells in sections of the ovaries, which contained large numbers of oocytes.

Fine hairs, 4 mm long, were present on the central portion of the upper lips of the late foetal and newborn specimens. There were two to three hairs on each side, 5 mm apart; some hairs were broken off at the base.

The elongated midline dorsal ridge, which replaces the dorsal fin, extended from the level of attachment of the flippers as far as the central notch in the flukes. It bore, projecting slightly from the surface, a series of white tubercles, arranged in a single line for the cranial 7-8 cm, then in lines of three for 15 cm, then in a single line for 4 cm. The caudal 15 cm of the ridge was devoid of tubercles.

Males

The testes and epididymides of ten males have been examined. They can be divided into two groups, a) males 104.5 to 154.5 cm in length and b) males 156.0 to 164.0 cm in length. In the younger group obtained in December (length 104.5 and 111.0), June (110.0), January (154.5) the weight of both testes and epididymides was from 18.1 to 35 g (average 24.4). The length of each testis varied from 6.3 to 20 cm and the thickness from 0.95 to 4.4 cm. The average diameter of the seminiferous tubules estimated from the better fixed specimens was 50 μ m. Most of the tubules lacked a lumen and possessed a lining composed only of spermatogonia and sustentacular cells. These animals were immature. Three, however, exhibited hypertrophy of the interstitial (Leydig) cells, which formed groups situated between the tubules. This hypertrophy was particularly marked in males 79-18 and 80-2, length 104.5 cm and 110 cm was less evident in male 80-7, length 111.0 cm and could not be detected in other males of this group or in the older animals.

In the second, older group the weight of both testes and epididymides had increased to 277-441 g (average 367g), an increase of fifteen times the average weight of testes in the younger group. The length of these testes was from 10 to 25 cm and the thickness had increased to as much as 7.5 cm. The seminiferous tubules varied from 80 to 110 μ m in diameter, their lining was composed of spermatocytes and no spermatids or spermatozoa were seen in the tubules. No spermatozoa were seen in any of the epididymides except in 79-7 obtained on 16 Jan. In this animal the epididymis contained occasional clumps of spermatozoa but the seminiferous tubules were inactive and devoid of spermatids.

Non-pregnant females

Three females were not pregnant. The smallest was 78-1, 140.4 cm in length, obtained on 17 Dec 1978. The right ovary weighed 0.9 g and was inactive, the left weighed 2.7 g and contained four corpora albicantia. Three of them were similar in size (5 x 5 x 5 mm) and the fourth was 4 x 4 x 4 mm. The three larger corpora had similar histological appearances. There were columns of degenerated luteal cells surrounding numerous convoluted, collapsed blood vessels, with larger perihelal blood vessels containing red cells. The smaller corpus albicans contained much fibrous tissue and acellular material in its outer half and appeared to be in a more advanced state of retrogression.

Female 79-9, 140.8 cm in length, had been kept in a pool for three weeks. It gave birth prematurely to a foetus 54 cm in length which was found dead in the pool so the exact date of birth is not known. The mother died about a week later on 18 March. The left horn of the

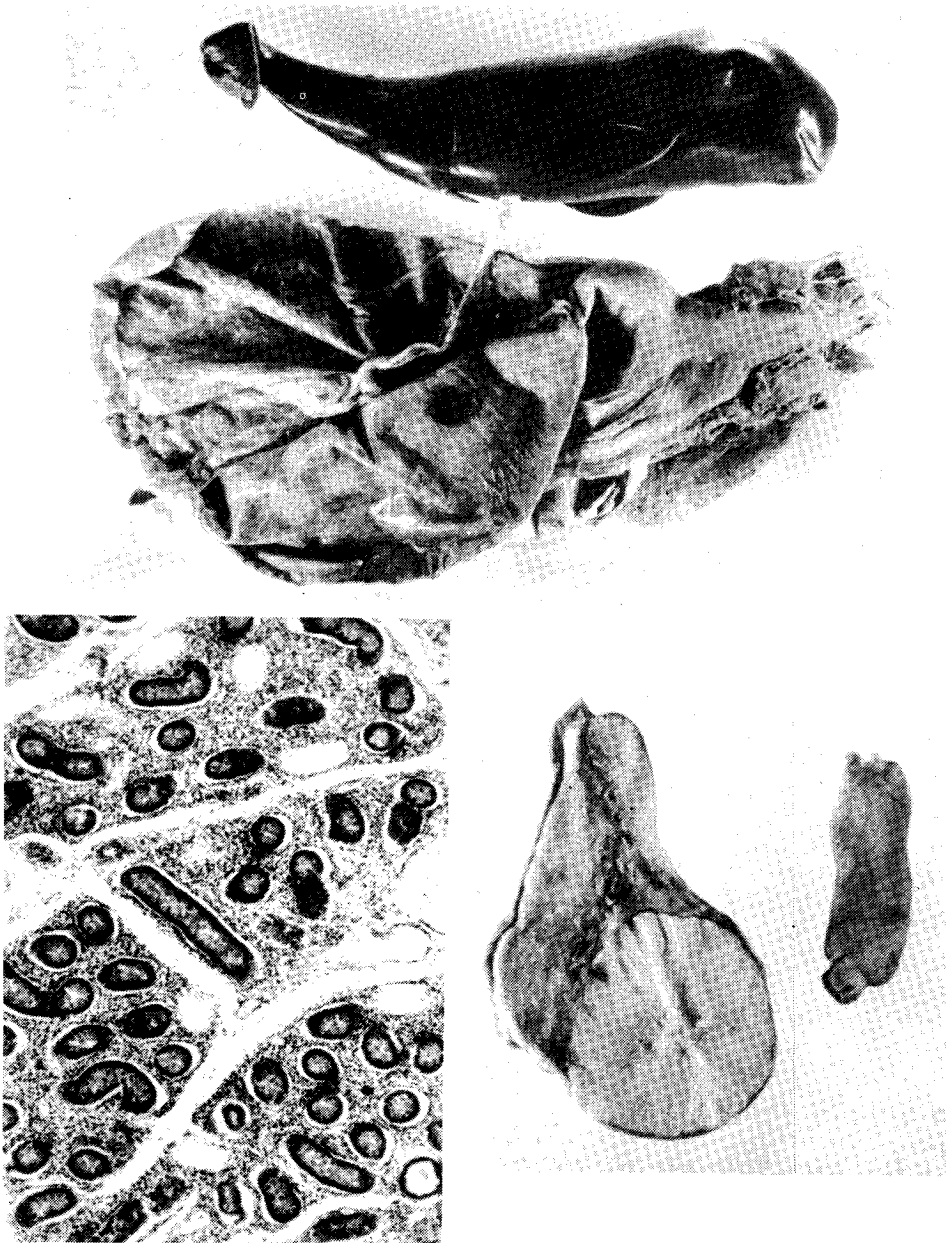


Fig. 1 (top) The uterus and 40 cm male foetus of female 81-1 (146.0 cm long), raised amniotic plaques and a hippomane can be seen on the everted aspect of the uterine horn. x 1/5
(Bottom left)

Photomicrograph of testis of the near-term male foetus 75.3cm in length of the pregnant female 80-1 (130cm in length). Note the hyperplasia of the interstitial cells. x 100
(Bottom right)

The ovaries of the pregnant female 79-6 (135.6cm in length); the foetus was 40cm in length. The left ovary contained a corpus luteum but the right ovary was small and inactive. x 1.2

uterus measured 85 x 40 x 21 mm and had obviously involuted rapidly, the right horn being 55 x 28 x 24 mm. The right ovary weighed 0.8 g (incomplete) and was inactive with no follicles or corpora. The left ovary contained a corpus luteum 15 x 13 x 12 mm in size. The luteal cells were 20 - 25 μ m in diameter and showed degeneration of the nuclei and extensive vacuolation of the cytoplasm. The blood vessels had collapsed and contained few red cells. The corpus was clearly retrogressing and was therefore a young corpus albicans seven to eight days old. There were no albicantia and no large follicles: this female, therefore, had been pregnant for the first time.

Female 78-2, 147 cm in length, was obtained on 22 December 1978. The ovaries weighed 1.4 g (left) and 2.6 g (right) but were damaged at removal: there was no evidence of corpora albicantia or of follicular growth. There was a spherical tumour, 1.65 cm in diameter and weighing 2.57 g, arising from the dorsal wall of the upper part of the vagina. It lay against the outer aspect of the cervix. On section it showed whorls of fibrous tissue and was a fibroma.

Pregnant females

Foetuses were recovered from the left uterine horns of seven pregnant females with the following lengths and dates: 40 cm (16 and 21 Jan, mothers 135.6 and 146.0 cm); 47 cm (16 Jan, mother 147 cm); 52.6 and 54.0 cm (26 March and 10 March, mothers 129.6 and 140.8 cm); 64.0 cm (7 March, mother 153 cm); 73.5 cm (18 March, mother's length unknown but estimated at over 130 cm). The ovaries of five of these animals were examined histologically. Female 79-6 (135.6 cm, with a 40 cm foetus) had a small inactive right ovary (0.95 g). The left ovary (not weighed) contained a corpus luteum 26 x 23 x 16 mm. There were no corpora albicantia. The corpus luteum was a solid structure divided into large lobules by narrow septa. The polyhedral luteal cells were up to 25 μ m in diameter and full of numerous minute vacuoles. There were few theca luteal cells with a diameter of 5 - 8 μ m.

Female 81-1 (146 cm, with a 40 cm foetus) had an inactive right ovary of 1.2 g. The left ovary weighed 8.4 g and possessed a corpus luteum 23 x 22 x 20 mm as well as three corpora albicantia 9 x 9 x 5, 6 x 5 x 5, 6 x 5 x 5 mm. The corpus luteum resembled that of 79-6, and the corpora albicantia were similar to those of 80-1 (see later).

Female 79-3 (147 cm, with a 47 cm foetus) had an inactive right ovary of 1.0 g. The left ovary contained a large corpus luteum 27 x 26 x 16 mm which was fully active with luteal cells over 30 μ m in diameter and full of minute vacuoles. It also contained at least five corpora albicantia 9 x 9 x 9, 6 x 5 x 5, 5 x 4 x 4 and two smaller ones found in the sections about 3 x 3 x 3 mm (see later).

Female 79-11 (153 cm, with a 64 cm foetus) had a small right ovary of 1.5 g which contained a few follicles 1.0 mm in diameter. The left ovary possessed a corpus luteum 26 x 21 x 16 mm and externally five corpora albicantia 12 x 9 x 8, 9 x 9 x 4, 8 x 7 x 4, 6 x 6 x 4, 6 x 6 x 3 and also a collapsed follicle 11 x 9 mm were distinguishable.

The corpus luteum was well-vascularized with an extensive capillary network surrounding groups of luteal cells and large vessels entered the gland from the periphery. The luteal cells were 25-30 μ m in diameter, with large nuclei and a granular cytoplasm containing numerous minute vacuoles.

The five corpora albicantia had average diameters of 9.7, 7.3, 6.3, 5.3 and 5.0 mm; a sixth much smaller one, not visible on the ovarian surface, was found in the section. The largest corpus, as 81-1 and 79-3, consisted of numerous, radially arranged, convoluted blood vessels all showing degenerative changes and enclosed by septa of fibrous tissue. More fibrous tissue was present in the peripheral part of the corpus surrounding large thick-walled blood vessels devoid of a lumen and there was a large core of fibrous tissue in the centre of the corpus. The other four corpora exhibited a structure but with more advanced degeneration of the blood vessels, reduction in the relative amount of fibrous tissue and the appearance of masses of acellular material. The sixth corpus was only 0.5 mm in diameter and consisted only of a

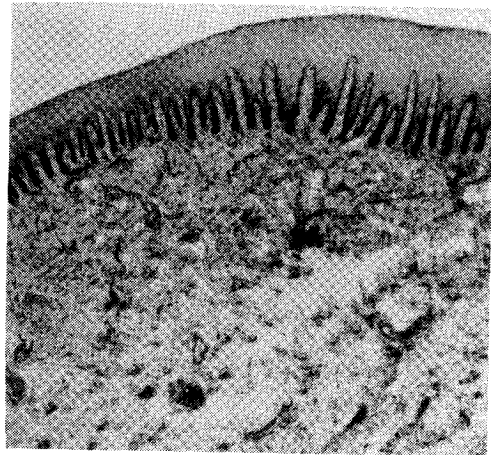
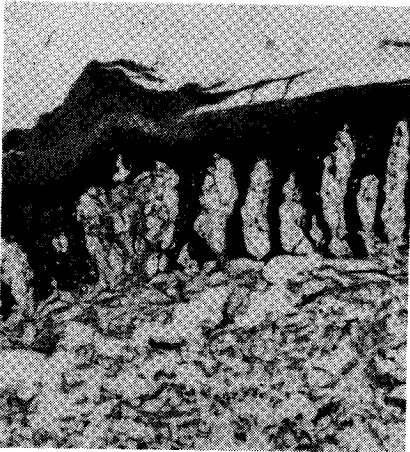
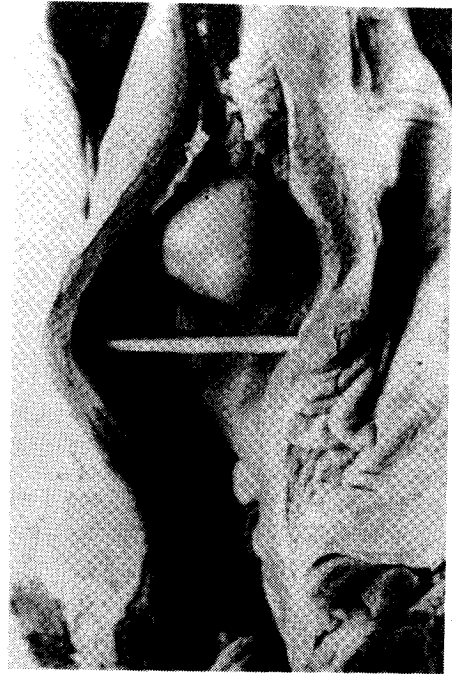


Fig. 2 (Top left) Small spherical bodies containing fibrous tissue marking a form of follicular atresia in the ovary of female 79-6; similar bodies were present in all ovaries of adult animals. x 100

(Top right) A spherical tumour 1.65 cm in diameter attached to the dorsal vaginal wall close to the cervix in female 78-2 (147 cm in length).

(Bottom left) Photomicrograph of a section from one of the dorsal spines of a *Neophocaena* female 131 cm in length which had been in captivity in Indonesia for three days before death. Specimen was provided by Mr John Allen. x 35

(Bottom right) Photomicrograph of a tubercle from the dorsal ridge of the young female 80-2 (length 68.3 cm). Note the difference between the tubercle and the dorsal spine. x 35

central core of cellular material containing a few fibroblasts surrounded by large obliterated blood vessels.

Female 80-1 (length over 130 cm, foetus 73.5 cm) had a right ovary of 5.0 g containing eight corpora albicantia 10 x 8 x 5, 7 x 6 x 5, 6 x 6 x 6, 6 x 5 x 5, 5 x 3 x 3, 4 x 4 x 4, 4 x 4 x 4, 3 x 3 x 3 mm. The left ovary possessed a large corpus luteum 33 x 28 x 16 mm. It was the only pregnant animal to exhibit past activity in the right ovary.

The corpus luteum was the largest in the series and resembled those of other pregnant females except that there was a relative increase in connective tissue between the groups and columns of large luteal cells. The corpora albicantia formed a series which decreased in size but which exhibited relative increase in fibrous tissue content. All had a central fibrous core and in their pattern of degenerating blood vessels and fibrous septa exactly resembled those in Plate 5 of HARRISON and MCBREARTY (1973-74). It is impossible to state whether these corpora albicantia were all related to previous pregnancies but, although the exact length was not recorded, it was a large female (weight 73.5 kg; 17 kg heavier than any other pregnant animal). Four of the pregnant females had small right ovaries which appeared inactive in that they lacked large follicles and corpora. The cortex of these ovaries, however, contained numerous apparently healthy oocytes as well as many small atretic follicles.

All ovaries but especially those of younger females displayed a characteristic type of follicular atresia. Follicles reached a diameter of 0.05 to 0.2 mm, then appeared to collapse together with degeneration of the oocyte; the zona pellucida persisted but later it also collapsed and eventually disappeared. The membrana granulosa and theca interna cells degenerated and were replaced by fibrous tissue to form encapsulated spherical bodies of varying size lying in the deeper part of the ovarian cortex.

The uterus and placenta

The details of the anatomy of the bicornuate uterus were exactly as described by HARRISON and MCBREARTY (1973-74). There were no vaginal folds (ores tincarum). In the pregnant specimens the conceptus occupied the left uterine horn and the chorionic sac extended through the common uterine chamber in to the smaller right horn as far as its tip. In female 81-1 the left horn contained a 40 cm foetus and was 25 cm long to where it joined the 14 cm long right horn.

The placenta was uniformly diffuse and epitheliochorial in type. The umbilical cord and neighbouring regions of the amnion were spotted with pigmented amniotic plaques 0.5 - 1 mm across. Hippomanes of a dark brown colour and firm consistency, measuring up to 3 mm across, were present.

Discussion and summary

CHEN *et al.* (1979) considered that birth in the Yangtse form of *Neophocaena* takes place from February to April. Three pregnant females (129 - 140 cm in length) captured in March were kept in a pond and soon gave birth to calves about 55 cm long. From mid-April until May adults that are suckling carry their calves on the hind part of their back while swimming. The present observations suggest that the calves born in captivity were premature and that body length at birth is more probably from 68 to 74 cm. Female 79-11 was 153 cm in length and pregnant with an almost fully grown foetus of 64 cm. Using OHSUMI's (1966) argument that a cetacean neonate is 45% of its mother's body length, length at birth in *Neophocaena* would be 68.8 cm. The more likely time of birth is April to early May.

Sexual maturity in females can certainly be reached at a length of 129 cm, which could mean that, when compared with other odontocetes of similar birth length and size, a female *Neophocaena* could become pregnant in its fourth year of life. Females with lengths of 135 and 140 cm had, however, become pregnant for the first time, assuming of course that the absence of corpora albicantia in the ovaries means they had not previously been pregnant. These

females could have grown faster, or could have taken longer to reach sexual maturity, or for some reason had not been mated.

In males there is a marked increase in testicular weight from an average of 24.4 g for both testes and epididymides in animals 104 - 154 cm in length to an average of 367 g in animals 156 - 160 cm in length. Although no evidence of active spermatogenesis was observed in the seminiferous tubules, some clumps of spermatozoa were seen in the epididymis of a male in January. Sexual maturity in males might therefore be reached at a length of 156 cm but with a seasonal cessation of spermatogenesis during winter months to complicate full testicular maturation. Unless there is a faster growth rate in young males they would seem to reach maturity several years later than females. HARRISON and MCBREARTY (1973-74) suggested from very limited material that in the Indus Delta *Neophocaena* became sexually active at less than 141 cm in females and between 140 and 150 cm in males.

The hypertrophy of interstitial tissue in the testes of the near-term foetus and some of the young males resembles that seen in male foetuses of horses and in near-term male foetuses of pinnipeds (HARRISON and KING, 1980) but in the young male *Neophocaena* the stimulus must be endogenous. It might reflect increased anterior pituitary activity leading to the development of male secondary sexual characteristics before the onset of sexual maturity. It is difficult to find interstitial cells in the testes of the longer males over 156 cm.

The only evidence regarding the time of mating and the length of gestation is that deduced from the length of foetuses during different months of the year (40 - 47 cm in January, 52-73 cm in March, 68 cm in May). If foetal length is plotted against date and using the same arguments given by HARRISON *et al* (1981) in the case of *Pontoporia*, a possible length of gestation is ten months for *Neophocaena* and mating could occur during the hot summer months of June and July. The duration of lactation is not known.

A fibroma was present on the posterior vaginal wall in one female. Tumours of any kind are rare in cetaceans (HARRISON and KING, 1980): in this female the fibroma could have been a cause of infertility.

A type of follicular atresia was seen in all ovaries which resulted in the formation of encapsulated spherical masses of fibrous tissue, varying in size from 0.05 to 0.2 mm, and often almost acellular. These atretic bodies appear to migrate to the basal region of the ovarian cortex but it is not known for how long they persist.

The corpora lutea had a uniformly similar histological structure in all the pregnant females. The gland persists in an active state until parturition and is well vascularized with an extensive capillary plexus. Theca luteal cells are present in all corpora but are not conspicuous and do not show the pronounced characteristics described in *Pontoporia* by HARRISON *et al.* (1981).

The corpus luteum at term has a mean diameter of nearly 26 mm: within some eight days of birth it can shrink rapidly to a mean diameter of 14 mm. The corpora albicantia of pregnant females could be divided into four main groups on the basis of their mean diameters, their occurrence in a sequence of sizes and in histological characteristics; the demarcation of each group is not clear cut. Group I was 8 - 10 mm in mean diameter, group II 6 - 7 mm, group III 4 - 5 mm and group IV were 3 mm and less in mean diameter. Each group was characterized by a decrease in cellular elements, a relative increase in fibrous tissue and acellular material as well as by its dimensions. The last group consists of corpora albicantia resembling dead scars with no recognizable cells. The maximum number of corpora albicantia in one animal was eight, all in the right ovary, with a corpus luteum as with all other pregnant females in this series in the left ovary. The age of the females is not known, nor is it certain that a female can have nine pregnancies during her lifetime. Corpora albicantia appear to persist even though they shrink to some 3 mm in mean diameter and there is no evidence that they are completely absorbed after four years as in *Pontoporia* (HARRISON *et al.*, 1981).

The presence of three small corpora of similar size and characteristics, with a fourth smaller one, in a non-pregnant female obtained in December when others of similar size (140 cm) were pregnant, does suggest that not all mature females become pregnant each year. The

presence of two or more corpora of the same size in any one animal could mean (unless the corpora are very small) that not all corpora are necessarily related to a successful pregnancy, as has been emphasized by HARRISON and MCBREARTY (1973-74). Premature birth occurred in three females subjected to stress of captivity, a fourth female abandoned its newborn calf and a fifth in this series possessed no corpora albicantia although it was of a mature length (and possibly infertile due to an obstructing vaginal tumour). If fecundity of *Neophocaena* in the Yangtze river is declining, a limited mating season, infertility of females due to unsuccessful mating, and stressful disturbance of the breeding stock by river traffic are possible deleterious factors needing investigation. It would also be advantageous to establish the structure of the total population.

Protection of the species is essential and is being enforced with the approval of all conservationists. The Institute of Hydrobiology is considering ways of introducing positive breeding policies to ensure survival of the two species *Lipotes* and *Neophocaena*. This might be accomplished by establishing reserves for protection of the breeding stock.

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