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Assessment of the Reproductive Cycle of the Indo-Pacific Humpback Dolphin, *Sousa chinensis*, Using Ultrasonography

Fiona Brook,[1] Evan H. T. Lim,[2] Frederic H. C. Chua,[2] and Bruce Mackay[2]

[1]*Department of Optometry & Radiography, The Hong Kong Polytechnic University, Kowloon, HKSAR*
[2]*Underwater World Singapore, Sentosa, Singapore*

Abstract

This project was aimed to collect data about the ovarian cycle of the Indo-Pacific humpback dolphin, *Sousa chinensis*, using sonographic examination of the ovaries and serum hormone level assessment. The ovaries of two nonpregnant females were assessed at least once weekly, and one pregnant female was assessed when possible. A total of 15 normal ovarian cycles, one episode of follicular atresia, and one episode of a luteinized cystic follicle were detected using ultrasonography and serum progesterone levels. Two more cycles were detected on serum progesterone levels but not on ultrasonography. The mean ovarian cycle, mean follicular phase, mean follicle diameter measured just before ovulation, mean luteal phase, and average diameter of the corpus luteum were determined. Data showed an irregular pattern of ovarian cycling, varying between and within subjects. Serum progesterone and estradiol levels were measured one to three times per month in two females and intermittently in one other female. Recorded progesterone and estradiol levels during the follicular phase, luteal phase, and anestrus are reported. Progesterone levels during pregnancy are also included.

Key Words: Indo-Pacific humpback dolphin, *Sousa chinensis*, reproduction, ovaries, ovarian cycle, sonography

Introduction

Aspects of the general biology of many species of Delphinidae have been studied over the past century; however, despite concerns about conservation and sustainability, detailed studies of reproductive physiology have been lacking. Several species of dolphins have been maintained in captivity for a similar length of time, affording more easily accessible subjects for research, but only recently have scientists begun to investigate reproductive performance and parameters in any detail.

Now, more is known about the reproductive cycle in the female of a few species. Killer whales (*Orca orcinus*), appear to ovulate regularly throughout the year (Robeck et al., 1993; Walker et al., 1988). Small cetacean species, such as the dusky dolphin (*Lagenorhyncus obscurus*) (Van Waerebeek & Read, 1994) and the harbor porpoise (*Phocoena phocoena*) (Neimanis et al., 2000; Read & Hohn, 1995) appear to have a clearly limited, seasonal pattern of reproduction; however, medium-sized dolphin species, such as the bottlenose dolphin (*Tursiops* sp.) and the Indo-Pacific humpback dolphin (*S. chinensis*) do not seem to have such an easily definable pattern, with reproduction often being described as "diffusely seasonal." Data from both wild and captive populations show that births may occur at any time of year (Cockcroft, 1989; Jefferson, 2000; Joseph et al., 2000; Urian et al., 1996; Wells, 2000).

The maintenance of bottlenose dolphins (*Tursiops truncatus* and *T. aduncus*) in captivity enabled a closer study of their reproductive anatomy (Brook et al., 2002; Harrison, 1969; Harrison et al., 1972; Harrison & McBrearty, 1977; Kasuya & Marsh, 1984; Perrin et al., 1976; Perrin & Reilly, 1984) and physiology (Kirby, 1990; Kirby & Ridgway, 1984; Sawyer-Steffan & Kirby, 1980; Sawyer-Steffan et al., 1983; Schroeder, 1990; Yoshioka et al. 1986). The anatomy of the uterus and ovaries is reported and is similar in all cetaceans studied (Robeck, 1994). Serum progesterone (P) data also were similar and indicated an irregular pattern of ovarian cycling in *Tursiops*, which was difficult to predict (Sawyer-Steffan & Kirby, 1980; Yoshioka et al., 1986). Data showed that estrus varied markedly between and within individuals. Generally, P levels of <1.0 ng/ml were determined to indicate anestrus. Elevated levels (3.0-34.0 ng/ml) indicated ovulation and the luteal phase of the cycle, but did not provide useful information about follicular development and the time of ovulation. P levels

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of 1-3 ng/ml were classified as indeterminate (Kirby, 1984; Kirby & Ridgway, 1984; Sawyer-Steffan & Kirby, 1980; Sawyer-Steffan et al., 1983; Schroeder, 1990; Yoshioka et al., 1986). Studies of serum total estrogen (E_t) levels in *T. truncatus* showed no variation between anestrus and estrus (Kirby, 1990; Kirby & Ridgway, 1984; Sawyer-Steffan & Kirby, 1980; Sawyer-Steffan et al. 1983; Yoshioka et al., 1986). Yoshioka et al. (1986) reported serum estradiol (E_2) levels of < 70 pg/ml during anestrus and 125-200 pg/ml during estrus. Although contributing useful information about reproductive physiology, investigations of serum steroid hormone levels did not allow direct assessment of ovarian activity in live animals.

More recently, ultrasonography allowed observation of real time morphologic changes in the ovaries of *T. aduncus* and correlation of ovarian cycle phase with serum P and E_2 (Brook, 1997, 2000). P levels were < 0.2-0.9 ng/ml during anestrus and < 0.2-18.5 ng/ml during estrus. E_2 levels were 8.4-106.3 pg/ml during anestrus and 11.2-379.2 pg/ml during estrus. These levels are similar to those reported for *T. truncatus*. This was the first time real time ovarian events were correlated with serum hormone data in dolphins.

Some aspects of the reproductive biology of the Indo-Pacific humpback dolphin have been studied in the wild in South Africa (Cockcroft, 1989; Karczmarski, 1996, cited in Jefferson, 2000) and Hong Kong (Jefferson, 2000; Parsons, 1997); however, it is difficult to collect sufficient data about reproductive physiology from wild animals, and little detailed information is known about reproduction in *S. chinensis*. These studies reported similar results in that births were observed throughout the year, although more newborn calves were seen in spring/summer. Gestation length has been estimated at about 11 months, and females are believed to attain sexual maturity at 9-11 years of age (Cockcroft, 1989; Jefferson, 2000).

Underwater World Singapore (UWS) opened their Dolphin Lagoon in 1999 and is one of two facilities known to maintain the Indo-Pacific humpback dolphin in sufficient numbers for breeding. The group consists of two adult males and three adult females. UWS is committed to controlled breeding and sustainable management of their population, as well as the collection of sound scientific data from the species. This project to study the ovarian cycle using ultrasonography began in 2002; the aim was to assess and monitor reproductive status and patterns in the females. The specific objectives of the study were to establish the normal sonographic appearance of the ovaries in Indo-Pacific humpback dolphin, determine whether sonography could identify and monitor folliculogenesis, correlate real time ovarian changes with serum hormone levels, and determine any seasonal influence on reproductive status.

Materials and Methods

The Dolphin Lagoon at UWS was created by fencing off a saltwater bay on the coast of Sentosa Island, Singapore. The lagoon was subdivided by pontoons with attached netting into four main areas. Capacity is approximately 30 million liters of natural seawater, with varying depths of up to 3 m. The lagoon water undergoes routine monthly testing, including total fecal coliform count, *Salmonella*, *Burkholderia pseudomallei*, and *Vibrio cholerae*.

Subjects

Three adult female Indo-Pacific humpback dolphins were the subjects for this study. Of the three animals, Pann was known to be sexually mature, having given birth at least once before (Table 1). Han and Euang (Figure 1) were reportedly born in captivity, but their exact age is not reliably documented. Neither is known to have conceived or given birth. Previously recorded serum progesterone levels available for Han and Euang indicated they were sexually mature in 2001, at 10-11 years of age. Both animals were housed separately from mature males since their arrival at UWS in 1999. They were in the same section of the lagoon and

Table 1. Details of age, size, and reproductive history in 2001 of female Indo-Pacific humpback dolphins at Underwater World Singapore

Dolphin	Age in 2002	Body length	Body weight	Reproductive history
Pann	15+ yr (estimated)	247 cm	170 kg	Stillbirth – 18 February 2001 Live birth – 6 November 2002
Han	11 yr	229 cm	148.5 kg	-
Euang	11 yr	223 cm	132.5 kg	-

underwent the same training sessions and interaction schedules. Details of the age and size of the three females are given in Table 1. Weights were measured using a poolside scale, during voluntary slide-outs by the dolphins.

Diet consisted of locally available yellowstripe scad (*Selaroides leptolepis*) and bigeye scad (*Selaroides crumenophthalmus*); daily amounts per animal vary from 7 to 11.5 kg. Fish is supplemented daily with multivitamins, ascorbic acid, cod liver oil, hesperidin, and a herbal liver supplement.

Serum Steroid Quantification

Operant conditioning for blood collection during voluntary presentation of the tail enabled regular samples to be collected. The protocol for collecting blood samples from the females was the same as reported for the Indo-Pacific bottlenose dolphin (Brook, 1997) in that blood was collected from the large fluke veins, with voluntary presentation of the tail by the dolphins. Serum steroid levels were evaluated at the O & G Research Laboratory of the National University of Singapore. Progesterone was assessed by radioimmunoassay (RIA) (O & G Research Laboratory, National University of Singapore) and estradiol levels by a chemiluminescent immunoassay (CLIA) (Roche Diagnostics Corporation, Indianapolis, Indiana, USA).

Ultrasound Protocol

All ultrasound examinations were performed using an Aloka SSD 900 ultrasound unit in conjunction with a 3.5 mHz curvilinear transducer. Images were recorded on videotape/thermal printing paper. All examinations were performed under voluntary behavior, with the dolphins remaining in the water, supported by the trainers (Figure 2). Weekly ultrasonographic examinations of the ovaries were performed, using methods developed for the bottlenose dolphin. Both ovaries were assessed at each examination and the ovarian cortex examined for any signs of folliculogenesis. When a developing follicle was seen, scans were carried out more frequently to assess follicular growth and dimensions at ovulation. Ovulation was identified when the follicle was not detectable on the next scan and validated by a subsequent rise in serum progesterone level, indicating the presence of a functioning corpus luteum. The size, appearance and duration of the corpus luteum were also monitored. Any follicular atresia, follicular cysts, or retained corpora lutea were also recorded (Brook, 1997, 2000, 2001).

Results

It was possible to visualize the ovaries and to identify folliculogenesis, ovulation, and development and regression of the corpus luteum in the Indo-Pacific humpback dolphin using sonography.

On 2 November 2001, just before the study, mating activity was observed between Pann and Jumbo, one of the males. Ultrasonography several weeks later confirmed a pregnancy. During the pregnancy, Pann did not cooperate for blood sampling and rarely for ultrasound examinations. When she did cooperate for ultrasound, it was for very short periods and ovaries were rarely visualized. During these examinations, a large (approximately 25 mm) corpus luteum was seen in the right ovary, but it was not possible to measure this accurately. No follicular activity was detected. Pann gave birth to a healthy, male calf on 6 November 2002 and has not been scanned regularly since. The main study has, therefore, concentrated on the younger females, Han and Euang.

Sonographic Appearances of the Ovaries

The ovaries can be identified in the lateral aspect of the abdominal cavity. They appear as ovoid structures, with a hyperechoic central hilus, surrounded by a relatively hypoechoic cortex (Figure 3). Measured ovarian lengths were 37-48 mm; both lateral and dorsoventral diameters were about 20 mm. The appearance of the ovaries was similar in all three females.

The ultrasonographic appearance of a developing follicle was an ovoid to rounded, echolucent structure in the cortex of the ovary (Figure 4a). Developing follicles were identified at a diameter of > 4 mm. Often, more than one follicle developed simultaneously, although only one dominant follicle progressed to ovulation. The site of

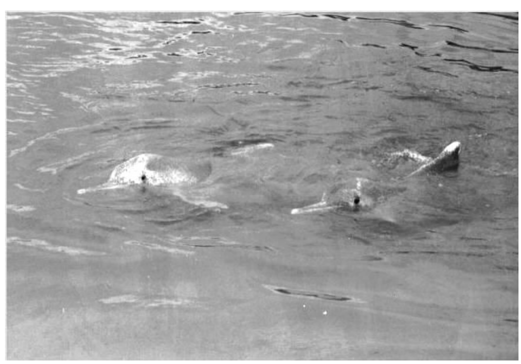

Figure 1. Han and Euang, two female *Sousa chinensis*, at Underwater World Singapore

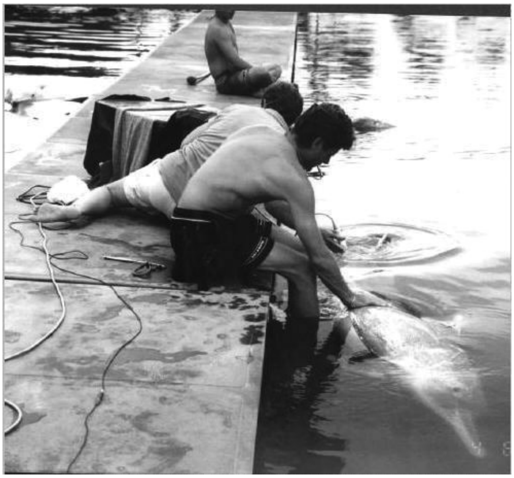

Figure 2. Ultrasound scanning at UWS. The ovaries are examined with voluntary behavior of the dolphins.

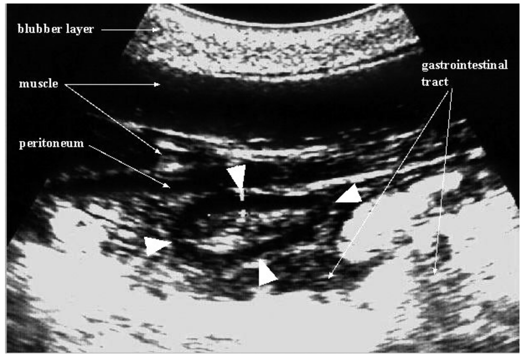

Figure 3. Sonographic appearance of an ovary in *Sousa chinensis*

ovulation could be identified by visualization of a collapsed follicle or corpus haemorrhagicum (Figure 4b). The normal corpus luteum appeared as a rounded, isoechoic, or hyperechoic structure (Figure 4c) and could be identified reliably on sonography by a week after ovulation.

One episode of follicular atresia was observed in Euang in May 2002 (Table 2). A 13-mm follicle was identified in the left ovary on 17.5.02; this increased to 21 mm by 25 May 2002; however, no ovulation occurred. This was confirmed by a low serum P level (0.29 ng/ml) on 4 June 2002. On the same date, a new 15-mm follicle was identified in the same ovary. This follicle increased in size and ovulated three to four days later. The atretic follicle slowly decreased to a diameter of 8 mm by 11 June 2002, after which it resolved. One episode of a luteinized follicular cyst was also noted in Euang in March 2003 (Table 2). The cystic follicle was identifiable by the larger size, thicker walls, and the presence of a septum within it. These sonographic features, plus the rise in serum progesterone, were considered to be consistent with a luteinized follicular cyst.

Sonographic Assessment of the Ovarian Cycle

It was not possible to scan as regularly as planned nor always on the same day as blood samples were collected, and some data were missed (Table 2).

The mean ovarian cycle length (determined by the time between observed, consecutive ovulations) was 29.9 + 2.85 days (range 28-36 days; n=7). The mean follicular phase, as assessed by sonography (i.e., from the appearance of a 5-mm follicle to ovulation), was 14.5 + 0.80 days (range 14-16 days; n=7), and the mean follicle diameter measured just before ovulation was 20.1 + 1.10 mm (range 18-22 mm; n=8). The mean luteal phase, as assessed by sonography (i.e., from ovulation to regression of the corpus luteum) was 14.0 + 1.41 days (range 12-16 days; n=5). Corpora lutea reached an average maximum diameter of 20.5 mm before regressing (range 18.5-22.5 mm; n=5). Follicle and CL diameters during one cycle are shown in Figure 5.

From January 2002 to August 2003, a total of 12 normal ovarian cycles were recorded in Euang. These occurred at the end of January/beginning

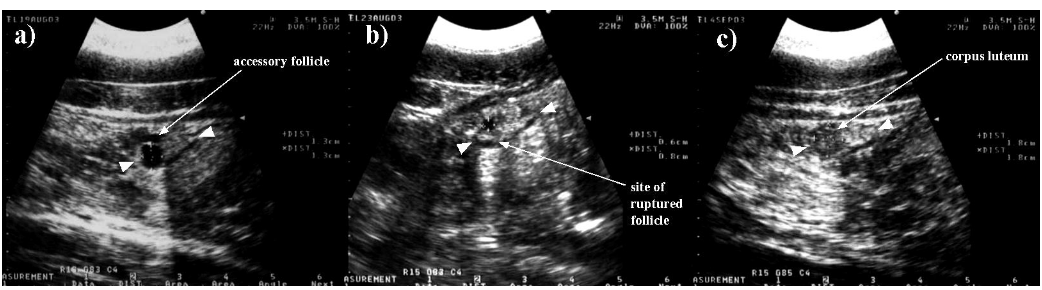

Figure 4. These sonograms show (a) developing follicle, (b) site of ovulation, and (c) a corpus luteum in *S. chinensis.*

Table 2. Comparison of ovarian activity with serum progesterone and estradiol levels recorded in three female Indo-Pacific humpback dolphins; CL = corpus luteum

Date	Estradiol (pg/ml)	Progesterone (ng/ml)	Reproductive status/ovarian cycle phase
EUANG			
8 Jan. 2002	-	0.36	No scan
5 Feb. 2002	-	2.1	No scan
12 March 2002	-	0.32	Follicular (13 mm follicle)
17-18 March 2002	-	-	OVULATION
18 March 2002	-	-	Corpus haemorrhagicum
19 March 2002	-	0.92	Early luteal
2 Feb 2002	<13.6	2.58	Luteal (18 mm CL)
10 April 2002	<13.6	0.51	Follicular (15 mm follicle)
14-15 April 2002	-	-	OVULATION
23 April 2002	<13.6	3.68	Luteal (18 mm CL)
7 May 2002	-	0.65	Follicular (9 mm follicle)
23 May 2002	-	-	Follicular (19 mm follicle)
4 June 2002	<13.6	0.29	Follicular (14 mm follicle)
13 June 2002	-	-	Follicular atresia (7 mm follicle)
18-19 July 2002	-	-	OVULATION
26 July 2002	<13.6	4.69	Luteal (19 mm CL)
6 Aug. 2002	20.73	0.09	Follicular (follicle not measured)
12-13 Aug. 2002	-	-	OVULATION
3 Sept. 2002	<13.6	0.07	Nil detected
1 Oct. 2002	15.56	0.18	Follicular (follicle 13 mm on 28 Sept. 2002)
9-10 Oct. 2002	-	-	OVULATION
7 Nov. 2002	<13.6	0.64	OVULATION
3 Dec. 2002	31.36	0.2	Follicular (15 mm follicle)
14 Dec. 2002	-	-	OVULATION
7 Jan. 2003	<13.6	0.2	Nil detected
4 Feb. 2003	<13.6	0.2	No scan
12 March 2003	<13.6	2.9	30 mm follicular cyst
26 March 2003	<13.6	0.2	17 mm residual follicular cyst
26-27 Apr. 2003	-	-	OVULATION
6 May 2003	<13.6	10.4	Luteal
21-26 May 2003	-	-	OVULATION
4 June 2003	<13.6	3.0	Luteal
24-26 June 2003	-	-	OVULATION
1 July 2003	<13.6	3.1	Luteal
23-24 July 2003	-	-	OVULATION
29 July 2003	-	-	22 mm CL
6 Aug. 2003	-	-	26 mm CL
12 Aug. 2003	<13.6	3.9	No scan
15 Aug. 2003	-	-	Follicular (8 mm follicle)
HAN			
2-6 Jan. 2002	-	-	OVULATION
8 Jan. 2002	-	2.22	No scan
5 Feb. 2002	-	0.41	No scan
12 March 2002	-	-	Nil detected
20 March 2002	-	-	Nil detected
2 Apr. 2002	0	0.52	Nil detected
10 Apr. 2002	-	-	Nil detected
23 Apr. 2002	16.0	0.49	Nil detected
17 May 2002	< 13.6	0.5	Nil detected
28 May 2002	<13.6	0.67	Nil detected
7 June 2002	0	0.25	Nil detected

Table 2 (cont.)

25-26 June 2002	-	-	OVULATION
12 July 2002	<13.6	1.8	Luteal (17 mm CL)
6 Aug. 2002	14.55	0.09	Nil detected
3 Sept. 2002	<13.6	0.08	Nil detected
1 Oct. 2002	<13.6	0.17	Nil detected
7 Nov. 2002	<13.6	0.13	Nil detected
3 Dec. 2002	<13.6	0.15	Follicular (6 mm follicle)
7 Jan. 2003	<13.6	0.17	Nil detected
11 March 2003	<13.6	5.9	Nil detected
1 Apr. 2003	<13.6	1.6	No scan
7 May 2003	<13.6	-	No scan
28 May 2003	-	-	Luteal (21 mm CL) – ? ovulation date
5 June 2003	<13.6	-	No scan
20 June 2003	15.86	-	No scan
1 July 2003	<13.6	0.5	Nil detected
12 Aug. 2003	<13.6	1.5	Luteal (17 mm CL 15 Aug. 2003) – ? ovulation date
PANN			
20 June 2000	-	10.63	Pregnant
4 July2000	-	12.83	Pregnant
25 July 2000	-	11.01	Pregnant
1 Aug. 2000	-	8.62	Pregnant
15 Aug. 2000	-	9.4	Pregnant
7 Nov. 2000	-	11.01	Pregnant
11 Dec. 2000	-	19.15	Pregnant
18 Feb. 2001	-	-	Stillbirth
23 March 2001	-	1.4	No scan
3 Apr. 2001	-	7.3	No scan
3 May 2001	-	4.0	No scan
17 July 2001	-	0.34	No scan
9 Oct. 2001	-	2.13	No scan
6 Nov. 2001	-	0.44	With mature male
5 Dec. 2001	-	4.9	Pregnant
6 Nov. 2002	-	-	Live birth; healthy male calf

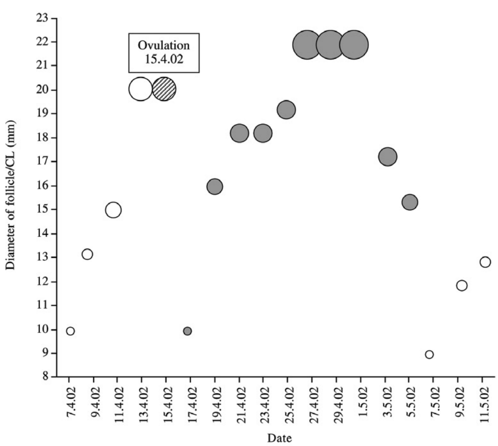

Figure 5. Illustration of follicle and corpus luteum development during one ovarian cycle in an Indo-Pacific humpback dolphin; white dots = follicle; grey dots = corpus luteum.

In contrast, only five cycles were identified in Han. One in January 2002, followed by five months of anestrus, and one in June 2002, followed by eight months of anestrus. She cycled again in March, May, and August 2003. Of these ovulations, three (67%) were from the left ovary, and the origin of two were not identified.

Serum Progesterone and Estradiol Levels

Behavior was not consistent, and it was not always possible to collect blood samples when planned; however, at least one sample was collected each month, except for once in Han. Results are shown in Table 2, with corresponding ultrasonographic findings, and in Table 3. Serum progesterone (P) levels for Han (n=18) and Euang (n=21) were consistent with observed ovarian activity and, as expected, were highest when a corpus luteum was

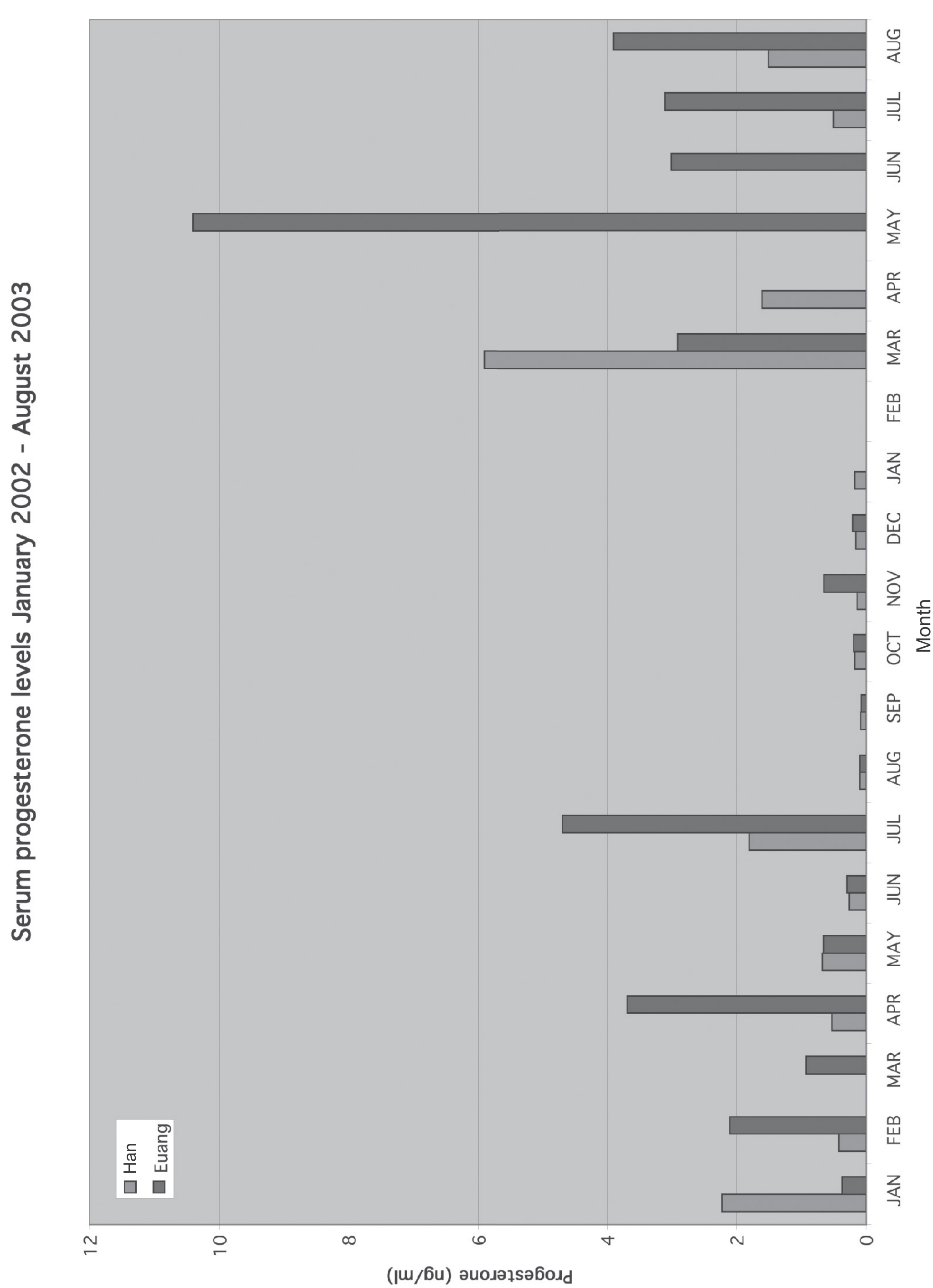

Figure 6. Serum progesterone levels recorded for Han and Euang between January 2002 and August 2003

Table 3. Serum levels of 1. progesterone [ng/ml] and 2. estradiol [pg/ml] during different phases of the reproductive cycle in three female Indo-Pacific humpback dolphins

Dolphin	Anestrus	Follicular phase	Luteal phase	Pregnancy
Euang	1. 0.07-0.2	1. < 0.09-0.65	1. 0.92-10.4	-
	2. <13.6-20.73	2. <13.6-31.36	2. <13.6	-
Han	1. 0.09-0.67	1. 0.15	1. 1.5-5.9	-
	2. 0-16.0	2. <13.6	2. <13.6	-
Pann	-	-	-	1. 4.9-19.15
				-

seen (Figure 6). Serum P levels did not appear to rise prior to ovulation. Recorded P levels ranged from 0.07-10.4 ng/ml in Euang and 0.09-5.9 ng/ml in Han. Levels recorded during follicular phases were 0.09-0.65 ng/ml and during luteal phases were 0.92-10.4 ng/ml. When no ovarian activity (anestrus) was observed on sonography, serum P levels were 0.07-0.67 ng/ml. Only one blood sample was collected from Pann during this study; however, further data were available from a previous pregnancy (total 8 samples), showing P levels ranging from 4.9 to 19.15 ng/ml (Table 3).

Serum estradiol (E_2) levels (Figure 7) were erratic and did not correlate with observed follicular development or ovulation. Recorded levels were low, ranging from < 13.6 to 31.36 pg/ml in Euang (detected in 20% of samples; n=18) and from 0 to 16.0 pg/ml in Han (detected in 30% of samples; n=19). Levels recorded during follicular phases were < 13.6-31.36 pg/ml, during luteal phases levels were < 13.6 pg/ml, and during anestrus were < 13.6-20.73 pg/ml (Table 3).

Discussion

Sonographic examination provided a useful and reliable method for monitoring ovarian activity in the Indo-Pacific humpback dolphin. The sonographic appearances of the ovaries, developing follicles and corpora lutea, and the ovarian cycle length were very similar to those described for the Indo-Pacific bottlenose dolphin (Brook, 1997, 2000, 2001). Follicular atresia has also been reported in this species (Brook, 1997), and luteinized follicular cysts have been observed (Brook, unpublished).

Serum P levels were useful to identify the presence of a corpus luteum and so usually indicated an ovulation. Recorded P levels during different phases of the ovarian cycle were similar to those reported for *T. aduncus* (Brook, 1997, 2000). Long-term records of increased serum P levels provide a useful retrospective of ovarian activity and any seasonal pattern of reproduction in these animals, provided that sampling is done at least twice per month, or within two weeks following ovulation, during a luteal phase, in order not to miss elevations.

Serum E_2 levels were low and did not correlate with actual events within the ovary or those levels reported previously in other dolphin species (Brook, 1997, 2000, 2001; Kirby, 1990; Yoshioka et al., 1986). This is likely because the number and frequency of samples were not enough to reflect true E_2 levels. Few long-term studies of serum E_2 levels in dolphins have been reported, and data collected have not been adequate for accurate interpretation (Kirby, 1990; Yoshioka et al., 1986). Estradiol levels are known to fluctuate markedly, with many sequential blood samples needed to illustrate activity over a 24-hour period in other mammals (Pauerstein et al., 1978). This is not advisable in dolphins, due to the risk of inducing phlebitis or alienating the animals. It has yet to be shown that serum E_2 levels are useful as an indicator of reproductive activity in dolphins.

These two females had an irregular pattern of ovarian cycling, with between one and four consecutive cycles recorded, interspersed with periods of anestrus of one to eight months. This is similar to data available for female bottlenose dolphins kept separated from males. In long-term studies of *T. aduncus*, using the same methods, between one and eleven consecutive cycles were seen, with periods of anestrus of one to 27 months (Brook, 1997, 2000, 2001). Results of a three-year study of serum hormone levels in female *T. truncatus* indicated two to seven consecutive ovulations, followed by periods of up to seventeen months when no ovarian activity was recorded (Yoshioka et al., 1986). In *T. aduncus* females, there was a relationship between the age of the female and the number of consecutive cycles and length of anestrus. Younger animals (i.e., less than 15 years of age) tended to cycle more frequently and had shorter periods of anestrus between periods of ovarian activity (Brook, 1997, 2000, 2001). Euang showed a similar pattern, consistent with the premise that repetitive ovarian cycling is more common in younger females; however, Han

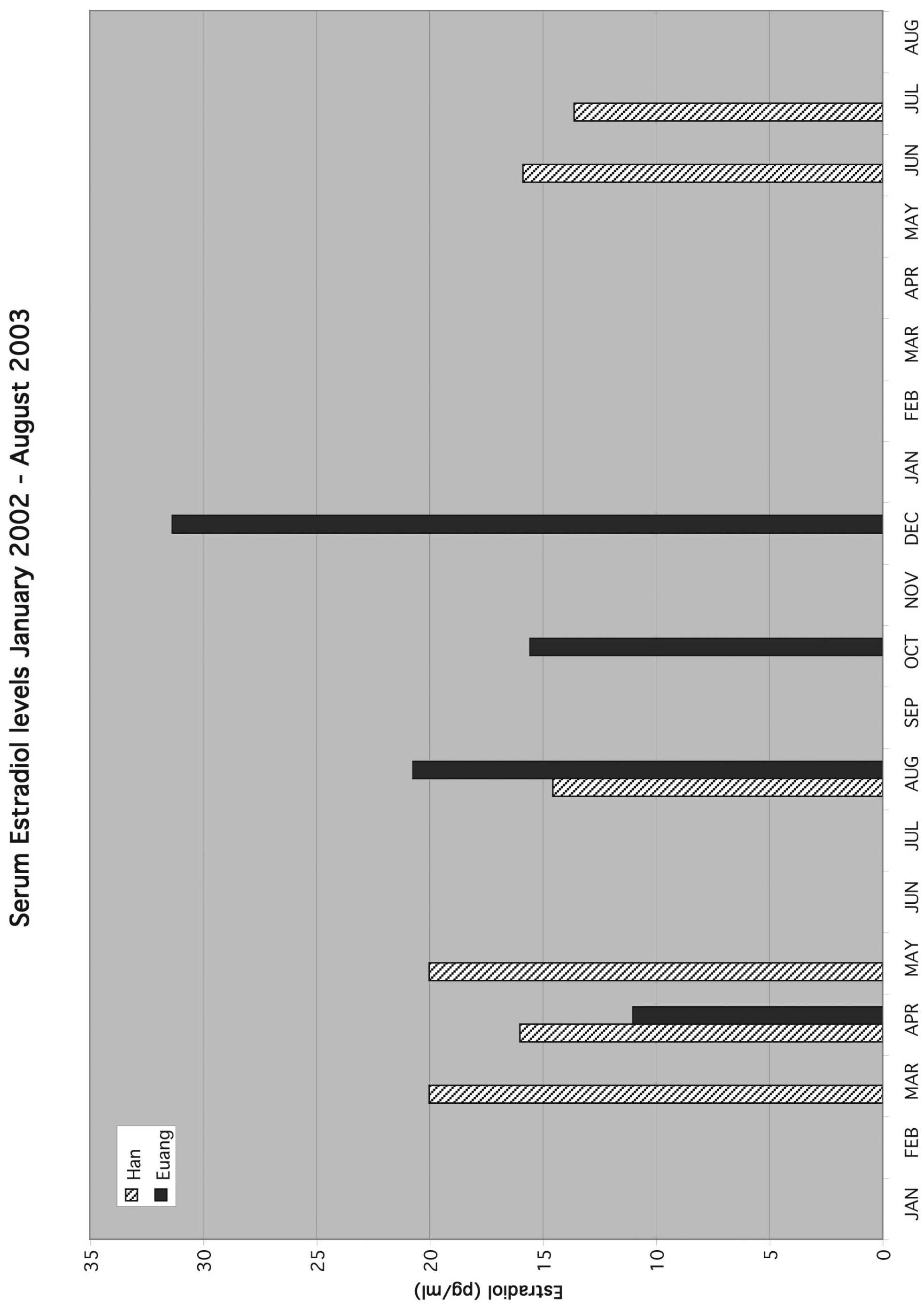

Figure 7. Serum estradiol levels recorded for Han and Euang between January 2002 and August 2003

showed a slightly different pattern, cycling once and then showing no activity for between one and eight months. These two females were the same estimated age and were maintained under exactly the same conditions. Reproductive activity in dolphins is influenced by many extrinsic factors (Leatherwood & Reeves, 1990; Perrin & Reilly, 1984). Further research is required to determine why the ovarian cycling pattern in these two females was different and what are the influencing intrinsic factors.

Ovarian activity was observed in every month of the year, except for September. Pann, the oldest female in the group, became pregnant in November 2001, indicating that ovulation and conception may occur during the "winter" months in captive animals, as has been observed in wild populations. More births occur in wild humpback populations during spring/summer (Cockcroft, 1989; Jefferson, 2000). One explanation is that this may be because prey is more plentiful at these times, thus encouraging reproduction (Urian et al., 1996); however, captive dolphins do not have to deal with variations in nutrition, so seasonal prey availability might impact on reproduction less than in their wild counterparts. Urian et al. reported that the timing of reproduction in captive *T. truncatus* shows greater variation than in the wild, although they also stated that wild born dolphins, despite long periods in captivity, showed a similar pattern of reproductive activity as their population of origin. Reproduction in some species of dolphins is obviously influenced by many factors, both extrinsic and intrinsic, and more research is needed to investigate these factors and possible differences between captive and wild animals and between different captive and wild groups.

Exact gestation length is unknown in this species, but has been estimated from fetal size and length at birth in wild animals to be about 11 months (Cockcroft, 1989; Jefferson, 2000). Although the date of ovulation is not known exactly, the low serum P level in Pann on 6 November 2001 indicated ovulation had not occurred, despite the observed mating of Pann and Jumbo on 2 November 2001. It is likely that Pann was in estrus and probably had a developing follicle at that time. An elevated P level (4.9 ng/ml) on 5 December 2001 indicated a luteal phase/pregnancy. The subsequent birth of a live, healthy calf on 6 November 2002, is consistent with a gestation length of 11 to 12 months. Closer study and controlled breeding of this species, as has been achieved in captive *T. aduncus* (Brook, 1997; Kinoshita et al., 2000), should enable the exact length of gestation in the Indo-Pacific humpback dolphin to be determined.

This ongoing study has provided the first scientifically documented real time data about reproductive physiology in female Indo-Pacific humpback dolphins. The information obtained from these animals should contribute to the sustainable breeding of animals in captivity and provide a reference for those investigating wild populations.

Acknowledgments

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