

Seasonal Difference of Diurnal Variations in Serum Melatonin, Cortisol, Testosterone, and Rectal Temperature in Indo-Pacific Bottlenose Dolphins (*Tursiops aduncus*)

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Abstract

Serum melatonin, cortisol and testosterone concentrations, and rectal temperature from four captive male Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) (estimated age: 36 to 39 y) were measured at 3-h intervals over a 24-h period on the winter solstice, spring equinox and summer solstice in order to (1) investigate diurnal variations in melatonin, cortisol and testosterone concentrations, and rectal temperatures and (2) examine the seasonal changes in parameters for discussing mechanisms of adaptation to the external environment in cetaceans. Serum melatonin, cortisol and testosterone concentrations, and rectal temperature values ranged from < 1.6 (detection limit of the assay) to 23.3 pg/mL, 0.8 to 14.0 ng/mL, 0.8 to 23.0 ng/mL, and 35.5 to 37.3° C, respectively. Melatonin measurements were not consistent with expected diurnal rhythm patterns—that is, higher values during the dark phase and lower values during the light phase. However, cortisol and testosterone concentrations and rectal temperatures showed significant diurnal rhythms with acrophases occurring at about the same time in all the seasons. The highest amplitude of testosterone diurnal rhythms, which suggests the resumption of testicular endocrine function, was at the spring equinox, and average testosterone concentrations were the highest at the summer solstice. Serum cortisol concentrations and rectal temperatures were highest at the spring equinox (lowest water temperatures of the three seasons), and there were significant negative correlations between water temperatures and cortisol concentrations and rectal temperatures. The results suggest that changes in secretion patterns and levels of hormones, as well as changes in rectal temperature, are influenced by various factors such as environmental change,

seasonal breeding, and required metabolic energy in the captive dolphins.

Key Words: Indo-Pacific bottlenose dolphin, *Tursiops aduncus*, melatonin, cortisol, seasonal changes, diurnal rhythms, testosterone, cetacean, rectal temperature, environment

Introduction

Many activities, including seasonal breeding, migration, and daily locomotor behaviors in mammals are controlled by the circadian system. Circadian rhythms are endogenously generated and synchronized to the environmental light-dark cycle (reviewed by Hastings et al., 2007). Melatonin is a hormone produced in the pineal gland of vertebrates, and its production is governed by environmental light-dark cycles, with higher concentrations during the dark phase than the light phase (Reiter, 1991, 1993). The duration of nocturnal elevation in circulating melatonin in short days is longer than those in long days; therefore, melatonin is considered to act as a neuroendocrine transducer of environmental cues (Reiter, 1993). The existence of the pineal gland in cetaceans remains a controversial subject. The gland is generally known to be reduced or even lacking (Oelschläger & Oelschläger, 2009), although it is found in the humpback whale (*Megaptera novaeangliae*) (Gersh, 1938) and harbour porpoise (*Phocoena phocoena*) (Behrmann, 1990). The presence of some rhythms in cetaceans, such as diurnal locomotor activity rhythms in bottlenose dolphins (*Tursiops truncatus*) (Sekiguchi & Kohshima, 2003), diurnal physiological rhythms in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) and killer whales (*Orcinus orca*) (Suzuki et al., 2003), and seasonal breeding and migration

rhythms in baleen whales (Gaskin, 1982; Lockyer, 1984), are behaviors consistent with circulating melatonin endogenously produced from pineal or nonpineal sources in response to external photoperiodic cues. However, no reports have been published on melatonin in cetaceans or on their circadian/circannual timing systems. In this study, we investigated diurnal circulating melatonin rhythms, a crucial component in the mammalian circadian system in captive Indo-Pacific bottlenose dolphins.

Hormones, including melatonin, and body temperature are outputs of the circadian system that can be quantified. Diurnal fluctuations of circulating cortisol and testosterone levels as well as rectal temperatures have been reported in some terrestrial mammals. Cortisol, a glucocorticoid, is released from the adrenal cortex as an important component of many physiological functions (e.g., stress, metabolism, immunity) (Nieuwenhuizen & Rutters, 2008). Plasma/serum cortisol concentrations in terrestrial mammals increase just before the active phase and decrease in the opposite phase (Krieger & Aschoff, 1979; De Souza & Meier, 1987; Lefcourt et al., 1993; Irvine & Alexander, 1994). Diurnal rhythms in circulating testosterone, the male reproductive hormone, have also been described for various types of terrestrial mammals, and plasma/serum testosterone concentrations were reduced during the late active phase, and the daily peak was recorded prior to the active phase (Krieger & Aschoff, 1979; Perret, 1985; Omari et al., 1989; Garcia-Bonacho et al., 2000). As for rectal temperature, the diurnal rhythm is often associated with locomotor and foraging activities by species. Rectal temperatures in diurnal terrestrial mammals increased in the light phase, whereas those in nocturnal animals showed the opposite pattern (Wakamura & Tokura, 2002; Brown & Downs, 2006; Kinahan et al., 2007). In cetaceans, Judd & Ridgway (1977) reported no diurnal rhythm of serum testosterone concentrations in bottlenose dolphins by collecting blood samples every 20 min for 24 h. They considered that testosterone secretions might be influenced by stress because blood samples were obtained from dolphins kept on stretchers throughout the experiment. Basal rhythms of output parameters in cetaceans have been reported only in cortisol. The levels of cortisol in captive Indo-Pacific bottlenose dolphins and killer whales were lower at 1800 h and higher in the early morning, similar to terrestrial mammals (Suzuki et al., 2003). Although the blood samples were collected at different sampling intervals during March, June, and August, the authors did not discuss the relationship of hormonal changes to the circadian system or environmental factors such as photoperiod and

water temperature. We examined diurnal rhythms of serum cortisol, testosterone concentrations, and rectal temperature in addition to melatonin during the winter solstice, spring equinox, and summer solstice in captive Indo-Pacific bottlenose dolphins in order to examine the relationship between diurnal rhythms and environmental changes for elucidation of the mechanism of periodic control in cetaceans.

Materials and Methods

The present study was conducted in accordance with the *Guidelines for Proper Conduct of Animal Experiments* by the Science Council of Japan.

Sample Collection

We collected blood samples during the winter solstice (22-23 December 2007), spring equinox (20-21 March 2008), and summer solstice (21-22 June 2008) every 3 h over a 24-h period from four adult male Indo-Pacific bottlenose dolphins (individual no. 1: body length [BL] 263 cm, body weight [BW] 216 kg, *ca* 39 y old; no. 2: BL 259 cm, BW 189 kg, *ca* 36 y old; no. 3: BL 259 cm, BW 178 kg, *ca* 36 y old; and no. 4: BL 258 cm, BW 191 kg, *ca* 36 years old) at the Okinawa Churaumi Aquarium, Okinawa, Japan (26° N latitude, 127° E longitude). All four dolphins were taken into captivity in 1975, and they remained reproductively active until at least 2009 based on circulating testosterone concentrations (Yoshioka et al., 2010).

The dolphins had been trained sufficiently in the husbandry blood sampling method from the veins of tail flukes. They were maintained in Tanks A (individual nos. 1 & 2; 464 m³ capacity, 14 m wide, 14 m long, 3 m deep) and B (individual nos. 3 & 4; 1,200 m³ capacity, 16.25 m wide, 24.25 m long, 4 m deep) and were exposed to natural outdoor lighting and water temperatures. They received 10 kg/d of capelin, mackerel, flying fish, surf smelt, and squid. About 10 mL of blood was drawn at intervals of 3 h (at 0900, 1200, 1500, 1800, 2100, 0000, 0300, 0600, and 0900 h), and serum samples after centrifugation (3,000 rpm, 15 min, and 4° C) were frozen at -30° C until hormonal analyses could be performed. Dark phase blood samples were collected using a handheld flashlight covered with red cellophane. Rectal and water temperature values were measured with a Terumo Finer CTM-303 (Terumo, Tokyo, Japan) by inserting a ME-PDK041 heat sensitive probe (Terumo, Tokyo, Japan) at the time of sample collections. Illumination intensity was recorded with an illuminance meter (model T-1M, Minolta, Tokyo, Japan) and was measured three times during each sampling period. The photoperiods

were defined as follows: the light phase as the period in sunlight; the twilight phase as an hour before and after sunrise or sunset; and the dark phase as the opposite pattern of the light phase.

Hormone Assays

Serum melatonin concentrations were determined by the enzyme-immunoassay kit manufactured by IBL International (Catalog No. RE54021, Hamburg, Germany). Recovery in this test kit was examined by adding known amounts of melatonin (1.56, 3.13, 6.25, 12.5, 25, 50, 100 pg/mL) to serum specimens of dolphins ($y = 1.2961x + 11.486$, $r^2 = 0.9082$). Serum testosterone determinations were performed by enzyme-immunoassay validated for use with dolphins in Kita et al. (1999). Serum cortisol concentrations were measured by the time-resolved fluorometric immunoassay method using the DELFIA system (Suzuki et al., 2008). Assay sensitivity of melatonin, testosterone, and cortisol was 1.6 pg/mL, 0.078 ng/mL, and 0.16 ng/mL, respectively.

Data Analysis

Data were presented as mean \pm SE. Diurnal variations of serum hormones and rectal temperatures were indicated as a percentage of the daily individual mean for eliminating individual differences in accordance with Krieger & Aschoff (1979) and Perret (1985), in addition to the raw data, because these parameters fluctuate depending on physiological states of the individual. For analysis of diurnal rhythms and seasonal comparisons, we used one-way ANOVA followed by Tukey Kramer's test. Correlations between cortisol concentrations or rectal temperatures and water temperatures were tested by Spearman's correlation coefficient by

rank test. Statistical analysis was performed using the *Statcel 2 Excel* add-in software. To show the characteristic of the diurnal profile in three seasons, we calculated the acrophase (mean value of daily peak time in each individual) and amplitude (mean percentage of the maximum to minimum value of the daily individual means).

Results

Environmental Conditions

Day lengths during the winter solstice, spring equinox, and summer solstice were 10 h 27 min, 12 h 9 min, and 13 h 52 min, respectively. The highest illumination intensity recorded on Tanks A and B in the three sampling seasons were $32,367 \pm 484$ and $42,800 \pm 1,882$ lx in the winter solstice, $86,633 \pm 677$ and $87,067 \pm 296$ lx in the spring equinox, and $90,867 \pm 384$ and $98,200 \pm 153$ lx in the summer solstice, respectively. Illumination intensities showed a seasonal change arising from the altitude of the sun, and they were significantly lower in the light phase on the winter solstice than on the spring equinox or summer solstice (Table 1). The illumination intensity of the dark phase was about 0.1 lx with a range of 0 to 0.46 lx in all the seasons. Water temperatures showed the highest value in the summer solstice with no large daily fluctuations (Table 1).

Diurnal Variations

Table 2 and Figures 1 & 2 represent diurnal variations of the three hormones and rectal temperatures in three seasons. Serum melatonin concentrations (< 23.3 pg/mL) in all individuals and sampling times were the equivalent of diurnal levels of other mammals (Griffiths et al., 1979; Brainard

Table 1. Comparisons of mean illumination intensity and water temperature (mean \pm SE) experienced at different sampling points during the winter solstice, spring equinox, and summer solstice

| Sampling date | | | Winter solstice (22-23 December 2007) | Spring equinox (20-21 March 2008) | Summer solstice (21-22 June 2008) |
|--------------------------------|--------------------------------|----------------|------------------------------------------|--------------------------------------|--------------------------------------|
| Parameters | Day length (sunrise-sunset) | | 10 h 27 min (0711-1738 h) | 12 h 9 min (0629-1838 h) | 13 h 52 min (0532-1924 h) |
| Illumination intensity (lx) | Tank A | Light phase | $25,440 \pm 1,968^a$ | $52,967 \pm 6,828^b$ | $57,693 \pm 7,237^{b'}$ |
| | | Twilight phase | 1.91 ± 0.3 | $3,123 \pm 913$ | $2,303 \pm 38$ |
| | | Dark phase | 0.06 ± 0.0 | 0.17 ± 0.0^a | 0.05 ± 0.0^b |
| | Tank B | Light phase | $21,130 \pm 4,876^a$ | $54,900 \pm 6,939^b$ | $56,363 \pm 8,604^b$ |
| | | Twilight phase | 0.07 ± 0.0 | $1,975 \pm 884$ | $1,560 \pm 4.1$ |
| | | Dark phase | 0.08 ± 0.0 | 0.12 ± 0.0 | 0.10 ± 0.0 |
| Water temperature (°C) | Tank A | | 23.7 ± 0.0^A | 22.6 ± 0.1^B | 27.6 ± 0.1^C |
| | Tank B | | 23.4 ± 0.0^A | 22.2 ± 0.1^B | 27.4 ± 0.1^C |

Different letterings show significant differences between the seasons (ab: $p < 0.05$, ab': $p < 0.01$, AB, BC, and AC: $p < 0.01$). Light phase means period of sunlight; twilight phase means the hour before and after sunrise or sunset; and dark phase means the opposite pattern of the light phase.

Table 2. Individual values of maximum and minimum levels; daily peak time; and amplitude of melatonin, cortisol, testosterone, and rectal temperature profiles determined in the four animals experimented with in the winter solstice, spring equinox, and summer solstice

| Animal # | Winter solstice | | | | Spring equinox | | | | Summer solstice | | | | | | |
|------------------------------------|-----------------|------|------|---------------|----------------|------|------|------|-----------------|---------------|------|------|------|---------------|---------------|
| | Max. | Min. | Mean | Peak time (h) | Amplitude (%) | Max. | Min. | Mean | Peak time (h) | Amplitude (%) | Max. | Min. | Mean | Peak time (h) | Amplitude (%) |
| (a) Melatonin (pg/mL) | | | | | | | | | | | | | | | |
| 1 | ND | ND | 0.8 | -- | 0.0 | 1.8 | ND | 0.9 | 0300 | 113.7 | 1.9 | ND | 1.1 | 1500 | 107.5 |
| 2 | 23.3 | ND | 7.5 | 0900 | 298.9 | 5.4 | ND | 1.7 | 2100 | 267.6 | 9.0 | 2.0 | 5.2 | 1200 | 136.0 |
| 3 | 10.1 | ND | 3.4 | 1200 | 271.5 | 5.7 | ND | 4.1 | 2100 | 119.4 | 13.7 | 2.0 | 6.0 | 2100 | 195.3 |
| 4 | 5.8 | ND | 1.8 | 0900 | 274.1 | 6.8 | ND | 2.2 | 0900 | 272.4 | 10.8 | ND | 4.6 | 2100 | 218.3 |
| Mean | 10.0 | 0.8 | 3.4 | 1000 | 211.0 | 4.9 | 0.8 | 2.2 | 1330 | 193.3 | 8.9 | 1.4 | 4.2 | 1715 | 164.3 |
| SE | 4.8 | 0.0 | 1.5 | 0100 | 70.6 | 0.9 | 0.0 | 0.7 | 0430 | 44.3 | 2.2 | 0.3 | 1.1 | 0215 | 25.7 |
| (b) Cortisol (ng/mL) | | | | | | | | | | | | | | | |
| 1 | 14.0 | 0.9 | 4.3 | 0900 | 304.7 | 13.9 | 4.5 | 8.0 | 0900 | 115.8 | 5.2 | 2.0 | 3.1 | 1800 | 102.9 |
| 2 | 5.8 | 1.7 | 3.3 | 0900 | 125.9 | 13.1 | 3.5 | 6.3 | 0900 | 152.7 | 7.8 | 1.9 | 4.3 | 0300 | 138.6 |
| 3 | 3.0 | 0.8 | 1.9 | 0900 | 114.5 | 7.5 | 2.2 | 4.5 | 0900 | 117.9 | 7.9 | 2.4 | 4.4 | 0900 | 126.6 |
| 4 | 5.4 | 1.7 | 2.8 | 0900 | 130.6 | 7.6 | 3.4 | 5.5 | 0900 | 76.0 | 6.0 | 1.3 | 3.0 | 0900 | 157.4 |
| Mean | 7.1 | 1.3 | 3.1 | 0900 | 168.9 | 10.5 | 3.4 | 6.1 | 0900 | 115.6 | 6.7 | 1.9 | 3.7 | 0945 | 131.4 |
| SE | 2.1 | 0.2 | 0.4 | 0000 | 45.4 | 1.5 | 0.4 | 0.5 | 0000 | 15.7 | 0.6 | 0.2 | 0.3 | 0305 | 11.4 |
| (c) Testosterone (ng/mL) | | | | | | | | | | | | | | | |
| 1 | 7.5 | 3.6 | 5.4 | 0300 | 72.8 | 8.5 | 3.3 | 5.5 | 0600 | 95.1 | 12.3 | 6.7 | 8.8 | 0900 | 63.2 |
| 2 | 11.9 | 7.7 | 9.9 | 0600 | 42.4 | 15.7 | 6.8 | 10.4 | 0300 | 85.8 | 23.0 | 11.9 | 16.7 | 0900 | 65.8 |
| 3 | 12.0 | 5.8 | 8.5 | 0900 | 72.5 | 20.0 | 6.8 | 10.1 | 0900 | 130.4 | 19.1 | 5.9 | 10.6 | 0900 | 124.0 |
| 4 | 5.5 | 3.3 | 4.2 | 0900 | 52.1 | 4.0 | 0.8 | 2.3 | 0300 | 138.8 | 7.6 | 4.0 | 5.8 | 0900 | 62.2 |
| Mean | 9.2 | 5.1 | 7.0 | 0645 | 60.0 | 12.1 | 4.4 | 7.1 | 0515 | 108.2 | 15.5 | 7.1 | 10.5 | 0900 | 78.8 |
| SE | 1.4 | 0.9 | 0.5 | 0126 | 7.6 | 3.1 | 1.3 | 0.7 | 0126 | 10.6 | 3.0 | 1.5 | 0.8 | 0000 | 15.1 |
| (d) Rectal temperature (°C) | | | | | | | | | | | | | | | |
| 1 | 36.4 | 35.9 | 36.2 | 0900 | 1.4 | 36.8 | 35.9 | 36.2 | 1200 | 2.5 | 36.4 | 35.5 | 35.9 | 0900 | 2.5 |
| 2 | 36.7 | 36.0 | 36.3 | 1200 | 1.9 | 36.6 | 35.9 | 36.3 | 1200 | 1.9 | 36.5 | 35.8 | 36.1 | 0900 | 1.9 |
| 3 | 37.1 | 35.8 | 36.4 | 1200 | 3.6 | 36.8 | 35.9 | 36.4 | 0900 | 2.5 | 36.5 | 35.8 | 36.2 | 1200 | 1.9 |
| 4 | 36.9 | 35.8 | 36.4 | 0900 | 3.0 | 37.3 | 36.0 | 36.5 | 1200 | 3.6 | 36.5 | 35.7 | 36.0 | 1800 | 2.2 |
| Mean | 36.8 | 35.9 | 36.3 | 1030 | 2.5 | 36.9 | 35.9 | 36.4 | 1115 | 2.6 | 36.5 | 35.7 | 36.1 | 1200 | 2.2 |
| SE | 0.1 | 0.0 | 0.1 | 0051 | 0.5 | 0.1 | 0.0 | 0.1 | 0045 | 0.3 | 0.0 | 0.1 | 0.0 | 0207 | 0.1 |

Max. and Min. – highest and lowest values of daily change, respectively; Peak time – time of daily peak (i.e., acrophase); Amplitude – percentage of the maximum to minimum value of the daily individual means; ND – not detectable; and -- -- no diurnal change. For statistical analysis, ND was calculated as one-half of the sensitivity of assay (Hecker et al., 2004; Alvarez-Pedrerol et al., 2009).

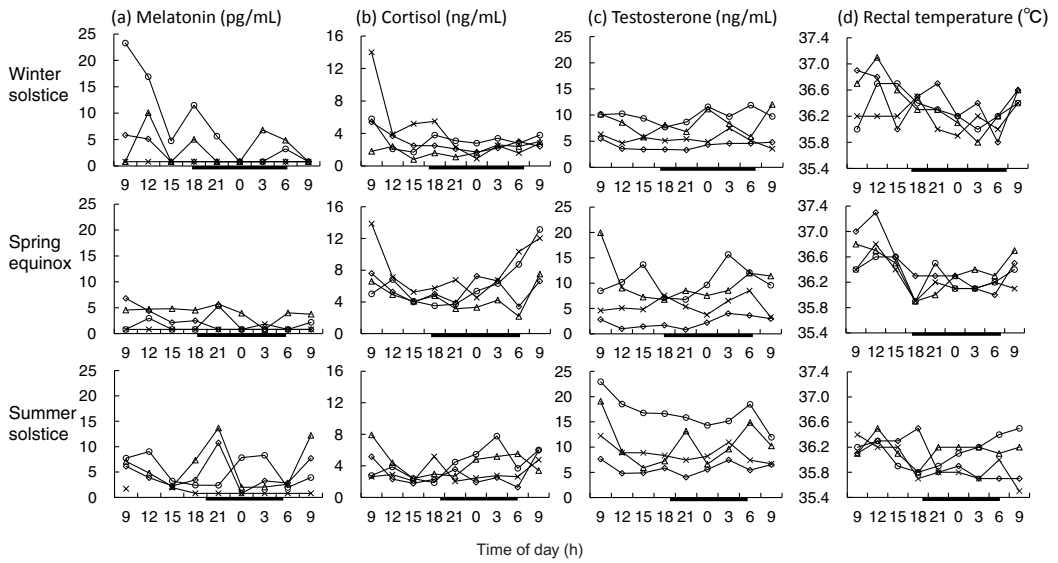


Figure 1. Diurnal variations of (a) melatonin, (b) cortisol, (c) testosterone levels, and (d) rectal temperature values in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) examined during the winter solstice, spring equinox, and summer solstice; crosses indicate hormonal levels and rectal temperatures in individual no. 1, circles in no. 2, triangles in no. 3, and squares in no. 4. Bar on the horizontal axis in each graph represents the dark phase.

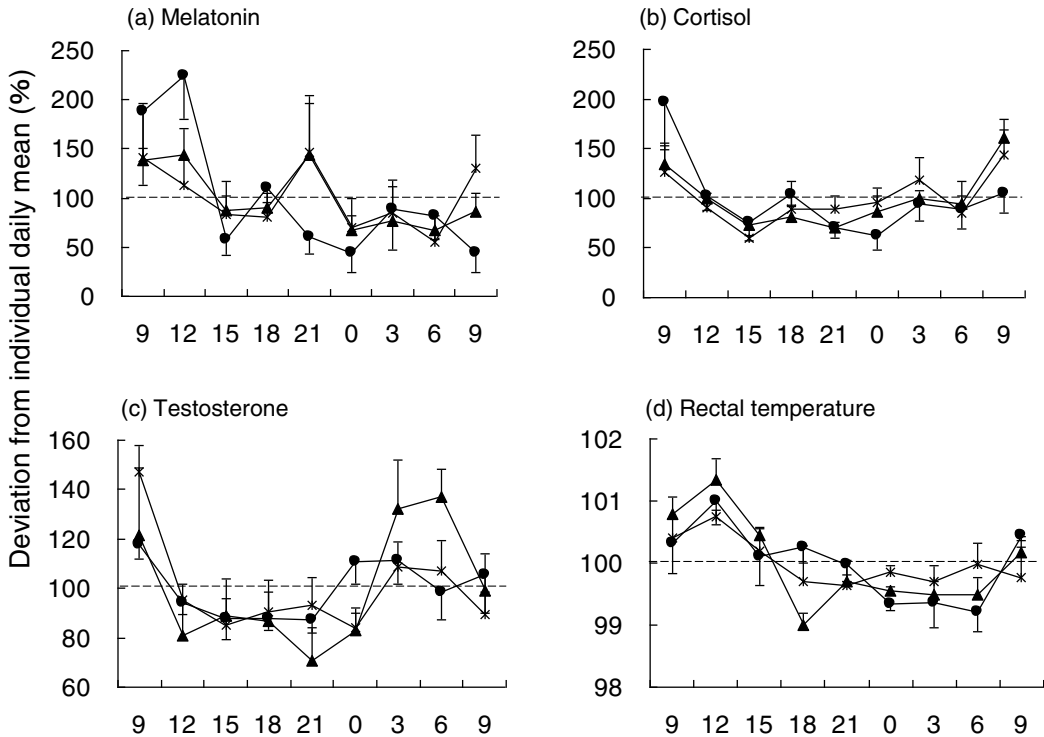


Figure 2. Diurnal variations of (a) melatonin, (b) cortisol, (c) testosterone levels, and (d) rectal temperature as a percentage of the daily individual mean (horizontal broken line) in Indo-Pacific bottlenose dolphins; circles indicate hormonal levels and rectal temperatures in the winter solstice, triangles in the spring equinox, and asterisks in the summer solstice.

et al., 1982; Earl et al., 1985; Bojkowski et al., 1987; Griffith & Minton, 1992), and no diurnal rhythm was observed throughout all the seasons (Figure 1a). However, changes in the mean percentage of melatonin concentrations showed highly variable results among the sampling times during the winter solstice (1200 h vs 0000 h, following 0900 h; $p < 0.05$) (Figure 2a).

Diurnal rhythms in serum cortisol concentrations were evident in most individuals, and they were higher at 0900 h than during the evening or at night (Figure 1b). The mean percentage of cortisol concentrations also showed high levels at 0900 h, decreased in the evening and night, and then returned to the initially high levels by the following morning (Figure 2b). Significant differences were detected for the following combinations: winter solstice – 0900 h vs 1500 and 0600 h ($p < 0.05$), 0900 h vs 2100 and 0000 h ($p < 0.01$); spring equinox – 1500 h vs following 0900 h, 2100 h vs following 0900 h ($p < 0.05$), 1800 h vs following 0900 h, 0000 h vs following 0900 h, and 0600 h vs following 0900 h ($p < 0.01$) (Figure 2b).

Serum testosterone concentrations varied greatly among individual animals (Figure 1c), although clear diurnal rhythms were observed. Beginning with the highest value at 0900 h, the mean percentage of testosterone levels gradually decreased toward 2100 h, and then increased toward early morning (Figure 2c). Significant differences were detected for the following combinations: summer solstice – 0900 h vs 1200, 1800, and 2100 h ($p < 0.05$), and 0900 h vs 1500, 0000, and following 0900 h ($p < 0.01$) (Figure 2c).

Diurnal rhythms of the rectal temperature of four animals in the winter solstice, spring equinox, and summer solstice showed a similar pattern of being higher in the daytime (Figure 1d). Mean percentage of rectal temperatures generally showed highest levels at 1200 h, decreased toward the evening and night, and then returned to high levels at 0900 h (Figure 2d). Significant differences were detected for the following combinations: winter solstice – 1200 h vs 0000, 0300, and 0600 h ($p < 0.05$); and spring equinox – 0900 h vs 0000, 0300, and 0600 h ($p < 0.05$), 0900 h vs 1800 h, 1200 h vs 1800, 2100, 0000, 0300, and 0600 h, and 1500 h vs 1800 h ($p < 0.01$) (Figure 2d).

Seasonal Comparison of Diurnal Variations:

Acrophase and Amplitude

Table 2 showed diurnal variations in the hormones and rectal temperatures as the acrophase and amplitude. The acrophase of melatonin was shown at various times irrespective of the phases of the photoperiod and season (Table 2a). In contrast, acrophases of cortisol, testosterone, and rectal temperature were observed at restricted time

zones without significant differences among seasons: from 0300 to 1800 h in cortisol, from 0300 to 0900 h in testosterone, and from 0900 to 1800 h in rectal temperature, respectively (Table 2b, c & d). Amplitudes of melatonin, cortisol, and rectal temperature did not show a significant seasonal difference (Table 2a, b & d). For testosterone, however, the amplitude in the spring equinox was higher than those in the winter solstice ($p < 0.05$) and summer solstice (Table 2c).

Seasonal Variations

Seasonal changes of daily mean concentrations in melatonin were not significantly different among the seasons (winter solstice: 3.4 ± 1.5 pg/mL, spring equinox: 2.2 ± 0.7 pg/mL, and summer solstice: 4.2 ± 1.1 pg/mL), while significant seasonal differences were observed in the other parameters (i.e., steroid hormones and rectal temperature). Daily mean concentrations of serum cortisol in the spring equinox (6.1 ± 0.5 ng/mL) were higher than in the winter (3.1 ± 0.4 ng/mL) and summer solstices (3.7 ± 0.3 ng/mL) ($p < 0.01$). For testosterone, daily mean concentrations in the summer solstice (10.5 ± 0.8 ng/mL) were higher than in the spring equinox (7.1 ± 0.7 ng/mL) and winter solstice (7.0 ± 0.5 ng/mL) ($p < 0.01$). Daily mean rectal temperature values in the summer solstice ($36.1 \pm 0.04^\circ$ C) were lower than in the spring equinox ($36.4 \pm 0.05^\circ$ C) and winter solstice ($36.3 \pm 0.05^\circ$ C) ($p < 0.01$). There were significant negative correlations between water temperature and cortisol (coefficient of correlation: -0.37) and rectal temperature (-0.33) ($p < 0.01$; see Figure 3).

Discussion

In the present study, absence of significant diurnal rhythms of melatonin was observed in all three sampling seasons, while cortisol and testosterone concentrations and rectal temperatures exhibited diurnal rhythms in captive Indo-Pacific bottlenose dolphins. In spite of variations in influencing factors such as day length and water temperature, the acrophase of cortisol, testosterone, and rectal temperature occurred at about the same time.

Previous studies showed that bright lights suppressed nocturnal secretion of melatonin from the pineal gland in humans (*Homo sapiens*) (Lewy et al., 1980; Bojkowski et al., 1987; McIntyre et al., 1989), Suffolk ewes (*Ovis aries*) (Earl et al., 1985), Japanese macaque (*Macaca fuscata fuscata*) (Nozaki et al., 1990), crossbred pigs (Griffith & Minton, 1992), Syrian hamsters (*Mesocricetus auratus*) (Brainard et al., 1982), and ground squirrels (*Spermophilus tridecemlineatus*) (Reiter et al., 1983). Minimum light intensity for melatonin suppression was over 300 lx in diurnal animals (Lewy

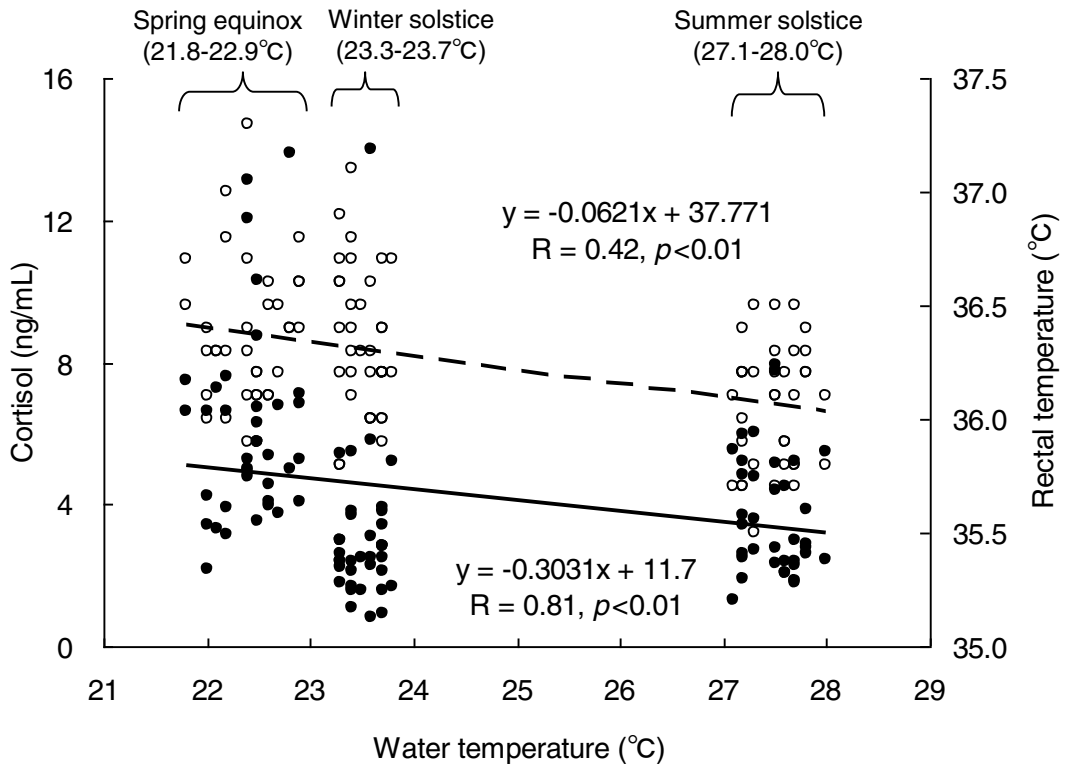


Figure 3. Relevancies of serum cortisol concentrations and rectal temperatures to water temperatures in four Indo-Pacific bottlenose dolphins; solid circles and the solid regression line indicate the relationship between concentrations of cortisol and water temperature, and open circles and the broken regression line indicate the relationship between rectal temperature and water temperature, respectively.

et al., 1980; Earl et al., 1985) and approximately 1 lx in nocturnal animals (Brainard et al., 1982). Light intensity during the dark phase in this study was about 0.1 up to 0.46 lx, much lower than 1 lx. It seems quite unlikely that light conditions suppress melatonin during the dark phase. The structure of the retina in cetaceans is similar to that of nocturnal animals, suggesting that cetaceans have scotopic vision (Supin et al., 2001), although there was a possibility that nocturnal secretion of melatonin was suppressed by a low intensity of light like 0.1 lx due to higher photosensitivity on the retina in Indo-Pacific bottlenose dolphins than nocturnal animals.

For cetaceans, the pineal gland might be in its rudimentary form except for some species (see "Introduction"), and it is doubtful that they have a functional pineal gland. The gland is also absent in crocodilians such as the American alligator (*Alligator mississippiensis*), which have no obvious daily melatonin rhythms (Roth et al., 1980). However, rhythms of locomotor activity and plasma corticosterone in American alligators showed synchronization to the light-dark cycle (Kavaliers &

Ralph, 1981; Lance & Lauren, 1984). Taniguchi et al. (1993) reported that the pineal gland of owls (*Strix uralensis*) (nocturnal birds) histologically degenerated, and plasma melatonin levels were very low during both midday (6 to 10 pg/mL) and midnight (16 to 38 pg/mL). Additionally, pineal cells of owls released virtually no melatonin over a 24-h period and did not respond to an exogenous stimulant agent (Taniguchi et al., 1993). So there is the possibility of absent melatonin rhythm in animals having a degenerated pineal gland. However, there have been no studies on other nonpineal or degenerated species—for example, edentates (Oksche, 1965), the Asian elephant (*Elephas maximus*), the African elephant (*Loxodonta africana*) (Shoshani et al., 2006), and the West Indian manatee (*Trichechus manatus*) (Ralph et al., 1985), and the functions and fluctuations of melatonin in those species remain unknown. Therefore, further studies are required to reveal the physiological mechanism, including melatonin rhythm, underlying the entrainment performance in cetaceans.

In Indo-Pacific bottlenose dolphins, diurnal pattern and levels in cortisol were similar to those

observed by Suzuki et al. (2003); that is, serum cortisol levels decreased in the evening and increased the following morning. In addition, serum testosterone and rectal temperature also increased in the early morning and daytime, similar to diurnal terrestrial mammals (Krieger & Aschoff, 1979; Wakamura & Tokura, 2002; Kinahan et al., 2007). Sekiguchi & Kohshima (2003) reported diurnal rhythm in behavioral activity in captive bottlenose dolphins, and the dolphins tended to be very active in the afternoon and inactive late at night. These diurnal rhythms should be considered to be affected by captive conditions in the aquarium as discussed by Sekiguchi & Kohshima (2003) and Suzuki et al. (2003).

Amplitude of testosterone diurnal rhythm during the spring equinox was higher than during the winter and summer solstices, and average testosterone concentrations were significantly higher during the summer solstice than during the winter solstice and spring equinox. According to reports that studied the peripheral metabolism, metabolic clearance rate, and binding protein capacity of testosterone in terrestrial mammals (Saboureau et al., 1982; Perret, 1985), an increase in amplitude of testosterone rhythm was regarded as the resumption of testicular endocrine function in seasonal breeding. Therefore, in Indo-Pacific bottlenose dolphins, it would be expected that testicular endocrine function began recovering in spring (i.e., the onset of breeding season), and then gradually activated toward the summer. This reproductive seasonality was in good agreement with circulating testosterone peaks during the spring and summer months for the four dolphins from this study (Yoshioka et al., 2010).

In a study of Alaskan harbor seals (*Phoca vitulina*) by Oki & Atkinson (2004), there was a daily rhythm of plasma cortisol concentrations only in the summer, and cortisol concentrations were slightly higher in the winter season with levels remaining steady throughout the day. One of the functions that cortisol plays is to maintain body temperature in homeotherms as hypothermia and death will occur if the adrenal gland is removed (Nieuwenhuizen & Rutters, 2008). The seal study suggested that cortisol needed to be continuously produced throughout the day in order to compensate for a higher homeostatic demand due to the colder environment during winter (Oki & Atkinson, 2004). In the present study, serum cortisol concentrations and rectal temperatures were highest in the spring equinox (lowest water temperatures of the three seasons), and there were negative correlations between water temperatures and cortisol concentrations and rectal temperatures in Indo-Pacific bottlenose dolphins. The seasonal changes in cortisol levels relating to

environmental temperatures are in agreement with results in many types of mammals, including killer whales (Suzuki et al., 2003), squirrel monkeys (*Saimiri sciureus*) (Schiml et al., 1999), chacma baboons (*Papio hamadryas ursinus*) (Weingrill et al., 2004), and humans (Walker et al., 1997). In a study of bottlenose dolphins in Sarasota Bay, Florida, located at about the same latitude as our study site, heat flux values of appendages in the winter were very low, and blubber thickness in the winter was significantly greater than in the summer (Meagher et al., 2008). These results suggest that cetaceans attempt to conserve heat in the winter irrespective of their geographic location, and that their physiological functions adapt to seasonal changes in their external environments.

Acknowledgments

We would like to thank Haruka Suzuki, Nami Takase, and Makio Yanagisawa, and all the staff of the Marine Mammal Section of the Okinawa Churaumi Aquarium for their invaluable assistance in handling animals and sample collections. We would also like to thank Aoi Nozawa of Nihon University for assisting in sample collection and cortisol measurement. This work was partially supported by the Japan Society for the Promotion of Science (JSPS) Grants-in-Aid for Scientific Research (B) (No. 19380112) to MY.

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