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Source: Zoological Science, 19(6) : 679-682

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.19.679

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ZOOLOGICAL SCIENCE **19**: 679–682 (2002)
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Annual Changes of Urinary Progesterone and Estradiol-17β of the Dugong (*Dugong dugon*) in Captivity

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ABSTRACT—Levels of urinary progesterone and estradiol-17β were measured twice a week in a female dugong, *Dugong dugon,* in captivity for two years from April 1996 to April 1998. The dugong showed 14 ovarian cycles during the period of study. Concentrations of progesterone ranged from 0.01ng/mg creatinine (Cr) to 1.94ng/mg Cr and the length of estrous cycle was 53.6±8.6 (mean±SEM) days based on intervals of urinary progesterone peak-to-peak measurements. Concentrations of urinary estradiol-17β ranged from 0.9pg/mgCr to 23.7pg/mgCr, and tended to peak just prior to elevations of progesterone during the first year of study. This is the first report demonstrates that the ovulatory cycle of the dugong is about 50 days. The present findings suggest that measurement of urinary progesterone is a useful method to detect ovarian cycle of the dugong in captivity.

Key words: Dugong, estrous cycle, urine, progesterone, estradiol-17β.

INTRODUCTION

The dugong, the only herbivorous marine mammal that is strictly marine, has a range extending through the tropical/subtropical waters of the Indo-West Pacific. It is listed as vulnerable by the IUCN(1996). Since the 1960s, aquarium and institutions in several countries including USA, Australia, Thailand, Indonesia, Japan and Singapore have attempted to keep dugong in captivity. Most attempts were unsuccessful. Most studies on reproduction in wild dugongs have been obtained from carcasses (Marsh *et al*., 1984a, b; Marsh, 1995; Boyd *et al*., 1999) or limited field observations of mating (Anderson, 1997; Preen, 1989). Dugongs have been kept in captivity at Toba aquarium since 1977. Mating behaviour between a male and a female dugong has been observed since 1995. During this time, the female has not conceived. Nowadays, through improvements in captive husbandry, hormonal levels based on urine analyses in female dugongs can be measured. In the present study, we monitored changes in urinary progesterone and estradiol-17β of a captive female dugong from April 1996 to April 1998.

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MATERIALS AND METHODS

Animal husbandry

The female dugong was orphaned at El Nido, north Palawan, Philippines and rescued on October 10, 1986 by the joint dugong research team comprising Toba Aquarium and the Philippine Government. She was named “Serena”, donated to Japan from the President of the Republic of the Philippines, and transported to Toba Aquarium in April 1987. At an estimated age of 5 months, “Serena” was fed infant formula and sea grass in the holding pen several times a day. She was very friendly, and quickly bonded with staff. By November 1997, she was 11years of age, measured 2.47m in body length and weighed 345kg. Environmental conditions during the period of study are shown in Fig. 1. The dugong tank consists of two indoor display tanks:(1) approximately 10m ×6.5m×3.5m depth (200m^3), (2) 9m×6.5m× 3.5m (180m^3), and a holding tank 5m×5m×1.5m in depth (37m^3) (Fig. 2). These three tanks are interconnected. Usually, the female “Serena” and the male “Jun-ichi” are kept separated, however, they can be given the opportunity to pair by operating interconnecting gates. In 1995, “Serena” (estimated 9 years of age) and “Jun-ichi” (estimated 17 years of age) paired, and their mating behaviour observed.

Sample Collection and Radioimmunoassays

Urine samples were collected from “Serena” twice weekly by floating her on the back, whilst applying pressure to urogenital area with fingertips (Fig. 3). The samples (less than 20ml) were frozen at –20°C until assayed. Progesterone and estradiol-17β were measured by the double antibody RIA system using ^{125}I-labeled radioligands (Taya *et al*.,1985). Antisera against progesterone (GDN 337; Gibori *et al*., 1977) and estradiol-17β (GDN 244; Korenman *et al*., 1974) were provided by Dr. G.D.Niswender (Animal Reproduction

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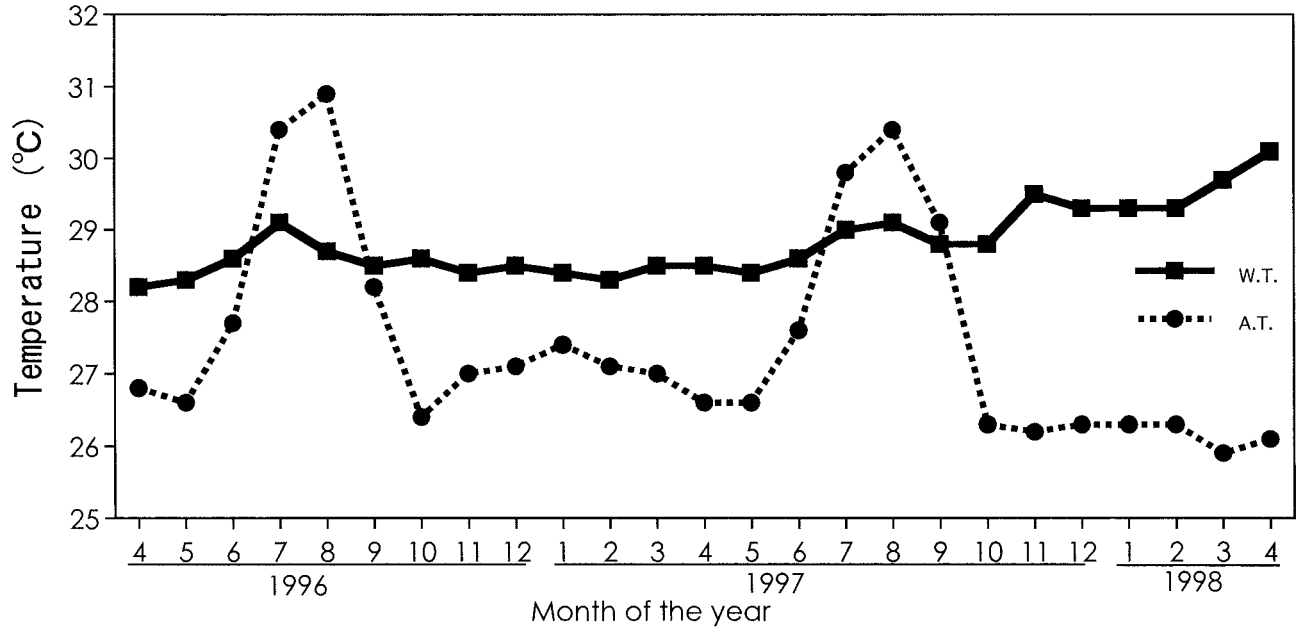


Fig. 1. Changes of mean water temperature (■) in the pool and air temperature (●) from April 1996 to April 1998.

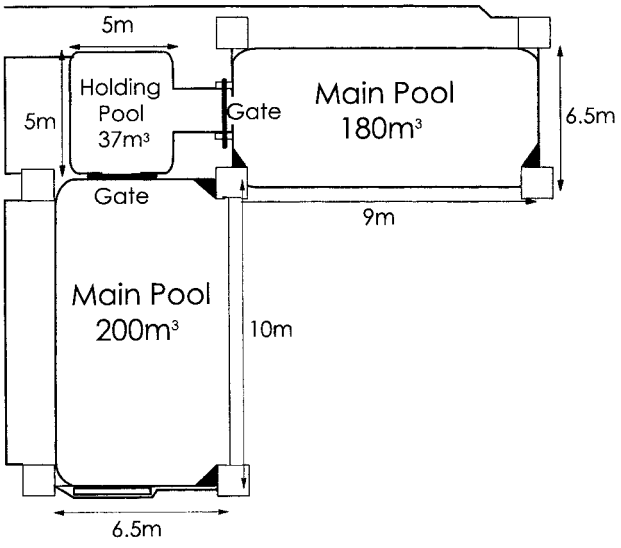


Fig. 2. Keeping pool of dugong in Toba Aquarium.

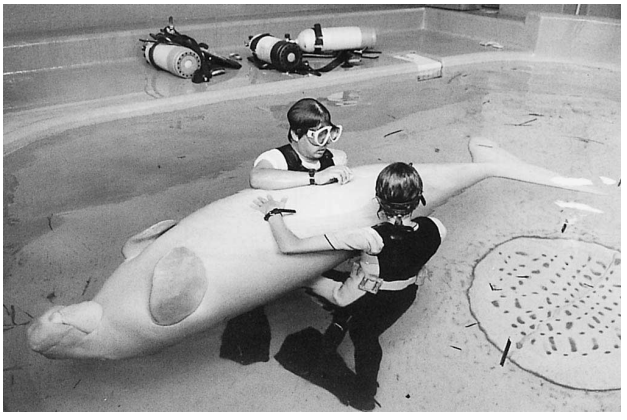


Fig. 3. The method for collecting urine sample from "Serena" by floating her on her back.

and Biotechnology Laboratory, Colorado State University, Fort Collins, Co, U.S.A.). The intra- and interassay coefficients of variations were 3.5% and 13.4% for progesterone and 4.8% and 5.8% for estradiol-17β, respectively. Urinary concentrations of progesterone and estradiol-17β were indexed using creatinine(Cr) concentrations to account for fluctuations in the dugong's fluid intake (Taussky,1954).

RESULTS

Urinary progesterone levels ranged from 0.01 to 1.94 ng/mgCr, with a total of 14 ovarian cycles from April 1996 through April 1998 (Fig. 4). The mean length of the estrous cycle was 53.6±8.6 (mean±SEM) days based on the intervals between urinary progesterone peaks (Fig. 5).

During the first year of the observation, urinary estradiol-17β levels ranged from 0.9 to 23.7pg/mg Cr and tended to peak just prior to elevations in progesterone (June, July and August in 1996 and January, February and April in 1997). However its cyclicity was not as distinct as progesterone.

The dugong's clitoris appeared 13 times just prior to elevations in progesterone. Its appearance did not always correspond to the peak of urinary estradiol-17β.

DISCUSSION

The cyclic changes in progesterone occurred continuously through the observation period, which is consistent with Marsh *et al.* (1984a)'s report that wild female dugongs

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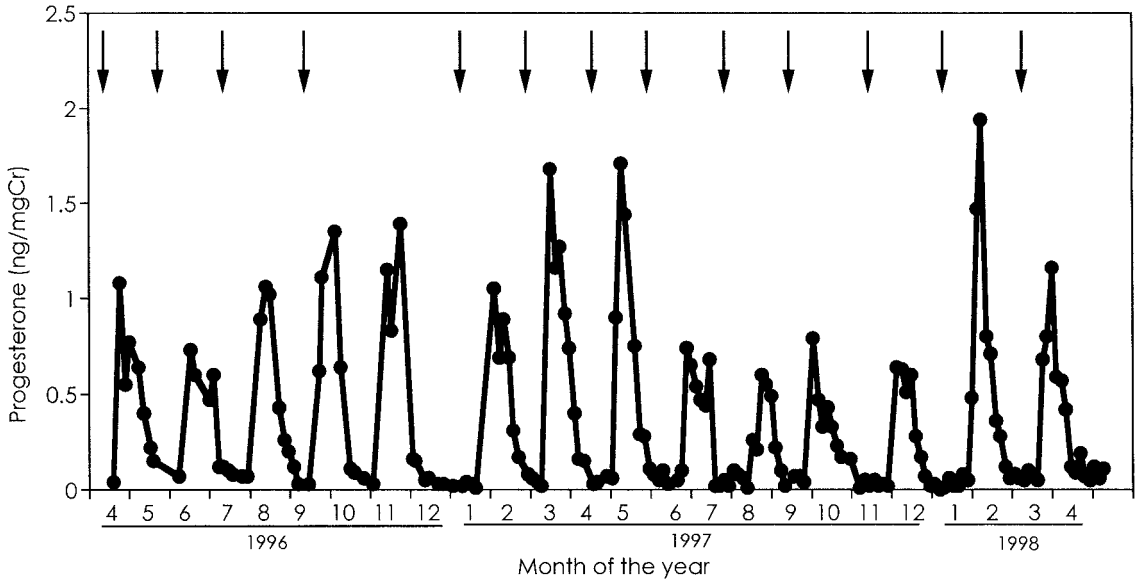


Fig. 4. Changes in the urinary concentrations of progesterone from April 1996 to April 1998. Arrows represent the day when the clitoris was observed.

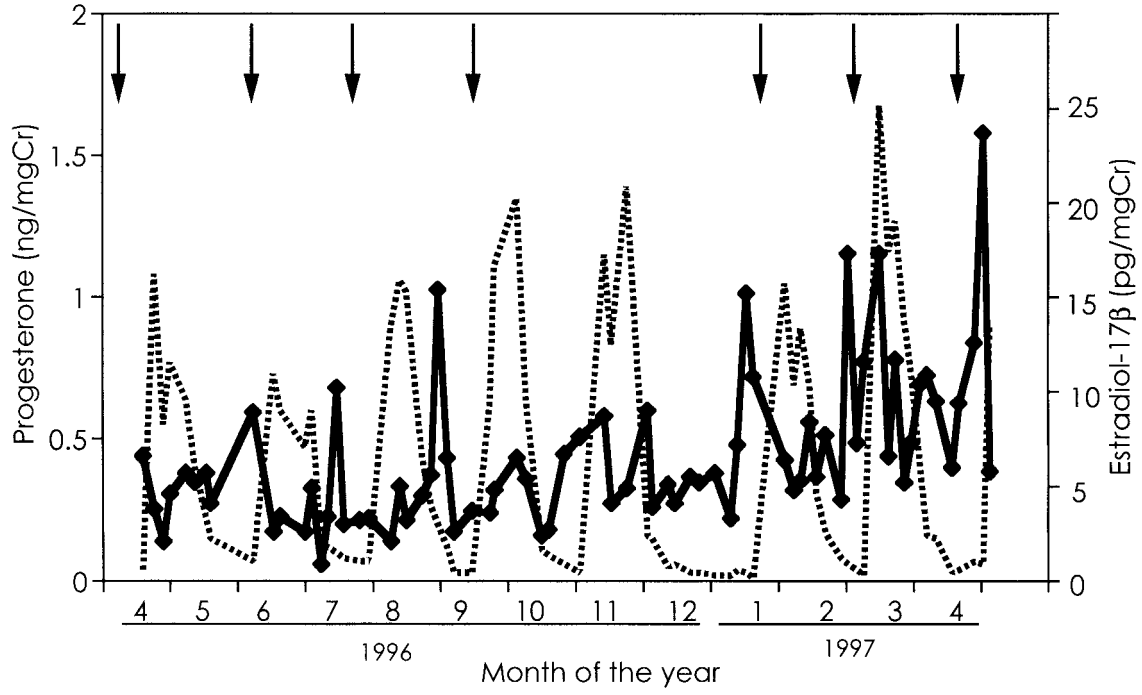


Fig. 5. Changes in the urinary concentrations of estradiol-17β (solid line) and progesterone (dotted line) from April 1996 to April 1997. Arrows represent the day when the clitoris was observed.

are polyestrous. However peak values of progesterone tended to be lower during summer time when the air temperature rose, although the water temperature did not change much. Day length might affect cyclicity in some extent in this species.

Concentrations of estradiol-17β, unlike progesterone did not show remarkable cyclic changes during the first year of this study. However, 6 peaks of estradiol-17β were observed at the intervals of 7 progesterone peaks. In future studies, it will be necessary to measure the concentrations of estradiol-17β more frequently by sampling urine at short intervals, and to observe in the context of estrous behaviour.

The clitoris was exposed between progesterone peaks except during the peak from October to December 1996. During the period of estradiol-17β measurement, the clitoris was observed 7 times. Appearances of the clitoris seemed

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to coincide with the low progesterone and the increase of estradiol-17β.

The female's behaviour changed during clitoris exposure. She swam constantly around the tank, rubbed her urogenital area against the wall, and lost her appetite. At the aquarium, the first cyclic change in the appearance of the clitoris was observed at 8 years of age, close to the minimum age (9 years) when the female dugong reaches sexual maturity (Marsh *et al*., 1984a). In contrast to the evidence of reproductive seasonality in wild dugongs (Anderson 1997; Boyed *et al*., 1999; Brownell *et al*., 1979, Marsh *et al*., 1984b; Marsh, 1995; Preen, 1989) there was no evidence of seasonality in captive dugongs.

ACKNOWLEDGMENTS

We acknowledge the assistance of Toba Aquarium Dugong staff members who have helped in collecting urine samples from "Serena". And we also thank Helene Marsh, School of Tropical Environment Studies and Geography, James Cook University, Australia, for assistance with the manuscript.

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(Received January 21, 2002 / Accepted March 25, 2002)

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