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Enhancing reproductive assessments of the Florida manatee *Trichechus manatus latirostris* by establishing optimal time period and inhibin B baseline concentrations

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ABSTRACT: The Florida manatee *Trichechus manatus latirostris* occupies coastal and riverine habitats that may influence the species' endogenous biological rhythms, including its reproductive potential. Inhibin B provides a biomarker of gonadal function and reproductive potential in humans and other eutherian mammals. This study examined the influence of size, sex, and time of year on inhibin B levels in manatees sampled among 3 habitats with varying degrees of environmental stress in Florida. Inhibin B levels in 38 males averaged (±SE) 4.90 ± 0.23 pg ml^{-1}; the average level in 31 females was 5.63 ± 0.46 pg ml^{-1}. Elevated patterns in inhibin B were exhibited between mid-March and mid-August corresponding to increased mating activity and testicular function, with significant differences in inhibin B levels between male and female manatees ($p = 0.03$) throughout the year. No significant differences in inhibin B were detected between low- and high-impacted sampling locations during winter, suggesting the potential influence of environmental stress on manatee reproduction may be best examined between mid-March and mid-August—the midpoint of the reproductively active, non-winter time period. Establishing temporal baselines for inhibin B values may be useful in assessing manatee reproductive status and potential conservation threats, shedding light on fertility potential, and enabling future assessment of the effects of stressors on reproduction in Florida manatees.

KEY WORDS: Florida manatee · Inhibin B · Reproduction · Conservation

1. INTRODUCTION

Ovarian granulosa cells and testicular Sertoli cells are the targets of follicle stimulating hormone (FSH) activity (Braunstein 2011) in mammals, and produce peptide hormones of the transforming growth factor beta (TGF-β) superfamily. These hormones have been extensively studied in humans (Hu et al. 2011, Suresh et al. 2011), and to the extent they have been studied in other mammals, their functions and modes of action appear to be consistent among species (e.g. Myers & Pangas 2010, Dow et al. 2011). The TGF-β superfamily includes hormones that affect reproductive success in both males and females, including inhibins, activins, follistatin, and anti-Müllerian hormone (AMH; Bilezikjian et al. 2006, Trombly et al. 2009). Of the various TGF-β superfamily peptides listed above, the 3 that have shown particular promise as diagnostic

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tools for assessing reproductive potential are AMH and inhibins A and B (Toulis et al. 2010, Karkanaki et al. 2011).

Initially studied to understand their role in reproduction and development in humans (Welt et al. 1999, Knight & Glister 2006), these peptide hormones have also been investigated in several terrestrial mammalian taxa, such as rodents (Buzzard et al. 2004, Kevenaar et al. 2006), bovines (Ireland et al. 2008), ovines (Campbell & Baird 2001), 3 species of marine mammals (Wilson et al. 2011, Schwierzke-Wade 2011, Lehnert et al. 2019), and 2 different echinoderms (Roudebush & Chosed 2019). The utility of measuring these hormones in a comprehensive evaluation of the conservation status of protected mammalian species has been considered (Marsh et al. 2011, Wilson et al. 2011), but seldom employed to further promote understanding of expression and function.

Levels of inhibin B reflect ovarian reserve and function during folliculogenesis in most female mammals (Roudebush et al. 2008), and levels in serum increase dramatically during folliculogenesis and are maximized just prior to ovulation. Inhibin B measurement can be a useful endocrine marker for monitoring male and female gonadal function (Meachem et al. 2001, Luisi et al. 2005, Dow et al. 2011) as well as a biomarker for overall health assessment (Lehnert et al. 2019). These hormones (AMH and inhibins A and B) are more sensitive markers of reproductive potential than other molecules (e.g. estradiol or testosterone; Roudebush et al. 2008).

The conservation status and reproductive biology of the Florida manatee *Trichechus manatus latirostris* have been considered (e.g. Hernandez et al. 1995, Larkin et al. 2005, Marsh et al. 2011). However, there have been only 2 TGF-β hormones examined to date in Florida manatees (AMH, Wilson et al. 2011; inhibin B, present study). The subspecies occupies coastal and riverine habitats, primarily in the southeastern United States. Although manatees are extremely adaptable, proximity to people has contributed to their exposure to a range of anthropogenic and environmental stressors that may influence endogenous biological rhythms, including but not limited to winter cold, chemical contaminants, watercraft-related noise, collisions with boats, harmful algal blooms, habitat alteration, and physical disturbance. The influences of anthropogenic stressors are difficult to assess in marine mammals, because of the complex logistics and legal limitations imposed on collecting tissue samples, and expressly prohibited experimental studies for protected species.

The Florida manatee is listed as threatened under the US Endangered Species Act. Thus, carefully validated diagnostic biomarkers, used to assess key biological processes, can be proxies for more invasive sampling to evaluate potential anthropogenic impacts. Biomarker and bioassay responses are readily quantifiable and can form a basis of long-term observational series to determine change over time (Langston et al. 2007). Among the possible sub-lethal consequences to manatees of exposure to single or cumulative stressors is reduced reproductive potential, which could impair population sustainability. Reproductive effects of stressors can be assessed, in part, by creating baselines for TGF-β peptide hormones, using presumably normal animals sampled throughout the year. This information could then be used for future studies using inhibin B to compare gonadal function and reproductive potential in individuals known to have been exposed to particular insults or stressors.

The objective of this study was to determine inhibin B levels in Florida manatees in order to characterize the relationship of concentrations of inhibin B to sex, body length (presumed maturity), winter and non-winter seasons (seasons defined by convention), and select representative environmentally stressed locations during different seasons. Manatees are generally considered sexually mature at a body length of ≥264 cm; individuals with a body length <264 cm are classified as immature (Marmontel 1995, Marmontel et al. 1996, Bonde et al. 2012). Manatees with a range of body lengths were sampled in this study to ensure that both mature and immature reproductive statuses would be assessed for both genders.

The gestation period for the Florida manatee is approximately 1 yr, and there appears to be a peak for breeding and calving in spring/summer, although breeding may occur at any time during the year. The division into winter (loosely identified for manatees as November/December–February/March) versus non-winter (mid-March–October) months is made because winter is typically a time of reduced testicular activity, reduced mating activity, and fewer births for Florida manatees than the collective non-winter months (Hernandez et al. 1995, Wright et al. 2002, Deutsch et al. 2003). In addition, large fetal manatees are reported primarily in late winter, spring, and early summer (Fig. 1). Thus, the most logical choice for testing seasonality of manatee reproductive potential as measured by inhibin B concentration centers on refining definitions for winter versus warmer breeding seasons.

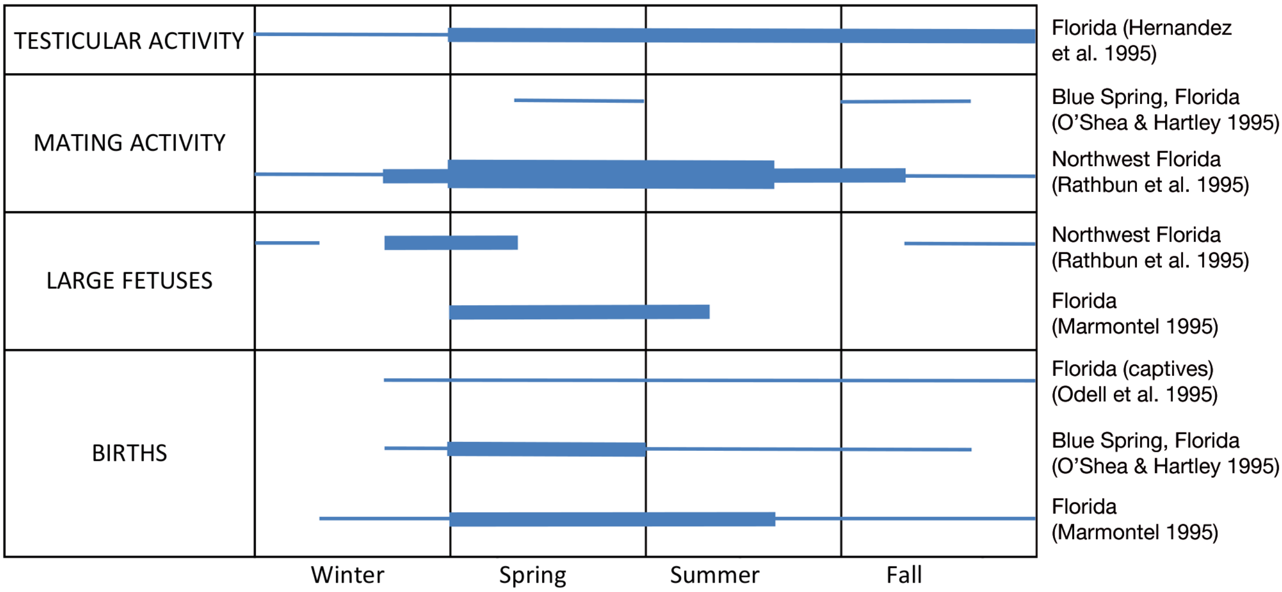

Fig. 1. Seasonal relationship of various reproductive measures in the Florida manatee. Horizontal line thickness corresponds to the degree to which a particular component occurs in specific seasons. Adapted with permission from Marsh et al. (2011)

Manatees were sampled from 3 different Florida locations representing a range of impacted environments (the low/non-impacted Crystal River; the moderately impacted Everglades National Park; and the highly impacted urban and agricultural Indian River Lagoon) to investigate means to assess the potential environmental impact effects on inhibin B levels (see Fig. 2). By establishing temporal baselines in manatees and documenting alterations associated with various biological conditions, scientists may ultimately be able to discern not only intra-annual patterns, but also variances in concentrations that may be due to possible influences of environmental stressors (e.g. contaminants, noise, habitat loss, etc.) on population-level reproductive potential and fitness.

2. MATERIALS AND METHODS

2.1. Animals

Florida manatee serum samples from 38 males and 31 females were obtained for analysis. The serum samples were collected during routine health surveys conducted by the US Geological Survey (USGS) Sirenia Project, and the Florida Fish and Wildlife Conservation Commission (FWC) from 2005–2011 at 3 locations in Florida (Fig. 2). Samples were taken during winter (defined here as 15 November–15 March) and non-winter time periods at Crystal River in the northwest (n = 32), during non-winter only for Everglades National Park in the south (n = 26), and during winter only in the Indian River Lagoon in Brevard County in the east (n = 11). Blood was obtained from the brachial vascular bundle of the medial pectoral flipper (Walsh & Bossart 1999, Stamper & Bonde 2012). Following collection, the whole blood was permitted to clot and then was centrifuged; serum samples were removed and subsequently stored at –80°C until analysis. Manatee demographic data collected for comparison with inhibin B concentrations included sex, collection date, total body length (cm), and geographic location of sampling (see Tables 1 & 2). Chronological age cannot be determined in living manatees.

Samples were taken from animals ranging in total body length from 154–324 cm, which would include both mature and immature reproductive status samples. The general health of each animal was evaluated prior to inclusion in the study; all individuals were considered to be healthy. Health status was determined by proxy with the standard clinical analysis of serum amyloid A (SAA) and is part of the routine USGS manatee health assessment to monitor for signs of inflammation, disease, and/or severe injury. No animals were included in this study if they did not have normal SAA values (Harr et al. 2006). Reproductive status for the sampled female manatees was not assessed via pregnancy test, and visual detection is only evident during the last trimester of pregnancy. However, none of the females appeared to be pregnant nor were there any mother/calf pairs observed.

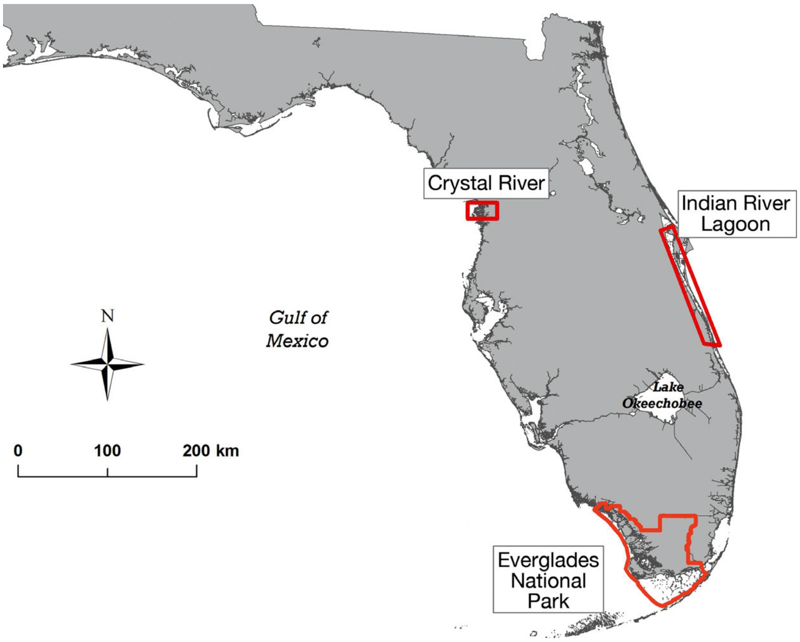

Fig. 2. Florida manatee sampling locations

2.2. Serum hormone analysis

Levels of inhibin B in the Florida manatee samples were determined by an enzymatically amplified 2-site immunoassay (GEN II ELISA; Beckman Coulter). The assay was validated for cross-reactivity with the Florida manatee by demonstrating parallelism between serial dilutions of manatee serum (neat, 1:2–1:64) and the inhibin B calibration standards. The dual monoclonal antibodies used were a highly characterized antibody pair that are specific to, and bind only to, the mature conserved region of inhibin B, and when validated, measured 100 % inhibin B in human, monkey, and rat. Inhibin A, activins A and B, AMH, follicle stimulating hormone, and luteinizing hormone were tested as potential cross-reactants and were found to be non-detectable. The limit of detection was 2.6 pg ml^{-1}.

Samples were processed in duplicate on a Dynex DS2 automated instrument (Dynex Technologies) following the manufacturer's instructions. Absorbance measured was directly proportional to the concentration of inhibin B. Standards were used to plot a log–log linear regression calibration curve of absorbance versus inhibin B concentration ($r^2 = 0.99$). The sample concentrations were calculated from the calibration curve fit equation. The assay sensitivity was 3.18 pg ml^{-1} and the intra- and inter-assay CVs were 1.71 and 1.81 %, respectively.

2.3. Statistical analysis

Statistical analyses were performed using R (R Core Team 2017); results were considered statistically significant at $p \le 0.05$. Serum inhibin B levels from manatees were analyzed to establish temporal baselines, based on 3 different factors: (1) sex; (2) body length (used as a proxy for designating maturity status); and (3) date of sample collection. Intra-annual patterns in manatee inhibin B concentrations were examined with a generalized additive model (GAM):

$$Y_{ij} = \mu + g(\text{day}) + \alpha_i + \beta(X_j - X) + \varepsilon_{ij} \quad (1)$$

where Y_{ij} is the inhibin B concentration of individual j of gender i (male or female); μ is the intercept term; g(day) is a nonparametric smoothing function for the day of the year; α_i represents the effect of gender i; β is the slope of the regression of inhibin B concentrations on manatee body length centered around the overall mean size of 265.71 cm ($X_j - X$); and ε_{ij} is the random unexplained error, which is assumed to be independent and normally distributed. Although model residuals exhibited minor heteroscedasticity, the inverse square-root transformations required to address this assumption were not applied to allow for model interpretability. To fit the smoothing function, the associated degrees of freedom ($k = 4$) were identified as the model yielding the minimum generalized cross-validation score. Preliminary data analyses did not detect differences in inhibin B levels among years, so year of sample collection was not included in the model. We considered incorporating the location of sample collection; however, as the limited temporal overlap of samples among locations produced spurious model results, we excluded location from the model to focus on temporal patterns.

Spatial differences in inhibin B concentrations were able to be tested between samples collected from the low/non-impacted Crystal River and the highly impacted Indian River Lagoon locations during winter using the general linear model:

$$Y_{hij} = \mu + \gamma_h + \alpha_i + \beta(X_j - X) + \varepsilon_{hij} \quad (2)$$

where model terms were as before, but with γ_h representing the effect of sampling location h. Again,

Table 1. Mean (±SE), quartile, and range for inhibin B levels (pg ml^{-1}), and mean (±SE) body lengths (cm) of all manatees sampled, by sex and the proposed periods for winter, peak reproductive activity (mid-March to mid-August), and transitional state (October; normally defined as a non-winter month) timeframes to distinguish peak inhibin B levels

Season	Sex	No. of samples	Mean length	1st quartile inhibin B	Mean inhibin B	3rd quartile inhibin B	Range inhibin B
Winter	F	19	271 ± 9	4.04	4.99 ± 0.42	5.25	3.18–11.77
	M	21	259 ± 7	3.50	4.25 ± 0.20	4.76	3.13–6.53
Mid-March to mid-August	F	7	268 ± 10	5.17	7.45 ± 1.54	8.11	4.22–15.63
	M	14	272 ± 11	4.51	5.96 ± 0.41	6.76	4.17–9.27
October	F	5	288 ± 6	4.97	5.50 ± 0.49	5.59	4.45–7.30
	M	3	205 ± 47	4.48	4.54 ± 0.09	4.62	4.37–4.65

inverse square-root transformations were not applied to address minor heteroscedasticity in model residuals to allow for model interpretability.

3. RESULTS

Measured inhibin B levels ranged from 3.13–9.27 pg ml^{-1} (mean ± SE = 4.90 ± 0.23 pg ml^{-1}) in males and 3.18–15.63 pg ml^{-1} (5.63 ± 0.46 pg ml^{-1}) in females (Tables 1 & 2). Model-based mean values of inhibin B for males (4.78 pg ml^{-1}) were 0.99 ± 0.46 pg ml^{-1} lower than for females (5.77 pg ml^{-1}; p = 0.03) after adjusting for inter-annual patterns and the effect of body length, albeit length did not exhibit a significant influence on inhibin B concentrations (p = 0.85). However, it is interesting to note that males in the lowest quartile of observed inhibin B values (<3.87 pg ml^{-1}) averaged 252 cm in length (below the commonly used 264 cm cutoff for identifying mature individuals), but were as long as 308 cm, whereas females in the lowest quartile of observed inhibin B values (<4.31 pg ml^{-1}), averaged 282 cm in length but were as long as 320 cm. Individuals in the highest quartile of observed inhibin B values (>5.58 pg ml^{-1} for both sexes) averaged 272 cm in total body length for males and 268 cm for females, but included individuals as small as a 203 cm male and a 246 cm female. These data highlight the importance of identifying the time period during which reproductive potential is best assessed, as size-based measures may not properly reflect maturity status.

Generally, mean inhibin B values were elevated from mid-March to mid-August (Fig. 3). Concentrations returned to overall mean levels by October, and were reduced in individuals sampled during December and January (Table 1). Based on the mid-March to mid-August time frame identified in this study, the highest quartile of observed inhibin B values was >6.76 pg ml^{-1} for males and >8.11 pg ml^{-1} for females. Overall inhibin B levels were not elevated during October, given that the highest quartile observed for samples collected in October was only >4.62 pg ml^{-1} for males and >5.59 pg ml^{-1} for females. Notably, mean inhibin B concentrations measured in samples collected from females in October were almost 2 pg ml^{-1} lower than females collected between mid-March and mid-August, even though the females from the October sampling were among the largest individuals (5 ind. averaging 288 cm).

Table 2. Mean (±SE) inhibin B levels (pg ml^{-1}) and body length (cm) of Florida manatees, by the proposed winter, peak (mid-March to mid-August), and separate October (normally defined as non-winter month) seasons and by sampling location

Location	Season	Sex	No. of samples	Mean length	Mean inhibin B
Indian River Lagoon	Winter	F	3	291 ± 17	5.39 ± 0.82
		M	8	262 ± 13	4.47 ± 0.29
Crystal River	Winter	F	16	267 ± 11	4.92 ± 0.49
		M	13	257 ± 7	4.11 ± 0.27
	October	F	1	286[a]	4.45[a]
		M	2	158 ± 4	4.48 ± 0.04
Everglades National Park	Peak	F	7	268 ± 10	7.45 ± 1.54
		M	14	272 ± 11	5.96 ± 0.41
	October	F	4	289 ± 7	5.76 ± 0.53
		M	1	298[a]	4.65[a]

[a]Only 1 sample, no mean or SE calculated

Manatees included in this study were sampled in environments representing a range of anthropogenic and environmental stressors: low/non-impacted Crystal River; moderately impacted Everglades National Park; and highly impacted Indian River Lagoon. The mean inhibin B levels within particular demographic groups were not significantly different between Crystal River and Indian River Lagoon during winter (p =

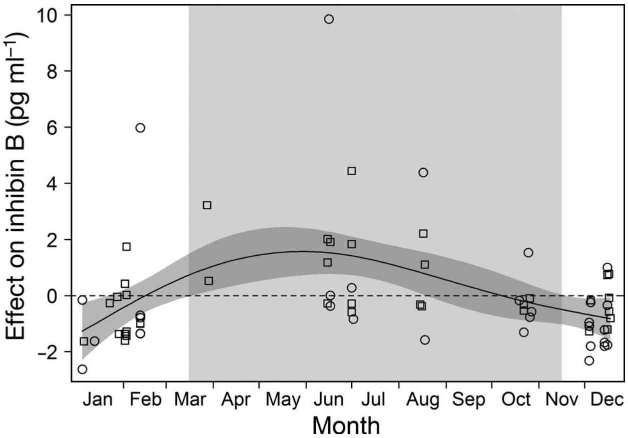

Fig. 3. Intra-annual patterns in inhibin B for Florida manatees sampled from Indian River Lagoon, Crystal River, and Everglades National Park, FL. Solid line: the smoothing function fit with the generalized additive model; dark grey shading: ±2 SE. Shaded box: the generally applied non-winter season for assessing reproductive potential; dashed line at 0: model-based mean inhibin B values for each sex (males: 4.78 pg ml^{-1}; females: 5.77 pg ml^{-1}), thus, the *y*-axis represents a deviation from that mean over time. Points: partial residuals for males (squares) and females (circles), indicating individual deviations from temporal patterns in inhibin B (without transformation) after adjusting for the effects of gender and body length

0.39), despite slightly higher inhibin B concentrations in individuals from Indian River Lagoon (Table 2). For this model, body size did not have a significant influence on inhibin B concentrations (p = 0.48), and male inhibin B concentrations were nearly significantly different than those of females (p = 0.07). Model-based means of males were 0.87 ± 0.46 pg ml^{-1} lower than females, a difference that is similar to the 0.99 ± 0.46 pg ml^{-1} difference reported by the GAM model, suggesting the number of sample sizes available during winter limited the model's ability to detect a significant gender effect.

4. DISCUSSION

In previous studies of the role of the inhibins in humans and select other mammals, it has been shown that inhibins primarily regulate gametogenesis by inhibiting the production and/or secretion of FSH from the anterior pituitary. In males, inhibin B is secreted by the prepubertal testis Sertoli cells during maturation, and the serum level of inhibin B is considered to reflect Sertoli cell function. Post maturation, production of inhibin B is dependent on FSH and the spermatogenic status. Serum inhibin B level may be an appropriate marker of the function and maturation of Sertoli cells in males and, as such, an indicator of maturity and an index of spermatogenesis (Meachem et al. 2001). In females, inhibin B is regulated by FSH stimulation, which induces proliferation and differentiation of granulosa cells from mammalian follicles. It is produced in the gonads, pituitary gland, placenta, and corpus luteum, and increases during the luteal-follicular transition, with peaks in the mid-follicular phase and again at ovulation. As with males, concentrations of inhibin B in females are low until maturation begins and then peak at full maturation but decrease as a function of senescence (Battistini et al. 2002, Crofton et al. 2002). Ultimately, the ovarian reserves of female mammals are expected to become depleted with age and as reproductive senescence naturally ensues in older individuals (Cohen 2004). Ovarian reserve can be evaluated by measuring inhibin B levels in serum (Welt et al. 1999).

While there are no studies validating that this hormone plays the same roles in manatees, in order to interpret the results from the present study, we infer that this is the case for most (if not all) mammals, including manatees (e.g. Hu et al. 2011). Data show that mean inhibin B levels (all body lengths, representing mature and immature animals combined) were 4.90 ± 0.23 pg ml^{-1} for males and 5.63 ± 0.46 pg ml^{-1} for females; among the lowest averages reported in mammals (e.g. Mann et al. 1997, Buzzard et al. 2004, Kumanov et al. 2006). Inhibin B levels were significantly higher (p = 0.03) in female manatees than in males, and linear models showed no significant relationship between body length (presumed maturity) and inhibin B levels for either sex (males: p = 0.46; females: p = 0.42). Schwierzke-Wade (2011) provided the only other inhibin B levels available for marine mammals; they were measured in bottlenose dolphin *Tursiops truncatus* but did not include evaluation of seasonal influences. In that study, levels of inhibin B were higher in female dolphins sampled from Florida and lower in those sampled in Georgia, and no relationship of body length to inhibin B level was observed (Schwierzke-Wade 2011). However, for another TGF-β family member, significant differences (p < 0.01) were seen with body length and AMH levels in male but not in female manatees (Wilson et al. 2011).

The levels of inhibin B were highest in both male and female manatees sampled between mid-March and mid-August (Tables 1 & 2), similar to the trend observed with AMH in manatees (Wilson et al. 2011). However, the generally accepted division into winter (November/December–February/March) versus non-winter (mid-March–October) seasons — characterized by levels of testicular activity, mating activity, and births for Florida manatees — may not be appropriate for monitoring of inhibin B levels of peak and non-peak identification for reproductive potential. Results for the month of October suggest that this period may be a transitional time for inhibin B levels in manatees rather than a reflection of the average values found in the other 2 seasons of winter and non-winter (Tables 1 & 2, Fig. 3). Therefore, for projects aiming to test inhibin B levels against seasonal baselines of manatee reproductive potential as measured by inhibin B concentration, the currently defined winter and non-winter time periods should exclude the month of October for sampling and analysis.

It seems likely that manatees breed rarely in winter based on general observations of reduced mating behavior (O'Shea & Hartley 1995, Rathbun et al. 1995, Reid et al. 1995). In males, reduced spermatogenesis (Hernandez et al. 1995), smaller testicular mass (Reynolds et al. 2004), and lower fecal testosterone levels (Larkin et al. 2005) have been documented (see Fig. 1). These results are consistent with the observation that the inhibin B level acts as a marker for gonadal function in male mammals and phase of estrous cycle in non-human female mammals (Knight & Glister 2006). One Everglades National Park male, measuring only 220 cm in body length, had a relatively high inhibin B value compared to its size (9.27 pg ml^{-1}), suggesting, perhaps, that this animal may have been precocious. To date, the smallest male manatee documented to have spermatogenic testes measured 237 cm long and was 2 yr old (Hernandez et al. 1995). Interestingly, in humans, inhibin B is a useful screening tool for diagnosing disorders of pubertal development, either delayed or precocious (Lahlou & Roger 2004).

Most births of wild manatees in Florida occur during the non-winter months (O'Shea & Hartley 1995, Rathbun et al. 1995, Reid et al. 1995). Inhibin B levels rise during folliculogenesis and peak just prior to ovulation in humans and some other mammals, suggesting that the point in the estrous cycle when the samples are collected may be an important consideration. If that is also true in manatees, it could explain the relatively high inhibin B levels (>10 pg ml^{-1}) of 3 presumed mature females possibly suggestive of folliculogenesis. However, although 2 were sampled during the non-winter peak breeding season in Everglades National Park, one was sampled in Crystal River during the winter period — a time of minimal breeding when many females undergo a seasonal depression in their reproductive hormones and reproductive capabilities (Fig. 1).

In human females, levels of inhibin A and B decline with age and, by approximately 1 yr before onset of menopause, neither inhibin can be detected (Øverlie et al. 2005). With regard to inhibin B levels declining in female manatees to undetectable levels if/as senescence approaches (as in humans), the lowest concentration measured in this study was 3.18 pg ml^{-1} in a 240 cm female (less than the conventional maturation length threshold of 264 cm). Thus, it seems plausible that no evidence of ovarian reserve decline was noted, an observation that suggests reproductive senescence may be unusual or non-existent in healthy manatees.

Although we were unable to test the influence of location on inhibin B levels throughout the year due to limited sample availability, no significant differences in inhibin B were detected between samples collected from the low/non-impacted Crystal River and the highly impacted Indian River Lagoon sampling locations during winter. Reproductive potential (as measured by inhibin B levels) may be robust to environmental disturbances, but we expect that potential site-specific differences in inhibin B levels associated with stressors such as toxicants and exposure to cold would be measurable if larger sample sizes were available from more locations during the time period that inhibin B concentrations are expected to be elevated. Specifically, it is important to consider whether exposure to endocrine-disrupting compounds such as polychlorinated biphenyls (PCBs) and organochlorine pesticide (OCP), common in human waste and Florida coastal waterways (Atkinson et al. 2003, Cox et al. 2004, Fair et al. 2010), may be causing some unusual circumstances with regard to reproductive hormone levels in manatees of either sex (e.g. Monsees et al. 2000, Fossi & Marsili 2003, Uzumcu et al. 2006). In fact in humans, the inhibin B level represents 'a very fast marker of testicular damage' and has been suggested as an important and rapid marker of spermatogenic disorders in populations exposed to testicular toxicants (Meachem et al. 2001, p. 568).

While there have been a number of studies on the links between contaminant exposure and adverse effects on reproduction in marine mammals, few have specifically evaluated the effects on sex hormones. Prior research has assessed the effects of stressors, including endocrine disruptors, on Sertoli cells and found increased inhibin B production (Monsees et al. 2000, Comhaire & Mahmoud 2003). A comparison of levels of known endocrine disruptors (i.e. PCBs) analyzed in bottlenose dolphin blubber collected from Florida sampling sites (Sarasota Bay and

Indian River Lagoon; Fair et al. 2010, Schwierzke-Wade 2011) were significantly lower than those sampled in Georgia (Turtle/Brunswick River; Balmer et al. 2011).

Knowing the status of potential reproduction impairment in wild populations can be useful for environmental health assessments and developing effective conservation strategies, especially for endangered and protected taxa. Baseline studies are becoming increasingly relevant with regards to evaluating potential effects of environmental stressors on the endocrine system of marine mammals. Since inhibin B analyses can help to assess the reproductive potential of humans and other mammals, creation of baseline values for this and related hormones in manatees may allow scientists and managers to relate these findings to a range of parameters including, but not limited to, genetic fitness, geographic location, nutritional status, exposure to biotoxins or contaminants, exposure to cold, or influences of disease. Policy makers, regulators, and resource managers need convincing evidence to support the possible sublethal effects of environmental and anthropogenic stressors on fertility of wild populations so that effective steps can be taken towards mitigating these stressors on critical biological functions where possible (O'Hara & O'Shea 2005). The capacity to evaluate the reproductive quality of individuals comprising a population allows scientists and managers to relate effects of environmental or anthropogenic stressors or other conservation risk factors to reproductive potential.

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