**Sexual maturation in male bowhead whales (Balaena mysticetus) of the Bering-Chukchi-Beaufort Seas stock**


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**ABSTRACT**

Since the mid-1970s, study has focused on reproductive biology of female bowhead whales, while little has been described for males. This study evaluates testicular morphology (mass and length) and histology in relation to body length to determine the onset of male sexual maturity. Mean testis mass and mean testis length were highly correlated. Body length and mean testis mass were significantly correlated and an inflection of increased testicular mass occurred at approximately 12.5-13.0m suggesting the onset of puberty, and also indicated by histologic findings. Biological variability and the fact that few male animals have been examined within this critical length cohort do not allow determination of the onset of maturity with higher precision. Too few mature males have been landed in spring to make statistical comparisons of testes mass with autumn-landed animals within specific size cohorts. Two large (15.7m and 17.7m) males landed in spring had relatively small inactive testes and were diagnosed as pseudocryptorchid; body length and mean testis length and seminiferous tubule diameter (STD) were not correlated with the other ‘normal’ whales. The smallest male confirmed as mature based on the presence of spermatozoa was 12.7m. The largest testses measured (combined mass 203kg) were from a whale landed in autumn. Mean STD for individual whales ranged from 33.3-170.9μ and increased with mean testis weight and whale length. The STD is similar within a testis regardless of region evaluated, with minor variability. Some variation was noted for transverse sections within a cross section for some whales but no pattern was evident.

KEYWORDS: AGE AT SEXUAL MATURITY; ARCTIC; BOWHEAD WHALE; MALE; REPRODUCTION

**INTRODUCTION**

More effort has been focused on the reproductive biology of female rather than male bowhead whales, *Balaena mysticetus*, of the Bering-Chukchi-Beaufort (BCB) Seas stock (Nerini et al., 1984; Koski et al., 1993; Tarpley et al., 1999a; b; c). Little has been published on male reproduction (Kenny et al., 1981; Medway, 1981; Koski et al., 1993; Tarpley et al., 1995), primarily because an understanding of the reproduction of females is more central to the modelling of populations in the context of various management strategies. However information on male reproduction and breeding behaviour (e.g. seasonality) can improve the models used in management and can provide valuable additional information on the general health of populations (e.g. in the context of Implementation Reviews – see IWC, In press). The present study presents information on: testicular morphology; histologic evidence of sexual maturation in relation to body length (as a proxy for age); and further description of testis appearance in two animals that were deemed pseudocryptorchid as previously described by Tarpley et al. (1995).

Confirmation of male sexual maturation requires determining the presence of spermatozoa either through seminal smears from fresh epididymides or histological examination of testis and epididymis tissue. When these data are collected in concert with data on testis size (length, width, depth and/or mass) for a large number of animals, it may become possible to predict whether animals are mature or immature from the size of testes alone. Testes of intermediate size will still need to be examined histologically to determine whether an animal is pubertal, or mature with recrudescence testes outside the breeding season (if seasonality occurs). In a synthesis of the existing bowhead whale literature, Koski et al. (1993) reported 13.0-13.5m as a mean length at sexual maturity for females and 12.0-13.0m for males, based primarily on males having a smaller body length at physical maturity. This study presents additional information on morphometrics of bowhead whales (i.e. standard length) and of their testes (length and mass); and histological indicators of sexual maturity (presence of sperm and seminiferous tubule diameter between and within testes) to estimate the body length range at sexual maturation.

**MATERIALS AND METHODS**

**Examination and tissue sampling of bowhead whales**

A subsistence fishery for bowhead whales is undertaken by Alaskan Eskimos (Inuit) during the spring and autumn migrations. Whales landed at Barrow and occasionally in other villages along the northern coast of Alaska were examined by North Slope Borough (NSB) Department of Wildlife Management (DWM) and/or Alaska Eskimo Whaling Commission (AEWC) personnel. Measurements were taken of standard body length, sex, and up to 43 morphological measurements including testicular mass, greatest testicular circumference, and/or length (pole to pole) for one or both gonads per animal. Where possible, epididymal or testicular fluid was collected. All tissue sections were saved in neutral buffered 10% formalin.

A triple balance beam was used to weigh testis < 3.0kg, a 45.3kg (+/- 0.45kg) spring scale for testis < 40kg, and a 136kg spring scale (+/-2.2kg) for larger testis. A transverse core sample (capsule to centre of cross section) from each pole (anterior and posterior) and mid-section (equatorial) plane (cross section) were taken and fixed in formalin for selected whales (Fig. 1). These three different planes or cross sections were further sub-divided from the capsular surface to the centre along the transverse core (Fig. 1). Transverse cores (or entire cross sections with core removed later) were taken from the posterior and anterior poles, and equatorial...
(mid-section) cross section early in the study (1980-1984) to determine if significant regional differences (between the three planes and within the cross-section or plane) existed. In later samples (1992-1998), only equatorial sections (mid-sections) were taken and sampled near the capsule, in the centre of the cross section, and equidistant between these two landmarks (three tissue samples per testis).

**Histological examination**

**Assessment of maturity**

Samples of testicular tissue were trimmed, embedded in paraffin blocks and thin sections were placed on a glass slide and stained with haematoxylin and eosin for microscopic examination following Tarpley et al. (1995). Histological examination was similar to that described by Clarke et al. (1994) and Tarpley et al. (1995) and included: (1) a morphological description; (2) measurement of ten seminiferous tubular cross section diameters (STD) per glass slide or site sampled; and (3) a count of the number of interstitial cells (i.e. Leydig cells) per high dry (40X) field (HDF). For each testis, Clarke (1956) used the mean of 10 tubule diameters; Clarke et al. (1994) found no statistical differences between measuring 10 and 20 tubules. For some whales, impression smears and/or wet smears were made from the epididymis and slides were examined under a light microscope (40X). A whale exhibiting ‘copious’ mature spermatocytes was considered to be sexually mature.

The STD was measured based on the opening in the stroma, as the epithelial cells were often slightly displaced from the supporting stroma and distorted due to processing. Two measurements were taken at right angles to one another across the tubule to determine whether the section was round and therefore a true cross section; only ‘round’ tubules were measured. Tubules that were closed, with an epithelium with no evidence of mitoses and an approximate tubular diameter of 60µ were considered immature. Tubules that were open with evidence of mitotic activity with a thickened epithelium and a diameter of approximately 130-140µ, were considered mature. Animals with intermediate (approximate diameter 85µ) sized tubules that were open but lacked mitotic activity with a flat epithelium were suspected to be pubertal.

**Site of sampling comparison**

Formalin-fixed sections of tissue for embedding into paraffin blocks were collected from the transverse core starting inside the capsule (outer connective tissue layer of the testis) and sequentially sampled from this point until reaching the centre of the testis (Fig. 1). These sections were labelled (A, B, C, etc.) sequentially until the centre was reached. For testes collected from 1980-1984, this procedure was conducted for the anterior and posterior poles, and the equatorial cross sections of the testis. For each site sampled (i.e. anterior pole section A) 10 different STDs were measured and the mean STD determined. This was done for 10 male whales landed in the spring, ranging in body length from 7.8-13.6m, and did not include the pseudohermaphrodites. Three different sites of the equatorial (mid-section) plane were examined for samples collected from 1992-1998; the central site, peripheral site and a site equidistant between these two sites (Fig. 1) and a mean STD for that testis is determined from 30 STD measurements (three sites with 10 STD measurements per site).

**Statistics**

For testes from whales sampled from 1980-1984, each cross section plane (anterior pole, posterior pole or mid-section) had the transverse core sequentially sampled from the periphery (capsule) to the centre and the STD values were compared by ANOVA within each plane (significance determined at a p < 0.05). This within-plane comparison was conducted to determine if STD varied significantly by location of the sample (i.e. centre versus closer to capsule) such that sampling site would affect assessment of ‘mature’ versus ‘immature’. The means of STD values within a plane were used to determine an overall mean and SD for each cross section or plane. ANOVA was used to determine if STD differed by plane (anterior pole vs posterior pole vs mid-section) within a testis. For testes for which STD was determined for three cross sections, an overall mean was also calculated. If two testes were assessed for an animal, the average of both testes was calculated to represent the overall animal STD (mean testis STD). Means, SD (standard deviation), ANOVA, Student’s t-test (Table 1) and figures were derived using Microsoft Excel 2000 for Windows. Regression analyses were performed on testis morphology using SPSS PC (7.5 for Windows). Models were fitted using the ‘curve estimation’ function.

**RESULTS**

**Gross morphology**

Testes weights were plotted versus whale length (1980-1998 whales) and a steep inflection of increased mean testes mass occurs at 12.5-13.0m body length (Fig. 2). Whale length was linearly related to the ln transformed mean testis mass (Table 2). Whale length versus mean testis length was compared using regression analyses with and without the two pseudohermaphrodites and in both cases the relationship was significant and positive. Removing the two male pseudohermaphrodites from the data achieved a better fit ($R^2$ increased from 0.68 and 0.79, to 0.91 and 0.91 for
non-transformed and ln transformed data, respectively) for whale length versus mean testis length (Table 2), and these data are presented in Fig. 3. A strong relationship was found between mean testis mass and mean testis length (Fig. 3). A strong relationship was found between mean testis mass and mean testis length (Table 2), and these confirmed pseudohermaphrodites fall outside the ‘normal’ male maturation curve for mean testis length versus body length (Fig. 3); and for mean STD (Fig. 5), as would be expected. Too few mature males have been landed in spring to make statistical comparisons of testicular mass with autumn-landed animals.

**Histomorphology**

An ANOVA was conducted to determine if a significant difference in STD occurred within each pole and equatorial (mid) cross section for each testis with respect to depth from the capsule along the transverse core sampled. Forty-three separate cross sections (anterior or posterior poles, and equatorial plane) were evaluated in this fashion and 25 cross sections (planes) showed a significant ($p < 0.05$) difference, while 18 did not ($p > 0.05$). However, no pattern was detected and the differences were small in magnitude (approximately 5 $\mu$m). Ideally, this examination should be conducted on pubertal whale testes.

The average STD for each pole and equatorial cross section was calculated from the averages determined at each depth for testes collected from 1980-1984. An ANOVA compared the means of each pole and equatorial plane for each testis and no significant difference was observed among the two poles and the mid-section for most of the testes evaluated (data not shown). Only two (of 16 tested) testes indicated a regional difference by plane ($p < 0.05$) where the mean STD for one ranged from 36.6-40.1 $\mu$m, and the other

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**Table 1**

<table>
<thead>
<tr>
<th>Whale class</th>
<th>Whale length (cm)</th>
<th>Mean testis mass (kg)</th>
<th>Mean length of testis (cm)</th>
<th>Mean STD ($\mu$m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All mature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1407.6</td>
<td>53.1</td>
<td>92.3</td>
<td>125.8</td>
</tr>
<tr>
<td>SD</td>
<td>105.2</td>
<td>28.8</td>
<td>19.2</td>
<td>24.5</td>
</tr>
<tr>
<td>No. of whales</td>
<td>18</td>
<td>13</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Range</td>
<td>1270-1660</td>
<td>11.3-101.6</td>
<td>59.0-134</td>
<td>88.0-170.9</td>
</tr>
<tr>
<td>All immature*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1059.7</td>
<td>4.27</td>
<td>40.4</td>
<td>51.8</td>
</tr>
<tr>
<td>SD</td>
<td>245.5</td>
<td>3.32</td>
<td>12.8</td>
<td>9.74</td>
</tr>
<tr>
<td>No. of whales</td>
<td>23</td>
<td>9</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td>Range</td>
<td>760-1770</td>
<td>0.44-9.8</td>
<td>20.4-58</td>
<td>33.3-69.1</td>
</tr>
<tr>
<td>All immature except pseudohermaphrodites</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1000.6</td>
<td>4.27</td>
<td>37.6</td>
<td>51.5</td>
</tr>
<tr>
<td>SD</td>
<td>152.7</td>
<td>3.32</td>
<td>11.6</td>
<td>10.1</td>
</tr>
<tr>
<td>No. of whales</td>
<td>21</td>
<td>9</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>Range</td>
<td>760-1260</td>
<td>0.44-9.8</td>
<td>20.4-58</td>
<td>33.3-69.1</td>
</tr>
<tr>
<td>Mature and active</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1442.5</td>
<td>62.0</td>
<td>101.6</td>
<td>132.5</td>
</tr>
<tr>
<td>SD</td>
<td>119.1</td>
<td>25.6</td>
<td>13.6</td>
<td>25.6</td>
</tr>
<tr>
<td>No. of whales</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Range</td>
<td>1270-1660</td>
<td>20.6-92.1</td>
<td>84-122</td>
<td>102.8-168.8</td>
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<tr>
<td>Mature and inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1379.6</td>
<td>42.3</td>
<td>86.7</td>
<td>122.5</td>
</tr>
<tr>
<td>SD</td>
<td>889.0</td>
<td>30.7</td>
<td>20.4</td>
<td>24.6</td>
</tr>
<tr>
<td>No. of whales</td>
<td>10</td>
<td>6</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Range</td>
<td>1290-1528</td>
<td>11.3-101.6</td>
<td>59-134</td>
<td>88.0-170.9</td>
</tr>
<tr>
<td>All mature v. all immature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P$ value**</td>
<td>$&lt; 0.0005$</td>
<td>$&lt; 0.0005$</td>
<td>$&lt; 0.0005$</td>
<td>$&lt; 0.0005$</td>
</tr>
<tr>
<td>Mature-active v. mature-inactive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P$ value**</td>
<td>0.130</td>
<td>0.124</td>
<td>0.092</td>
<td>0.336</td>
</tr>
</tbody>
</table>

*Includes two pseudohermaphrodites (Tarpley et al., 1995).

** Student’s $t$-test.

Note: for mature whales, ‘early mature’ was diagnosed for four whales. For immature whales, three were classified pubertal. These seven whales have intermediate STDs (mean 85.3 $\mu$m) and likely represent pubertal whales.

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Fig. 2. Mean testis mass (kg) versus whale body length (cm) for select bowhead whales landed in northern Alaska 1980-1998.
from 41.9-46.5μ. This indicates that mean STD by pole or mid-section does not vary significantly in most specimens; when it did it was only approximately 5μ.

Histological examination indicated that the tubules of immature whales were closed (did not have an obvious lumen), lined by an epithelium characterised by a single layer of prospermatogonia along the basement membrane, Sertoli cells closer to the centre of the tubule, and no evidence of mitoses (Fig. 6). Immature whales (mean whale length of sample 10.6m) had an average STD of 51.8μ (Table 1). Excluding the pseudohermaphroditic whales from the immature category results in a mean STD of 51.5μ (mean whale length of sample 10.0m).

The tubules in mature whales had an obvious lumen, the epithelium was thickened with evidence of mitotic activity, occasional spermatooza within the lumen, average STD was 125.8μ (Fig. 7) and average whale length was 14.1m (Table 1). Whales with similar findings, but a flat epithelium and no evidence of spermatooza were classified as suspected mature but inactive. Seven animals had intermediate sized tubules that were open but lacked mitotic activity with a flat

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**Table 2**

Regression analyses for morphologic measures of testicular mass (kg), length (cm), total whale length (cm), and seminiferous tubule diameters (STD, μ) for male bowhead whales.

<table>
<thead>
<tr>
<th>Morphology measured</th>
<th>n</th>
<th>R²</th>
<th>P value for F statistic</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis mass</td>
<td>32</td>
<td>0.89</td>
<td>&lt;0.0001</td>
<td>y=27.20+16.91*ln(x)</td>
</tr>
<tr>
<td>Mean testis length</td>
<td>36</td>
<td>0.93</td>
<td>&lt;0.0001</td>
<td>y=-7.75+0.008(x)</td>
</tr>
<tr>
<td>Testis length</td>
<td>44</td>
<td>0.68</td>
<td>&lt;0.0001</td>
<td>y=886.47+133.56*ln(x)</td>
</tr>
<tr>
<td>Mean testis length</td>
<td>44</td>
<td>0.79</td>
<td>&lt;0.0001</td>
<td>y=1,245+0.0022(x)</td>
</tr>
<tr>
<td>Mean testis length</td>
<td>42</td>
<td>0.91</td>
<td>&lt;0.0001</td>
<td>y=4.85<em>10^6</em>x^2.333</td>
</tr>
<tr>
<td>Seminiferous tubule diameter (STD)</td>
<td>38</td>
<td>0.59</td>
<td>&lt;0.0001</td>
<td>y=0.00128x^1.438</td>
</tr>
<tr>
<td>Mean testis weight</td>
<td>17</td>
<td>0.79</td>
<td>&lt;0.0001</td>
<td>y=44.50+21.80*ln(x)</td>
</tr>
<tr>
<td>Mean testis length</td>
<td>21</td>
<td>0.71</td>
<td>&lt;0.0001</td>
<td>y=172.65+63.59*ln(x)</td>
</tr>
</tbody>
</table>

1 P value for the ANOVA F-test that coefficient is different from 0.
2 Data were ln transformed prior to regression analyses.
3 Pseudohermaphrodites (‘pseudos’) were determined based on histology and morphology (Tarpley et al., 1995).
this paper describes the sexual maturation of males of the spermatozoa and changes in the germinative epithelium), whale length) and histological evaluation (STD, presence of intratesticular variation in STD does not affect the interpretation of testis maturity or activity if multiple sites are sampled. Whales have been defined as pubertal if immature and mature seminiferous tubules occur in the same testis section (Clarke et al., 1994). In some animals, detection was made of what would appear to be a mean STD that is lower than the large obviously mature males, but higher than clearly immature whales with small STD. These intermediate mean STDs (approximately 85μ) could represent a pubertal or testicular development phase.

Best (1969) showed that sperm whale testes mature from the centre to the periphery and thus location of samples should be considered in any maturation assessment. In our sample, this pattern was only seen in two of 16 whales by comparing cross sections but significant STD differences were found based on depth from the capsule in some sections of 25 of 43 whales. Given this, we recommend sampling from the centre to the periphery of the testis, especially for suspect pubertal animals. With an increased sample of pubertal whales, the centre to periphery maturation theory could be tested in the future. It was reported that on occasion (3-12%), only one testis had detectable sperm in the sperm capsule (Clarke et al., 1994) and attempts to evaluate both gonads per bowhead whale should be encouraged.

The largest bowhead whale testes measured in this study had a combined weight of 203.2kg in a 15.2m whale landed in the autumn. Testes of mature northern right whales are often larger, up to 2m long with a combined weight of BCB stock of bowhead whales. One method for estimating the onset of maturity is to determine at what point the testes mass to body length curve inflects sharply. Mean testes mass increases sharply once body length reaches approximately 12.7m, suggesting the onset of puberty can be less than 13.0m. In their review, Koski et al. (1993) noted that few data were available on male length at attainment of sexual maturity but predicted that it would occur at 12.0-13.0m. The results here concur with this; ‘pubertal’ or ‘early mature’ whales ranged in length from 11.5-13.5m. Durham (1972) reported that sexual maturity in the bowhead whale was estimated to occur at 11.5m but Marquette did not detail how this estimate was obtained. For females, Koski et al. (1993) reported values of 14.2m (landed whale data) and 13.0-13.5m (photogrammetric data). However, caution is needed when comparing data from landed whales to photogrammetric studies, as in our experience, stretching of the landed whale will probably increase length measurements. The shorter length at sexual maturation for males is not surprising since females of most baleen whales tend to be larger (Whitehead and Payne, 1981; Koski et al., 1993; George et al., 1999). The estimated age at sexual maturity as determined by stable carbon isotope analysis of the baleen is 17-20yr (Schell et al., 1989; Schell and Saupe, 1993). The estimate mean age for 13m whales using aspartic acid racemisation was about 25 years (George et al., 1999). The mean STD increased at a body length of approximately 13.0m paralleling an increase in testicular size at a slightly lower body length of about 12.7m. Maturation has been studied in many other whale species (see Boness et al., 2002). Comparisons to these studies of other species indicate male bowhead whales may have the oldest age at sexual maturation (George et al., 1999).

In some testes, a significant difference in STD was detected based on depth from the capsule or plane (anterior pole, posterior pole or equatorial) of sampling, but no clear pattern was evident and the difference was small (approximately 5μ). Thus, we conclude that intratesticular variation in STD does not affect the interpretation of testis maturity or activity if multiple sites are sampled. Whales have been defined as pubertal if immature and mature seminiferous tubules occur in the same testis section (Clarke et al., 1994). In some animals, detection was made of what would appear to be a mean STD that is lower than the large obviously mature males, but higher than clearly immature whales with small STD. These intermediate mean STDs (approximately 85μ) could represent a pubertal or testicular development phase.

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The largest bowhead whale testes measured in this study had a combined weight of 203.2kg in a 15.2m whale landed in the autumn. Testes of mature northern right whales are often larger, up to 2m long with a combined weight of
1.000kg or more. The relatively large testicular mass for right whales and to a lesser degree bowhead whales may involve a sperm competition reproductive strategy (Brownell and Ralls, 1986). In humpback whales, the paired testes weigh approximately 4.0kg at puberty and are heavier on breeding areas than in feeding areas (Nishiwaki, 1959; Chittleborough, 1965). The combined maximum weight of both testes in the gray whale at the height of the breeding season was approximately 68kg (Rice and Wolman, 1971). The much heavier testes and larger seminiferous tubules of gray whales during the southward migration compared to the northward migration and in the summer feeding areas indicate a marked seasonal sexual cycle with a peak in spermatogenic activity in late autumn and early winter (Rice and Wolman, 1971; Wolman, 1985). Sample size precluded comparisons of spring versus autumn-landed mature males here; bowhead whales are thought to breed in March (Koski et al., 1993).

In conclusion, based on testicular size (mass) and STD, male bowhead whales initiate significant testicular development at approximately 12.5-13.0m in length. In this study, seasonality could not be determined and Leydig cell (interstitial) assessment was found to be of very limited value given their cryptic nature. Future studies utilising special techniques to highlight interstitial cells (i.e. Leydig) and androgen measures in serum and other matrices may help to describe seasonally based changes in testicular activity and better define sexual maturity.

ACKNOWLEDGEMENTS

We greatly appreciate the cooperation of the many whaling captains and crews (Barrow Whaling Captains Association), and the Alaska Eskimo Whaling Commission (AEWC) for allowing us to sample landed whales for this study. We thank L.M. Philo, P. Nader, D. Ramey, V. Woshner, and many others for assistance with sample and data collection. We thank T. Albert, Charles D.N. Brower, and B. Nageak for supporting this study. We thank two anonymous reviewers for their constructive comments. C. Willetto prepared Fig. 1.

REFERENCES


