Abstract.–Serum progesterone and testosterone levels, as measured by radioimmunoassay, were used to estimate the mean length at attainment of sexual maturity (LSM) in a sample of 124 female and 31 male incidentally-killed Dall's porpoises *Phocoenoides dalli*. Females with serum progesterone levels greater than 1.34ng/mL were considered mature. The LSM for females was estimated at 169.0cm using Kasuya's "summation" technique. A technique to fit a two-phase regression model to the male data produced an estimated LSM for males of 183.0 cm. Overall, the estimates for the LSM in this study agreed well with previously published reports using histological and morphological measures of sexual maturity. Hormonal estimation of maturity is proposed as a rapid, inexpensive, and potentially non-lethal alternative technique in odontocete populations.

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Use of Serum Progesterone and Testosterone to Estimate Sexual Maturity in Dall's Porpoise *Phocoenoides dalli*

Jonathan L. Temte

Hatfield Marine Science Center, Oregon State University, Newport Oregon 97365
Present address: Department of Zoology, University of Wisconsin
Madison, Wisconsin 53706

Estimation of the mean age and length at the attainment of sexual maturity in odontocetes has traditionally relied upon morphological or histological parameters, thus necessitating whole animal preparations. Examination of reproductive tissue allows for the determination of maturity. When this information is coupled with age/length data, the age or length at attainment of sexual maturity for females and males in a population can be determined using a variety of methods (DeMaster 1984). These methods, however, implicitly require the collection of dead marine mammals in the field.

Several authors have used radioimmunoassay (RIA) to assess reproductive condition in living odontocetes. For example, RIA of progesterone has been used as an indicator of luteal function in *Tursiops truncatus*, *Delphinus delphis* (Kirby and Ridgway 1984, Sawyer-Steffan et al. 1983), and *Stenella longirostris* (Wells 1984). Likewise, measurements of serum testosterone by RIA have been used to assess male sexual condition in *T. truncatus* (Harrison and Ridgway 1971) and *S. longirostris* (Wells 1984).

Temte and Spielvogel (1985) demonstrated that serum progesterone concentration, as measured by RIA, was a good predictor of corpus luteum mean diameter in a sample of 32 incidentally-killed pregnant or lactating Dall's porpoises *Phocoenoides dalli* from the northwestern North Pacific Ocean. Furthermore, they noted that 17 sexually immature females (no corpora present in ovaries) had very low concentrations of serum progesterone (Table 1).

In this study the results of progesterone and testosterone RIA in 124 female and 31 male Dall's porpoises, respectively, were used to estimate the mean length at attainment of sexual maturity (LSM) in this species. The results are compared to those obtained using traditional histological techniques to demonstrate the effectiveness of this inexpensive and non-lethal technique.

Methods

Blood samples

During June and July of 1982, personnel of the National Marine Mammal Laboratory (NMFS) obtained blood samples from a total of 105 Dall's porpoises that were incidentally-killed in Japanese salmon gillnets in the North Pacific Ocean. The following groups were represented in the sample: 21 non-pregnant, non-lactating females in which the maturity status was not known (149–199 cm, standard body length); 30 pregnant females (167–200cm); 23 lactating females (167–200cm), and 31 males in which the maturity status was not known (101–210cm). Samples of whole blood were drawn and centrifuged using methods described

Table 1
Serum progesterone concentrations [P] in immature and mature female Dall's porpoises. Data from 1980 sample of Temte and Spielvogel (1985).

Status	No.	Mean [P] (ng/mL)	SD	Range of [P]
Mature	33	14.76	12.43	0.0–45.3
Pregnant (P)	24	19.40	11.31	0.9–45.3
Lactating (L)	8	2.63	3.75	0.0–11.4
non-P, non-L	1	0.49		
Immature*	17	0.27	0.46	0.0–1.3

* Excluding one immature female with a large follicle and 31.1 ng/mL progesterone.

by Temte and Spielvogel (1985). In these samples, however, serum was decanted into 1.5-mL plastic ultracentrifuge tubes and frozen at −20°C until assays were performed.

RIA for progesterone

Progesterone RIA was identical to that reported by Temte and Spielvogel (1985). This assay had previously been validated for porpoise serum, and chromatography had shown an absence of interference from other serum constituents. Triplicate volumes of porpoise serum (10 μL for pregnant, 50 μL for lactating, and 100 μL for non-pregnant, non-lactating females) were doubly extracted utilizing a 1:2 mixture of benzene and hexane (Sawyer-Steffan and Kirby 1980). The antiserum used was anti-progesterone-11-BSA, No. 1337 (Gordon D. Niswender). The intraassay coefficient of variation (COV) was 5.5%, while the interassay COV was 5.9%. The sensitivity of this assay was 0.1 ng/mL.

RIA for testosterone

The procedure for testosterone RIA was nearly identical to that for progesterone. Triplicate volumes of 25-μL porpoise serum were doubly extracted with 1:2 benzene:hexane. The antiserum used was anti-testosterone, No. s-250 (Gordon D. Niswender). The competitor was [^{3}H]testosterone (NET-553, New England Nuclear). The intraassay coefficient of variation (COV) was 6.3%, while interassay COV was 14.6%. The assay sensitivity was 0.3 ng/mL.

Maturity criteria for females

The mean serum progesterone for immature females reported in Temte and Spielvogel (1985) was 0.27 ng/mL. Assuming a normal distribution, 99% of immature females would be expected to have serum progesterone levels less than 1.34 ng/mL. Based upon this result, females with serum progesterone concentrations greater than 1.34 ng/mL were considered sexually mature. Whereas, this progesterone level is lower than the 3.0 ng/mL used by Kirby and Ridgway (1984) as an indicator of ovulation in *D. delphis* and *T. truncatus*, 94% of the immature females and 96% of the pregnant females reported in Temte and Spielvogel (1985) were correctly classified using this criteria.

Maturity criteria for males

Since samples were collected near the peak breeding period for Dall's porpoises (Newby 1982; see also Jefferson 1989), and considering the seasonal flux of testosterone in other odontocetes (Wells 1984), separation of immature and mature Dall's porpoises by testosterone level alone was theoretically possible in this study. Although Wells (1984) regarded serum testosterone concentrations of less than 8.0 ng/mL as baseline levels in *S. longirostris*, a natural break in the Dall's porpoise data occurred between 1.7 and 5.0 ng/mL. However, as tissue samples were not available for maturity assessment, no hormonal maturity criteria could be confirmed.

Estimation of LSM

The proportions of mature females in each 10-cm increment (135–225 cm) were calculated. Graphical analysis determined the length at which the cumulative probability of maturity, $\Sigma(m_i)$, equaled the cumulative probability of not being mature at that length or longer, $\Sigma(1-m_i)$. This method was first used by Kasuya (1972) and has been termed the summation technique by DeMaster (1984). It is recommended when small samples of size- or age-classes exist.

A small total sample precluded the use of the summation technique in males. As a consequence, a modification of the regression technique (see DeMaster 1984) was used to identify the LSM. Because testosterone concentrations demonstrated a discontinuity (see above), a continuous two-phase regression model (Yeager and Ultsch 1989; review by Nickerson et al. 1989) was used to objectively identify the transition point in the data. The transition point in the length represents the point at which the discontinuity occurred, or in this case, the LSM.

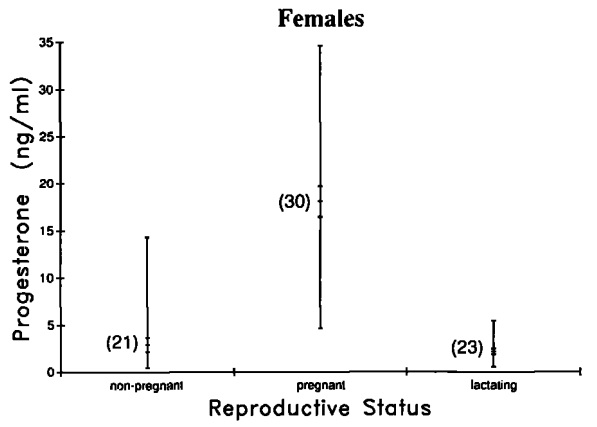

Figure 1
Serum progesterone concentrations for non-pregnant/non-lactating, pregnant, and lactating female Dall's porpoises. Vertical bars indicate ranges of values. Horizontal bars indicate means and standard errors. Sample sizes are in parentheses. Animals were collected June–July 1982 in the western North Pacific Ocean.

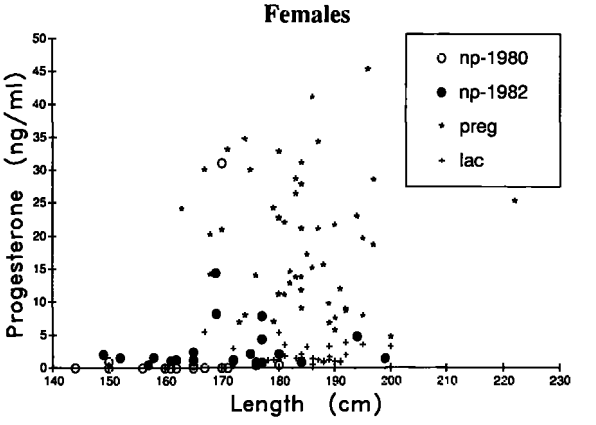

Figure 2
Serum progesterone concentration as a function of length in non-pregnant (1980 O; 1982 •), pregnant (*), and lactating (+) female Dall's porpoises.

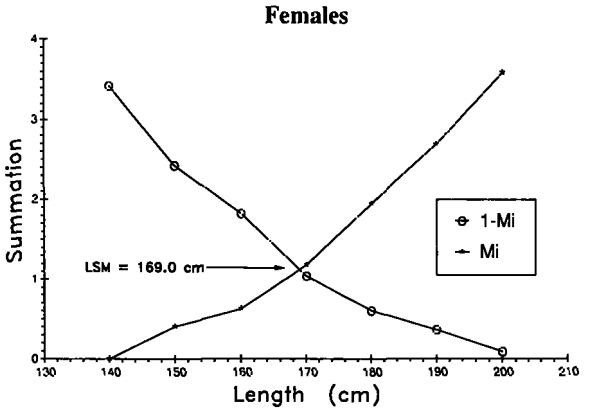

Figure 3
Summation technique for female Dall's porpoises based on progesterone values. Intersection of lines occurs when $\Sigma(m_i) = \Sigma(1-m_i)$.

Results

Comparison of 1982 and 1980 progesterone data

The results of the progesterone assays (1982 data: Fig. 1) were compared with results of Temte and Spielvogel (1985) (1980 data). No significant differences were found between the mean progesterone concentrations for pregnant females (1980: $n = 24$, $\bar{x} = 19.40$; 1982: $n = 30$, $\bar{x} = 18.09$; $P > 0.5$; Student's t-test), or for lactating females (1980: $n = 8$, $\bar{x} = 2.63$; 1982: $n = 23$, $\bar{x} = 2.17$; $P \gg 0.05$; Wilcoxson rank sum). Mean progesterone concentrations were significantly different for the two groups of non-pregnant, non-lactating porpoises (1980: $n = 19$, $\bar{x} = 1.91$; 1982: $n = 21$, $\bar{x} = 2.92$; $P < 0.01$; Wilcoxson rank sum). However, the mean length of the 1982 sample was significantly greater than that of the 1980 sample (1982: $\bar{x} = 171.2$ cm; 1980: $\bar{x} = 163.1$ cm; $P < 0.05$; Student's t-test), and the difference in mean progesterone could be due to a difference in the proportion of mature females. Therefore, the results of progesterone analysis in pregnant, lactating, and non-pregnant/non-lactating females from the 1982 sample were pooled with the results from the 1980 sample of Temte and Spielvogel (1985).

Females

Serum progesterone is plotted against length for 124 female Dall's porpoises (Fig. 2). The results demonstrate the incidence of high progesterone levels at lengths greater than 165 cm. These elevated levels were seen not only in pregnant females, but also in non-pregnant, non-lactating females, indicating possible ovulation. The summation technique provided an estimated LSM of 169.0 cm (Fig. 3).

Males

Serum testosterone displayed a marked increase with an increase in length. A natural break in the testosterone data occurred between 175 and 180 cm, when

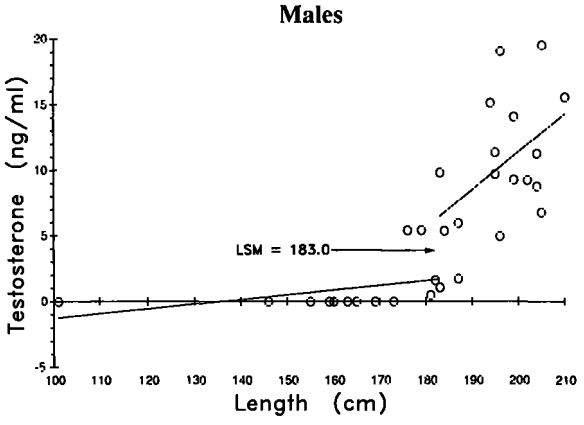

Figure 4
Serum testosterone concentration as a function of length for 31 male Dall's porpoises collected in 1982. Two-phase regression lines are shown.

testosterone increased from undetectable levels to relatively high levels. The best fitting two-phase regression model (Fig. 4) indicated that the transition point occurred at 183.0 cm. Hence, the LSM for males was estimated as 183.0 cm. The mean (± SE) testosterone concentration was 1.00 ± 0.52 ng/mL for males shorter than the LSM, and 10.46 ± 1.20 ng/mL for males longer than the LSM.

Discussion

Newby (1982), using the presence of ovarian scars and the 50th percentile method (see DeMaster 1984), estimated the LSM to be 170.5 cm for females in this western North Pacific population. The use of hormonal data alone provided a similar, but slightly lower, LSM estimate of 169.0 cm. However, based on the growth curves for females of this population (fig. 31 in Newby 1982), this 1.5-cm difference translates into small differences in ages.

The estimate for LSM in males of 183.0 cm is in very close agreement with the previously estimated LSM of 182.6 cm based on the 50th percentile method using testis-epididymal weight (Newby 1982). Direct comparison with previous studies are not possible due to the lack of variance estimates for LSM by the methods used. Nevertheless, the hormonal estimates of LSM provided in this study agree quite well with previous, non-hormonal methods.

Radioimmunoassay is a quick, economical, and accurate measure of reproductive condition. Small samples of blood (< 1 mL) provide adequate serum for replicate assays. Previous studies have shown the high predictive value of serum progesterone level to corpus luteum size and reproductive state (Temte and Spielvogel 1985, Kirby and Ridgway 1984, Sawyer-Steffan et al. 1983). Moreover, such methods could allow the rapid collection of samples for maturity status assessment from large numbers of animals in the field.

Hormonal assessment of reproductive status may prove to be a non-lethal technique to estimate population parameters such as LSM. It is, at present, more applicable to captive animal studies. However, situations in which animals are killed incidentally may provide opportunities for obtaining concordant blood and reproductive tissue samples. Such sampling could allow the direct comparison of hormonal and histological methods of estimating maturity and establish appropriate hormonal criteria for future studies.

Seasonality in breeding may well limit the usefulness of this method in males (see Perrin and Donovan 1984) because testosterone undergoes seasonal fluctuation. In addition, the presence of environmental toxins may interfere with steroid hormone production. For example, Subramanian et al. (1987) have shown a significant negative relationship between testosterone concentration and DDE residue level.

There is potential for using progesterone, testosterone, and other hormonal parameters, such as chorionic gonadotropin, FSH, and LH, in the estimation of sexual maturity and reproductive status in marine mammal populations. As we expand our reproductive database in the cetacea, we also need to correlate histological and morphological states with hormonal parameters. Research protocols which include 10-mL samples of fresh blood and assessment of hormonal state should be encouraged.

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