Analysis of seasonal changes in reproductive organs from Icelandic harbour porpoises (*Phocoena phocoena*)

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**ABSTRACT**

In this study, we analyse some aspects of the macro- and microscopical appearance of gonads of harbour porpoises (*Phocoena phocoena*) from Icelandic coastal waters. Sampling of animals bycaught in gillnets took place in the years 1991 to 1997 and covered the months from September to June. The differences in diameter of seminiferous tubules between samples from the peripheral and central parts of the testis indicate that histological changes associated with maturity begin in the core of the testis. The average tubule diameter was 49, 78 and 118 µm in immature, pubertal and mature animals respectively. The tubule size increased from 55 to 95 µm, coinciding with combined testis weight of 75 to 150 g, indicating the onset of puberty within this range of tubule size and testis weight. The estimated average diameter of tubules when an animal reaches maturity is 82.2 µm or 86.15 µm depending on the method used. The diameter of seminiferous tubules of mature and pubertal animals varies seasonally with a steady increase in the spring. However, lack of samples after mid-June makes estimation of the exact timing of mating impossible. In females, the follicle size of mature and immature animals of age 2 years and older shows seasonal variation, increasing in late winter or spring. The corpus luteum increases in size during the late pregnancy. The average size of the corpus albicans as a function of the total number of corpora albicantia for each animal, diminishes following the logarithmic equation $y = 4.49 - 0.447 \cdot \ln x$ ($y = \text{corpus size}, x = \text{number of corpora albicantia}$) but apparently they never disappear completely from the ovary. Ovarian activity was almost confined to the left ovary. Our results indicate parturition and copulation in the summer months from late June to August.

**INTRODUCTION**

The traditional way of determining the maturity stage of cetaceans is by analysis of the gonads. When an immature male porpoise reaches puberty and subsequently full sexual maturity, the testicle growth rate increases and the seminiferous tubules expand in diameter and become more elongated (Mackintosh and Wheeler 1929, Ridgway and Green 1967, Gambell 1968, Collet and Saint Girons 1984, Kasuya and Marsh 1984, Hohn et al. 1985, Sørensen and Kinze 1994). The microscopical character and size of cetacean tubules indicate when the animals have reached sexual maturity (Mackintosh and Wheeler 1929, Harrison 1969, Collet and Saint Girons 1984) but definitions of sexual maturity based on histological examinations vary somewhat between authors (Perrin and Donovan 1984, Perrin and Reilly 1984). Some use 3 stages of maturity based on the appearance of the seminiferous tubules in the testicles: immature, pubertal and...
Assessment of the maturity stage of females is generally easier as each ovulation and pregnancy leaves a permanent scar in the ovary, the corpus albicans. Based on the presence or absence of such scars, females are usually simply classified as either mature or immature (Perrin and Donovan 1984). Ovaries in many species of cetaceans do show an asymmetry in activity, meaning that one ovary (left or right) is more active than the other. This is often a species and age related characteristic (Oshumi 1964). In harbour porpoises ovarian activity is confined almost completely to the left one, but what causes this polarisation is not known (Harrison 1970, Fisher and Harrison 1979). Some studies have been done on various reproductive parameters of the harbour porpoises in the North Atlantic and connected waters (see Lockyer 2003), but knowledge on their reproductive biology in Icelandic waters has been very limited until recently (Ólafsdóttir et al. 2003). This study is a part of a larger project centred on the feeding ecology of the harbour porpoises (Víkingsson et al. 2003) in Icelandic coastal waters, itself part of a multispecies research program conducted by the Icelandic Marine Research Institute (MRI) in the years 1991 to 1997.

MATERIALS AND METHODS

Sampling
In the years 1991 to 1997 the MRI sampled harbour porpoises bycaught in gillnet fisheries and other fishing activities in coastal Icelandic waters. The nature of the sampling and dissection process varied according to landing location and availability of MRI staff. Some of the animals were dissected onboard the vessels by the fishermen and the samples taken, frozen and subsequently thawed and prepared at MRI. When MRI staff was available at the port of landing, the animals were dissected and samples frozen or fixed within hours, but if not available, the carcasses were transported to MRI in Reykjavík where dissected as soon as possible, or frozen whole. Normally these animals were kept on ice or at least in a cool place until dissected. The storage time of the samples in the freezer varied from a few days to several months. All samples for histological examination were fixed in 10% neutral buffered formalin. Thus, the histological samples vary somewhat with respect to the time lapse before freezing and/or fixation. The dissections at the MRI were done by standard routine (Anon. 1991) and included sampling for various studies such as feeding ecology (Víkingsson et al. 2003), biological parameters (Ólafsdóttir et al. 2003), genetics (Tolley et al. 2001), energetics, morphometrics and pollution.

Dissection process and histological analysis

Males
During dissection of males, both testes were weighed after removing the epididymis and a cross section taken from the mid-part along the longitudinal axis of one testis. After fixation, samples about 1 cm in diameter were taken, 1 from the peripheral (P) or near-surface part of the testis and 1 from the central part or core (C). From the smallest testis one combined sample, covering both the peripheral and central part was taken (PC sample). The fixated samples were dehydrated, embedded in paraffin, sectioned on a microtome in 10 µm slices, mounted on glass and stained with haematoxylin and eosin (H&E).

The stained sections were examined under a microscope equipped with an ocular micrometer and magnification up to 400X. Ten tubules were randomly chosen for measurements of diameter. In samples of poor quality (e.g. in terms of tubule circularity), the best 10 tubules were chosen. The sample was not used if fewer than 5 tubules were favourable for measurements. All measurements were made from the basement membrane or seminiferous epithelium if basement membrane was detached from the seminiferous epithelium, as this has shown not to be affected in slightly autolysed samples (Neimanis 1996 cited in Neimanis et al. 2000). Collet and Saint Girons (1984) found the sem-
iniferous epithelium useable up to 10 or 15 days after death. The mean size of the tubules in the sample was calculated from all (usually 10) measurements made. Each sample was assessed to 1 of 3 stages of sexual maturity from the appearance of the tubules and surrounding tissue: immature, pubertal and mature. The primary criteria were the relative size and appearance of the tubules and interstitial tissue. The detailed methods for assessing the sexual maturity in males followed in this study are found in Mackintosh and Wheeler (1929), Gambell (1968), Collet and Saint Girons (1984), Mitchell and Kozicki (1984), Hohn et al. (1985) and Sørensen & Kinze (1994). Because the seminiferous tubules are sensitive to post-mortem changes (Mackintosh and Wheeler 1929, Laws 1961, Gambell 1968) and at least several hours passed from catch or death of the animals until freezing or fixation of samples, no attempt was made to assess spermatogenesis in this study. This study is therefore confined to the seminiferous tubule size but Mitchell and Kozicki (1984) found this to be the best single histological character from which to judge sexual maturity.

When 2 samples were available from the same testis (P and C), the final maturity stage of the animal was determined using a combination assessment as summarised in Table 1.

### Females

During dissection of females, the uterus was carefully examined for the possible presence of a foetus. The ovaries were weighed and fixed intact in 10% formalin. In the laboratory, the ovaries were sectioned by hand in 1-3 mm sections. The diameter of the corpus luteum was measured in 3 dimensions and the average used to indicate size. The largest follicle, all corpora, and other ovarian scars (other bodies) were measured under a stereo microscope with an ocular micrometer using magnification up to 40X. Corpus albicans diameters were measured in 2 dimensions in the section where they appeared largest. The criteria for the classification of corpora and other bodies were based on Mackintosh and Wheeler (1929), Laws (1961), Marsh and Kasuya (1984) and Perrin and Donovan (1984). In this study, we assess each female as either immature or mature from the status of the ovaries. If a corpus luteum or corpus albicans was present in either of the ovaries, the animal was assumed mature. If no corpora or other ovarian scars indicating ovulation or a pregnancy were observed, the animal was assumed immature.

### RESULTS

#### Sampling

A total of 1,268 harbour porpoises were collected during the years 1991-1997. The sampling covered the months between September and June leaving 2 months, July and August (weeks 26 to 36) without any samples. The largest sample sizes were from March and April, coinciding with the peak of the gillnet fishery. Further information on the nature of the sample, including spatial and temporal distribution, sex ratios, etc. is provided by Vikingsson et al. (2003) and Ólafsdóttir et al. (2003).

---

<table>
<thead>
<tr>
<th>Maturity of sample C</th>
<th>Immature</th>
<th>Pubertal</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature</td>
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<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Pubertal</td>
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</tr>
<tr>
<td>Mature</td>
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<td>8</td>
<td>246</td>
</tr>
</tbody>
</table>

Table 1. Assessment of maturity status of male harbour porpoises based on combinations of maturity assessments of peripheral (P) and central (C) testis tissue samples. The results of this classification are also indicated (N = number of animals of each combination).
Males

Within testis variation in maturity and tubule size

Table 1 shows the results from assessments of reproductive status based on the analysis of peripheral (P) and central (C) testis tissue. In most cases, the same maturity stage was observed in both testis samples from a given individual. Only 4 animals that had immature tubules in P had mature tubules in C and no animals with mature tubules in P had immature tubules in C.

In Table 2, the difference in tubule diameter in PC, P and C samples is summarised by maturity stage. In the PC samples the sample maturity is equivalent to the maturity stage of the animal as there is only 1 sample from these animals. No mature animals were found among those where only 1 sample (PC) was taken and only 3 were pubertal. Table 2 also shows the combined average tubule size of P and C by final maturity stage of the animal and overall average tubule size by maturity stages. In immatures, there was practically no difference in tubule diameter between central and peripheral samples, while higher values were obtained for the central samples in pubertal and mature animals (Table 2). The difference was, however, statistically significant only in mature males (T-test, \( P < 0.05 \)).

The relationships between average tubule size of C-samples by tubule size of P-samples can be described by the following linear regressions:

- Immature: \( y = 1.6 + 1x \) \( (R^2 = 0.587, n = 48) \)
- Pubertal: \( y = 3.77 + 0.991x \) \( (R^2 = 0.784, n = 33) \)
- Mature: \( y = 62.7 + 0.527x \) \( (R^2 = 0.554, n = 143) \)

where \( y \) = tubule diameter (µm) in C and \( x \) = tubule diameter (µm) in P. The relationship between tubule size in P and C in mature animals is shown in Figure 1.

Average tubule sizes of P and C samples for all different combinations of reproductive classes are shown in Table 3. The difference in tubule size of P and C samples was found to be significantly different when C samples were judged to be mature and P samples as pubertal or mature (Table 3) (paired T-test, \( P < 0.05 \)).

Tubule size by maturity stage

The size of the seminiferous tubules increased dramatically when the animals reached sexual

<table>
<thead>
<tr>
<th>Sample location</th>
<th>Maturity</th>
<th>Average</th>
<th>Min</th>
<th>Max</th>
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<td>33</td>
<td>77</td>
<td>6.29</td>
<td>266</td>
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<td></td>
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<td>66</td>
<td>9.92</td>
<td>3</td>
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<td>81</td>
<td>6.82</td>
<td>70</td>
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</tr>
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<td>227</td>
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<td>270</td>
</tr>
<tr>
<td>P</td>
<td>Immature</td>
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<td>43</td>
<td>105</td>
<td>9.12</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Pubertal</td>
<td>82</td>
<td>24</td>
<td>179</td>
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<td>36</td>
</tr>
<tr>
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<td>Immature</td>
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<td>45</td>
<td>93</td>
<td>7.48</td>
<td>70</td>
</tr>
<tr>
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<td>80</td>
<td>34</td>
<td>177</td>
<td>25.14</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Mature</td>
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<td>68</td>
<td>208</td>
<td>20.84</td>
<td>270</td>
</tr>
<tr>
<td>Average P-C</td>
<td>Immature</td>
<td>54</td>
<td>45</td>
<td>93</td>
<td>7.48</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Pubertal</td>
<td>80</td>
<td>34</td>
<td>177</td>
<td>25.14</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Mature</td>
<td>118</td>
<td>68</td>
<td>209</td>
<td>20.84</td>
<td>270</td>
</tr>
<tr>
<td>Total average according to maturity stage</td>
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<td>33</td>
<td>93</td>
<td>7.18</td>
<td>336</td>
</tr>
<tr>
<td></td>
<td>Pubertal</td>
<td>78</td>
<td>34</td>
<td>177</td>
<td>24.94</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Mature</td>
<td>118</td>
<td>68</td>
<td>209</td>
<td>20.84</td>
<td>270</td>
</tr>
</tbody>
</table>
maturity. As summarised in Table 2 and 4, the average size increased from 49 µm in immature to 118 µm in mature animals. In pubertal animals the size was intermediate at 78 µm. The difference between the size of seminiferous tubules for the 3 maturity stages was significant in all cases (T-test, \( P < 0.05 \)). The size range of the tubules does, however, overlap between all the maturity groups (see Table 2). Average tubule size in the PC samples was 47 µm in immature animals and 59 µm in pubertal animals, which was found to be significantly different (T-test; \( P < 0.05 \)).

**Tubule diameter at maturity**

The percentage of each maturity stage for seminiferous tubule diameter groups of 5 µm is given

<table>
<thead>
<tr>
<th>Table 3. Average seminiferous tubule diameter (µm) in peripheral (P) and central (C) testis tissue samples, grouped by histological maturity assessment of P and C samples and results of paired T-tests between tubule size of the P and C samples in each combination (( * = P &lt; 0.05 )).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maturity of sample C</strong></td>
</tr>
<tr>
<td><strong>Maturity of sample P</strong></td>
</tr>
<tr>
<td>Immature</td>
</tr>
<tr>
<td>Pubertal</td>
</tr>
<tr>
<td>Mature</td>
</tr>
</tbody>
</table>

Results of paired T-test between sizes of P and C at 0.05 significant level (\( * = P < 0.05 \))

<table>
<thead>
<tr>
<th><strong>Results</strong></th>
<th>Immature</th>
<th>Pubertal</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Pubertal</td>
<td>NS</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Mature</td>
<td>NS</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1. Relationship between average seminiferous tubule diameters in center (C) and peripheral (P) samples for mature animals.**
in Fig. 2 and maturity class proportions are also given in Table 5. The proportion of pubertal animals increases rapidly at tubule diameters above 60 µm, reaches a peak at 70-80 µm and decreases abruptly thereafter (Fig. 2 and Table 5). The proportion of mature animals increases from 33% to 71% between tubule sizes of 80 and 85 µm (Fig. 2). Using the method of DeMaster (1984), we found that the size of the seminiferous tubule, when the proportion of mature males reaches 50% is 82.2 µm (DeMaster 1984, Ferrero and Walker 1999). Another possible way to calculate the size of seminiferous tubules at maturity, is by applying the non-parametric “Sum of fraction immature” method of Hohn as used by Sørensen and Kinze (1994), to the tubule information. Although this method was developed for estimation of age at sexual maturity, in principle, it can be applied to estimate tubule size at sexual maturity. By this method, age at sexual maturity is estimated as the sum of the fraction immature in each age-class in which both immature and mature occur (indeterminate age class) (DeMaster 1978, 1984) and then added to the age of the first age-class where mature animals are found (Sørensen & Kinze 1994). Here we apply this method to the tubule information with 2 maturity classes, immature (including pubertal) and mature. As each tubule size class consists of an interval of 5 µm (as opposed to one year in the original method), the sum of fraction immature has to be multiplied by 5 before being added to the minimum tubule size (70µm) where mature animals are detected. By this method, we estimated the mean size of seminiferous tubules at sexual maturity to be 86.15µm (70 + 5 x 3.23) (Table 5).

**Tubule size vs testis weight**
The relationship between total testis weight and average tubule size is illustrated in Figure 3. Associated with increase in testis weight from 75 g to 150 g is a sudden growth spurt in the average tubule diameter from 55 to 95 µm.

**Tubule size by catch week**
Analysis of seminiferous tubules by catch week is illustrated in Figure 4. There seems to be a pronounced increase in average tubule size of mature animals during the first half of the year (before the breeding season) and a decrease during autumn. Thus, the mean diameter increased

![Fig. 2. Percentage of each maturity stage by tubule size.](image)

Harbour porpoises in the North Atlantic
from around 110 µm in weeks 10 to 15 to around 200 µm in weeks 22 to 23. The overall increase in average tubule diameter during late winter-spring in mature males can be described by the linear regression:

\[ y = 94.99 + 2.50x \quad (R^2 = 0.226, \ n = 17) \]

(\(x = \) catch week, \(y = \) tubule diameter in µm). It should be noted that regressions on tubule diameter by catch week use the average values for each week, so significance tests are not appropriate. During the second half of the year, the average tubule size decreased again, from 140 and 170 µm in weeks 38 and 39 respectively to about 111 µm in week 51. Using linear regression on the average diameter, the decrease can be described by:

\[ y = 184 - 1.15x \quad (R^2 = 0.062, \ n = 13) \]

When skipping a single outlier (circled in Fig. 4) in week 52 we found the decrease in average tubule size in mature animals to follow the linear regression:

\[ y = 259.4 - 2.92x \quad (R^2 = 0.455, \ n = 12) \]

(Alt. regression for matures shown in Fig. 4).

In immature animals, on the other hand, no seasonal variation was noted (Fig. 4), either during spring or during autumn.

**Females**

**Ovarian asymmetry**

In the total sample, there was only one observation of ovarian scar in the right ovary. This scar was identified as corpus abberantia and originated from a mature animal caught on April 3. The left ovary contained one 3.6 mm corpus albicans of the “Old” type.

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**Table 4.** Comparisons of seminiferous tubule size (µm) by maturity stage in harbour porpoises and some other species of similar size.

<table>
<thead>
<tr>
<th>Area</th>
<th>Immature (range)</th>
<th>Pubertal (range)</th>
<th>Mature (range)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iceland</td>
<td>49 (33-93)</td>
<td>78 (34-117)</td>
<td>118 (68-209)</td>
<td>This study</td>
</tr>
<tr>
<td>Northwest Atlantic</td>
<td>48 (37-64)</td>
<td>124 (91-176)</td>
<td></td>
<td>Gaskin et al. 1984</td>
</tr>
<tr>
<td>Danish waters</td>
<td>60 (50-80)</td>
<td>85 (80-89)</td>
<td>189 (80.9-119)</td>
<td>Sørensen and Kinze 1994</td>
</tr>
<tr>
<td>Bay of Fundy</td>
<td></td>
<td></td>
<td></td>
<td>Neimanis et al. 2000</td>
</tr>
<tr>
<td>English and Wales water</td>
<td>47</td>
<td></td>
<td>118</td>
<td>Karakosta et al. 1999</td>
</tr>
</tbody>
</table>

**Other species**

<p>| Japanese Finless porpoises | (43-73) | 121 | (109-242) | Shirakihara et al. 1993 |
| Dall’s porpoise           | 59      |     | 173       | Ferrero and Walker 1999 |
| Common dolphin            | (40-60) | “about 50” | (100-250) | Collet and Saint Girons 1994 |</p>
<table>
<thead>
<tr>
<th>Tubule size group (µm)</th>
<th>n Immature</th>
<th>n Pubertal</th>
<th>n Mature</th>
<th>n Total</th>
<th>Pn Immature</th>
<th>Pn Pubertal</th>
<th>Pn Mature + Pubertal</th>
<th>Pn Mature</th>
</tr>
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<td>35</td>
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<td>1</td>
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<td>13</td>
<td>0.92</td>
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</tr>
<tr>
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<td>83</td>
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<td>1.00</td>
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</tr>
<tr>
<td>55</td>
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<td>0.02</td>
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<td>0.18</td>
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<td>0.00</td>
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<tr>
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<td>1</td>
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<td>0.80</td>
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**Sum of fracture immature (70+5*3.23):** 86.15 µm
The largest follicle found in the right ovary was 1.96 mm and the largest follicle in left ovary was 2.36 mm. Average size of the largest follicle in left ovaries of immature animals was 2.13 mm ($s = 1.480, n = 123$) and 1.05 ($s = 0.774, n = 123$) in the right ovaries. By paired T-test, they were found to be significantly different ($P < 0.05$). In mature animals, the average size of the largest follicle in left ovaries was 1.61 mm ($s = 0.792, n = 55$) and 1.13 ($s = 0.311, n = 55$) in right ovaries. They were also found to be significantly different (paired T-test, $P < 0.05$).
When comparing the average size of the follicles in left ovaries of immature (2.13 mm) and mature animals (1.61 mm), the size was significantly different (T-test, $P < 0.05$). The size of follicles of the right ovaries, 1.05 and 1.13 mm in immature and mature respectively, was on the other hand not significantly different (T-test, $P > 0.05$).

**Follicle size by week**

As most of the sampling took place in 2 distinct periods leaving several weeks without any samples, the material can be divided into 2 periods; week 4-25 (period 1) and week 37-52 (period 2), (Figs. 5 and 6a). When comparing follicle size between these 2 periods we found the follicles from left or right ovaries not to be significantly different in any of the maturity groups (T-test, $P > 0.05$).

Figure 5 shows the relationship between follicle diameter of left (5a) and right (5b) ovary and catch week in mature animals. A linear regression lines are shown.

**Fig. 5.** Seasonal variation in follicle diameters of left (a) and right (b) ovaries in mature animals by catch week and of left (c) and right (d) ovaries in immature animals two years of age or older. Linear regression lines are shown.

When comparing the average size of the follicles in left ovaries of immature (2.13 mm) and mature animals (1.61 mm), the size was significantly different (T-test, $P < 0.05$). The size of follicles of the right ovaries, 1.05 and 1.13 mm in immature and mature respectively, was on the other hand not significantly different (T-test, $P > 0.05$).

**Fig. 6.** Relationship between Corpus luteum diameters and catch week (a) and foetus weight (b). Linear regression lines are shown.
sion of follicle diameter on week number for mature animals gave the following relationship:

Left ovary in period 1:
\[ y = 0.951 + 0.049x \]  
\( R^2 = 0.039, \ n = 54 \)

Left ovary in period 2:
\[ y = 8.537 - 0.153x \]  
\( R^2 = 0.753, \ n = 9 \)

Right ovary in period 1:
\[ y = 0.861 + 0.019x \]  
\( R^2 = 0.043, \ n = 47 \)

Right ovary in period 2:
\[ y = 3.593 - 0.055x \]  
\( R^2 = 0.428, \ n = 8 \)

\( x \) = catch week, \( y \) = follicle diameter in mm).

The largest follicle in left ovaries in period 1 was 5.1 mm (week 14) and 3.0 mm in period 2 (week 38). The largest follicle size of the right ovary was 2.0 mm (week 14) and 1.6 mm (week 42) in period 1 and 2 respectively.

A linear regression of follicle diameter on week number for immatures revealed no seasonal variation. In order to include only animals that are approaching sexual maturity (pubertal) in the analysis, a linear regression was performed on all immature females aged 2 years and older (Fig. 5c and d).

Left ovary in period 1:
\[ y = -0.606 + 0.218x \]  
\( R^2 = 0.152, \ n = 40 \)

Left ovary in period 2:
\[ y = 6.785 - 0.103x \]  
\( R^2 = 0.050, \ n = 8 \)

Right ovary in period 1:
\[ y = 1.115 + 0.008x \]  
\( R^2 = 0.001, \ n = 40 \)

Right ovary in period 2:
\[ y = 6.623 - 0.125x \]  
\( R^2 = 0.330, \ n = 8 \)

\( x \) = catch week, \( y \) = follicle diameter in mm).

The largest follicle in left ovaries in period 1 was 5.1 mm (week 14) and 3.0 mm in period 2 (week 38). The largest follicle size of the right ovary was 2.0 mm (week 14) and 1.6 mm (week 42) in period 1 and 2 respectively.

Seasonal variation in corpus luteum size

Figure 6a shows the size of the corpus luteum by catch week. The 2 largest corpora lutea (27.3 mm and 23.7 mm) were from animals sampled during late spring (week 21 and 22). The increase in size of the corpus luteum in the first half of the year can be described by the linear regression:

\[ y = 16.39 + 0.251x \]  
\( R^2 = 0.234, \ n = 51 \)

\( y \) = corpus luteum diameter in mm, \( x \) = week number). There was no clear trend in the size of corpus luteum during autumn.

No significant difference in corpus luteum size was found between first time ovulators and the “more experienced” females (repeated ovulators) between weeks 12 to 22 (T-test, \( P > 0.05 \)).

Corpus luteum size by foetus weight

Figure 6b shows the relationship between foetus weight and corpus luteum size. The relationship can be described by the linear regression:

\[ y = 18.9 + 0.398x \]  
\( R^2 = 0.19, \ n = 48 \)

\( y \) = mean corpus luteum diameter in mm and \( x \) = foetus weight in kg). Unfortunately, there were no foetuses between the weights of 0.25 and 1.3 kg in the sample, so the development of the corpus luteum during the early phase of pregnancy is uncertain. The 2 largest corpora lutea could indicate an exponential growth of the corpus luteum during the last stage of pregnancy (Fig. 6b), but the data are too scanty during this period for further analysis.

Decrease of corpus albicans by time

The low sample size of mature females, except in March and April, prevented analyses of seasonality in corpus albicans size.

To study the regression rate of the corpus albicans we calculated the total average diameter categorised by total number of corpora albicantia in each animal (Fig. 7 upper line). The decrease in average corpus albicans diameter can be described by the logarithmic regression:

\[ y = 4.49 - 0.447 \cdot \ln x \]  
\( R^2 = 0.583, \ n = 13 \)

\( y \) = corpus albicans size in mm, \( x \) = number of corpora albicantia). In the same way, the decrease of the smallest corpora albicantia in the ovaries can be described by the logarithmic regression (Fig. 7 lower line):

\[ y = 3.11 - 0.267 \cdot \ln x \]  
\( R^2 = 0.159, \ n = 13 \).
DISCUSSION

Sampling

Unfortunately, sampling was unevenly spread throughout the season. No porpoises were obtained during July and August and the total sample size was very small during May to September. This was due to the seasonal nature of the gillnet fishery around Iceland that was the main source of samples for this study. The effort of these and other fisheries that might be expected to involve bycatch of porpoises (e.g. lumpfish) peaks in late winter or spring and is much lower during the summer. The sample size in the latter half of the year is also very small compared to late winter and early spring. The total lack of samples, usable for this study, from a period lasting 11 weeks (26 to 36) and the low sample size in the adjacent weeks is particularly unfortunate as it seems to include the main mating and calving season (Ólafsdóttir et al. 2003).

Males

Quality of samples

In mammals, the fixation of the testicular samples should preferably take place within a few minutes from death in order to gain maximum information from the sample (Mackintosh and Wheeler 1929). This is because of the sensitivity of testicular tissue to post-mortem changes, as autolysis starts almost immediately after death (Laws 1961). Time from death until fixation or freezing of the histological sample is therefore reflected in the quality of the fixed sample. In the present study, no direct comparison was made between the quality of freshly fixed and frozen testis samples or samples stored for a shorter or a longer time in the freezer. Storage in a freezer for an extended period has been shown to affect the gonadal tissue and the quality of tubules (Neimanis 1996 cited in Neimanis et al. 2000).

In a study on the southern sei whale, Gambell (1968) did not attempt any assessment of spermatogenesis as the time from death to sample fixation ranged from 4 to 44 hrs (16 hrs on average). In our case, post mortem time until fixation or freezing varied from around 24 hrs up to about one week in a few extreme cases. Furthermore, freezing may also have affected

**Fig. 7.** Relationship between corpus albicans diameter and total number of corpora albicantia in mature animals. (●) average size, (▼) minimum size. Line shows regression described by the equation for average size.
the testicular tissue. In addition, the time for thawing and subsequent fixation of samples from a whole animal takes several days, so the chances for achieving reliable results on assessing spermatogenesis and spermatozoa production are low. No attempt was therefore made to assess sperm production. In the case of blue whales, Mackintosh and Wheeler (1929) found, however, that although the fixation of their samples occurred from 4 hrs after death, good fixations could be achieved. Read and Hohn (1995) and Hohn et al. (1996) used Berg’s stain with good results to detect spermatozoa in autolysed samples. Such studies could be of great value in defining the peak period of copulation in Icelandic harbour porpoises, however it would require more samples from the period June to September (weeks 22-40). Despite sub-optimal conditions for fixation with regard to detection of spermatogenesis, most of our samples were suitable for assessment of maturity stage and measurements of tubule diameter and all samples favourable in terms of tubular circularity were used.

Relationship between tubule size at the periphery and centre of testis.
In addition to the traditional divisions of “immature” and “mature”, Aguayo in 1963 (cited in Best 1969), proposed a third maturation stage from the appearance of the seminiferous tubules in maturing sperm whales (Physeter macrocephalus). This group, called pubertal or maturing, contains animals where the testis tissue shows characteristic features of both mature and immature animals. Later Best (1969) adopted this classification in a study on reproduction characters of the sperm whales and found that the central part of the testicles matured at a body length about 10 feet (3 m) shorter than did the peripheral tissue. In contrast, no difference was found in maturity between different locations of the testis in a study on short-finned pilot whale (Globicephala macrorhynchus) (Kasuya and Marsh 1984).

In our study, there are indications that histological changes associated with maturity appear earlier in the core than near the periphery of the testis (Tables 1, 2 and 3). This is similar to the conclusion of Best (1969) for sperm whales. Our study does not indicate a differentiation within the testis tissue in immature and pubertal animals (paired T-test, \( P > 0.05 \)). However, in mature animals, (Table 2 and Fig. 1), the mean diameter of central seminiferous tubule was significantly larger than in peripheral testis tissue (paired T-test, \( P < 0.05 \)). When the animals have reached maturity, the central tubules appear to grow at about half the rate of the peripheral so that they have reached similar size at a tubule diameter of 130-140 µm (Fig. 1). The onset of differentiation between C and P in testicular maturity is not clear from the present results (Fig. 1). A confounding factor in this respect is the combination of seasonal variation (Fig. 4) and the uneven temporal distribution of the samples, in particular the total lack of samples from the breeding season that appears to be in July-August off Iceland (Ólafsdóttir et al. 2003). Thus, a possible growth spurt in tubule diameter during or immediately before the breeding season would not be detected in the present study.

Seasonality in tubule size
Seasonal fluctuations in the diameter of seminiferous tubules have been demonstrated in many cetacean species (Fountain and Barrette 1997, et al. 1984, Hohn et al. 1985, Kasuya and Marsh 1984, Neimanis et al. 2000), although this does not seem to be the case in all species (Best 1969).

The diameters of seminiferous tubules in mature harbour porpoises have not been shown to be related to age but are strongly related to date of capture (Gaskin et al. 1984). In the present study, no seasonal variation in tubule diameter is apparent for immature males while the mature animals show an increase during spring and a decline during autumn. For pubertal animals, when ignoring a single peculiar outlier, we see a very steep line indicating rapid growth of the seminiferous tubules from a size similar to that in immature animals in the spring, to the size of those in mature animals few weeks later (Fig. 4). This indicates that puberty (as here defined in terms of rapid histological changes in the testis) of Icelandic harbour porpoises starts in the early spring (in weeks 10 to 12 (March)). Measuring the hormonal level of pubertal animals might throw further light on this. Hormonal studies on male porpoises in captivity have
revealed pronounced seasonal fluctuations in plasma testosterone levels, with increased levels in spring and early summer (Desportes et al. 2003). The blood serum testosterone level of North Atlantic fin whales increased fourfold from June to August (Kjeld et al. 1992) and the testosterone level of Hawaiian male spinner dolphin (Stenella longirostris) showed seasonal variation (Wells 1984). The sudden increase of tubule size in pubertal animals in this study fits well with the timing of the peak in hormonal level in the study of Desportes et al. (2003). Due to a lack of samples, we were unable to monitor the subsequent histological development during summer. Porpoises classified as pubertal during spring may mate for the first time during the summer, but it is also possible that the pubertal animals in the spring still have pubertal characteristics in the autumn, but then with larger tubules as seen in the few animals showing tissue characteristics of puberty during the autumn (Fig. 4). This would imply that the testicles do not completely reach the status of mature animals until late winter the following year.

Fisher and Harrison (1970) concluded that testicular activity in the North Atlantic harbour porpoises increases from May, reaching a peak in late July and then decreases by mid-August. Our information agrees with their findings, but again the total lack of samples from late June until mid-September hampers our conclusions in this respect. In Danish harbour porpoises, Sørensen and Kinze (1994) found that the size of seminiferous tubules peaks in late June to mid-July. The decrease in tubule size during the autumn was, however, not as steep as that found by Gaskin (1984). Read and Hohn (1995) found maximum testicle weight in late June and early July. From our limited autumn data we can suggest a prolonged period of testicular activity until week 44 (late October) as the tubule size appears to be stable between week 38 and 44, after which the size declines further. The large variability in tubule size within periods also suggests a rather temporally dispersed breeding period. This is in good agreement with Harrison (1970) who refers to Slijper (1962), suggesting that the harbour porpoise in the North Atlantic has a period of mating extending into the months September and October, and the findings of newborn calves in Portuguese waters in January and March (Sequeira 1996). Harrison and Ridgway (1971) have also suggested multiple breeding seasons or a diffused pattern of seasonality in bottlenose dolphins (Tursiops truncatus).

**Tubule size by maturity stage**

Gambell (1968) found that the tubule size of pubertal southern sei whales covers nearly the whole range recorded from the smallest immature to the largest of the mature animals. Our findings are in general similar, but the tubule size of pubertal animals is clearly connected to season. The largest seminiferous tubules in pubertal animals do not however reach the maximum size of those in mature porpoises (Table 4). The size of seminiferous tubules in different maturity classes of Icelandic harbour porpoises lies within the range observed in other studies on porpoises and other cetacean species of similar size (Table 4). Our results show, however, a somewhat wider range than in other studies.

The incidence of pubertal animals in the smallest tubule size group can be explained by the fact that if only one tubule of the 10 (or fewer) measured is considered mature and all other tubules in the sample are very small and immature, the sample will be judged pubertal and consequently the animal as pubertal or mature depending on results from the overall assessment from both P and C samples (see Table 1).

Considering the proportions of pubertal males (Fig. 2) and testes weight (Fig. 3) in relation to tubule diameter, there is an abrupt increase in both parameters at tubule sizes between 55 µm and 95 µm, after which there is a slower but steady increase in both parameters. We can conclude that this sudden rise in seminiferous tubule size demonstrates changes associated with the onset of sexual maturity. This is consistent with the findings of Lockyer (1995) for harbour porpoises in British waters.

**Females**

**Quality of samples**

No apparent difference was noted between ovaries fixed fresh and ovaries that were frozen before fixation. It is however possible that corpora become less detectable in ovaries that are fixed after they have frozen and would there-
fore need staining to be as detectable as in freshly fixed ovaries. Further comparison between freshly fixed ovaries and frozen and fixed ovaries could help in providing further recommendations for preparation, dissection and analysis of ovaries.

**Ovarian asymmetry**

Many studies have shown strong polarity or asymmetry in ovarian activity of cetaceans. In odontocetes, the left ovary is the more dominant while in mysticetes the polarisation is less prominent or absent (Ohsumi 1964, Harrison 1970), although a few exceptions have been reported. Considering the accumulation rate of corpora in left and right ovaries, Oshumi (1964) suggests categorising harbour porpoises in the Type III class together with *Lagenorhynchus*, *Tursiops*, *Delphinus* and *Stenella*, where “remarkable difference of corpora accumulation rate occurs between the left and right ovary”.

The right ovary of harbour porpoises is usually sub-mature and non-functional (Gaskin et al. 1984). Fisher and Harrison (1970) found 1 specimen with a corpus albicans in the right ovary. Kaarstad (MS 1993) found 2 animals with corpora in the right ovary, while no corpora were found in the left ovaries of these animals and Sørensen and Kinze (1994) found one animal with all corpora in right ovary. In the present study, only one incidence of corpora (identified as corpora abberantia, and thus not considered to be indicative of pregnancy) was found in the right ovary of a 20-year-old female, one of the oldest sampled. The left ovary of that animal contained one corpus albicans. While the present study generally confirms earlier findings of strong polarity of ovarian activity in harbour porpoises, we cannot, because of the small sample size of old females, conclude anything about the possibility of increased activity of the right ovary later in life.

**Follicle size**

Ovulation occurs when the enlarged follicle ruptures and releases an egg, but expansion of the follicle occurs shortly before the ovulation (Perrin and Donovan 1984). The size of the largest follicle in non-pregnant animals indicates how close to ovulation the animal is. The size of the largest follicles in mature ovaries varies between cetacean species. Follicles up to 10 cm in diameter have been found in blue whales and 6 cm in Southern Hemisphere fin whales (Mackintosh and Wheeler 1929). Ferrero and Walker (1999) found that in Dall’s porpoise the maximum follicle diameter was found in June with average size of 1.7 mm and the largest follicle 9.5 mm in diameter.

A recently ruptured follicle after ovulation is easily noticeable on the surface of the ovary by its bloody stained hole (Laws 1961). Mackintosh and Wheeler (1929) found that in the ovary of southern fin whales there are many follicles visible but only one of large size, implying that there is only one ovum shed at a time. In contrast, Gaskin et al. (1984) found up to 15 corpora albicantia in animals 4 and 5 years of age, suggesting multiple ovulation without fertilisation in younger harbour porpoises. No recently ruptured follicle of ovulation was observed in our sample.

In mature animals we found both the largest (5 mm) follicles and the smallest corpus luteum (16.7 mm) in week 14 (late March to beginning of April). In a study by Fisher and Harrison (1970) the largest follicle of the immature animals was 6 mm and found in week 30. Additionally they found no follicles of the mature animals larger than 4.5 mm in diameter, which was from the left ovary of a lactating non-pregnant animal, caught in week 33, whose left ovary contained an 18 mm corpus luteum. Read (1995) found the 2 smallest corpora lutea, both 11 mm, in weeks 25 and 26.

The largest follicles found by Kaarstad (MS 1993) were 8 mm from porpoises sampled in spring and summer, indicating that birth and copulation in Norwegian and Swedish waters takes places from mid-May to mid-July. Ovulation is expected to occur from July to mid August in Norwegian and Swedish waters (Kaarstad MS 1993). Sørensen and Kinze (1994) found the largest follicle (14.5 mm) in Danish waters in July. In the western North – Atlantic, both Gaskin et al. (1984) and Fisher and Harrison (1970) found the largest follicles in early July. In a study on harbour porpoises in the Bay of Fundy, the largest follicle, 10.3 mm in diameter, was found in mid June, while the sizes of follicles of animals sampled later in the
In our sample of mature and pubertal animals (including immatures aged 2 years and older), only 2 follicles were 5 mm or larger. This could indicate that the maximum follicle size before rupture is close to 5 mm, but it is more likely, that the late growth of follicles was missed in our sample.

Immature animals aged 2 years and older (pubertal) exhibited clear seasonal variation in follicle size in the left ovary but our results for the right ovary are inconclusive. In this group 2 clusters of mean follicle sizes can be distinguished in the data collected in late winter and spring (Fig. 5c), one over ca 2 mm in diameter and another under ca 1.6 mm. Assuming that the first cluster represents first time ovulators, comparison with follicle development in the left ovary of mature porpoises (Fig. 5a) suggests that ovulation takes place earlier in the season in pubertal females (first time ovulators) than in mature animals that have ovulated more than once. The fact that the large follicles are found over an 11 week period (weeks 14 to 25, Fig. 5c) in pubertal animals also suggests a prolonged mating season in pubertal animals, probably starting earlier than in the fully mature animals. Although the right ovary is generally not functional in ovulation, it shows some sign of increase in follicle size in the spring (Fig. 5d).

Alternatively, this apparent difference between pubertal and mature females might be explained by a difference in the growth rate of follicles in the period immediately preceding ovulation. However, lack of sampling around the peak of the breeding season prevents any firm conclusions on this.

**Corpus luteum**

In some mammals (horse, elephant, Norway rat, etc.) accessory corpora lutea are commonly found. They can form at the same time as the "corpus luteum of pregnancy" or can even be formed later during pregnancy as in mares. These accessory corpora lutea are also found in cetaceans and are most common in odonto-cetes of the genera *Delphinapterus* and *Monodon*, where they occur in 12% of pregnant females (Brodie 1972, Perrin and Donovan 1984). They can either form from ovulated or unovulated follicles and their histology and function is identical to the corpus luteum of pregnancy (Laws 1961). In this study no individuals had more than one corpus luteum. No incidents of "corpus luteum of ovulation" (Marsh and Kasuya 1984, Perrin and Donovan 1984, Read 1990) were observed in the present study as foetuses were found in all animals with a corpus luteum.

In both fin and blue whales, the corpus luteum increases in size for few weeks at the beginning of pregnancy, after which they apparently shrink slightly (Mackintosh and Wheeler 1929). Gambell (1968) and Laws (1961) on the other hand did not detect such changes in their studies on southern hemisphere sei whales and humpback whales. Laws (1961) found that the corpus luteum of fin and humpback whales reaches its maximum size in late pregnancy. Our study is inconclusive regarding the development in corpus luteum size during the early pregnancy because of the total lack of foetuses between 20 and 40 cm. In addition, the sample includes only 7 foetuses less than 20 cm. It appears that the size of the corpus luteum is relatively constant (after a presumed growth spurt during the first weeks of pregnancy) until late winter or spring when the corpus luteum increases in size (Figure 6(a)). Our findings are similar to those of Laws (1961) for fin and humpback whales, suggesting that the corpus luteum grows for one or two months after which growth ceases until late pregnancy.

The newly formed corpus luteum should easily be distinguished from the later stages by its thin outer membrane and the numerous blood vessels beneath it (Laws 1961). Gaskin et al. (1984) found no significant difference between corpus luteum size of porpoises at different reproductive stages but Read et al. (1990) found the corpora lutea of visible foetuses to be significantly larger than those of non-visible foetuses, 20.8 and 18.5 mm respectively. The average size of 3 newly formed corpora lutea of ovulation in harbour porpoises sampled in Swedish and Norwegian waters during summer.

Harbour porpoises in the North Atlantic

136
was 15.0 mm, while *corpora lutea* from pregnant animals were significantly larger at 22.8 mm (Kaarstad MS 1993). In a study by Fisher and Harrison (1970), the smallest *corpus luteum* found, originating from a recently impregnated lactating female, measured 15 mm in diameter in week 28. The smallest *corpus luteum* associated with a foetus (64.5 cm long) in the present study was 16.5 mm, and the majority of *corpora lutea* in our study were in the range of 17 to 20 mm.

Within the North Atlantic, considerable variation in the time of parturition has been demonstrated. In the Gulf of Maine, Read and Hohn (1995) found mature females that had recently given birth in June. In Danish waters, calving peaks in early July (Kinze 1990) and a small sample from Portuguese waters suggests an earlier and less defined calving season as newborn calves were found in January and March (Sequeira 1996).

No newly formed *corpora lutea* were observed in our study so the rupture of follicles and formation of the *corpus luteum* must have started during the summer period when no samples were available.

*Decrease in size of the corpus albicans over time*

Mackintosh and Wheeler (1929) concluded from their study on *corpora albicantia (lutea) b* in blue whales that the rate of shrinkage of the *corpus albicans* decreased as the *corpus albicans* became smaller. In blue whales, the *corpus albicans* regressed from around 5 cm to 3-4 cm in first 10 months. Laws (1961) found that the modal diameter of the *corpus albicans* in southern fin whale stabilises at 2 cm. Our findings are rather unclear. As can bee seen from the smallest *corpora albicantia* on Figure 7 it appears that the rate of shrinkage decreases when the total number of *corpora albicantia* increases and they become smaller and the size could stabilise somewhere between 1.5 and 2 mm.

The harbour porpoise is relatively short lived animal, the maximum age reported from Icelandic waters being 20 and 16 years for females and males respectively (Ólafsdóttir et al. 2003). When there is only one *corpus albicans* found in the ovaries the average size of *corpora albicantia* is 4.51 mm (smallest found was about 3 mm). When the total number of *corpora albicantia* has reached 18 (animal age 20), the average size of the *corpora* is still about 3 mm (minimum *corpora* 1.67 mm) (Fig. 7). There is thus no reason to expect the *corpus albicans* to disappear from the ovaries in Icelandic harbour porpoises. However, we cannot preclude the possibility that *corpora albicantia* can get so small in very old animals that they can be missed without using staining or microscopic examination or by slicing the ovaries finer than 1 mm.

**GENERAL CONCLUSIONS**

Despite the shortage of data during the summer months, from the seasonal distribution in size range of *corpora lutea* and follicles as well as the seasonal variation in size of seminiferous tubules, we can conclude that parturition and conception takes place mostly in the months of June to August. Additional evidence for this conclusion comes from the seasonal distribution of foetus sizes and testis weight (Ólafsdóttir et al. 2003), from which it was concluded that Icelandic harbour porpoises give birth sometime between June and the beginning of September. In this study, near-term foetuses (75-80 cm) (Leatherwood and Reeves, 1983, Gaskin et al. 1984, Lockyer 1995) were found in June, indicating that parturition begins in that month. Considerable variability was, however, detected in foetal size as well as in the characteristics of the reproductive organs in both males and females. This variability indicates that the periods of parturition and conception range over at least 1 to 2 months. Further studies on seasonal and age specific changes in the gonads of the harbour porpoises in Icelandic waters would help clarify these and other matters. In this respect, sampling during the breeding season would be most important.

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