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Sex Hormones and Reproductive Status of the North Atlantic Fin Whales (*Balaenoptera physalus*) During the Feeding Season

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Abstract

Reproductive fin whales (*Balaenoptera physalus*) (448 females and 278 males), classified by anatomical/histological methods, were studied for serum sex hormones. Of the 207 females classified as pregnant by anatomical methods, 95% had progesterone (P) levels higher than 9.0 nmol/l with a near symmetrical distribution of $\log_{10}$ P-levels around the mean of 1.55 (35.5 nmol/l geometric mean). More than half of the sexually immature females (n = 157) had P-levels ≤ 0.1 nmol/l. Nonpregnant mature cows were older on average than pregnant cows, suggesting the beginning of reproductive senescence in females before the age of 30 y. The mean serum testosterone (T) of mature males (3.1 nmol/l) was significantly higher than that of immature males (1.0 nmol/l). In mature males, T-levels were positively associated with testicular size, as well as time (day-count) during the summer whaling season. For fin whales, serum P- and T-levels agreed closely with anatomical studies of reproduction and may be decisive when anatomical indexes fail. Furthermore, the serum T-level appears to be an excellent index for monitoring the latter part of the annual male reproductive cycle.

Key Words: fin whale, *Balaenoptera physalus*, testosterone, progesterone, pregnancy, *corpus luteum*, testicles, reproductive senescence, changing hormone levels, North Atlantic

Introduction

The fin whale (*Balaenoptera physalus*) is the second largest in the baleen whale family, Balaenopteridae, and attains weights of around 70 metric tonnes in the northern hemisphere (Víkingsson et al., 1988), with its counterpart in the southern hemisphere still heavier at about 10 to 15% longer. Like most baleen whales, fin whales migrate seasonally every year to higher latitude feeding areas and breed about every other year at lower latitudes as reviewed by Lockyer (Boyd et al., 1999). The mating activity of the northern fin is believed to peak during the months of December and January and, with a gestation period of 11 to 12 mo, the peak calving period is thought to be during the months of November and December each year (Lockyer, 1984; Boyd et al., 1999).

The reproductive status of both the North Atlantic and the Antarctic fin whale has been studied extensively in connection with earlier whaling activities (Laws, 1961; Lockyer, 1984). Information on the sexual condition of females has mainly been obtained by examination of the *corpora lutea* and *corpora albicantia* in the ovaries. Under the assumption that the ovarian scars (*corpora albicantia*) are permanent, the breeding history of an individual can be inferred. Through age determination by earplug layers (Lockyer & Sigurjónsson, 1992), the age at sexual maturity has been established (Lockyer, 1984). Pregnancy has been confirmed by anatomical/histological examination of the uterus and the presence of fetuses, the increasing size of which with time has been used to back-calculate the conception time and the gestation period (Lockyer, 1984; Boyd et al., 1999).

The sexual condition of the males has been assessed by studying histological characteristics of the testes, including the width of the seminiferous tubules by microscopic examination and by the weight of the testes (Laws, 1961; Lockyer & Sigurjónsson, 1992). While Gambell (1968) did not find a seasonal variation in testes size for the Antarctic sei whale (*B. borealis*), Laws (1961) and Lockyer & Sigurjónsson (1992) reported a seasonal trend for the Antarctic and the North Atlantic fin whale, respectively, with increased testes weight in the months close to the beginning of the mating season. No significant change in the mean weight of the ovaries with time during the hunting season has been reported for any

reproductive females, however, even in spite of considerable fattening (Lockyer, 1987; Víkingsson, 1995) caused by ingestion of great volumes of food throughout the summer feeding season (Víkingsson, 1997; Kjeld, 2003).

After classifying 722 fin whales into reproductive categories or classes by the above anatomical methods, we now also have measured the serum sex hormone concentrations and studied their differences among categories, their changes with time during the hunting season, and their association with other available data.

Materials and Methods

Sample Material

Blood samples were obtained at sea from a total of 797 whales, but only 726 of these were anatomically classified—448 females and 278 males. The whales were caught west and southwest off the coast of Iceland from June until the end of August or at the beginning of September during the summers of 1981 to 1988. The collection and use of postmortem blood samples and the stability of steroid hormones in serum have been described (Kjeld et al., 1981; Kjeld, 2001). Within about 15 min after the animal's death, the skin of the fluke was dried with a cloth followed by its lateral third being cut off; blood from the wound was collected into plastic test tubes. The samples were centrifuged at 2,000 rpm at sea and the supernatant serum kept frozen at -80° C until measured about 6 mo and sometimes 2 y later. Fetuses were collected from the uteri of the whales by biologists at the whaling station who recorded their sex and length. Until 1986, however, a number of fetuses were lost due to the whalers' practice of cutting open the whales' bellies at sea for the purpose of cooling the meat. Sometimes one or both ovaries and part of the uterus were lost at sea due to this practice, making the diagnosis of pregnancy by anatomical indicators uncertain. In several of these cases, reproductive status still could be assessed by histological examination of the uterine mucosa (Lockyer & Smellie, 1985; Lockyer & Sigurjónsson, 1992).

Anatomical/Histological Measurements

The anatomical measurements used to decide the reproductive status of the female fin whales were conducted according to earlier reports on fin (Mackintosh & Wheeler, 1929; Laws, 1961) and sei whales (Gambell, 1968). Recovered ovaries were inspected, weighed, and sectioned, and the presence of *corpora lutea* (CL) and *corpora albicantia* (CA) was recorded and their longest diameter measured. The CLs also were removed and weighed. To confirm pregnancy in females with CL and a slit uterus without a fetus, the width of the uterine cornua was measured and also the thickness of the *stratum compactum* of the uterine mucosa (Lockyer & Smellie, 1985). By these anatomical methods, female whales were classified into five reproductive categories: (1) immature, (2) anestrous, (3) pregnant, (4) lactating nonpregnant, or (5) undetermined.

For males, the respective methods of the above cited references were used. The testicles were weighed and then sectioned for histological examination. The width of open seminiferous tubules and their relative preponderance in the testicles was studied under a microscope. Spermatogenesis and the presence of spermatozoa in the tubuli were recorded. From these studies, the males were divided into three categories: (1) immature, (2) pubertal (intermediate), or (3) mature (Masaki, 1976). These histological techniques are further described in more recent publications on studies of toothed whales (Collet & Saint Girons, 1984; Sørensen & Kinze, 1994; Halldórsson & Víkingsson, 2003). By these methods, males were classified into four reproductive categories: (1) immature, (2) pubertal, (3) mature, or (4) undetermined. Total body length was measured from the tip of the snout to the central fluke notch in a straight line on the flensing platform.

Age Determination

The age of the whales was determined by the ear plug method (Purves, 1955; Lockyer, 1984). The technique is based on the identification and counting of light and dark laminae (each pair representing 1 y of age) present in the core of the plug, either with the naked eye or by using a low-power magnifying lens.

Radioimmunoassays (RIAs)

RIAs with solvent extraction and internal standards were chosen to measure the hormones. Precision of RIAs is generally less than that of enzyme immunoassays and ELISA assays, but RIAs are robust, and the extraction step avoids possible matrix effects from the little known serum of whales. The assays for the total (protein bound and free) sex hormone concentrations in serum have been described with regard to their sensitivity and specificity (Kjeld et al., 1992).

Briefly, the testosterone antiserum used was raised in rabbits against testosterone -3-carboxymethyl-oxime-bovine serum albumin. It had a 66% cross-reaction with 5α – dihydrotestosterone, but only 3% and 2% with 5α – androstane – 3β, 17β – diol and 5α – androstane – 3α, 17β – diol, and < 0.7% for a number of other structurally related steroids. The cross-reaction of 5α – dihydrotestosterone was not considered a problem because it

is an androgen of which testosterone is the main precursor and, in human serum, known to be ten times lower in concentration. After the addition of tritium labeled internal T standard, serum samples (0.5 ml) were extracted with six volumes of diethyl ether, which was evaporated at 40° C under a gentle air stream followed by redissolution of the dried extract in 0.5 ml of assay buffer. The assay had a mean inter-assay imprecision of 14% for a sample with T concentration of 3.6 nmol/l and intra-assay imprecision of 8% for a sample with T concentration of 4.9 nmol/l. The detection limit of the assay was 0.1 nmol/l. Mean recovery of the internal standard was 82% (CV = 6).

The progesterone assay has been described (Kjeld et al., 1980), but was used without the chromatography. Instead, after adding the internal standard to 0.5 ml serum samples, they were extracted in eight volumes of petroleum ether (boiling range 40 to 45° C; BDH, Poole Dorset, UK) to reduce the effects of more polar (hydroxylated) interfering compounds (Johansson, 1969). Thus, the average extraction efficiencies of the 11α- and 11β-hydroxyprogesterones were 2 and 9% compared to 78% (CV = 14) for progesterone. Mean inter- and intra-assay imprecision (CV) in the progesterone assay was 18 and 12%, respectively, for a serum pool with a concentration of 6.7 nmol/l. Lower detection limit was 0.1 nmol/l.

For the estradiol assay, a highly specific and sensitive antiserum raised against estradiol-6-carboxymethyloxime-bovine serum albumin was used. Structurally related steroids such as estriol, estrone, and ethynyl-estradiol had a cross-reaction of 0.4, 0.2, and 0.16%. Serum samples of 0.4 ml were extracted in 4.0 ml of diethyl ether, which was evaporated at 40° C under a gentle air stream. The assay had a mean inter- and intra-assay imprecision of 16 and 9%, respectively, for a serum pool of 141 pmol/l. The detection limit for this assay was 15 pmol/l. To reduce the effect of the high inter-assay imprecision, assays were kept large or about 80 to 90 tests per assay when possible.

Conversion factors for the hormone concentrations between the S. I. and the conventional units are as follows: testosterone, nmol/l × 0.288 = ng/ml; progesterone, nmol/l × 0.315 = ng/ml; and estradiol, pmol/l × 0.272 = pg/ml.

Statistical Methods

Scatterplots were used for description of associations between measured variables. Quantitative assessment was made by the product moment correlation coefficient or Kendall's correlation. The Student's two-tailed *t*-test was used to compare categories by their means if appropriate. Otherwise, the Mann-Whitney U test was used for comparison. A linear regression model was adapted to the $\log_{10}$ of the T-levels related to the days (daycount) of the hunting season counted from the 1st of June. The chi-squared (X^2) test was used to compare proportions in categories. Linear trend in proportions was tested by the X^2 test for trend. In figures, the near symmetrical distributions of the $\log_{10}$-transformed concentrations of serum P and T are given (Armitage et al., 2002), but in the tables, concentration values have not been transformed. The level of significance was set at $\alpha = 0.05$.

Results

Females

The number of females in each category is shown in Table 1. Also shown are the means and SDs for serum P and body length, the medians and lower and upper quartiles of the age, and the number of individuals from each category found within three different P-level intervals. These serum P-level intervals are based on the P-level distribution shown in Figure 1 for mature female whales. From the female whales, two additional and overlapping categories of pregnant females were extracted, as shown in the last two rows of Table 1. Among the anatomically classified categories, the pregnant category had by far the highest mean serum P-level (37.4 nmol/l), with 196 (94%) of its P-levels above 9.0 nmol/l. In the last column of Table 1, there are 3 and 2 individuals, respectively, from the immature and anestrous categories with serum P-levels above 9.0 nmol/l (i.e., with 10.5, 12.6, and 23.2 nmol/l and with 12.0 and 50.0 nmol/l, respectively). These extreme values were not included when computing the mean serum P-levels and SD for the respective categories as they were regarded to be of limited use due to errors believed to have occurred during the processing of data or specimens (see below and footnote to Table 1).

Immature females were most numerous ($n = 93$) in the lowest serum P-range ($p \leq 0.1$), but 36 (53%) of the anestrous mature females also had P-levels at the detection limit of the assay (Table 1). Two "anatomically pregnant" females were recorded with P-levels at the lower limit of the assay. Eighty-six of the 90 females with fetuses detected make up about 42% of the 207 anatomically classified pregnant category, but four of these 90 females were measured with serum P-levels < 1.1 nmol/l (Figure 1). When the 279 sexually mature (anatomical classification) females were divided into pregnant ($n = 199$) and nonpregnant ($n = 80$) categories by the serum P-levels (dividing value 9.0, see above), it became apparent that the nonpregnant cows were older. Also, when divided into 5-y age categories, the proportion of the nonpregnant females increased significantly with age (X^2_{trend} =

Table 1. By anatomical methods, 448 female fin whales were classified into five categories; pregnant females were further classified: (1) those with fetus detected and (2) those with P ≥ 9.0. Mean levels of serum P (SD) and body length are given, as well as the median of the age with lower and upper quartiles. The number of individuals of each category which are found in three different intervals of serum P-levels also are given (i.e., ≤ 0.1, 0.1 < P < 9.0, and ≥ 9 nmol/l).

Female reproductive categories	*n*	Mean serum P (SD), nmol/l	Mean body length (SD), m	Median age (lower-upper quartiles), y	P ≤ 0.1	0.1 < P < 9.0	P ≥ 9.0
Immature	157	0.80 (1.4)[a]	17.48 (1.1)	7 (4-9)	93	61	3[b]
Anestrous	68	1.06 (1.8)[a]	19.3 (1.0)[c]	15 (10-21.5)[d]	36	30	2[b]
Pregnant	207	37.4 (21.1)[c]	19.4 (0.92)	13 (10-17)	2	9	196
Lactating	4	0.8 (1.04)[c]	20.4 (0.91)	17 (12.8-20.75)	1	2	1[b]
Undetermined	12	2.1 (1.9)	19.3 (1.1)	13 (11-18)	1	4	7[b]
Fetus detected	90	38.8 (21.7)	19.5 (1.0)	13 (10-18)	1[b]	3[b]	86
Serum P ≥ 9.0	209	39.1 (20.0)	19.4 (0.93)	13 (10-17.5)	0	0	209

[a] The mean serum P and SD do not adequately reflect the characteristics of the serum P-levels of the category because more than 50% of the values are at the detection limit of the assay (0.1 nmol/l).
[b] Not included in calculations of the mean P-levels for the respective category
[c] Significantly different from value just above (*t*-test)
[d] Significantly different from value just above (Mann-Whitney U test)

5.22, $p = 0.022$), rising from 20% in the youngest category to 50% by the age of 35 y. The distribution of age in both categories was slightly skewed to the right, however, and to avoid confounding influence from maturing females, all females ≤ 8 y in each category, 10 anestrous and 35 pregnant, were skipped before using the Mann-Whitney U test to compare ages in the two mature categories. The test showed a significantly higher age for the nonpregnant category (U = 3459.5, $p = 0.006$).

The distribution of the $\log_{10}$-transformed serum P-levels of the female fin whales classified as mature (anestrous, pregnant, and lactating nonpregnant) by the anatomical methods is shown in Figure 1. The hatched part of the columns signifies the 86 cases with P-levels ≥ 9 nmol/l and fetuses detected in the uterus. The $\log_{10}$ P-levels of pregnant females > 0.95 (9.0 nmol/l) were almost symmetrically distributed about their mean value 1.545 (39.4 nmol/l geometric mean) and the mode of 1.57 (37.1 nmol/l) with quartiles of 1.42 and 1.68 nmol/l. About 98.5% of this category is made-up of females classified as pregnant by the anatomical methods. Serum P-levels did not increase significantly with either age or size of the pregnant females nor did they increase with the time of the season or the lengths of the fetuses. An increase in mean serum P-levels was associated ($p < 0.001$) with increasing weight (range: 0.2 to 1.8 kg) and diameter (62 to 175 mm) of the CL, however, which in turn were not associated with the size of the pregnant females.

Males

The serum T-levels of the male fin whales ranged from the lower detection limit of the assay (0.1 nmol/l) to 40.2 nmol/l. The mean serum T-levels and ranges for each reproductive category are shown in Table 2. The last two columns of the table show the number of serum T-levels, which are at the assay limit (≤ 0.1 nmol/l) or at an arbitrarily chosen higher level (≥ 3.0), thus giving some idea about the distribution of values. The undetermined category contains six males that could not be definitely classified. The distribution of age was skewed, and its medians for the categories are given with the lower and upper quartiles. The Mann-Whitney U test confirmed that immature males were younger (U = 647.5, $p < 0.001$) than the pubertal ones, which in turn were younger than the mature males (U = 1547, $p < 0.001$).

The symmetrical distribution pattern of the $\log_{10}$-transformed T-levels of immature and mature males is illustrated in Figure 2. There is a substantial overlap of values in the two categories, and a number of the mature whales have T-levels at the lower limit of the assay. The mature males, however, had significantly higher ($p < 0.001$) mean T-levels than the two immature categories. Male fin whales older than 40 y had lower mean T-levels than those between 20 y and 40 y, but not significantly (Mann-Whitney U test: U = 149.5, $p = 0.53$).

Serum T-levels, however, showed a tendency for positive association with the testes weight in both the immature and pubertal categories, but only for the mature category (range: 2.2 to 52.0 kg) was the association significant (r = 0.35, $p < 0.001$).

The serum T-levels of the males were compared to the time of the hunting season (daycount). While the immature and pubertal males showed weak but

nonsignificant increase with time, the T-levels in mature whales rose significantly ($p < 0.001$) with the daycount (Figure 3). Twelve mature whales, eight of which were caught in the earlier half of the hunting period, had T-levels at the lower limit of the assay (0.1 nmol/l) and were not included in the regression analysis. Neither testes weight nor body length nor the ratios of testes to body weight were associated with the daycount in any of the three reproductive categories.

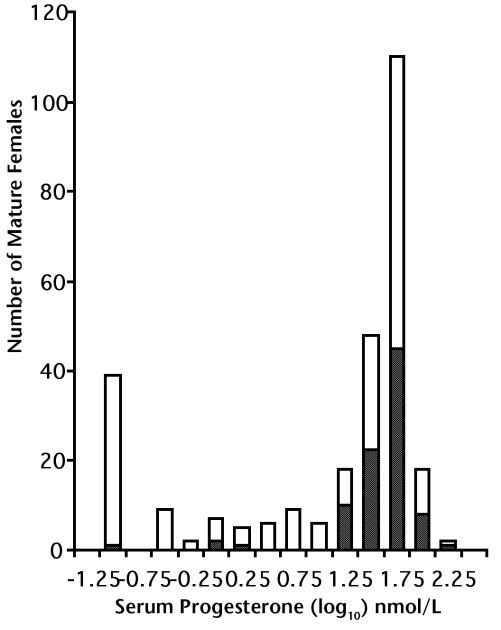

Figure 1. The frequency distribution of the log_{10}-transformed serum progesterone (P) levels of the female fin whales judged to be sexually mature by the anatomical methods; a nearly symmetrical distribution of the pregnant P-levels (> 9.0 nmol/l) is apparent with a mean of 1.545 (35.1 nmol/l serum P) and a median of 1.57 (37.1 nmol/l serum P) with quartiles at 1.42 and 1.68 nmol/l, respectively. The hatched parts of the bars denote the P-levels of the 90 females recorded with fetuses present, four of which had P-levels ≤ 1.1 nmol/l (log_{10} 1.1 = about 0.041). The widths of the concentration intervals on the X-axis are 0.25 (about 1.8 nmol/l) with the upper limit (tick mark) to the right of the concentration value on the axis.

Serum Estradiol (E2), P, and T in Males and Females

Serum estradiol (E2) levels were measured randomly in 34 pregnant, 26 anestrous, and 5 immature female fin whales. The mean (SD) levels were 58.3 (53), 42.3 (33), and 42.8 (10) nmol/l, respectively. Serum E2 levels were also measured in 41 randomly selected males, and a mean E2 value in 27 mature whales was 72 (± 44) pmol/l. No significant correlation with serum T-levels was found and no difference was found between E2 levels in the reproductive categories of either sex by the Mann-Whitney U test. However, the median serum E2 level in mature males was higher (U = 212, p = 0.005) than that in immature females.

Serum T-levels were measured randomly in 70 pregnant, 63 anestrous, and 20 immature females. The mean (SD) levels were 0.6 (1.3), 0.5 (0.8), and 0.3 (0.5) nmol/l, respectively. No significant differences between means in the categories were found by the Mann-Whitney U test. Similarly, serum P-levels were measured in 71 mature, 9 pubertal, and 27 immature males, finding means (SD) of 1.5 (1.6), 1.5 (1.8), and 1.8 (2.2) nmol/l, respectively. Serum P-levels did not differ significantly between male categories.

Discussion

Sample

Because of strict regulations involving minimum size limits and protection of cows with calves, our sample of whales was selected with respect to size and sexual status in general. Furthermore, the short hunting season from June to August allows us to study only a limited part of the yearly cycle of

Table 2. Male fin whales classified by anatomical methods into four reproductive categories; mean serum T-levels (range) and body length are given, as well as the median of the age with lower and upper quartiles. The number of individuals in each category with serum values of T ≤ 0.1 and T ≥ 3.0 nmol/l are also given.

Male reproductive categories	*n*	Mean serum T (range), nmol/l	Mean body length (range), m	Median age (lower-upper quartiles), y	T ≤ 0.1	T ≥ 3.0
Immature	74	1.0 (0.1-4.2)	17.1 (15.2-19.5)	7 (5-8)	5	2
Pubertal	40	1.0 (0.1-4.3)	17.9 (16.5-20.1)[a]	10 (8-12.25)[b]	7	3
Mature	158	3.1 (0.1-40.2)[a]	18.4 (16.8-20.1)[a]	14 (11-20)[b]	12	43
Undetermined	6	1.5 (0.6-4.5)	18.8 (17.7-19.8)	21 (20-22)	0	1

[a] Significantly different from the value next above (*t*-test)

[b] Significantly different from the value next above (Mann-Whitney U test)

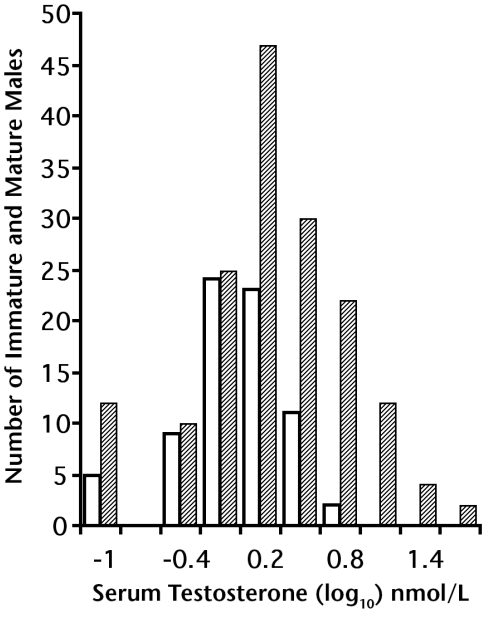

Figure 2. The frequency distributions of $\log_{10}$-transformed serum testosterone (T) values of 74 immature (open bars) and 158 mature (solid bars) male fin whales as classified by anatomical/histological methods; a substantial overlap of T-levels of the two categories is seen, but there was a significant difference between the means of the categories.

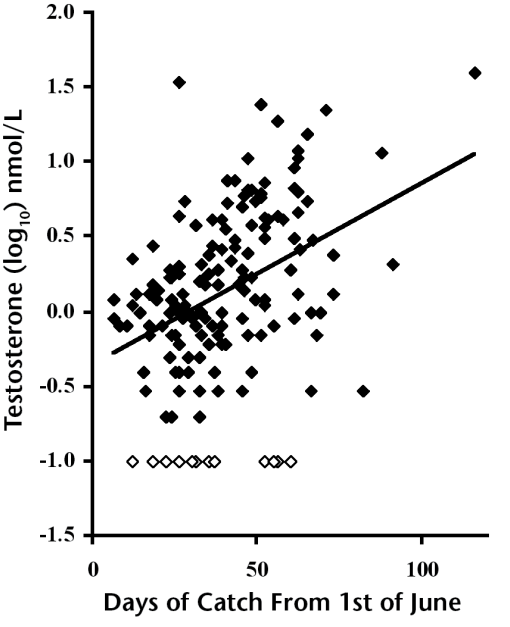

Figure 3. Log_{10}-transformed serum T-levels of 158 mature male fin whales are plotted against the daycount (days of catch). The equation for the regression line was $\log_{10}$ T-levels = 0.012 daycount – 0.349. Twelve males had T-levels at the detection limit of the assay (0.1 nmol/l; open diamonds, not in calculations), eight of which were caught in the earlier half of the hunting period.

hormonal changes in the whales. Added to this is the fact that only one preliminary report on serum sex hormones in fin whales has been published to date (Kjeld et al., 1992), so there are no data for comparison from other places at different times of the year. This report, therefore, represents the first data on serum sex hormones in relation to the anatomically assessed sexual condition of the North Atlantic fin whale. Reports on hormone levels in reproductive endocrinology of baleen whales are few and mostly recent (Fukui et al., 1996; Kjeld et al., 2003).

The samples were taken postmortem at sea, generally after a 0- to 30-min chase. Earlier studies indicated that the chase time, the time of day of the catch, or the time from the death of the animal until the blood specimen is taken do not influence the blood sex hormone concentrations perceptibly (Kjeld et al., 1992; Kjeld, 2001).

Females

The pregnant category among the anatomically classified female fin whales had by far the highest serum P concentrations, which after $\log_{10}$-transformation, showed practically a symmetrical distribution. Of the immature females, 59% were at the lower detection limit of the P assay (0.1 nmol/l), with unknown distribution of P-levels. Sixty-eight mature, anestrous females had about half (n = 36) of their serum P-levels at the detection limit of the assay. The rest of the P-levels of this anestrous category were evenly distributed. Seven of 12 female whales in the undetermined category had serum P-levels > 9.0 and were probably pregnant. Changes in pregnant and nonpregnant serum P values have been reported for several female odontoceti in captivity—for example, the killer whale (*Orcinus orca*) (Walker et al., 1988; Robeck et al., 1993), the bottlenose dolphin (*Tursiops truncatus*) (Yoshioka et al., 1986), and the humpback dolphin (*Sousa chinensis*) (Brook et al., 2004). All three species appeared to show three- to four-times higher P values in pregnancy than during ovulation. A high serum P-level during the feeding season is indeed likely to be a reliable index of pregnancy. A large survey on the fin whale (Lockyer & Sigurjónsson, 1992) found that an active CL is almost always coincident with pregnancy. The present study on serum P-levels agrees closely with that.

Increased P-levels were associated with increased CL weight. This association agrees with a finding reported for heifers (Kastelic et al., 1990).

Neither P-levels nor CL weight changed significantly with the daycount within the time frame available in this study, nor did they change with body size of the females or that of their fetuses. The reason, therefore, for the variability of the CL's sizes and serum P-levels in individual pregnant females remains unknown and might be controlled either by the implantation or the maternal tissues. Serum P-levels are of interest in this respect as the P produced in the CL has recently been suggested to have a role as a genomic and nongenomic mediator of the action of gonadotropins (Peluso, 2003; Stouffer, 2003) along with its role in making it possible for the fertilized egg to implant, be nourished by, and grow in the uterine endometrium.

A clear age difference became apparent when the two female categories of sexually mature whales, defined by serum P-levels as pregnant and nonpregnant, were studied. The nonpregnant category of mature cows had a higher median age than the pregnant one, and the proportion of nonpregnant females grew significantly with increasing age. The Mann-Whitney U test showed a significantly higher age for the nonpregnant category when the female whales of 8 y and younger were excluded from the test. The sexual maturation of the North Atlantic female fin whale has been reported to be between the ages of 9 to 11 y (Lockyer, 1984; Kjeld et al., 1992; Boyd et al., 1999). By eliminating females < 9 y, the remaining sample should better represent our studies. This particular age difference is indeed a finding of considerable interest, which has, to our knowledge, not been reported in whales before or in other wild animal species that we know of. This reflects not only on the reproductive years of the species in question but also its lifespan. It indicates that fin whale cows older > 25 to 30 y have a tendency for reduced fecundity compared with the younger ones—that is, the older females are either not conceiving as often as the younger ones or are suffering an increased fetal loss, or both, suggesting reproductive senescence.

Conversely, the nonpregnant elderly cows might live a little longer than the often pregnant ones as Lockyer has suggested that pregnancy may be a risk factor for the survival of cows (Boyd et al., 1999). In aging women, reduced fertility has been attributed to follicular atresia with fewer ovulations, and to chromosomal abnormalities with increased fetal loss as a result of aging oocytes (O'Connor et al., 1998). In old mares, the pregnancy rate was significantly lower and embryo-loss rate greater compared to younger ones (Carnevale & Ginther, 1992). In aging pregnant rats, serum P-levels did not diminish, suggesting that uterine failure might contribute to the loss of fertility (Miller & Riegle, 1980).

The age range of the anestrous females of 5 to 70 y in the present specimens seems compatible with a longest lifespan for the fin whale of about 70 to 80 y since reduction of fecundity generally begins during the middle of the lifespan of the species (vom Saal et al., 1994). Extension of life beyond fecundity could be advantageous for the species' survival (Pfeiffer, 1990). Herein, for instance, old whales might serve as guides during long travels, assist communication relays in the vastness of the oceans, or provide added care of the young; however, readability of age diminishes in old fin whales (Lockyer et al., 1977), and the fecundity of old females remains to be explored further in a large sample such as that used by Lockyer & Sigurjónsson (1992), where this particular subject could be addressed specifically.

Serum P concentrations and their changes during pregnancy differ widely in different species. In women and ewes, serum P-levels increase up to six times (to about 580 and 44 nmol/l, respectively) from the first few weeks of pregnancy until about a week before parturition (Bassett et al., 1969; Tulchinsky et al., 1972), and serum E2 concentrations in women rise in similar fashion. In mares, serum P-levels are at about 45 nmol/l from the second to the fourth month and then fall to 10 times lower values (Holtan et al., 1975; Atkins et al., 1976). Female one-humped camels (*Camelus dromedarius*) and the Asian elephant (*Elephas maximus*) both have serum P-levels of about 10 to 15 nmol/l during most of their 56 and 93 weeks pregnancies, respectively (Skidmore et al., 1996; Niemuller et al., 1998).

Perhaps not surprisingly, some errors, clerical or specimen mix-ups, seem to have crept into our work and could not be controlled by the researchers. These errors seem to be few, however, mostly below 4%, and should not seriously affect the results. The most conspicuous error was to find that four of the 90 females recorded to have been carrying fetuses had serum P-levels of ≤ 1.1 nmol/l. The somewhat arbitrary serum P limit used for deciding pregnancy should not have made much difference; if that limit was brought down from 9.0 to 6.5 nmol/l, only three more pregnancies would have been assumed in the mature females or about 1%.

Males

Serum T concentrations in the male fin whales were widely scattered from 0.1 to 40.2 nmol/l. Even after the males had been classified into three reproductive categories by the histological/anatomical methods, much overlap was still apparent between T-levels in the categories (Table 2). Mature males had significantly higher means than the pubertal and immature ones, however, but that difference was not significant during the first 45 d of the

hunting season. This, of course, emphasizes the advantage of having samples from the male fin whales for a longer period of the year. Thus, a mature captive male bottlenose dolphin showed an annual rhythm in T values, rising to values well over 100 nmol/l before the autumn breeding season (Schroeder & Keller, 1989) at which time the separation of serum T-levels between the mature and immature animals would be greatest.

Mean serum T concentrations of the mature male fin whales (3.10 nmol/l) were a little lower than in mature male sei whales (4.8 nmol/l) off Iceland during summer (Kjeld et al., 2003), but four times higher than in mature male minke whales (*Balaenoptera acutorostrata*) off Norway (0.73 nmol/l) during a similar time of the year (Kjeld et al., 2004). Log_{10}-transformed serum T-levels in both mature and immature male fin whales showed a near symmetrical distribution, and both had between 6 and 8% of the individuals lying at the detection limit of the serum T assay. When serum T-levels of the three male reproductive categories were compared with the daycount, the immature and pubertal categories showed weak association, but the T-levels of the mature males showed a significant increase with the daycount. Thus, serum T increased about 2.3 times every 30 d from the beginning of June and, hence, assuming the same rate of increase, at the end of November, the mean serum T-levels in the male fin whales can be predicted by extrapolation to be about 70 nmol/l. A similar rise in serum T concentrations was observed for North Atlantic sei and minke whales during a comparable time period (Kjeld et al., 2003, 2004), but not for the Antarctic minke whale (*B. bonaerensis*) (Yoshioka & Fujise, 1992; Fukui et al., 1996; Iga et al., 1996).

Increased weight of the testicles was associated with increasing T-levels in the mature whales and increased body weight of all three male categories. Testicular weight of the mature whales or its proportion of the body weight did not increase significantly with the daycount during the summer hunting season in our material. Lockyer & Sigurjónsson (1992), however, found a significant increase in testicular weight of mature males between early June and late September, using a much larger sample than presented here, and with the month of September added to the observation period. A seasonal increase in testes weight, together with increased serum T-levels, has been described for various seasonal breeders, herbivores, and predators, from temperate as well as circumpolar areas (Blottner et al., 1990; Bronson & Heideman, 1994; Howell-Skalla et al., 2002).

In 16 mature bulls over 30 y of age, serum T-levels of ≤ 0.3 nmol/l were twice as frequent as in the younger ones, but this difference was not significant. If studied at a time closer to the main mating month, difference in T-levels between young and old males might have become more apparent. This may not have been tested in a similar manner in seasonal breeders before (vom Saal et al., 1994), but it agrees with reduced steroidogenesis of the testes and isolated Leydig cells in aging rats (Zirkin et al., 1997).

The mating activity of the fin whale is thought to peak in December (Lockyer, 1984; Boyd et al., 1999). In seasonal breeders such as the bottlenose dolphin and terrestrial herbivores, serum T concentrations generally reach a peak or an elevated plateau before the rutting begins, at which time the T-levels begin to fall (Schroeder & Keller, 1989; Bronson & Heideman, 1994). In this study, there was little indication that the rise of serum T-levels was coming to a halt at the end of the hunting season. There is no known indicator to tell what height serum T concentrations need to reach before the males of different species can play their due role for the procreation of that species, however. For the dolphins and herbivores cited above, T-levels changed from a mean of about 10 and 6 to a mean of 180 and 50 nmol/l in about 2 to 3 mo, respectively. Thus, it seems that our results agree with a peak rutting time in December for the North Atlantic fin whale.

Concluding Remarks

Thus far, only a few papers have been published on serum concentrations of reproductive hormones in baleen whales. Pioneering studies by biologists, however, need to be followed up and expanded by the tools available to modern reproductive endocrinology. It is important to understand if and how these relatively little known, inaccessible, pelagic mammals might be reproductively endangered in a changing world.

Because serum P-levels discern between pregnant and nonpregnant females, a possibility is presented to diagnose pregnancy via nonlethal methods by obtaining blubber specimens at sea for P measurements (Mansour et al., 2002). Since serum T-levels, especially in late autumn, can discern between the sexes in many cases, they could similarly be used to decide on the sex of the whales. With more sensitive and specific antisera in the assays, bringing the detection limits into the picomole range, these measurements should have greater diagnostic power.

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