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Reproductive hormone monitoring of dugongs in captivity: Detecting the onset of sexual maturity in a cryptic marine mammal

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ABSTRACT

Determining the reproductive status of long-term captive animals is essential because the onset of sexual maturity and reproductive activity may necessitate changes in husbandry requirements. This study reports on the first multi-year reproductive hormone monitoring program for captive dugongs of both sexes using feces. Fecal samples were collected from one male (*Pig*) over 9 years (4–13.2 y of age; $n = 288$ samples, 0.8 ± 0.1 samples per week from July 2007 to February 2012) and one female (*Wuru*) over 7 years (from neonate to 6.9 y; $n = 171$ samples, 0.5 ± 0.1 samples per week from July 2007 to February 2012), and from one solitary female dugong (*Gracie*) over 10 months (10.5–11.3 y of age; $n = 54$ samples, 1.1 ± 0.2 sample per week from September 2008 to June 2009). Using enzyme-immunoassay, fecal progesterone (fP) and estradiol-17β (fE) concentrations were assayed in the two captive females, and testosterone (fT) concentration in the captive male, and compared these to concentrations in wild dugongs. Female *Wuru* exhibited increasing fP concentrations at 5+ y, indicating early onset of ovarian cycling typical of non-pregnant adult females. Female *Gracie* maintained basal fP concentrations consistent with wild immature dugongs, indicating that she had not reached puberty by 11 y. Nutritional plane may account for differences in age at sexual maturity in these female dugongs. At age 3–4 y, *Wuru* had fE concentrations 1.4 times greater than maximum concentrations recorded in all wild females, and these concentrations were coincident with a period of rapid weight gain. For the male *Pig*, increasing fT concentrations at 9 y provided early indications of puberty. *Pig*'s tusks erupted by 11 y, and sexual maturity (indicated by spermatic semen) was confirmed by 12.8 y. Identification of sexual maturation prompted two trials of a male contraceptive treatment using the GnRH agonist, deslorelin (9.4 mg administered in 2010 and 15.6 mg in 2011). Testosterone production was not significantly suppressed by these dosages, and treatment did not terminate sperm production at week 10–11 post-implantation, even at the larger dose tested. Routine analysis of fecal hormones was helpful for making reproductive management decisions regarding individual captives and in guiding the long-term captive management of this cryptic species.

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1. Introduction

Management of reproduction is a critical component in the husbandry of wildlife in captivity. As captive-held animals grow and mature, their biological, social, and environmental needs evolve, so that requirements such as adequate provisions, social conditions, enrichment, and functional enclosure space may need revision. Decision-makers need to be well informed in order to preempt the necessary actions required to contend with the growth, maturity, and sexual activity of animals held in long-term captivity. Anticipating the approach of an animal's sexual maturity can be challenging, particularly when dealing with a wildlife species not commonly kept in captivity and difficult to study in the wild.

The dugong (*Dugong dugon*) is an elusive, entirely aquatic marine mammal that attains an adult body length of up to 3.2 m, weighs up to 600 kg (Lanyon et al., 2010), and has a lifespan of 70 years or longer (Marsh, 1995). Dugongs have a wide distribution across the tropical Indo-Pacific region and are listed globally as vulnerable to extinction (Marsh, 2008). Oceanaria in several countries have housed dugongs at various times, with individuals entering captivity as either small (< 2.0 m body length) captured dugongs (Jones, 1960; Aung, 1967; Oke, 1967; Lanyon and Marsh, 1995a) or more recently as rescued unaccompanied calves (Elliott et al., 1981; Anon., 1995; Blanshard, 2000; Chua et al., 2001; Wakai et al., 2002; Adulyanukosol et al., 2004; Blanshard, 2006). Early attempts to rear calves and juvenile dugongs were mostly short-lived (typically < 6 month survival in captivity; reviewed by Adulyanukosol et al., 2004), with many captive dugongs dying prematurely from malnutrition or disease (Aung, 1967; Oke, 1967; Elliott et al., 1981; Adulyanukosol et al., 2004). Prior to the 1980 decade, the greatest longevity of captive-held dugongs was recorded in a pair of young males (1.6 m and 1.96 m long at capture) kept for 11 years in India (Jones, 1960; Nair et al., 1975). More recent husbandry practices, however, have been successful in extending the longevity of captive dugongs, so that current facilities need to plan for later life history stages including adult maturity (Anon., 1995; Blanshard, 2000; Chua et al., 2001; Wakai et al., 2002; Blanshard, 2006).

The onset of sexual maturity is delayed in wild dugongs, with both sexes exhibiting a protracted period of immaturity, probably due to a combination of phylogeny, intrinsic life history traits, and/or environmental factors including marginal nutrient availability (Marsh and Kwan, 2008). Furthermore, there is considerable variation in age at first reproduction both among and within dugong populations (Marsh and Kwan, 2008). Through examination of reproductive tracts from recovered carcasses, it has been established that females do not bear their first calf until they are at least 6 years but in many cases up to 17 years old, and males may attain sexual maturity anywhere between the ages of 4 and 13 years (Marsh et al., 1984; Kwan, 2002). Because the relationship between absolute age as determined by tusk growth layer groups (Marsh, 1980) and body size is variable (Marsh et al., 1984; Kwan, 2002), body length is not always a reliable indicator of maturity. In subtropical Moreton Bay, Australia, wild male dugongs reach reproductive maturity at body lengths > 2.4 m and females > 2.5 m (Burgess et al., 2012a, 2012b), which is over 80% of full asymptotic growth potential at ~3 m long. In contrast, precocial maturation has been recorded at body lengths closer to 2 m (i.e., mature males from 1.9 m long and pregnant females from 2.1 m) for dugongs in some parts of the tropics (Kwan, 2002). There is evidence that variation in age to maturity of wild dugongs may be nutritionally based (Lanyon, 1991; Kwan, 2002; Marsh and Kwan, 2008). The seagrass diet of the dugong is not only generally low in nutrients and energy (Lanyon et al., 1989; Lanyon, 1991) but varies spatially and temporally in both abundance and quality (Preen, 1992; Lanyon and Marsh, 1995b). It is likely that given food of greater nutritional quality, growth to sexual maturity may be accelerated in the dugong (Marsh and Kwan, 2008) as occurs in other wild and captive mammals (Sæther, 1997; Lindström, 1999).

Determining when an animal becomes sexually active is beneficial to managing behavioral changes, such as mating and associated aggressive behavior. Basic reproductive management (such as contraception and pregnancy detection) is particularly important for facilities where individuals of different sexes are housed together, which is a commonplace arrangement in order to accommodate several large, aquatic mammals at one facility. Furthermore, as male dugongs mature, a pair of permanent incisors erupts as protruding tusks in the upper jaw (Marsh, 1980; Marsh et al., 1984; Burgess et al., 2012b). These tusks are thought to have a reproductive function for the breeding male and are capable of inflicting rake injuries on conspecifics (Preen, 1989; Athousis, 2012). Therefore, the maintenance of reproductively mature dugongs in oceanaria may be problematic due to the possibility of aggressive and physical conflicts similar to those observed among wild adult dugongs (Preen, 1989). To date, no dugongs have been successfully rehabilitated back into the wild (Blanshard, 2000, 2006), and therefore, facilities holding dugongs (particularly those rescued as calves) need to have appropriate management practices in place to monitor the reproductive development of individuals.

Hormone monitoring using non-invasive sampling is a useful tool for the examination of basic reproductive processes in captive species (Schwarzenberger et al., 1996) and is considered integral to the captive management of wildlife (e.g., Brown, 2000). Previously, Toba Aquarium in Japan has successfully monitored the reproductive cycle of a single female dugong using urinary hormone concentrations (Wakai et al., 2002). However, urine sample collection required this dugong to be pliant to both inversion at the water's surface and then urogenital stimulation during frequent monitoring. Feces are the easiest samples to collect for monitoring programs of excreted hormones because they can be collected non-invasively. The practical utility of collecting voided feces from large aquatic animals has provided a means of assessing reproductive state in other captive sirenian species, including the Florida manatee *Trichechus manatus latirostris* (Larkin et al., 2005) and Amazonian manatee *Trichechus inunguis* (Amaral et al., 2009). Moreover, concentrations of reproductive hormones in fecal samples are reliable indicators