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Diurnal Variation of Testosterone in Beluga Whale Blow

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Abstract

Studies on the concentrations of certain hormones in cetaceans and their effects on cetacean biology are useful to determining physical conditions such as age, sex, and reproductive status of individuals. Hormone concentrations can be determined through blood samples, but collection of blow (exhaled respiratory vapor) has proven to be a noninvasive method of obtaining samples that can be analyzed for hormone concentrations. The existence of diurnal variation in hormone concentrations greatly affects the value of what time of day samples are collected. Previous studies have found that other mammalian species exhibit diurnal variation of testosterone and that for many species the secretion of the hormone is dependent on sleep cycles. Cetaceans are unique in that they engage in a method of sleep called unihemispheric slow wave sleep. This allows for half of their brain to sleep while the other half remains awake, therefore they are never fully asleep. The aim of this study was to determine whether or not beluga whales housed at an aquarium expressed diurnal variation in their testosterone concentrations. Blow was collected from two male belugas at three different times throughout the day. A competitive enzyme immunoassay (EIA) was used to determine the testosterone concentrations. It was found that the relationship between time of day and testosterone concentration was not significant and there was not a significant difference in concentration between the collection times that were tested. This suggests that diurnal variation of testosterone secretion is not occurring in these beluga whales during daylight hours.

Introduction

The behavior and physiological functions of many species of cetaceans are still unknown due to their relative inaccessibility in the ocean. For this reason, many recent studies have focused on increasing understanding of the effects of hormones on processes within cetaceans such as reproduction and stress, and using that information to gain insight into other concepts of cetacean biology. Access to cetaceans in zoos and aquariums has allowed for the collection of blood, and also more recently respiratory exhales called “blow”, which can be used to study hormones. Since the knowledge of hormone concentrations in animals is useful in determining other physical conditions such as age, sex, and reproductive status, the existence of diurnal cycles in cetaceans could significantly affect the importance of what time of day samples are collected. Use of these collection methods in cetaceans housed in

aquariums allows for noninvasive study of diurnal variations in reproductive hormones that otherwise cannot be evaluated in wild cetaceans.

Other studies on reproductive hormones in various organisms have shown cases of diurnal variation. For instance, estradiol, the biologically active form of estrogen, expresses diurnal rhythms of secretion during the menstrual cycle in humans (Bao et al., 2003). Progesterone does not exhibit diurnal variation but a derivative, 17-hydroxyprogesterone, does (Strott et al., 1969). In rats (Kinson and Liu, 1973), wild stallions (Kirkpatrick et al. 1976), bulls (Thibier, 1976), and man (Barberia et al., 1973) analysis of plasma testosterone indicated patterns of increased testosterone levels in the morning and decreased testosterone levels in the evening. In humans, it has been found that diurnal testosterone secretion is dependent on sleep cycles (Wittert, 2014). Testosterone peaks at around 3 hours of sleep, remaining at this level until waking, at which point secretion follows a general pattern of decrease throughout the day, with a few increasing bursts, until it really begins to increase when sleep resumes.

Cetaceans are unique in that they engage in an unusual method of sleep termed unihemispheric slow-wave sleep (USWS). USWS allows half of their brain to remain active while the other half rests. Episodes alternate between the two hemispheres. This likely enables them to be continuously aware of their environment, return to the surface to breathe, and maintain thermogenesis while sleeping (Lyamin et al., 2013). In adult male belugas, 26 episodes of USWS were recorded over a two-day period, each episode lasting between 10 and 81 minutes. The fact that cetaceans are never fully asleep, and rest in periodic episodes, is a cause for consideration whether or not this behavior affects the occurrence of diurnal testosterone secretion seen in other mammals.

Although cetaceans exhibit a unique sleep method, cetaceans housed in aquariums are often most active throughout the day and rest at night (Sekiguchi and Kohshima, 2003). This resting behavior is likely the result of aquarium schedules. Therefore the sleep schedules of these cetaceans are similar to those of terrestrial mammals such as humans that are active during the day and sleep at night. It seems likely then that if testosterone secretion is dependent on sleep cycles, then testosterone secretion in cetaceans housed in aquariums would follow a similar pattern as terrestrial mammals, reaching a peak in the morning hours and reaching a nadir in the evening hours. Because the existence of diurnal

variation greatly affects the value of what time of day samples are collected, determining whether or not diurnal variation is occurring in the secretion of testosterone in beluga whales is a crucial to the goal of being able to determine reproductive condition for an unknown whale using hormone analysis from blow samples.

Methods

In this study, blow was collected from two male beluga whales, a juvenile and an adult, at the Mystic Aquarium in Mystic, Connecticut. The belugas are trained to exhale by signal of the trainer. Blow was collected from both males three times a day at 9:15 am, 11:00 am, and 2:15 pm for a period of four days over the course of two weeks. A total of 24 samples were collected (12 samples from each whale).

Blow samples were collected using nylon stretched across petri dishes. After collection, the nylon was removed and placed in a conical tube with a stopper at the bottom and these were stored at 80 °C. The tubes were then thawed and centrifuged for 30 minutes at3500 RPM to spin off any excess fluid and the mass and volume were recorded. Processed samples were stored at 80 °C until the time of the assay. Before assaying each sample, they were thawed and centrifuged for 10 minutes in a microcentrifuge at 10,000 RPM to remove any particulates.

A competitive enzyme immunoassay (EIA) was used to determine the concentrations of testosterone based on the color change that results during the reaction (Cayman Chemical, Ann Arbor, MI). This assay had previously been validated for use with beluga blow samples (Richard et al., unpublished results). Samples were assayed at a 1:2 dilution, requiring 55 µl of sample. A spectrophotometer was used to measure the degree of absorbance, keeping in mind that the darker the color, the greater the absorbance and the lower the concentration of hormone in the sample. Each sample was assayed in duplicate. The average testosterone concentration for both whales at each time of collection was calculated and used to analyze the pattern of diurnal variation. A repeated measures ANOVA was used to assess the relationship between collection time and testosterone concentration. The difference in concentrations between each of the collection times was assessed using a pairwise t-test.

Results

The two male belugas in the study had similar ranges in testosterone concentration (Fig. 1). When the average testosterone concentration for each of the collection times was calculated, there was not a similar pattern of secretion between the two whales (Fig. 2) The juvenile male, Juno's concentration peaked at 11:00 am. The mature male, Naluark's concentration decreased continuously throughout the entire period of collection. When the concentration values at each of the collection times are viewed for the four days separately, it is evident that the concentration values were all within 50 pg/ml of each other, except for an outlying value for each of the whales (Fig.3 and Fig.4) The repeated measures ANOVA gave a p-value of 0.389, indicating that the relationship between time and testosterone concentration were not significant. The pairwise t-test indicated that there was no significant difference in testosterone concentrations between any of the collection times.

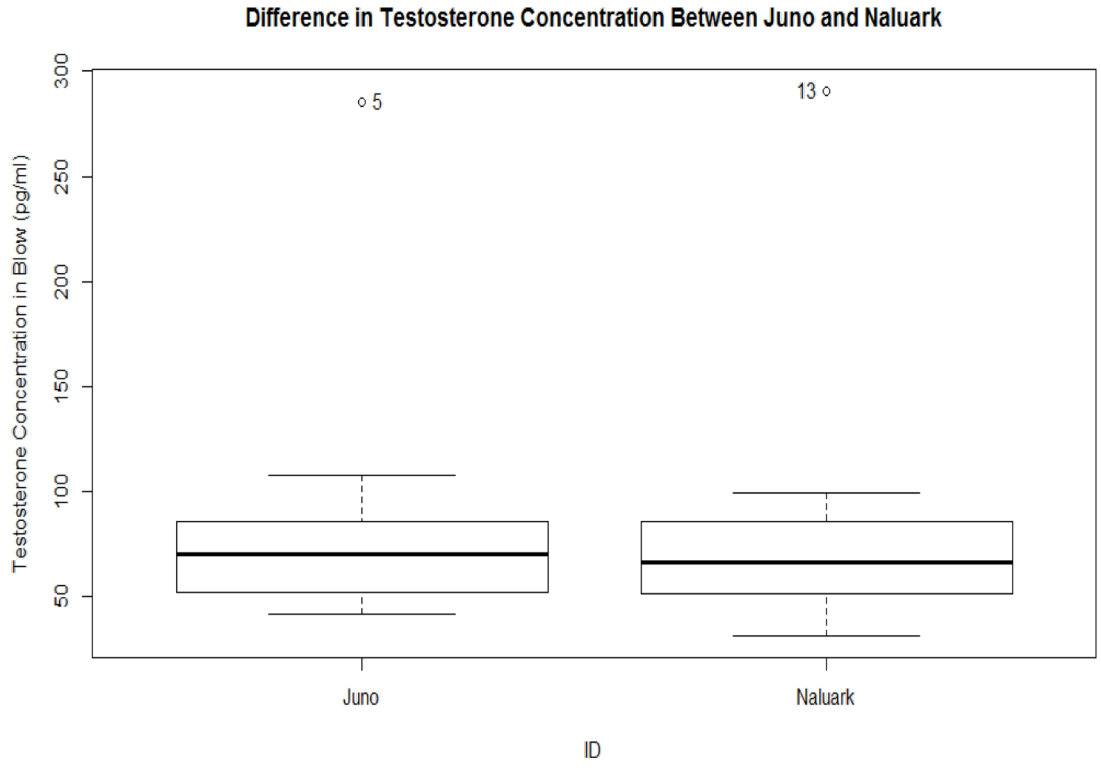

Figure 1. The ranges in testosterone concentration for the beluga whales, Juno and Naluark. Juno is a juvenile male and Naluark is a mature male. The bold line indicates the median value for each of the ranges in testosterone concentration.

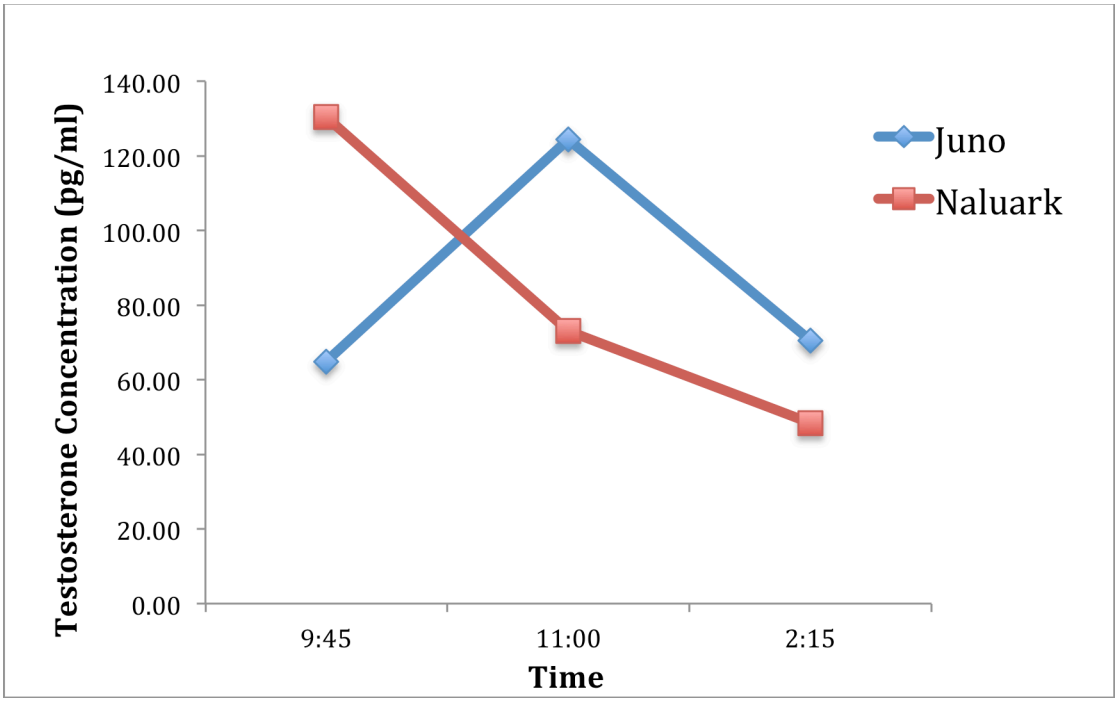

Figure 2. The average testosterone concentrations (pg/ml) in the blow of two different male beluga whales collected at three different times of day. Samples were collected on four separate days.

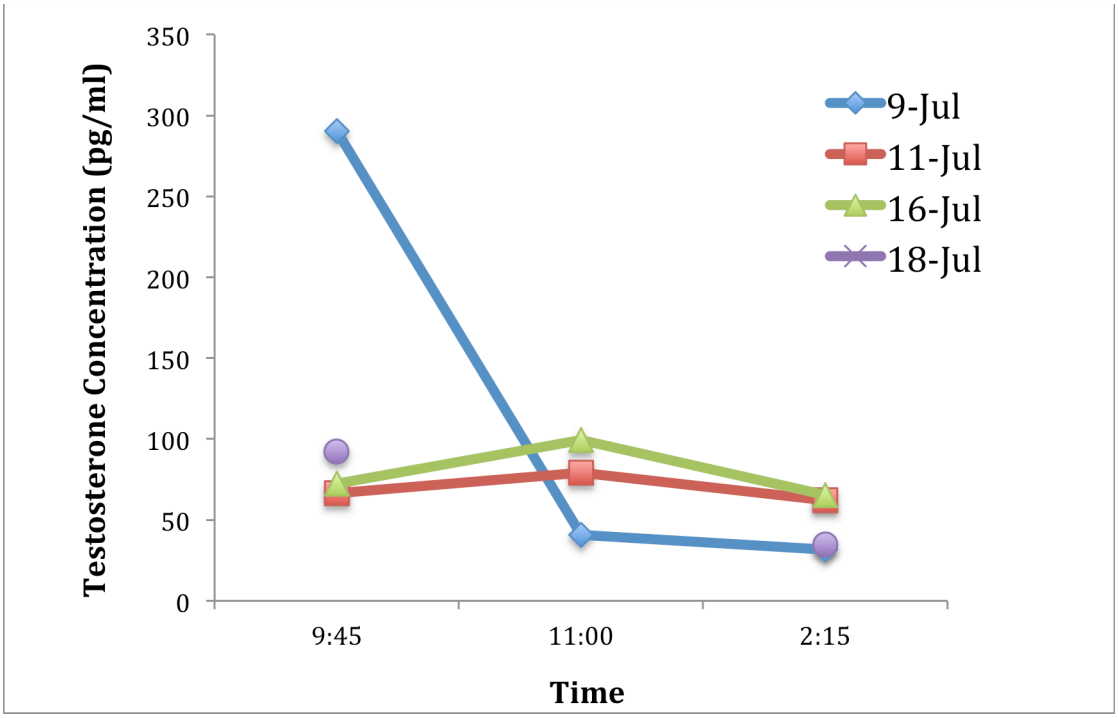

Figure 3. Testosterone concentrations (pg/ml) of Naluark, a mature male beluga whale, for each of the individual samples collected. Each line represents a day of sample collection. The 11:00 am data point for July 18th was not available as there was an insufficient volume of sample collected. The 9:45 am sample on July 9th is an outlying value.

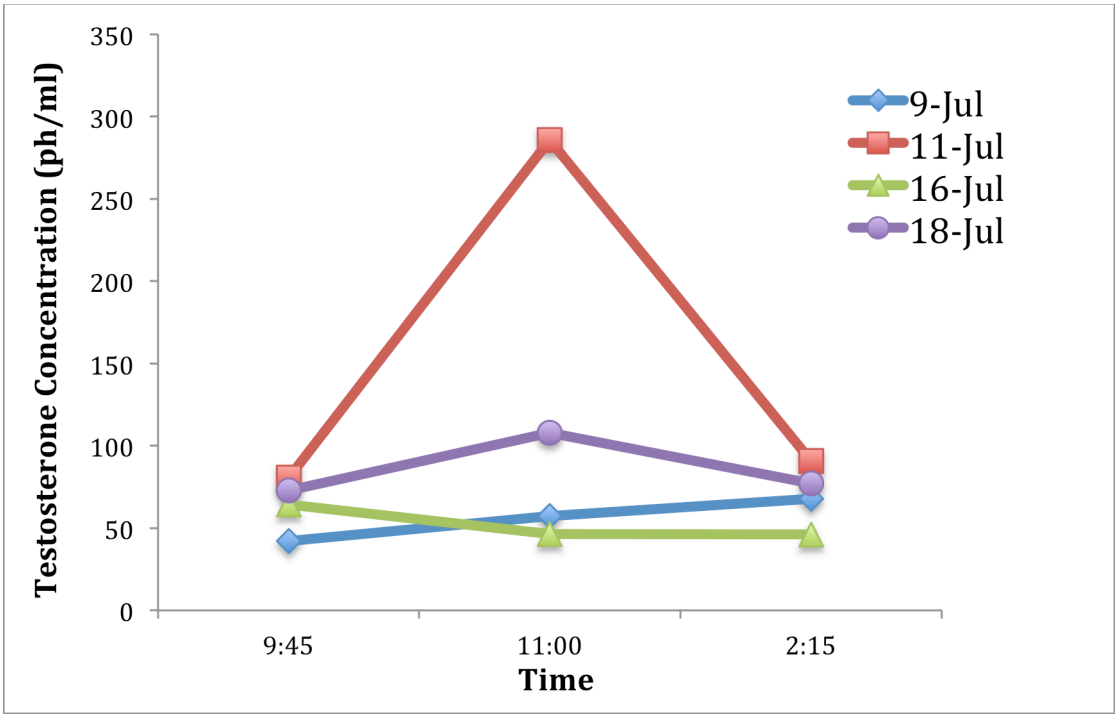

Figure 4. Testosterone concentrations (pg/ml) of Juno, a juvenile male beluga whale, for each of the individual samples collected. Each line represents a day of sample collection. The 11:00 am sample on July 11th is an outlying value.

Discussion

The fact that the ranges in testosterone concentration were similar between Juno and Naluark is interesting given that Naluark is mature and Juno had yet to reach maturity at the time of the study. Since testosterone is a hormone produced in the testes, a greater the amount of testicular volume should produce more testosterone. Since Naluark's testes were three times larger than Juno's, it would be reasonable for Naluark's testosterone concentrations to be greater than Juno's, but this does not seem to be the case. Given that their concentrations are so similar, despite them being at different maturity level, it is concerning whether or not this method could be used to determine maturity level in an unknown whale outside the breeding season. Within the breeding season there is a detectable difference in testosterone concentrations between juvenile and mature males, with mature males having significantly higher concentrations (Rolland et al., 2005).

Disregarding the outlying data points, testosterone concentrations were very close at each of the collection times and there was no significant relationship between time of collection and testosterone concentration. This would indicate that diurnal variation in

testosterone secretion is not occurring, at least not within the time span studied. It is possible that testosterone concentrations changes in blood are too small to be detected accurately in blow. It is also possible that their unique method of sleep (USWS) plays more of a factor in their testosterone secretion than the period of the day in which they are resting and when they are active, which would indicate that the original hypothesis was incorrect.

Other studies have explored the existence of diurnal variation of hormones in cetaceans. There is no evidence of diurnal variation of catecholamines – epinephrine, norepinephrine, and dopamine – in Indo-Pacific bottlenose dolphins (*Tursiops abuncus;* Suzuki et al., 2012). There is also no diurnal variation of the hormone melatonin (Funasaka, 2011). Secretion of catecholamines and melatonin are both dependent on sleep cycles. Cortisol secretions on the other hand do exhibit diurnal variation in both bottlenose dolphins and orcas (*Orcinus orca*), with concentrations highest in the morning hours and lowest throughout the night (Suzuki et al., 2003). What is significant is that cortisol secretion is not dependent on sleep cycles. The results of all of these studies seem to indicate that hormone secretions that are influenced by sleep do not demonstrate diurnal variation in cetaceans, where as those that are independent of sleep cycles do show diurnal variation patterns.

Despite the fact that the results of this study indicated diurnal variation in the secretion of testosterone was not occurring, future research would be advantageous to determine the full picture of testosterone secretion in belugas housed in aquariums. Future studies should collect a larger number of samples over a longer period of time, and to collect samples at later times in the day. This could help to determine how or if a trend is occurring in testosterone secretion over an entire 24-hour span or during certain reproductively relevant periods such as the breeding season.

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