

# Changes in the Ovaries of the Short-Finned Pilot Whale, *Globicephala macrorhynchus*, with Age and Reproductive Activity

H. MARSH

Zoology Department, James Cook University, Townsville, 4811, Australia

AND T. KASUYA

Ocean Research Institute, University of Tokyo, Japan\*

## ABSTRACT

Macroscopic examination of the ovaries of 298 specimens of *Globicephala macrorhynchus* obtained from a driving fishery off the Pacific Coast of Japan was supplemented by histological and histochemical study of a representative series of ovarian structures. The patterns of follicular development and atresia and corpora-luteum development and regression are described. The atresia of macroscopic follicles was frequently accompanied by thecal luteinization producing conspicuous corpora atretica which did not persist. We could not separate corpora lutea of pregnancy from those of 'ovulation'. The corpus luteum persisted throughout the gestation period but with apparent change(s) in lipid metabolism. Some corpora albicantia regressed fully within two years, but the rate of regression probably varied with hormonal status. Corpora albicantia persisted for life as a record of ovulations.

Almost all the whales were aged using dentinal and/or cemental growth-layer groups. The age-specific decline in the pregnancy rate was paralleled by a decline in the ovulation rate, and infertile ovulations were more common in older females. Females ceased to ovulate before age 40 years. About 25% of mature females had senescent ovaries which were severely depleted of oocytes (presumably because of the exceptionally high atresia rate) and histologically similar to post-menopausal human ovaries. Female short-finned pilot whales in this population thus had a definite post-reproductive phase in their life cycle.

## INTRODUCTION

The ovaries of various cetaceans have been studied as indices of relative age and terminal reproductive status and as records of reproductive history. However, with few exceptions, scientists have had no direct information on the species' reproductive physiology or life history. Thus the interpretation of cetacean ovaries has been rather like running an experiment without a control.

Research methods have been determined largely by specimen availability. Harrison and his co-workers have made detailed macroscopic and histological examination of the ovaries of mostly fairly small samples of a large variety of odontocete species (e.g. Harrison, 1949, 1972; Harrison, Boice and Brownell, 1969; Harrison and Brownell, 1971; Harrison and Ridgway, 1971; Harrison, Brownell and Boice, 1972; Harrison and McBrearty, 1973-74, 1977; Collet and Harrison, 1981). This approach gives precise information about each ovary, but interpretation has usually been hampered by sample size and/or lack of information about the ages of the animals.

Interpreting ovaries on the basis of macroscopic examination alone is less precise, but much less time-consuming, and has been the usual method of studying ovaries from a large series of conspecifics obtained from a fishery. Interpretation of these data has been facilitated by an independent estimate of each animal's absolute age in some studies (e.g. Ohsumi, 1965; Kasuya, Miyazaki and Dawbin, 1974; Perrin, Coe and Zeifel, 1976; Miyazaki, 1977; Perrin, Holts and Miller, 1977), but not others (e.g. Chittleborough, 1954; Sergeant, 1962; Gambell, 1968, 1972).

Laws (1958, '59, '61), Best (1967), Fisher and Harrison (1970), Hirose, Kasuya, Kazihara and Nishiwaki (1970),

\* Present address: Far Seas Fisheries Research Laboratory, Ordo, Shimizu-shi, Shizuoka-Ken 424, Japan.

Zimushko (1970) and Harrison, Bryden, McBrearty and Brownell (1981) have combined the advantages of both these approaches. They have macroscopically examined the ovaries of a large series of conspecifics and have used histology as a supplementary tool. Unfortunately, these studies were hampered by a lack of absolute-age data.

In this study, we have attempted to maximize the information value of the ovaries from each pilot whale by examining them macroscopically and, where necessary, histologically. An independent estimate of the whale's absolute age was subsequently used to enhance our ability to interpret these observations.

## MATERIALS AND METHODS

The ovaries examined were collected from 298 specimens of *Globicephala macrorhynchus* caught in the driving fishery off the Pacific Coast of Japan between 1975 and 1981 in the following months: January, February, May, June, July, October and December. Each whale was assigned an accession number which is a hyphenated combination of the school number and the number of the animal within the school, e.g. whale 13-24 refers to whale 24 in School 13. The methods used to collect and preserve specimens from each whale are outlined in our companion paper (Kasuya and Marsh, 1984). The ovaries from 298 whales from Schools 7, 9 to 18, 20, 22 to 27 (Kasuya and Marsh, loc. cit.) were examined by Marsh (those from Schools 7 and 17 were also checked by Kasuya) without knowledge of the age estimate for each whale, which was obtained by Kasuya by counting dentinal and/or cemental growth layers as described by Kasuya and Matsui (1983).

Each formalin-fixed ovary was trimmed of its bursa and weighed to the nearest 0.1 g. The number of corpora lutea, corpora albicantia, and corpora atretica were

Table 1  
The characteristics on which the macroscopic classification of corpora albicantia was based

| Characteristic                    | Class of corpus albicans   |   |  |
|-----------------------------------|--|---|--|
|                                   | Young  | Medium  | Old  |
| <i>External structure</i>         |  |   |  |
| Protuberance from ovarian surface | As small corpus luteum   | Usually slight except for stigma                                    | Stigma only  |
| Shape                             | As small corpus luteum   | May be round or flattened against surface                           | Irregular, may be round or flattened against surface |
| Stigma                            | Obvious as on corpus luteum  | Smaller than young corpus albicans                                  | White, usually puckered plaque                       |
| Nature of surface                 | Smooth   | Smooth  |  |
| <i>Internal structure</i>         |  |   |  |
| Trabeculae                        | Obvious  | Less obvious  | Not visible  |
| Periphery                         | Obvious  | Obvious   | Traced with difficulty                               |
| Colour                            | Pale orange to white   | Usually white, may have orange/brown pigment especially near centre | White, may have trace of brown pigment               |
| Blood vessels                     | Mainly around periphery  | Relatively much more obvious  | Form bulk of structure                               |
| Avascular connective tissue       | Forms bulk of structure (although still vascular in very young CA) | Much less   | Very reduced, may be almost absent                   |

counted by hand-slicing the cortex and medulla at one- to two-mm intervals. The hilar region was left intact to hold the slices together.

On the assumption that the macroscopic appearance of a corpus albicans was likely to be a more reliable index of its age than its diameter would be, each corpus albicans was classified as young, medium, or old according to the characteristics outlined in Table 1 before it was measured.

Three diameters of all corpora lutea, corpora albicantia, corpora atretica, and the largest follicles present in each ovary were measured to the nearest 0.1 mm with vernier calipers. The mean diameter of each structure was calculated as the cube root of the product of the three diameters.

Each sample for histology was dehydrated through a graded series of ethanols, cleared in xylene, embedded in *Paraplast* and sectioned at 5  $\mu$ m. One section from each sample was stained with *Mayer's* haemalum and *Young's* eosin-erythrosin (Marsh, Heinsohn and Spain, 1977); another with either a variant of Gomori's trichrome (Gomori, 1950), or van Gieson's stain (Curtis, 1905) with celestin blue haemalum.

A cryostat was used to cut frozen sections of selected formalin-fixed follicles, corpora lutea, corpora albicantia, and corpora atretica at 8  $\mu$ m. These sections were stained for lipids with a modification of Herxheimer's method using Oil-Red 0 and Sudan IV (Drury and Wellington, 1967); or with haematoxylin and eosin as above.

The ovaries of the whales in School 17 were examined first. During the macroscopic examination of these ovaries we sampled a wide range of structures for histology. Each of these ovaries was then re-examined along with the corresponding histological slides. This provided a check on our macroscopic identifications and a firm basis for the subsequent macroscopic examination of the remaining ovaries, which was also supplemented with histology as required. After all the ovaries had been

sliced and examined, the corpora albicantia count for each was rechecked macroscopically. This was facilitated by marking each corpus albicans with a pin as it was counted.

The Non-Linear Regression Program from IMSL (Anon., 1975) was used to fit the curve describing the relationship between corpus count and age.

## RESULTS

### Weight of ovaries

#### *Immature whales*

The ovaries of immature specimens of *G. macrorhynchus* are lozenge-shaped organs about 3 cm long by 0.8 cm high by 1.5 cm wide in a three-month old animal and about 3.5 cm long by 1 cm high by 2 cm wide in a nine-year old female approaching maturity. In our sample of 37 immature females, the weight of both ovaries (Figs 1 and 2) ranged from 2.5 g (at 0.5 year) to 11.5 g (8.5 years old).

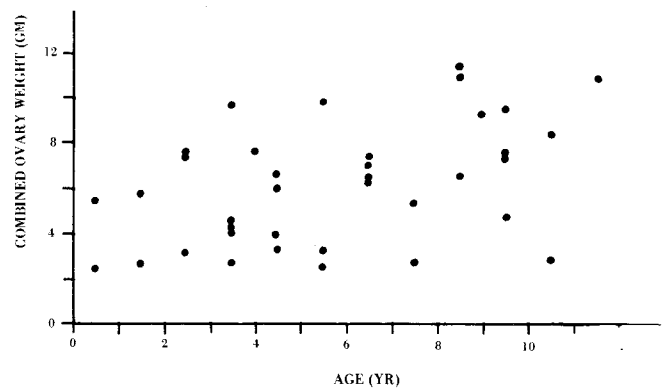


Fig. 1. Scatterplot of combined ovary weight against age for immature females of *G. macrorhynchus*.

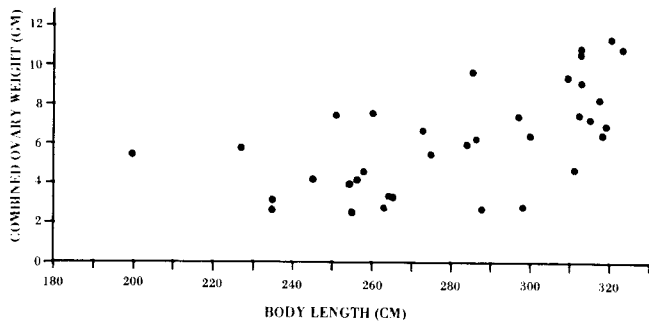


Fig. 2. Scatterplot of combined ovary weight against body length for immature females of *G. macrorhynchus*.

(mean ( $\bar{x}$ ) 6.2 g; standard deviation ( $s$ ) 2.6 g). Even though there was considerable variation in ovary weight for animals of the same age (Fig. 1) or body length (Fig. 2), combined-ovary weight was positively correlated with both factors. (Age:  $r = 0.45$ ;  $P < 0.01$ ; Body length:  $r = 0.63$ ,  $P < 0.001$ ).

*Mature whales*

The ovaries of mature whales were usually readily distinguishable from those of immature animals by the presence of the obvious surface scars of the corpora albicantia (CAs) (e.g. Fig. 3E). The size of mature ovaries varied greatly with age and reproductive status.

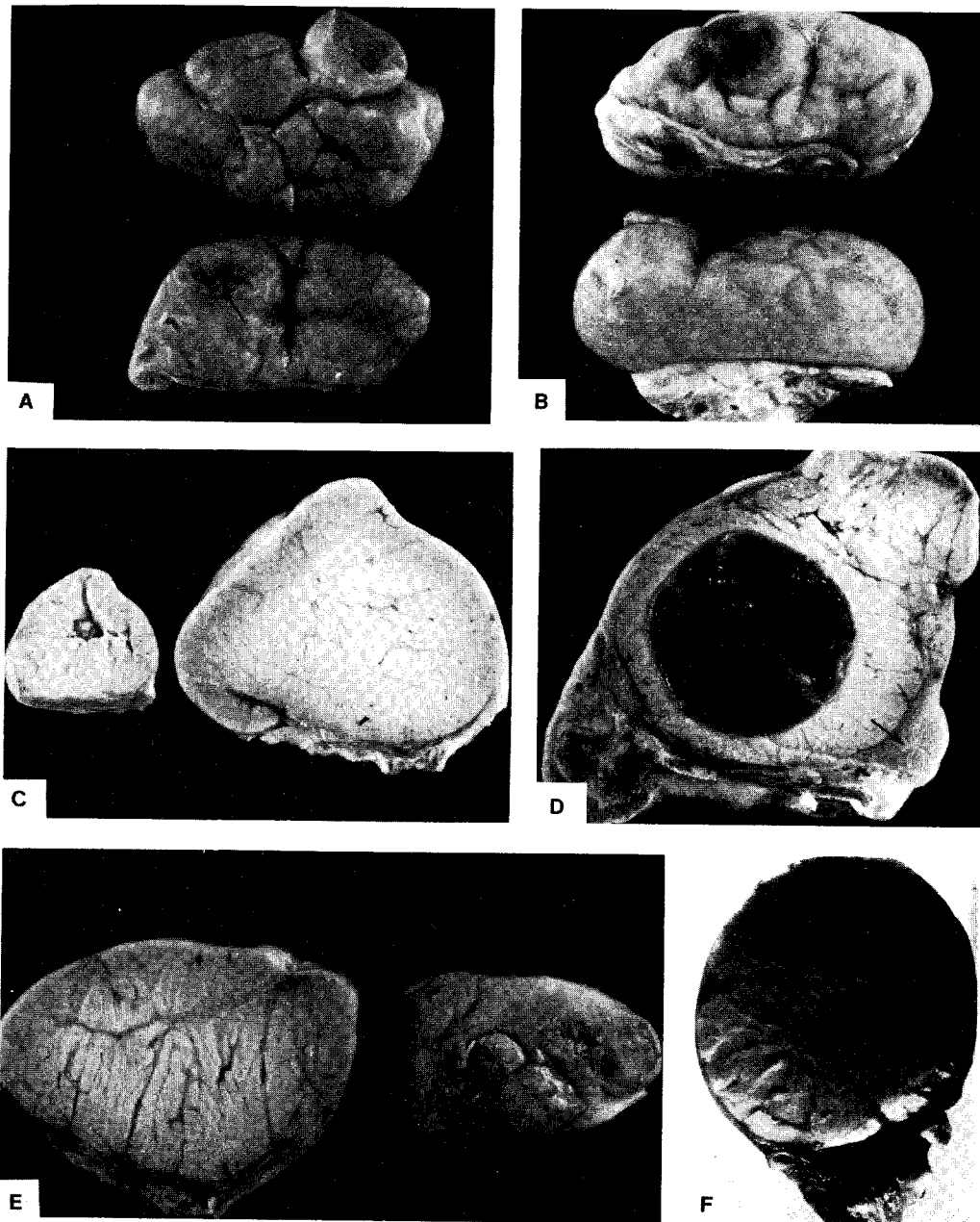


Fig. 3. Ovaries of *G. macrorhynchus* actual size. A. Ovaries of resting Whale 13-35 (11.5 years). The upper (left) ovary contained a large follicle (mean diameter 13.6 mm): the lower (right) ovary, a recent rupture point (arrowed) and a medium corpus albicans (at right end). B. Ovaries of lactating Whale 13-36 (21.5 years). The stigma of a corpus atreticum *a* can be seen in the upper (right) ovary. A young corpus luteum of 'ovulation' is visible at the left end of the lower (left) ovary. C. Median slices through a young corpus luteum of 'ovulation' (right) in the right ovary of lactating Whale 10-1 (22.5 years) and a corpus luteum of late pregnancy (fetal length 146 cm) in the left ovary of Whale 9-101 (23.5 years). D. Median slice through a young corpus luteum of early pregnancy (fetal length 1.2 cm) with an unusually large jelly-filled centre. An adjacent medium corpus albicans is arrowed (from Whale 9-79, 14.5 years). E. Ovaries of Whale 9-113 (23.5 years) which was pregnant with a 3-mm fetus. The corpus luteum (median slice at left) was in the right ovary and was already well-established. The left ovary contained one young and six old corpora albicantia; five of these are visible in the photograph. F. Median-slice through a large corpus luteum of 'ovulation' in the right ovary of resting Whale 25-32 (18.5 years).

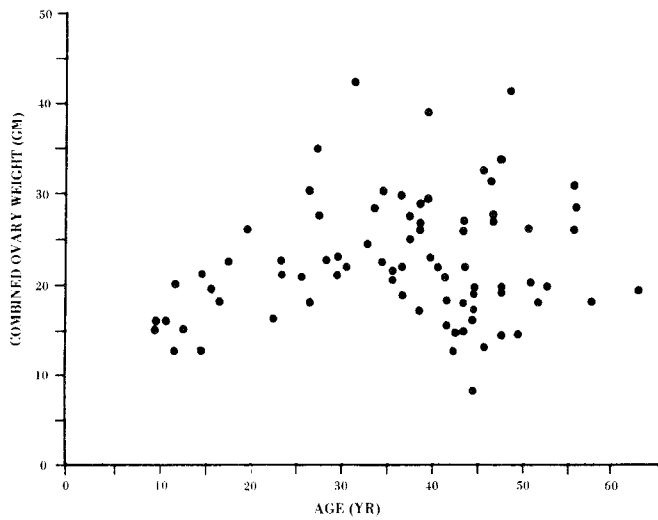


Fig. 4. Scatterplot showing the combined ovary weights of resting females of various ages. Note the lack of a generalized reduction in ovary weight for females over 40 years old, all of which were classified as post-reproductive.

For the 69 pregnant whales, the weight of both ovaries ranged from 32.7 g (in a newly-mature 8.5-year old animal) to 109.3 g (in a 22.5-year old female with a particularly large corpus luteum (CL) that was 44.9 mm in mean diameter) ( $\bar{x}$  56.8 g;  $s$  15.8 g). The pregnant ovaries were significantly heavier ( $t$  test;  $P < 0.01$ ) than those from 14 females with a CL but no detectable fetus; the latter ranged from 16.6 g to 79.4 g in combined weight ( $\bar{x}$  42.9 g). The mean weight of the smaller ovary (i.e. the ovary without a CL) of 78 pregnant whales was 10.5 g ( $s$  4.82 g). This was significantly heavier than the smaller ovary of 128 whales which were neither pregnant nor ovulating (i.e. no CLs or large follicles present) ( $\bar{x}$  9.1 g,  $s$  3.3 g) ( $t$  test;  $P < 0.05$ ). This result suggests that even the inactive ovary undergoes some enlargement during pregnancy.

The weights of both ovaries of the 55 lactating females (excluding those with a CL) ranged from 9.3 g to 50.5 g ( $\bar{x}$  23.2 g;  $s$  7.8 g), significantly lighter ( $t$  test;  $P < 0.0005$ ) than the pregnant ovaries but not significantly different in weight ( $t$  test;  $P > 0.2$ ) from the ovaries of 80 resting

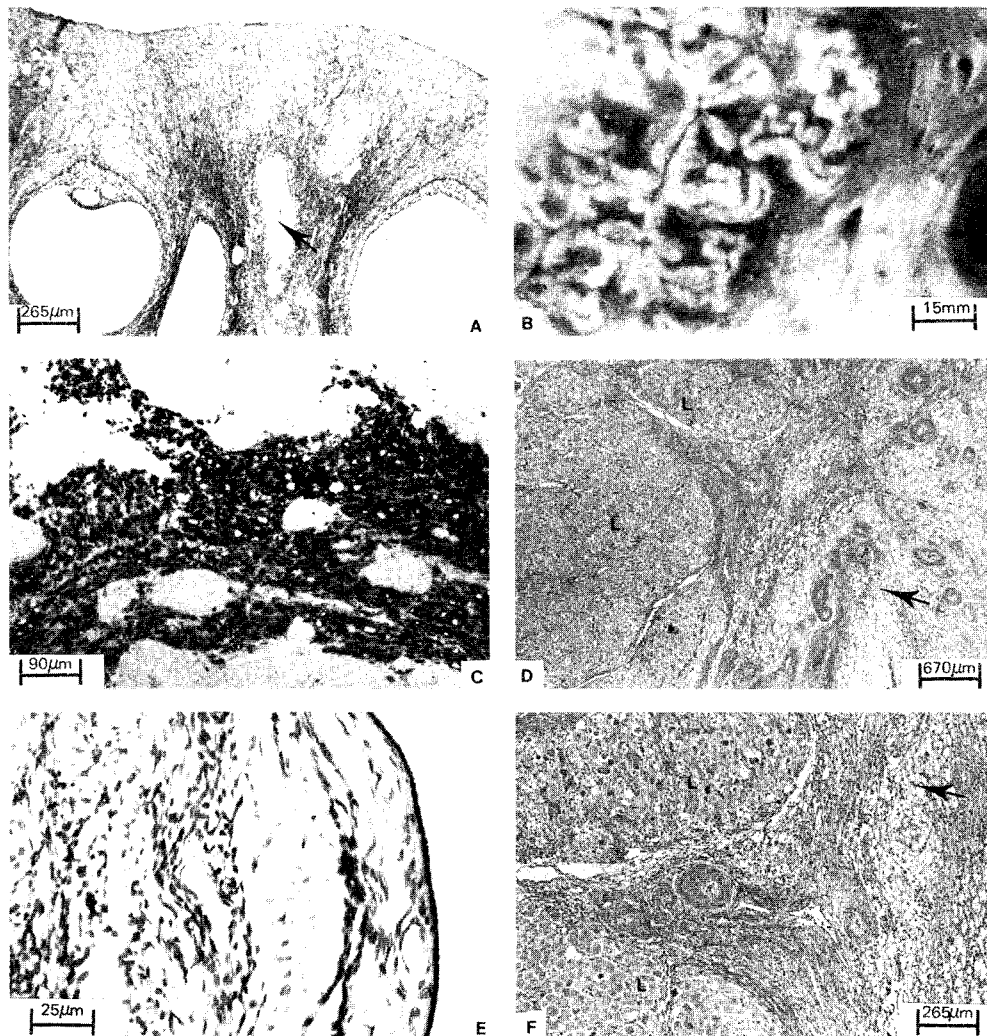


Fig. 5. Different forms of follicular atresia in *G. macrorhynchus* (see also Fig. 11B). A. Cortex of the ovary of immature Whale 17-33 (4.5 years) showing numerous primordial and antral follicles and one corpus fibrosum (arrowed) stained with haematoxylin and eosin. B, D, F. Corpus atreticum a (mean diameter 6.2 mm) in the ovary of lactating Whale 17-17 (15.5 years). B. Part of a median slice showing the irregular outline of the corpus. D, F. Histological preparations stained with Gomori's trichrome showing that some lobes of the well-vascularized corpus are still composed of luteal cells (L) while others (arrowed) are undergoing 'fatty' degeneration. C. Part of a corpus atreticum b in the ovary of resting Whale 17-33 (40.5 years) stained for lipid with Oil-Red O. E. The wall of one of three cystic follicles in the ovary of resting Whale 25-31 (34.5 years) where granulosa and theca cells have been replaced by fibrous tissue. Stained with haematoxylin and eosin.

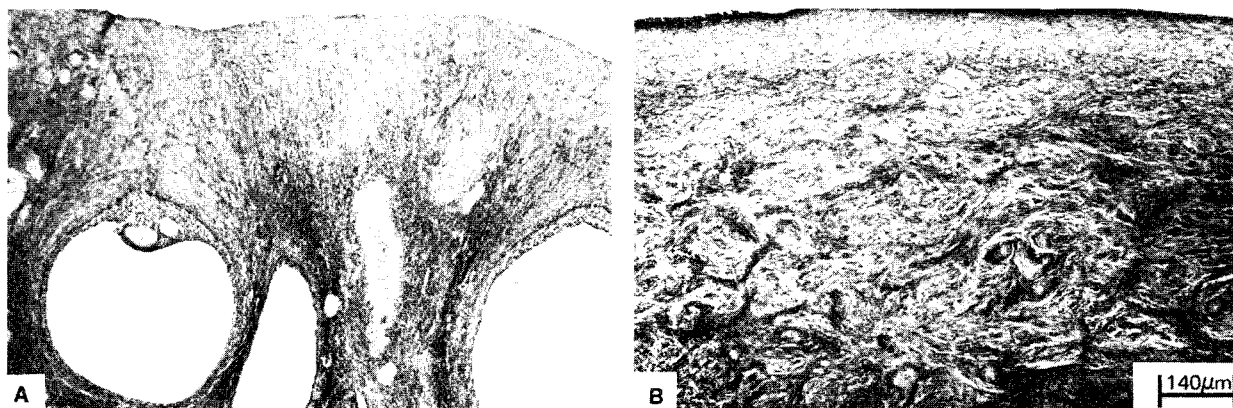


Fig. 6. Age-related changes in the histology of the ovarian cortex in *G. macrorhynchus*. Both photos same scale. A. Part of the cortex of immature Whale 17-33 (4.5 years) showing numerous primordial and antral follicles. Stained with haematoxylin and eosin. B. Cortex of lactating Whale 17-41 (47.5 years) and probably post-reproductive. No follicles can be seen. The cortex is reduced in thickness and composed mainly of fibrous tissue. Stained with van Gieson and celestin blue haemalum.

females (excluding those with a CL) which ranged from 8.4 g to 42.5 g ( $\bar{x}$  22.3 g;  $s$  6.72 g). The combined ovary weight of resting females (Fig. 4) was positively correlated with the age of the whale for animals less than 30 years old ( $r = 0.65$ ,  $P < 0.01$ ) but was highly variable in animals older than this. Both ovaries of some of the females over 40 years appeared shrivelled and had a combined weight of less than 15 g (e.g. No. 17-24 aged 44.5 years had a combined ovary weight of 8.4 g). However, we have no evidence for a reduction in the ovary weight of all old females, and the average combined ovary weight of 44 females older than 40 years was 21.8 g ( $s$  7.0 g) which was not significantly different from the average combined ovary weight ( $\bar{x}$  23.9 g;  $s$  6.7 g) of 31 females (without a CL or young CA) aged between 20 years and 40 years ( $t$  test;  $P > 0.1$ ).

**Follicular development**

Primordial follicles occurred scattered throughout the periphery of the cortex in young specimens of *G. macrorhynchus* (Figs 5A, 6A). The mean of the diameters of 10 such follicles was 58  $\mu\text{m}$ , and the mean size of the oocyte was about 20  $\mu\text{m}$ . Follicle growth followed the usual pattern outlined in Appendix B of Perrin and Donovan (1984). The smallest follicles with an obvious antrum had a diameter of 150 to 200  $\mu\text{m}$ . By this stage, the granulosa layer was several cells thick and the capillaries in the theca interna were obvious. In macroscopically-visible (i.e. > 1 mm in diameter) antral follicles, the theca interna and theca externa were much more clearly differentiated. As most of the antral follicles larger than 1 cm in diameter that we examined histologically were atretic, we have no information on whether the granulosa and theca cells start to luteinize before the follicle ruptures.

A frequency histogram of the diameter of the largest follicle present in 297 pairs of ovaries is presented in Fig. 7. No macroscopic follicles were seen in 44% of ovary pairs; the proportion of mature animals with no macroscopic follicles being greater in old animals (Fig. 8).

In immature animals, there is a seasonal cycle of follicle

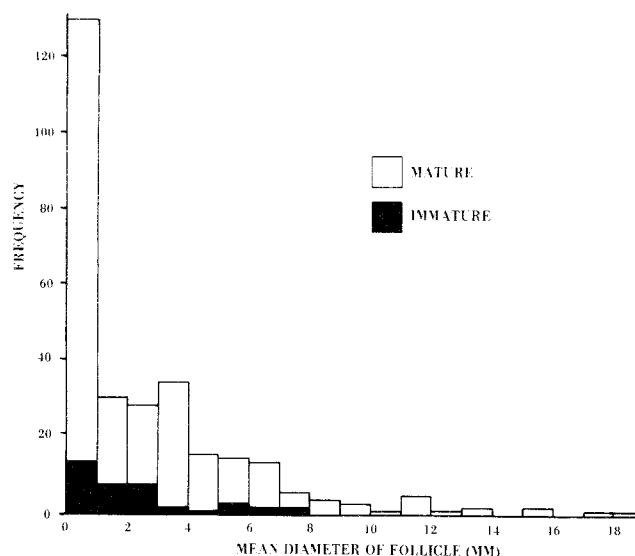


Fig. 7. Frequency histogram showing the size distribution of the largest follicle present in immature and mature ovaries of *G. macrorhynchus*.

growth. Although immature animals had follicles up to 7.1 mm in diameter (Fig. 7) (e.g. No. 24-31 aged 3.5 years), the presence of follicles greater than 4 mm was limited to the period 31 May to 23 July inclusive (Fig. 9).

In immature females, many follicles tended to be enlarging, so that the surface of the ovary was covered with bulges. In older females approaching oestrus, fewer follicles tended to enlarge. The three whales which had a corpus luteum of 'ovulation' ('CLO') but no macroscopic follicles were all estimated to be between 36 and 38 years old.

The frequency histogram illustrating the maximum follicle diameter of whales at different stages of pregnancy (Fig. 10) indicates that the presence of large (> 5 mm diameter) follicles was limited to animals with fetuses less than 50 cm long. At all stages in the gestation period, there tended to be some whales with no macroscopic follicles and some with medium-sized follicles (1 to 5 mm in diameter).

Macroscopic follicles below 8 mm in diameter were

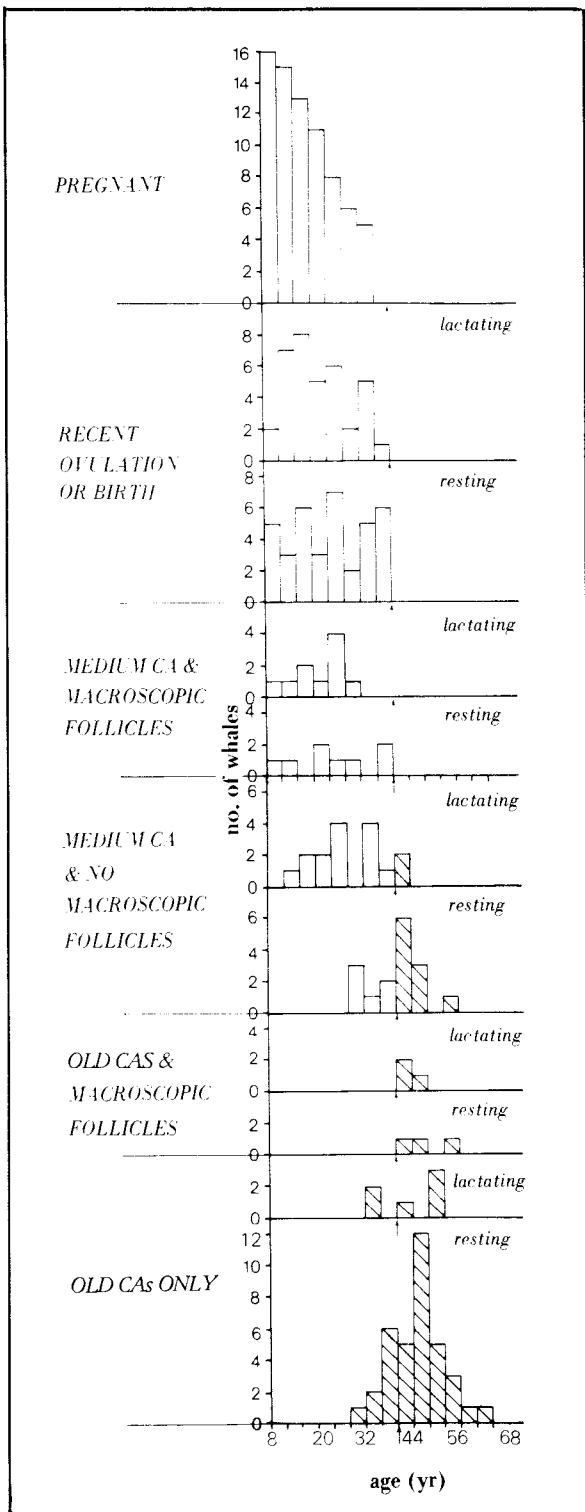


Fig. 8. Reproductive status and ovarian condition of 245 mature females of *G. macrorhynchus* aged from 8.5 to 62.5 years. The hatching represents the whales that we classified as post-reproductive. All 'non-pregnant' pilot whales with a corpus luteum or young corpus albicans (CA) have been classified as 'recent ovulation or birth'. Macroscopic follicles were discounted if they were obviously atretic (even without histology). Animals classified as having old corpora albicantia (CAs) only had no macroscopic follicles.

observed in some of the lactating females in all months for which specimens were available (Fig. 9). Larger follicles were observed in only four lactating females, all

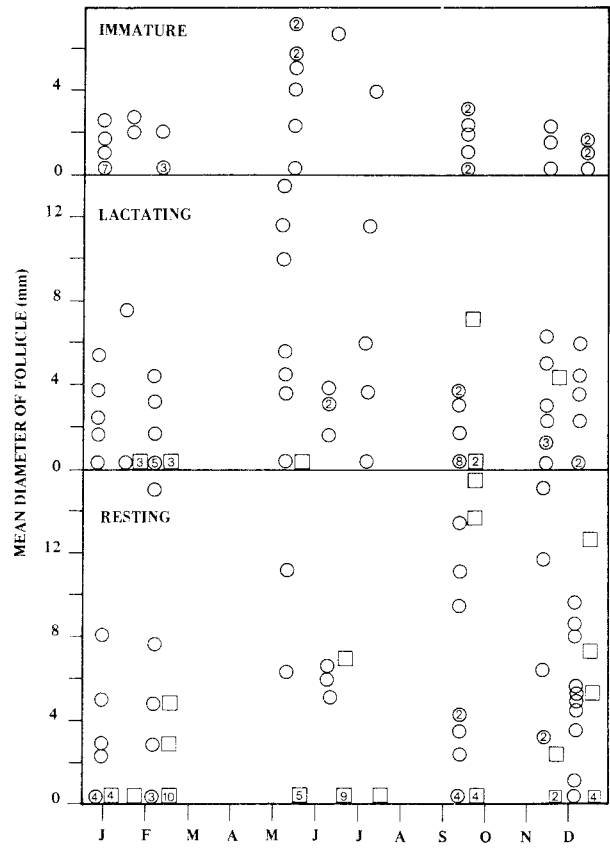


Fig. 9. Seasonal changes in the diameter of the largest follicle present in immature, lactating and resting pilot whales. Data for postreproductive (□) animals are presented separately from the remainder (○) as the large follicles present in these whales tend to be atretic. □ or ○ 1 whale, ▢ or ⊙ n whales.

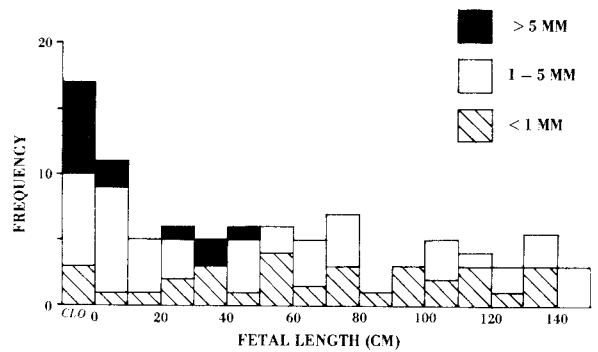


Fig. 10. Frequency histogram of the mean diameter of the largest follicle in *G. macrorhynchus* ovaries with corpora lutea of 'ovulation' and at various stages of pregnancy.

of which were examined in the period 31 May to 23 July inclusive, the same period in which the largest follicles (> 4 mm in diameter) were observed in immature whales.

This seasonal pattern was much less clear in resting females (Fig. 9). Macroscopic follicles 8 mm in diameter and smaller were observed in some animals in all months. Larger follicles were not limited to the May–July period but were also observed in some animals in February, October and December. This pattern persisted even when we distinguished between the reproductive and post-reproductive females using the criteria outlined below.

Many of the largest (> 10 mm) follicles were thick-



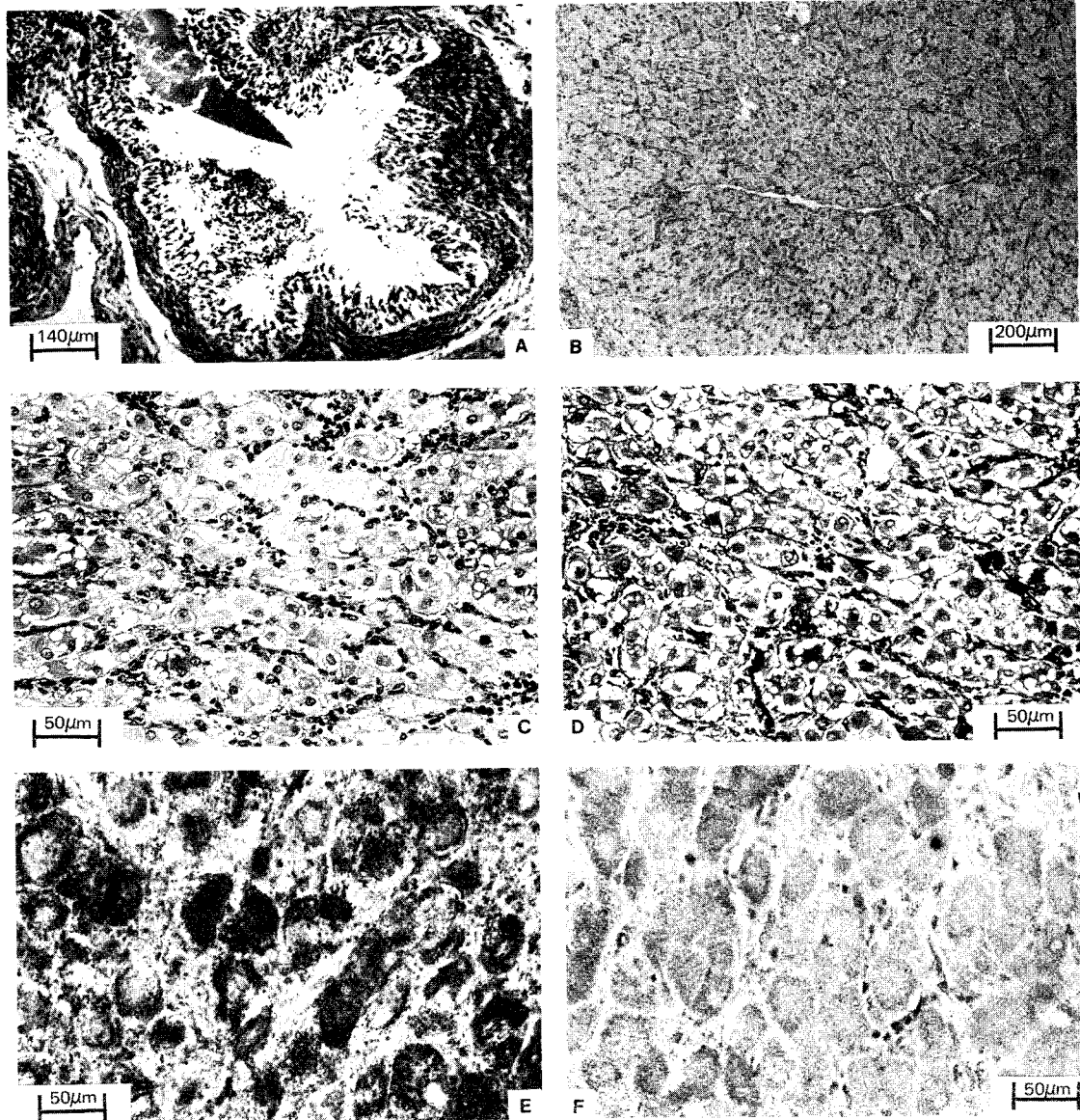


Fig. 11. Histology of corpora lutea in *Globicephala macrorhynchus* ovaries. A. Recently ruptured follicle in the right ovary of Whale 13-35 (aged 11.5 years) (see also Fig. 3A). The granulosa and theca layers are very developed and there has been some haemorrhage into the antrum. (Haematoxylin and eosin). B. Small accessory corpus luteum (3.6 mm in diameter) in the left ovary of Whale 17-31 (10.5 years) which was pregnant with a 23.3-cm fetus. Note the primary and secondary luteal cells. This ovary also contained one normal-sized corpus luteum (31.3 mm in diameter) and another accessory corpus luteum (11.0 mm in diameter). Neither accessory corpus luteum had a stigma. (Haematoxylin and eosin). C, D. Sections from a large corpus luteum of 'ovulation' (45 mm in diameter) in the right ovary of Whale 25-32 (18.5 years) stained with haematoxylin and eosin (C) and Gomori's Trichrome (D). The large pale primary luteal cells with vacuolated cytoplasm lie in rows within the network of secondary luteal cells which have darkly staining nuclei. The tissue is highly vascular and the erythrocytes (arrowed) are clearly visible in (D). E, F. The lipid metabolism of a corpus luteum appears to change during pregnancy. The primary luteal cells in (E) (Whale 13-28, 19.5 years, with 6.3-cm fetus) are packed with lipid granules and droplets in contrast to those in (F) (Whale 9-102, 26.5 years, with 124-cm fetus). Stained with Oil-Red O and Sudan IV.

walled and obviously atretic. It is very difficult to (1) be sure without histological examination that a large follicle is not atretic, and (2) measure large antral follicles (Fig. 3A) accurately. We cannot therefore make a definite statement about the usual size of follicles at ovulation. However, our observations that all follicles were below 8 mm in diameter for the immature period of seven or more years (Kasuya and Marsh, 1984) and that follicles larger than this were seasonally limited in lactating females suggest that a follicle probably stays at or below about 8 mm in diameter before the pre-ovulation growth spurt.

**Follicular atresia**

Coupled with the normal follicular development cycle is the concurrent normal phenomenon of follicular atresia. This is not necessarily simply a degenerative process. The metamorphosis of the follicle wall into a different kind of probably functional tissue occurs without ovulation as a normal and essential event in the ovarian cycle (Weir and Rowlands, 1977). Atresia may lead to the development of secondary interstitial tissue (which we did not study in *G. macrorhynchus*) and accessory corpora lutea, both of which are probably hormonally active (Weir and

Table 2  
Details of specimens of *G. macrorhynchus* with accessory corpora lutea

A. Accessory corpora lutea of pregnancy

| Accession no. | Age (years) | Foetal length (cm) | Mean diameter(s) accessory corpora lutea (mm) |     |
|---------------|-------------|--------------------|---|-----|
| 17-31         | 10.5        | 23.3               | 11.0  | 3.2 |
| 25-2          | 11.5        | 29.5               | 7.0   | 3.9 |
| 18-27         | 21.5        | < 0.5              | 7.0   |     |
| 17-8          | 32.5        | 57.5               | 7.1   |     |
| 20-8          | N/A         | 63                 | 5.0   | 3.9 |

B. Accessory corpora lutea in post-reproductive ovaries

| Accession no. | Age (years) | Mean diameter(s) of accessory corpora lutea (mm) |     |     |
|---------------|-------------|--|-----|-----|
| 26+27-43      | 36.5        | 4.4  |     |     |
| 10-15         | 37.5        | 6.5  |     |     |
| 13-31         | 40.5        | 10.2   |     |     |
| 22-3          | 40.5        | 3.6  | 3.1 |     |
| 22-12         | 41.5        | 5.4  | 4.0 |     |
| 9-106         | 41.5        | 6.4  |     |     |
| 9-98          | 43.5        | 4.2  | 3.9 | 3.0 |
| 24-12         | 48.5        | 7.6  |     |     |
| 17-1          | 55.5        | 4.2  |     |     |

Rowlands, 1977). Atresia may lead to the development many more become atretic.

Follicles may become atretic at any stage of their development (Byskov, 1979). We did not study the atresia of small follicles in *G. macrorhynchus*, as this did not appear to modify the macroscopic structure of the ovary. However, the atresia of medium and large follicles is conspicuous and presents a variety of appearances depending on the stage of the follicle and the hormonal status of the whale at the time it begins. These are discussed below.

*Atresia without luteinization*

*Corpora fibrosa*: The atresia of follicles in immature females and of some medium-sized follicles in mature specimens occurred without luteinization, i.e. without accumulation of lipids in the granulosa or theca cells. Small corpora fibrosa (Fig. 5A) were derived from the basement membrane of atretic medium-sized follicles. These fibrous bodies appeared to be slowly resorbed and were not evident in large numbers in the cortex of old whales (e.g. Fig. 6B).

*Cystic follicles*: Many of the large follicles we examined histologically (especially those from old females) had become cystic (e.g. Fig. 5E). In some cases the walls were obviously unusually thick even when examined macroscopically, the granulosa and theca cells having been replaced by fibrous tissue.

*Atresia with luteinization*

Atresia of Graafian follicles of various sizes often progressed via different stages of luteinization. These will be discussed separately below.

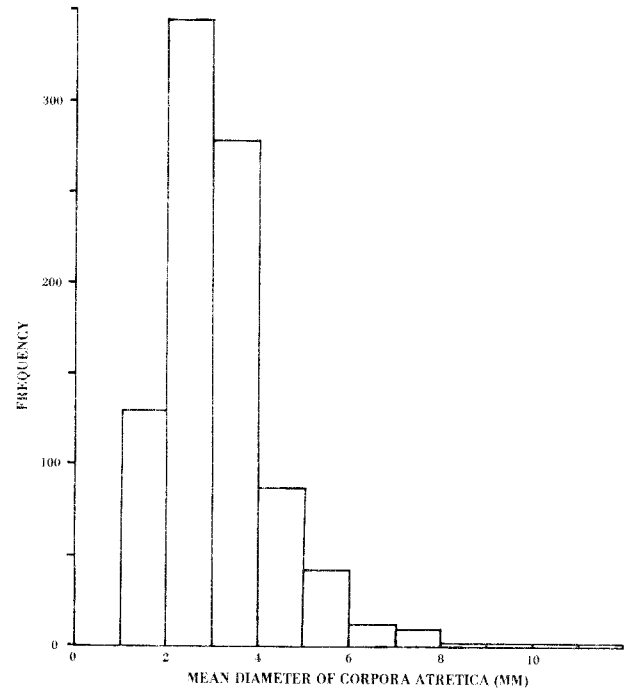


Fig. 12. Frequency histogram showing the size distribution of corpora atretica b in *G. macrorhynchus*.

*Accessory corpora lutea*: In several whales we found one or more follicles that had behaved as if ovulation had occurred but without the egg being released, thus forming an accessory corpus luteum (accessory CL) on which no stigma was visible. Details of the animals in which accessory CL were found are summarized in Table 2. The ovaries of five animals (aged 10.5 to 32.5 years) contained accessory corpora lutea of pregnancy which were 5.0 to 11.0 mm in diameter (Fig. 11B). The ovaries of nine old females aged from 36.5 to 55.5 years also contained yellow structures without stigmata which were 3.0 to 10.2 mm in diameter and histologically identical to corpora lutea, even though these ovaries showed no signs of having ovulated recently.

*Corpora atretica b*: We observed irregularly-shaped masses of yellow-brown pigmented tissue ranging from 1 to 12 mm in mean diameter (modal mean diameter 2 mm to 3 mm) (Fig. 12) in about 75% of the mature ovaries examined. None were seen in immature ovaries. A total of 938 such bodies were recorded in 235 pairs of mature ovaries. The surface trace associated with these structures was slight or absent.

Histological study indicated that these bodies were formed from atretic follicles of various sizes in which lipid accumulation had started in some of the granulosa cells and in the cells of the theca interna. As atresia continued, the granulosa cell members became reduced by lysis and phagocytosis with concomitant collapse of the follicle. During the atretic differentiation, few thecal cells became necrotic. Rather they had hypertrophied and accumulated lipid droplets (Fig. 5C), undergoing a type of 'fatty' degeneration. The resulting lipid pigment (which resembled ceroid) was pale yellow at first and readily soluble in fat solvents but gradually oxidized, becoming darker and more insoluble. Best (1967) reported similar bodies in



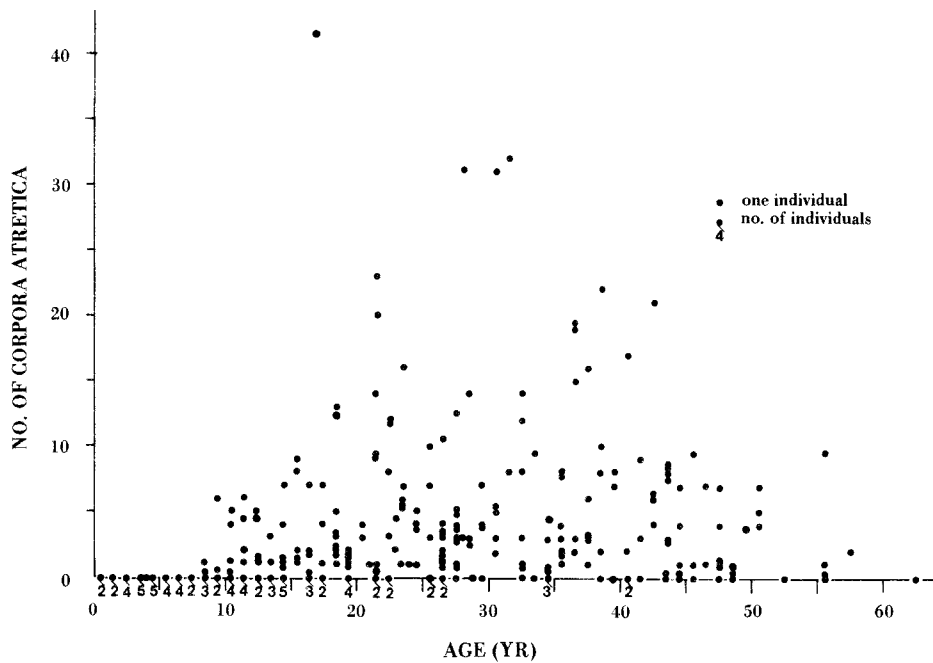


Fig. 13. Scatterplot of corpora atretica *b* number against age for *G. macrorhynchus*.

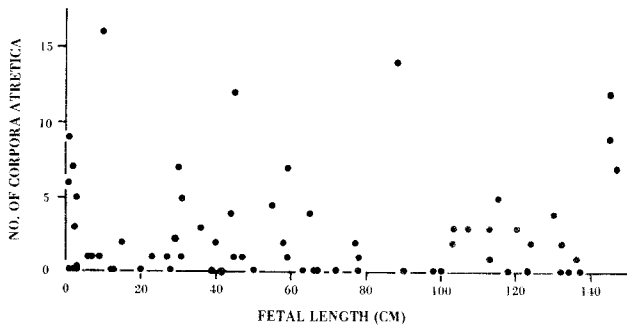


Fig. 14. Number of corpora atretica *b* present per pair of ovaries of *G. macrorhynchus* at various stages of pregnancy.

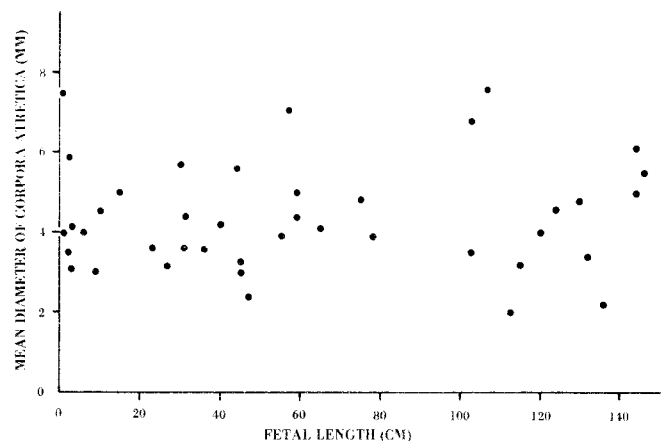


Fig. 15. Scatterplot showing the size of the largest corpus atreticum *b* present in the ovaries of *G. macrorhynchus* at various stages of pregnancy.

sperm whale, *Physeter macrocephalus*, ovaries and called them 'corpora atretica *b*'. We have followed his terminology.

Fig. 13 illustrates the relationship between corpora-atretica count and age of the whale. Animals with one or more corpora atretica *b* were less likely to be aged less than 20 years ( $\chi^2$ ,  $P < 0.01$ ) or more than 40 years ( $\chi^2$ ,  $P < 0.05$ ) than between 20 and 40 years.

When the incidence of corpora atretica *b* was studied within an age group of whales, their presence/absence was found to be independent of pregnancy status ( $\chi^2$ ,  $P > 0.5$ ; both for whales aged less than 20 years and between 20 and 36 years).

Corpora atretica *b* were present in pilot whales at all stages of pregnancy (Fig. 14), and the likelihood of an animal having one or more was independent of the stage of pregnancy ( $\chi^2$ ,  $P > 0.05$ ). There was no evidence that the maximum diameter of corpora atretica *b* changed during the gestation period (Fig. 15). The distribution of corpora atretica *b* within a pair of ovaries was also independent of their current activity as measured by the presence of a corpus luteum or young corpus albicans ( $\chi^2$ ,  $P > 0.05$ ). There was no significant difference in the

proportion of ovaries with corpora atretica *b* between the periods December–February and June–July ( $\chi^2$ ,  $P > 0.05$ ), suggesting that their occurrence was independent of the time of year.

Within a pair of ovaries, the ovary with the larger number of CAs was also likely to have more corpora atretica *b* ( $\chi^2$ ,  $P < 0.05$ ).

The number of corpora atretica *b* per ovary pair ranged from 0 to 43 ( $\bar{x}$  4); most whales had less than 10 (Fig. 13). Animals with one or more such bodies had an average of 5.4 per ovary pair. Table 3 details the reproductive status of the 23 whales with more than 10 corpora atretica *b*. Nearly half of this group had a corpus luteum of 'ovulation' or large follicles suggesting that when large-scale atresia with luteinization occurred, it tended to be around the time of oestrus and in pilot whales older than about 17 years. The oldest whale with 10 or more corpora atretica *b* was aged 42.5 years, suggesting that these bodies do not persist.

Table 3

Details of the ovaries of 23 specimens of *G. macrorhynchus* (age 17.5 to 42.5 years) with more than 10 corpora atretica *b*. (*R* = resting, *L* = lactating)

| Structures present in ovaries                            | No. of whales                   |   |
|--|---------------------------------|---|
|  | Total mature whales in category | No. with > 10 corpora atretica <i>b</i> |
| Corpus luteum of pregnancy (fetal length 10.5 to 144 cm) | 77                              | 5                                       |
| Corpus luteum of 'ovulation'                             | 17                              | 5 <i>R</i> * + 1 <i>L</i>               |
| Macroscopic follicles > 5 mm in diameter                 | 32†                             | 3 <i>R</i> * + 1 <i>L</i>               |
| Macroscopic follicles 1 to 5 mm in diameter              | 40†                             | 1 <i>R</i>                              |
| No macroscopic (i.e. > 1 mm in diameter) follicles       | 88†                             | 5 <i>R</i> * + 2 <i>L</i>               |

\* One whale also with one corpus atreticum *a*.

† Excluding animals with a corpus luteum.

*Corpora atretica a*: Other yellow bodies which were histologically similar to corpora atretica *b* but better-defined and usually larger structures were also observed in the ovaries of *G. macrorhynchus* (Fig. 5B). These irregularly-shaped bodies were placed superficially in the ovary and often had an obvious stigma (Fig. 3B) suggesting that they had originated from ruptured follicles. Best (1967) described similar structures in sperm whale ovaries. He called them 'corpora atretica *a*' and we have followed his terminology (for a discussion of alternative terminologies, see Appendix B of Perrin and Donovan (1984)).

Fig. 5(D, F) illustrates the start of the process of degeneration of one of these bodies. We have not observed extensive fibrous replacement of the luteal cells like that seen in fin whale (Laws, 1961), sperm whale (Best, 1967) or minke whale (Appendix B, Perrin and Donovan, 1984) corpora atretica *a*. We therefore consider that it is likely that most degenerate pilot whale corpora atretica *a* eventually virtually disappear from the ovary, leaving at most a surface scar. Thus they are probably less likely to be confused with CAs than the corresponding structures which have been described from the ovaries of large whales.

Corpora atretica *a* (ranging from 3.2 mm to 13.3 mm in mean diameter;  $\bar{x}$  7.1 mm; *s* 2.6 mm) were found in the ovaries of 23 pilot whales. The reproductive status of the whales whose ovaries contained these bodies is detailed in Table 4. Thirteen of these animals had ovulated recently (two were pregnant with a small fetus; 10 had a 'CLO', and one a young CA). More than half of the animals with a 'CLO' also had a corpus atreticum *a*. Five animals with large follicles in their ovaries suggesting approaching oestrus also had a corpus atreticum *a*. Whale 9-116, which had given birth the day before her death, had seven old corpora atretica *a* (all with obvious stigmata) in her left ovary, which also contained the corpus luteum.

We did not include corpus atretica *a* in our corpora counts, as we consider that they resulted from follicle ruptures which were not followed by normal corpus-luteum development.

Table 4

Details of the ovarian status of 23 *G. macrorhynchus* females (age 11.5 to 40.5 years) with at least one corpus atreticum *a*. (*R* = resting, *L* = lactating)

| Structures present in ovaries                             | No. of whales                   |                                    |
|---|---------------------------------|------------------------------------|
|   | Total mature whales in category | No. with corpus atreticum <i>a</i> |
| Corpus luteum of pregnancy (foetal length < 5 mm to term) | 77                              | 5                                  |
| Corpus luteum of 'ovulation'                              | 17                              | 5 <i>R</i> + 5 <i>L</i> *          |
| Macroscopic follicles (> 5 mm in diameter)                | 32†                             | 4 <i>R</i> + 1 <i>L</i>            |
| Young corpus albicans                                     | 51†                             | 1 <i>R</i> *                       |
| Medium corpus albicans                                    | 50†                             | 1 <i>R</i> * + 1 <i>L</i> *        |

\* One corpus atreticum *a* degenerating.

† Excluding animals with a corpus luteum.

### The corpus luteum

A corpus luteum (CL) (*sensu* Appendix B of Perrin and Donovan, 1984) was observed in the ovaries of 95 pilot whales. A fetus was found in the uterus of 73 of these animals, confirming that these were CLs of pregnancy (CLPs). Another two whales with a CLP had recently aborted; two more had recently given birth. All four were from School 9, which was held for several days between capture and slaughter (Table 1, Kasuya and Marsh, 1984). A cystic placental mole (Jubb and Kennedy, 1977) presumably derived from the remains of a very small embryo (Dr P. Ladds, Department of Tropical Veterinary Science, James Cook University, pers. comm.), was found in the uterus of another whale with a CL (No. 25-1, 28.5 years).

No fetus could be found in the uteri of the remaining 17 whales. We shall refer to the CLs of all these animals as 'CLOs' even though some may have been from animals which had recently aborted a fairly small fetus or which contained a very small embryo missed at dissection.

#### The corpus luteum of 'ovulation'

Fourteen 'CLOs' (excluding collapsed, recently ruptured follicles, e.g. Fig. 3A) ranged from 12.5 mm to 45 mm in mean diameter ( $\bar{x}$  25.1 mm). As can be seen from Fig. 1, all but four were below the size range of the CLPs. All had a conspicuous stigma (Fig. 3B). In cross-section most of the ochre-coloured 'CLOs' were loose-structured, the cords of glandular tissue being separated by hollow fissures (Fig. 3C). However, some 'CLOs' (e.g. the 45-mm diameter CL of Whale 25-32 (18.5 years) (Fig. 3F) and the 40-mm diameter CL of Whale 24-9 (37.5 years)) were compact structures similar morphologically and histologically to all but the youngest CLPs. Thus we were unable to separate all 'CLOs' from CLPs using criteria of size or morphology. Five of the 'CLOs' were found in lactating whales (age range 21.5 to 37.5 years) and 12 in resting whales (age range 11.5 to 39.5 years).

As discussed below, we have no evidence of re-ovulation by any specimen of *G. macrorhynchus* more than 40 years old. The proportion of lactating and resting females less than this age with a 'CLO' is significant.

Table 5

Seasonal incidence of corpora lutea of 'ovulation' ('CLO'). (All animals over 39 years old excluded, but animals from School 9 whose ovaries were examined in the field only (see Kasuya and Marsh, 1984) included

| Month     | School no(s)   | Number of whales |       |            |       | % with 'CLO'<br>(Lactating and resting) |
|-----------|----------------|------------------|-------|------------|-------|---|
|           |                | Lactating        |       | Resting    |       |   |
|           |                | With 'CLO'       | Total | With 'CLO' | Total |   |
| January   | 7, 11          | 0                | 5     | 2          | 8     | 15.4                                    |
| February  | 12, 16, 22, 23 | 0                | 13    | 0          | 10    | 0                                       |
| May/June  | 24             | 2                | 7     | 3          | 5     | 41.7                                    |
| June/July | 9, 10          | 3                | 17    | 12         | 24    | 36.6                                    |
| October   | 13, 25, 26, 27 | 1                | 12    | 5          | 10    | 27.3                                    |
| December  | 14, 15, 17, 18 | 0                | 16    | 0          | 19    | 0                                       |

higher in May/June (41.7%) June/July (36.6%) and October (27.3%) than in the other months for which data are available ( $\chi^2$ ,  $P < 0.001$ ) (Table 5).

*The corpus luteum of pregnancy*

The 77 CLPs ranged in mean diameter from 30.4 mm to 47.5 mm ( $\bar{x}$  37.6 mm;  $s$  3.4 mm; mode 37.5 mm). Although there is no evidence for a consistent change in the mean diameter of CLPs throughout the gestation period (Fig. 16), we tested whether CLPs tended to shrink during early pregnancy by examining the relationship between fetal length and the mean diameter of the CLP for 17 whales, each of which had a fetus 20 cm or less in length. However, no change was detected, as neither the linear nor quadratic relationship between these two variables was significant ( $r = -0.39$ ,  $P > 0.20$ ; Regression ANOVA: neither Regression Mean Square (MS) or Curvature MS was significantly different ( $P > 0.10$ ) from the Residual MS).

Besides being larger than most 'CLOs', the appearance of all but the youngest CLPs (Fig. 3E) was different in cross-section, the luteal tissue being held more closely together by the more extensively developed connective tissue network. However, the stigma was generally still very obvious although morphologically highly variable.

About 15% of CLs had a 'jelly-filled' centre.

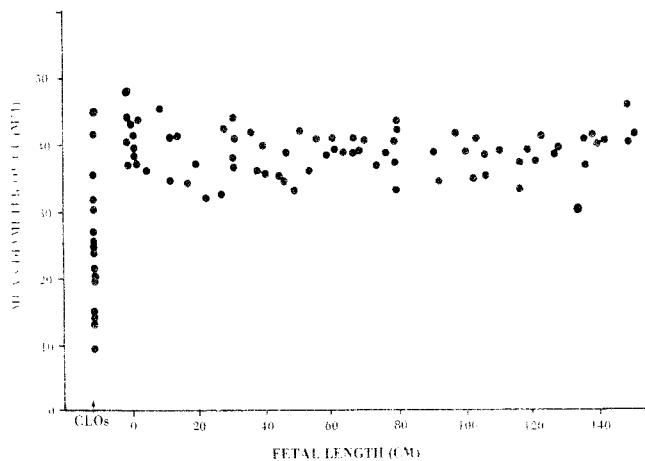


Fig. 16. Scatterplot of the diameters of corpora lutea of 'ovulation' ('CLO') (including two recently ruptured follicles) and of corpora lutea of pregnancy through the gestation period.

exceptionally up to 30 mm in diameter (Fig. 3D) but usually much smaller. This was formed from fibrin resulting from blood entering the antrum as the follicle ruptured. The 'jelly' was replaced by fibrous connective tissue in older CLs.

Only three pregnant whales were simultaneously lactating. Their ovarian condition is summarized in Table 6.

*Development of the corpus luteum*

The following description of the histological appearance of the developing CL was built up from the examination of two recently-ruptured follicles, eight 'CLOs' and 13 CLPs (from whales with fetuses 3 to 146 mm long).

The two recently-ruptured follicles we studied histologically were 8 mm and 15 mm in maximum diameter. Ovulation had been followed by haemorrhage from the blood vessels of the theca interna. Luteinizing cells lining the antrum were interspersed with erythrocytes and fibrinous material (Fig. 11A). The theca interna and membrane granulosa were clearly separated by a distinct basement membrane and were both deeply folded. The granulosa cells each had a rounded nucleus about 7  $\mu$ m in diameter, surrounded by varying but small amounts of eosinophilic granular cytoplasm, which was irregularly

Table 6

Details of ovaries of three specimens of *G. macrorhynchus* which were simultaneously pregnant and lactating

| Accession no. | Age (years) | Fetal length (cm) | Estimated <sup>1</sup> length of pregnancy (days) | Mean diameter CA (mm) |        |     |
|---------------|-------------|-------------------|---|-----------------------|--------|-----|
|               |             |                   |   | Young                 | Medium | Old |
| 26-8          | 12.5        | 19.3              | 98  |                       |        | 7.9 |
|               |             |                   |   |                       |        | 7.4 |
|               |             |                   |   |                       |        | 5.8 |
| 25-38         | 13.5        | 39                | 156   |                       | 9.2    |     |
| 10-12         | 34.5        | 20                | 100   |                       | 10.3   | 7.7 |
|               |             |                   |   |                       |        | 5.8 |
|               |             |                   |   |                       |        | 5.5 |
|               |             |                   |   |                       |        | 5.0 |

<sup>1</sup> Calculated from formula derived from Laws (1959).

$$\text{Length of pregnancy in days} = \left\{ 452 \times 0.91 \times \frac{\text{F.L.}}{139.5} \right\} + (452 \times 0.09)$$

(F.L. = fetal length).

positive for lipid. Cell boundaries were difficult to see. The theca cells had round to oval nuclei about  $10\ \mu\text{m}$  long and cytoplasm which did not stain for lipid. Both layers were much more developed than in large, intact follicles.

Two morphologically-distinct types of luteal cells (Fig. 11C) were observed in all the fully-formed CLs we examined histologically. We refer to these as primary and secondary luteal cells *sensu* Mossman and Duke (1973). Similar cells have been referred to as granulosa and theca luteal cells by various authors (e.g. Harrison, 1949; Harrison *et al.*, 1981). We have avoided this terminology, as it is impossible to verify the origin of these cell types without a more complete series.

The secondary luteal cells (Fig. 11C) formed a lattice which radiated throughout the CL and was continuous with an interrupted layer of cells around the periphery. These cells followed the trabeculae into the substance of the CL and were obvious where they surrounded capillaries. The secondary cells were fusiform. Each had a distinct ovoid, basophilic nucleus about  $5$  to  $8\ \mu\text{m}$  long and a small amount of cytoplasm which did not stain with fat stains, even though it tended to be vacuolated in older CLs (e.g. old 'CLPs' from animals with fetuses  $> 15$  cm long). The primary luteal cells occurred in rows within the network of secondary luteal cells and connective-tissue fibres. Primary luteal cells (Fig. 11C) were rounded or polyhedral, ranging from about  $25$  to  $50\ \mu\text{m}$  in diameter, and contained a pale-staining, rounded nucleus about  $10\ \mu\text{m}$  in diameter with a prominent nucleolus. Cells containing two nuclei were sometimes seen in young CLs.

In young CLs, the cytoplasm of the primary luteal cells tended to be lipid-rich and granular (Fig. 11E). In animals with small embryos (3 to 10 cm long), an increasing proportion of the lipid component of the cytoplasm of the primary luteal cells tended to be concentrated in large droplets up to  $15\ \mu\text{m}$  in diameter. In older CLPs (from animals with fetuses 15 to 146 cm long) the cytoplasm stained palely or not at all with Oil-Red 0 and had a fairly uniform appearance (Fig. 11F). These results suggest that the lipid metabolism of the primary luteal cells of a CLP alters when the fetus is between 10 and 15 mm long. In our material, this was the most obvious change in the primary luteal cells to occur during pregnancy. Other changes may have occurred but if so, they were masked by inadequate fixation.

The connective-tissue strands separating areas of luteal tissue (Fig. 3C) are highly developed in all but the youngest CLPs. This correlates with the more compact appearance of older CLPs. All CLs had a very well-developed blood supply with an extensive network of capillaries (Fig. 11D).

### The corpus albicans

In this paper, we refer to regressing and regressed CLs as corpora albicantia (CAs) irrespective of their colour. We have recorded and measured 1,737 CAs from *G. macrorhynchus* ovaries, up to 18 per pair, the modal number being six (Fig. 17). Each CA was classified as young, medium or old according to the criteria outlined in Table 1. As regression is essentially a continuous process, these categories are somewhat arbitrary, but we consider them a useful guide to the state of regression.

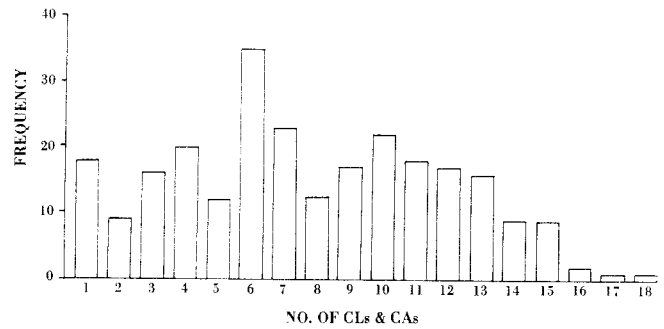


Fig. 17. Frequency histogram showing the distribution of corpora counts (number of corpora lutea (CLs) plus corpora albicantia (CAs)) for *G. macrorhynchus*.

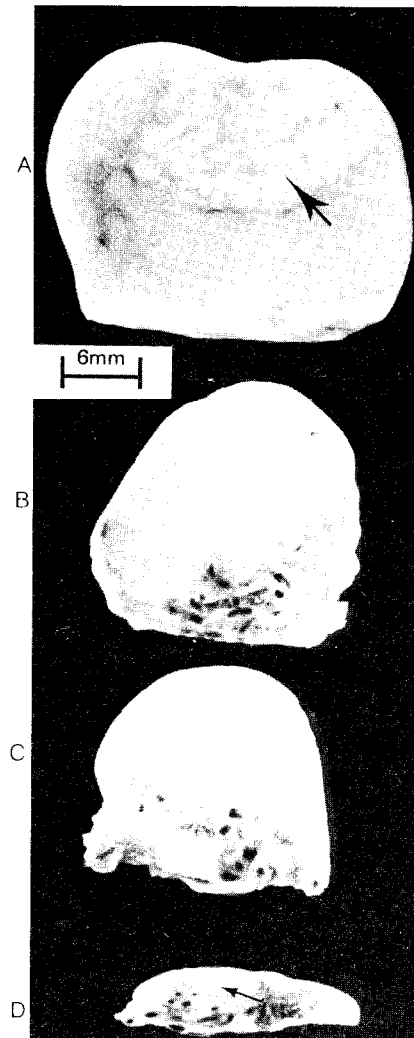


Fig. 18. Median slices through various corpora albicantia to show the pattern of regression. A. Initial stage in the regression of a corpus luteum of 'ovulation', some lobes (arrowed) were still active (Whale 13-23, 32.5 years). B. Young corpus albicans of pregnancy from 9-100 (aged 10.5 years and lactating). C. Slightly older corpus albicans from 9-95 (aged 30.5 years and lactating). D. Old corpus albicans (arrowed) also from Whale 9-95.

Eighty CAs were classified as young (Figs. 18A, B, C; 19A). Their size distribution is shown in Fig. 20, the range of mean diameters being from 8.5 to 28.5 mm ( $\bar{x}$  15.2 mm;  $s$  3.27 mm). These young CAs can easily be distinguished from CLs on the basis of colour (young CAs

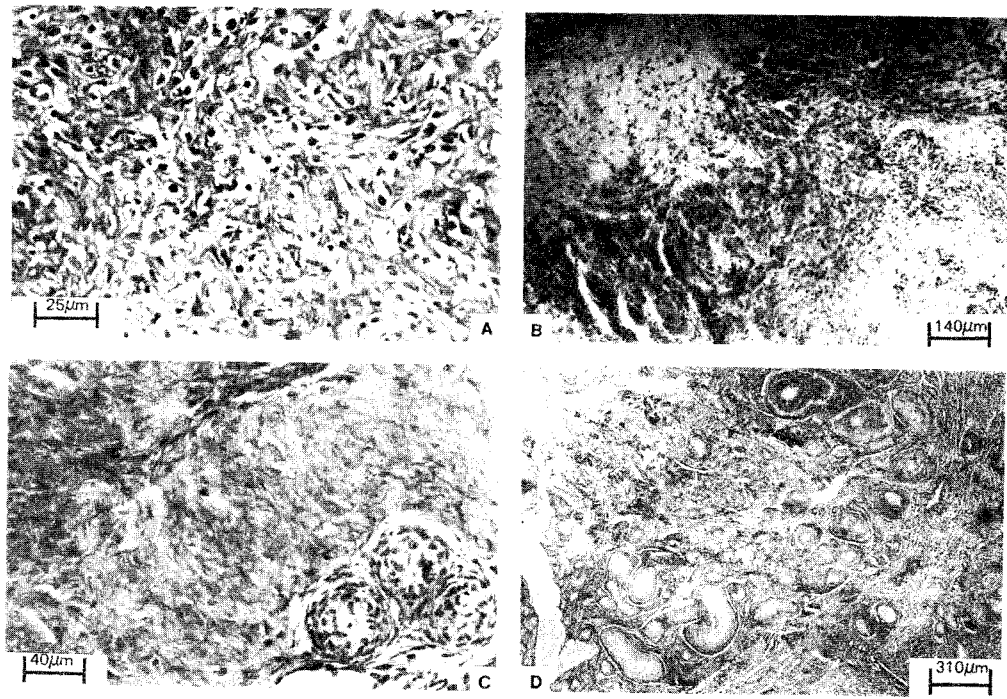


Fig. 19. Histology of regressing corpora albicantia in ovaries of *G. macrorhynchus*. A. Histology of regressing lobe of young corpus albicans of Whale 13-23 shown in Fig. 18A. The primary luteal cells have been replaced by cellular fibrous tissue but some presumed secondary luteal cells with dark nuclei are still visible (Gomori's trichrome). B. Macrophages filled with lipid in the brown central area of a medium corpus albicans (10.5 mm in diameter) in the ovary of Whale 11-21 (14.5 years). (Haematoxylin and eosin). C. Hyaline connective tissue forms the bulk of this medium corpus albicans (11.9 mm in diameter) in the left ovary of pregnant Whale 17-44 (16.5 years). D. Part of one of the 12 old corpora albicantia of lactating Whale 26-12 (48.5 years). This corpus albicans, which was 7.5 mm in diameter, consisted mainly of thick-walled blood vessels (van Gieson and celestin-blue haemalum).

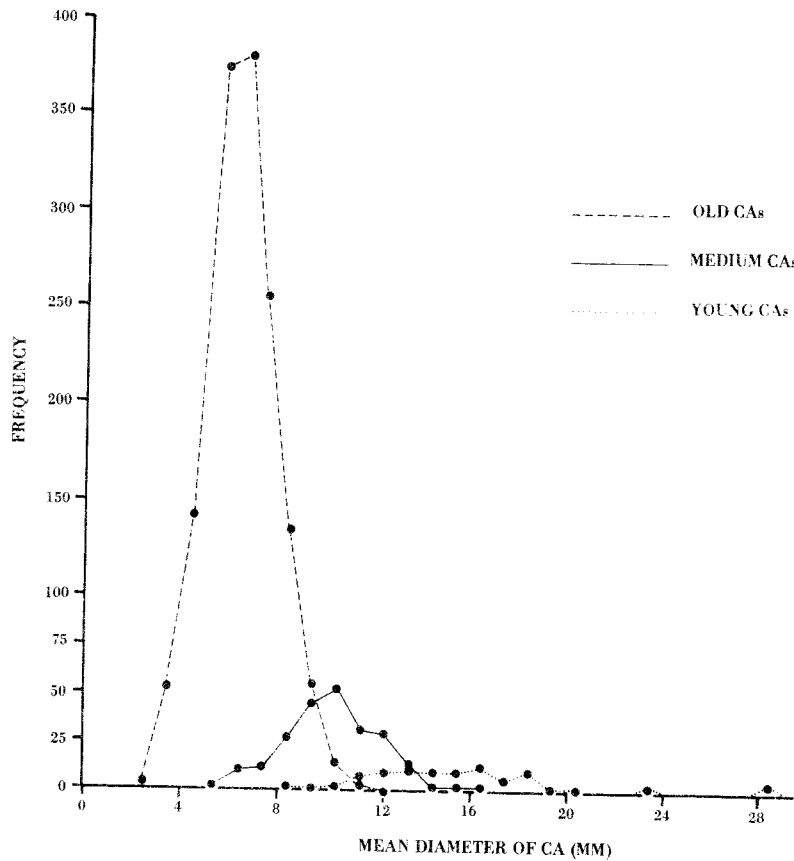


Fig. 20. Size-frequency distributions of young, medium and old corpora albicantia (CAs) in ovaries of *G. macrorhynchus*.

Table 7

Details of whales on which the histological comparison of young corpora albicantia derived from corpora lutea of pregnancy and presumed ovulation was based. (P = pregnant; L = lactating; F.L. = fetal length)

| Accession no.                          | Age (years) | Reproductive status | Number of corpora |           |            |         |
|--|-------------|---------------------|-------------------|-----------|------------|---------|
|  |             |                     | CL                | Young CAs | Medium CAs | Old CAs |
| <i>Corpora of pregnancy</i>            |             |                     |                   |           |            |         |
| 9-100                                  | 10.5        | L                   |                   | 1         |            |         |
| 9-99                                   | 11.5        | L                   |                   | 1         |            |         |
| <i>Probable corpora of 'ovulation'</i> |             |                     |                   |           |            |         |
| 9-92                                   | 10.5        | P (F.L. 137 cm)     | 1                 | 1         |            |         |
| 9-115                                  | 22.5        | P (F.L. 1.2 cm)     | 1                 | 2         | 0          | 7       |

are pale orange or white; CLs are ochre) and texture (CAs are much more fibrous), as well as size. Two hundred and thirty-one medium CAs (Figs. 3D; 19B, C) were measured. They ranged from 5.5 mm to 16.5 mm in mean diameter ( $\bar{x}$  10.4 mm;  $s$  1.99 mm) (Fig. 20) and were significantly smaller than the young CAs ( $t$  test;  $P < 0.0005$ ). The 1,426 CAs classified as old (Figs. 3E, 18D; 19D) ranged from 2.5 mm to 12 mm in mean diameter ( $\bar{x}$  6.4 mm;  $s$  1.5 mm) (Fig. 20). The mean diameter of the old CAs was significantly less than that of medium CAs ( $t$  test;  $P < 0.0005$ ).

#### *The pattern of regression*

The CLs of the two whales from School 9 that had recently given birth (one of them only the day before its death) were macroscopically and histologically indistinguishable from the CLPs of late pregnancy. The youngest CA in our series was a regressing 'CLO' 23.5 mm in diameter (Figs 18A; 19A), in some lobes of which a large proportion of primary luteal cell were still recognizable, although few had visible nuclei and these were all pyknotic. Fibrous replacement of the primary luteal cells was obviously progressing rapidly and many fibroblasts were visible. In the remaining lobes, most of the primary luteal cells had been replaced by cellular fibrous tissue (Fig. 19A) within which occasional primary luteal cells and groups of presumed secondary luteal cells were still visible. This young CA was a very vascular structure with an extensive network of capillaries and larger vessels. The trabecular arrangement of the former CL was still obvious. The other young CAs (e.g. Fig. 18B, C) were all slightly older and less vascular, and the density of cells was much lower.

We compared the macroscopic structure and histology of several young CAs. Two were definitely derived from CLPs, the other two were probably derived from 'CLOs' (see Table 7). We could not separate the two groups.

The connective tissue of the CAs classified as medium was shrunken and hyalinized, making the blood vessels appear much more prominent (Fig. 19C). Some blood vessels were occluded; most contained erythrocytes. The trabeculae were less obvious.

In old CAs (Fig. 19D), the shrinkage and hyalinization of the connective tissue had progressed even further. The trabeculae were no longer obvious, and thick-walled blood vessels made up the greater part of the structure. However, many of the blood vessels were patent.

The histological structure of all 12 CAs of Whale 26-12, which was 48.5 years old, corresponded to the above description of old CAs. As discussed below, our data suggest that (1) CAs persist and (2) this whale had probably not ovulated for at least nine years. As this animal was lactating, we presume she had been pregnant at least once and conclude that the bodies we have classified as old CAs represent the end-point in the degeneration of all CLs.

Some CAs in all three categories had a brown central area full of lipid-laden cells (presumably macrophages) (Fig. 19B). The lipid inclusions were histochemically and morphologically similar to those in corpora atretica, and we suggest that they may have derived from secondary luteal cells, which are probably of thecal origin (see Harrison *et al.*, 1981) and which have undergone 'fatty' degeneration.

#### *The rate of regression*

We have studied the rate of regression of CAs by considering the ovaries of sexually mature whales less than 13 years old (Table 8). As pointed out by Kasuya and Marsh (1984) our sample suggests that short-finned pilot whales begin to ovulate at a minimum of about 7.5 years (see No. 7-17, Table 8). The youngest animals with old CAs were Nos. 14-15 and 15-25, both aged 9.5 years. We therefore conclude that it is possible for a CL to degenerate into an old CA within two years.

However, it is likely that the degeneration rate is dependent on hormonal status. Fig. 21 shows the diameters of the largest young CA observed in various females at different stages of pregnancy. Assuming that ovulation does not occur during pregnancy (which is estimated to last 14.9 months (Kasuya and Marsh, 1984)) it is obvious that the degeneration of a young CA must be greatly reduced or halted during this time, a conclusion supported by histological study of the young CAs present in the ovaries of Whales 9-115 (pregnant with a 1.2-cm fetus) and 9-92 (pregnant with a near-term fetus) (Table 7).

It is also likely that the rate of regression is slower in old animals, although we have no data bearing directly on this. As discussed below, the oldest pregnant whales we have examined had an expected age of parturition of 35.5 years, and we have no evidence of ovulation occurring in whales older than 40 years. Yet medium CAs were recorded in animals as old as 55.5 years (Table 9 and



Table 8  
Details of the ovaries and reproductive status of *G. macrorhynchus* females 12.5 years and younger

| Accession no.                            | Reproductive status | Fetal length (cm) | Estimated time <sup>2</sup> since last ovulation (days) | Mean diameter CA (mm) |              |                           |
|--|---------------------|-------------------|---|-----------------------|--------------|---------------------------|
|  |                     |                   |   | Young                 | Medium       | Old                       |
| <i>8.5 years (3 immature; 4 mature)</i>  |                     |                   |   |                       |              |                           |
| 9-122                                    | P                   | 3                 | 50 <sup>1</sup>   | 15.1                  | 11.2<br>8.8  |                           |
| 26-9                                     | P                   | 40.5              | 160 <sup>1</sup>  |                       |              |                           |
| 17-2                                     | P                   | 66.5              | 237 <sup>1</sup>  |                       |              |                           |
| 7-17                                     | P                   | 90                | 306 <sup>1</sup>  |                       |              |                           |
| <i>9.5 years (5 immature; 5 mature)</i>  |                     |                   |   |                       |              |                           |
| 14-15                                    | P                   | 100               | 336   |                       | 10.4         | 7.3                       |
| 24-25                                    | P                   | 132               | 430 <sup>1</sup>  |                       |              |                           |
| 10-14                                    | L                   |                   | > 452 <sup>1</sup>                                      |                       | 11.1         |                           |
| 15-25                                    | R                   |                   |   | 13.3                  | 10.2         | 5.6                       |
| 26-21                                    | R                   |                   |   | 17.8                  |              |                           |
| <i>10.5 years (2 immature; 9 mature)</i> |                     |                   |   |                       |              |                           |
| 9-114                                    | P                   | 1                 | 43.6 <sup>3</sup>                                       | 15                    |              |                           |
| 10-8                                     | P                   | 2.8               | 49 <sup>1,3</sup>                                       |                       |              |                           |
| 17-31                                    | P                   | 23.3              | 109   |                       | 12.2         |                           |
| 15-15                                    | P                   | 65.5              | 234   |                       | 15.4         | 11.5<br>7.2<br>7.0<br>5.0 |
| 15-47                                    | P                   | 78                | 271 <sup>1</sup>  |                       |              |                           |
| 11-16                                    | P                   | 100               | 336 <sup>1</sup>  |                       |              |                           |
| 9-92                                     | P                   | 137               | 445   | 12.7                  |              |                           |
| 9-100                                    | L                   |                   | > 452 <sup>1</sup>                                      | 20.3                  |              |                           |
| 14-3                                     | R                   |                   |   |                       | 11.8<br>10.8 | 7.5                       |
| <i>11.5 years (1 immature; 7 mature)</i> |                     |                   |   |                       |              |                           |
| 25-2                                     | P                   | 29.5              | 128   |                       | 14.0<br>8.0  | 3.7                       |
| 12-5                                     | P                   | 31                | 132   |                       | 10.5         | 9.6                       |
| 18-47                                    | P                   | 50                | 188 <sup>1</sup>  |                       |              |                           |
| 9-99                                     | L                   |                   | > 452 <sup>1</sup>                                      | 18.5                  |              |                           |
| 20-2                                     | R or L              |                   |   | 16.7                  | 9.2          |                           |
| 13-35                                    | R (ovd)             |                   | several   |                       | 11.6         | 4.5                       |
| 11-5                                     | R                   |                   |   | 16.9                  |              |                           |
| <i>12.5 years (6 mature)</i>             |                     |                   |   |                       |              |                           |
| 26-8                                     | P/L                 | 19.3              | 98  |                       |              | 7.9<br>7.4<br>5.8         |
| 15-44                                    | P                   | 75                | 262   |                       | 11.3         |                           |
| 23-4                                     | P                   | 78                | 271 <sup>1</sup>  |                       |              |                           |
| 26+27-42                                 | L                   |                   | > 452   |                       | 13.3<br>7.6  | 6.7                       |
| 13-29                                    | L                   |                   | > 452   | 15.5                  | 9.2          | 8.3                       |
| 16-10                                    | R                   |                   |   |                       | 12.5         | 7.9<br>6.2<br>5.0         |

<sup>1</sup> Pregnant at first ovulation.

<sup>2</sup> Calculated from formula derived from Laws (1959).

$$\text{Length of pregnancy (in days)} = \left( 452 \times 0.91 \times \frac{\text{F.L.}}{139.5} \right) + (452 \times 0.09)$$

<sup>3</sup> Probably over-estimated.

P = pregnant; L = lactating; R = resting; R (ovd) = resting with large follicles; P/L = pregnant and lactating; F.L. = fetal length.

Fig. 8). The rate of resorption of CAs is also known to be reduced in post-menopausal human ovaries (Peters and McNatty, 1980).

*Evidence for the persistence of corpora albicantia*

If old CAs disappeared from the ovary, we would expect their size distribution to be negatively skewed and the modal value to decrease with age. Figs 20 and 22 indicate

that neither of these expectations was fulfilled in *G. macrorhynchus*. The mean diameter of the smallest CA in each ovary pair also showed no evidence of changing after about age 20 years (Fig. 23). As discussed below, we have no evidence for ovulations occurring in animals over 40 years old. Thus for example, No. 24-7 (aged 62.5 years) probably had not ovulated for more than 20 years yet had ten old CAs clearly visible in her ovaries. On the

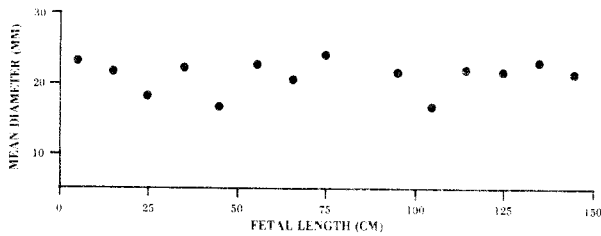


Fig. 21. Diameter of the largest young corpus albicans present per pair of ovaries of *G. macrorhynchus* at various stages of pregnancy.

basis of all this evidence, we concluded that CAs persist in this species.

The frequency distribution of corpus counts (Fig. 17) is somewhat at odds with this conclusion. For if the CAs do not disappear, and if the population of whales is stable and our sample unbiased with respect to age, we would expect the modal number of corpora to be one (rather than six as in Fig. 17) and the frequency to fall off with increasing numbers of corpora (which it does but only after the mode). There are several explanations for the frequency distribution in Fig. 17. Firstly, our sample of

whales is biased with respect to age. Females under 15 years old are under-represented in our sample from the largest school (School 9) and the reproductive status of the sample is not representative of the population (see Kasuya and Marsh, 1984). Further, the range of ages at which sexual maturity is reached (7 to 12 years), the reduction of ovulation rate with increasing age (Fig. 25), the wide individual variation both in ovulation rate (Table 8 and Fig. 25) and in the age at which ovulation declines and ceases (see Fig. 8) will also affect the frequency distribution of corpus counts. In view of these confounding influences, we conclude that we would be unjustified in rejecting the conclusion that CAs persist on the basis of Fig. 17 alone.

#### Bilateral ovarian activity

Even though ovulation appeared to take place from any point on the surface of either ovary, more ovulations occurred from the left ovary in animals of all ages (Fig. 24). The first ovulation occurred from the left ovary in eight whales and from the right in four whales. (The difference is not significant,  $\chi^2$ ,  $P > 0.25$ ). Of 1,521 CLs

Table 9  
Results of histological examination of ovarian cortex to obtain an index of follicle abundance

| Accession no. | Age (years) | Reproductive status <sup>1</sup> | Follicle abundance ranking <sup>2</sup> | Age of youngest CL or CA <sup>3</sup> | Macroscopic follicles present | No. of corpora atretica and accessory CLs found on macroscopic examination |
|---------------|-------------|----------------------------------|---|---------------------------------------|-------------------------------|--|
| 17-33         | 4.5         | Im                               | 4                                       | —                                     |                               | 0  |
| 17-9          | 4.5         | Im                               | 4                                       |                                       | ✓                             |  |
| 17-31         | 10.5        | P                                | 4                                       | CLP                                   | ✓                             | 1  |
| 17-5          | 14.5        | R                                | 4                                       | young CA                              | ✓                             |  |
| 17-3          | 15.5        | R                                | 3                                       | young CA                              | ✓                             | 1  |
| 17-17         | 15.5        | L                                | 3                                       | medium CA                             | ✓                             | 9  |
| 17-35         | 17.5        | L                                | 3                                       | young CA                              | ✓                             | 1  |
| 7-22          | 22.5        | R                                | 3                                       | 'CLO'                                 | ✓                             |  |
| 17-18         | 22.5        | L                                | 3                                       | medium CA                             | ✓                             | 3  |
| 23-18         | 24.5        | L                                | 3                                       | young CA                              |                               | 5  |
| 17-37         | 24.5        | L                                | 3                                       | medium CA                             | ✓                             | 4  |
| 24-22         | 26.5        | L                                | 3                                       | medium CA                             |                               | 1  |
| 7-8           | 27.5        | L                                | 3                                       | young CA                              | ✓                             | 4  |
| 17-13         | 27.5        | R                                | 3                                       | young CA                              | ✓                             | 12   |
| 23-3          | 29.5        | R                                | 1                                       | old CA                                |                               | 4  |
| 17-29         | 35.5        | L                                | 3                                       | young CA                              | ✓                             | 1  |
| 17-20         | 38.5        | R                                | 1                                       | old CA                                |                               | 8  |
| 16-9          | 40.5        | R                                | 3                                       | medium CA                             |                               | 17   |
| 22-12         | 41.5        | R                                | 1                                       | medium CA                             |                               | 9  |
| 9-85          | 43.5        | R                                | 1                                       | old CA                                | ✓                             | 8  |
| 17-24         | 44.5        | R                                | 1                                       | old CA                                |                               |  |
| 22-9          | 44.5        | R                                | 1                                       | old CA                                |                               |  |
| 24-12         | 45.5        | R                                | 1                                       | old CA                                |                               | 9  |
| 24-19         | 46.5        | R                                | 1                                       | old CA                                |                               | 1  |
| 24-1          | 47.5        | R                                | 1                                       | old CA                                |                               | 7  |
| 15-5          | 47.5        | R                                | 1                                       | old CA                                | ✓                             |  |
| 17-41         | 47.5        | L                                | 1                                       | old CA                                | ✓                             | 1  |
| 15-4          | 55.5        | R                                | 1                                       | medium CA <sup>4</sup>                | ✓                             |  |
| 17-1          | 55.5        | R                                | 1                                       | old CA                                | ✓                             |  |
| 24-7          | 62.5        | R                                | 1                                       | old CA                                | ✓                             | 2  |

<sup>1</sup> Im = immature; P = pregnant; L = lactating; R = resting.

<sup>2</sup> 1: < 2 follicles per 10 fields each 2.7 mm<sup>2</sup>; 2: < 10 follicles per 10 fields; 3: 10 > 50 follicles per 10 fields; 4: > 50 follicles per 10 fields.

<sup>3</sup> CLP = corpus luteum of pregnancy; 'CLO' = corpus luteum of 'ovulation'; CL = corpus luteum; CA = corpus albicans.

<sup>4</sup> Classified as medium on macroscopic examination; mean diameter 9.5 mm.

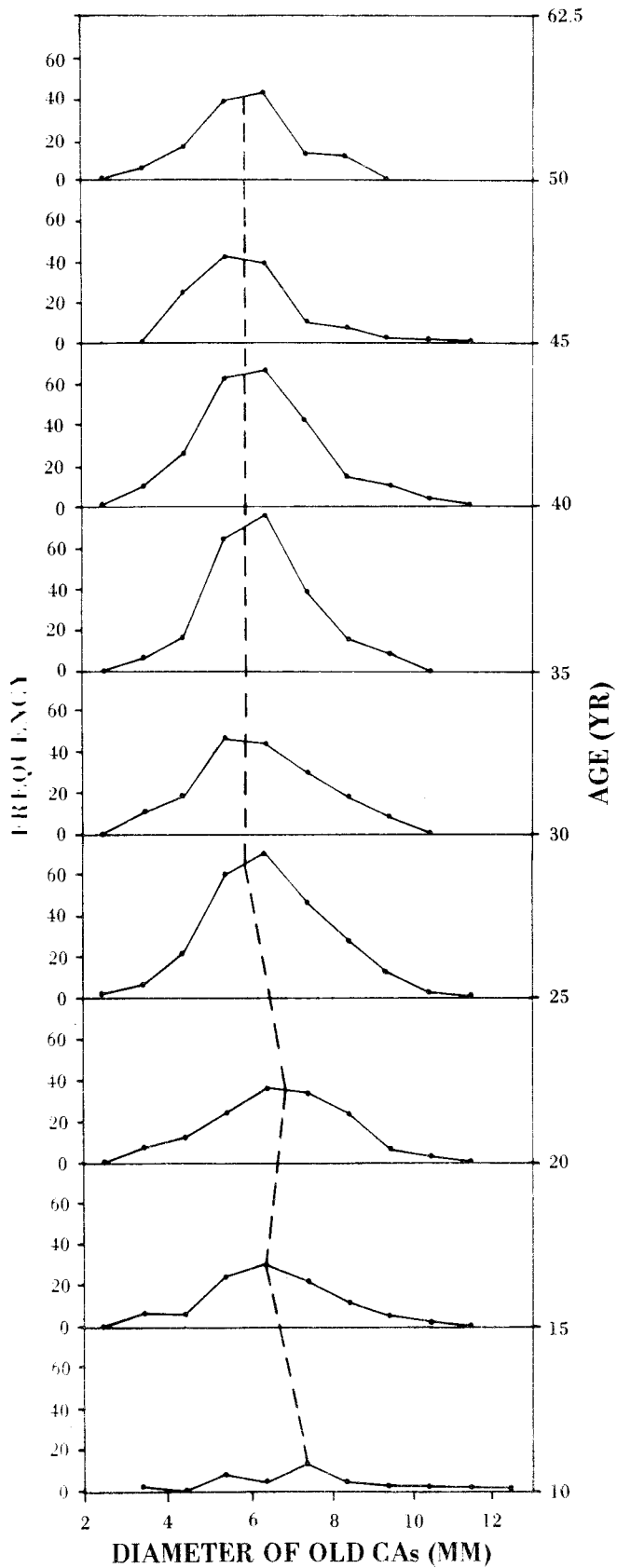


Fig. 22. The size distribution of old corpora albicantia (CAs) in the ovaries of *G. macrorhynchus* at various ages. The dotted line traces the change in the mode.

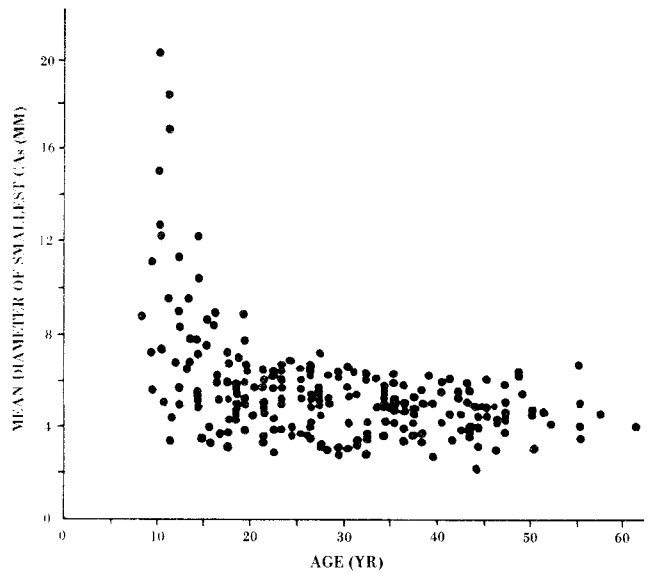


Fig. 23. Scatterplot showing the size of the smallest corpus albicans (CA) in the ovaries of *G. macrorhynchus* at various ages.

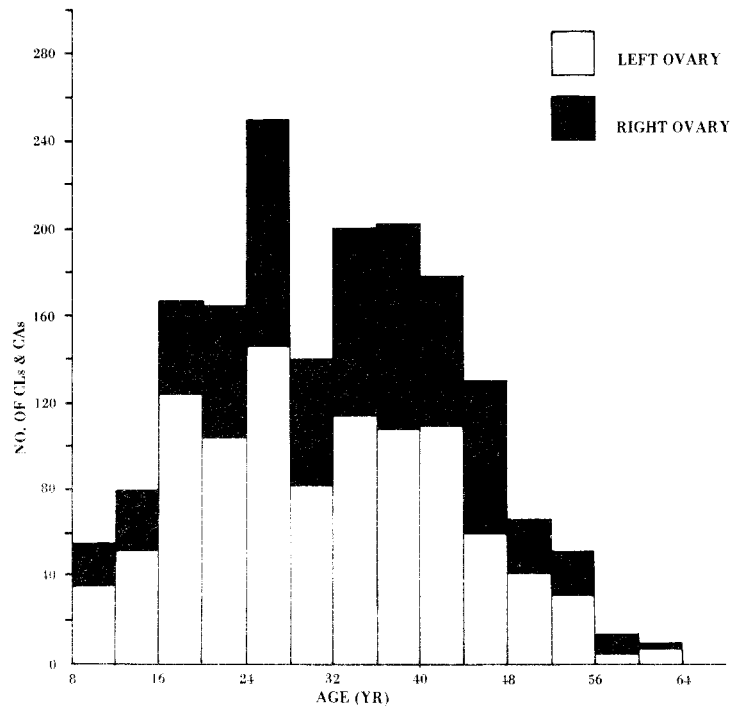


Fig. 24. Numbers of corpora lutea (CLs) and corpora albicantia (CAs) in the left and right ovaries of *G. macrorhynchus* at various ages.

and CAs scored, 61% were found in the left ovary, the proportion being significantly higher (71%) in animals less than 20 years old than in older whales (59%) ( $\chi^2$ ,  $P < 0.001$ ). However, there was no difference in the bilateral distribution of corpora between animals aged between 20 years and 40 years and those older than 40 years ( $\chi^2$ ,  $P > 0.05$ ), providing further evidence that CAs are not lost from the ovary.

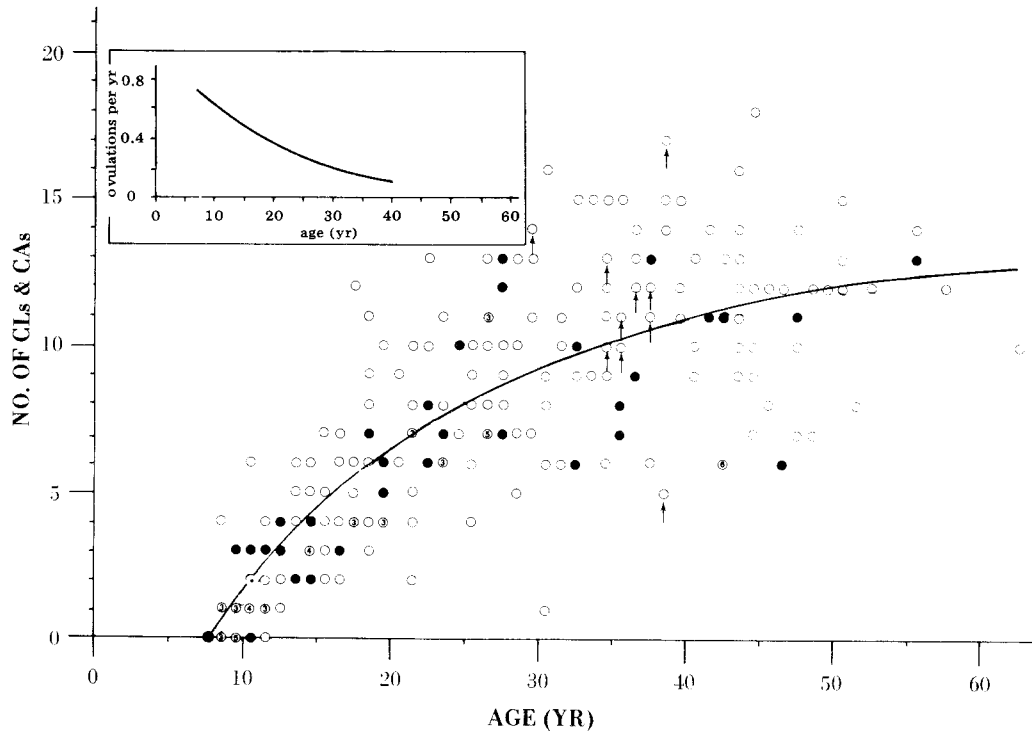


Fig. 25. Relationship between corpus count (number of corpora lutea (CLs) plus corpora albicantia (CAs)) and age in *G. macrorhynchus*. The fitted regression line is

$$y = 13.39 - 19.65 (0.95)^x$$

where  $y$  is the number of corpora at age  $x$ .

○ one whale; ● two whales; ⊙ whales

The arrows indicate the whales less than 40 years old which have been classified as post-reproductive. (All whales more than 40 years old were also classified as post-reproductive). Inset: change in ovulation rate with age calculated using the formula

$$\frac{dy}{dx} = 1.008 (0.95)^x \text{ up to age 40 years.}$$

Where  $dy/dx$  is the number of ovulations per year at age  $x$ . The mean of the corpora count at each age is given in Table 10.

**Ovulation rate**

Even assuming that CAs persist as a record of ovulations, estimating the rate of accumulation is difficult because of (1) variation in the age at attainment of sexual maturity; (2) change in ovulation rate during an individual's reproductive life span and (3) individual variation in the accumulation rate. All of these factors contribute to the scatter in the plot of number of corpora (CLs plus CAs) against estimated age (Fig. 25).

As discussed by Kasuya and Marsh (1984) and illustrated in Table 8, females of *G. macrorhynchus* ovulate for the first time between the ages of 7 and 12 years. The ovulation rate obviously changes with age (Fig. 25) and, as discussed below, we have no evidence of recent ovulations occurring in any whale over 40 years old. The number of corpora in 45 whales 40 years old and older was independent of age ( $r = 0.12, P > 0.2$ ). The spread of values for the number of corpora counted in the ovaries is considerable irrespective of the age of the whale (Fig. 25; Table 10). The magnitude of the standard deviation of corpus counts for a given age is highly variable (probably due to the small sample sizes) but shows no systematic increase after about age 17 years.

The individual variation in the accumulation rate is illustrated by Table 8, which summarizes the details of 31

Table 10  
Mean ( $\bar{x}$ ) of corpus counts (corpora lutea and corpora albicantia) *G. macrorhynchus* females of various ages

| Age (years) | Corpus count |           | Age (years) | Corpus count |           | Age (years) | Corpus count |
|-------------|--------------|-----------|-------------|--------------|-----------|-------------|--------------|
|             | $n^*$        | $\bar{x}$ |             | $n^*$        | $\bar{x}$ |             |              |
| 8.5         | 7            | 1.0       | 24.5        | 3            | 9.0       | 40.5        | 3            |
| 9.5         | 10           | 0.9       | 25.5        | 5            | 7.4       | 41.5        | 3            |
| 10.5        | 11           | 1.8       | 26.5        | 11           | 9.0       | 42.5        | 4            |
| 11.5        | 8            | 1.9       | 27.5        | 9            | 10.1      | 43.5        | 7            |
| 12.5        | 6            | 2.8       | 28.5        | 4            | 8.8       | 44.5        | 5            |
| 13.5        | 5            | 3.8       | 29.5        | 4            | 11.3      | 45.5        | 2            |
| 14.5        | 10           | 3.5       | 30.5        | 5            | 8.0       | 46.5        | 3            |
| 15.5        | 5            | 4.2       | 31.5        | 3            | 9.0       | 47.5        | 5            |
| 16.5        | 6            | 4.2       | 32.5        | 7            | 9.7       | 48.5        | 2            |
| 17.5        | 6            | 5.8       | 33.5        | 2            | 12.0      | 49.5        | 1            |
| 18.5        | 8            | 6.9       | 34.5        | 7            | 9.4       | 50.5        | 3            |
| 19.5        | 8            | 5.5       | 35.5        | 7            | 9.4       | 51.5        | 1            |
| 20.5        | 2            | 7.5       | 36.5        | 5            | 11.4      | 52.5        | 1            |
| 21.5        | 8            | 6.3       | 37.5        | 5            | 11.0      | 55.5        | 3            |
| 22.5        | 6            | 8.5       | 38.5        | 4            | 12.8      | 57.5        | 1            |
| 23.5        | 7            | 7.3       | 39.5        | 3            | 12.7      | 62.5        | 1            |

\*  $n$  = Sample size.

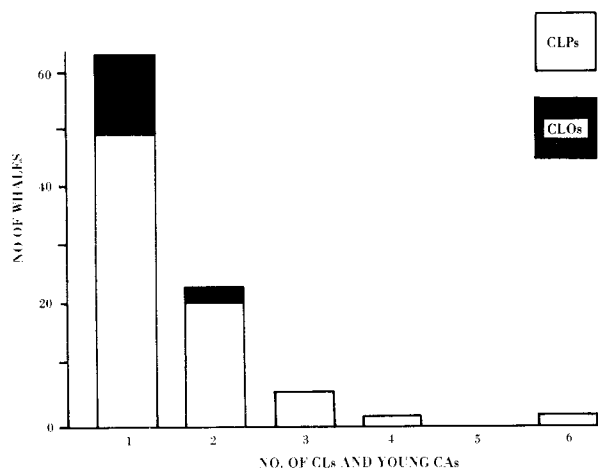


Fig. 26. Frequency histogram of the number of recent ovulations (corpora lutea (CLs) plus young corpora albicantia (CAs)) in female *G. macrorhynchus* with at least one corpus luteum. CLP = corpus luteum of pregnancy; 'CLO' = 'corpus luteum of ovulation'.

newly-mature whales aged between 8.5 and 12.5 years inclusive. Although 14 of these animals had only 1 corpus, one of the youngest (No. 9-122, 8.5 years) had four; another (No. 15-15, 10.5 years) had six. Fig. 26 provides further evidence of individual variation in the ovulation rate. Counts of the number of CLs and young CAs in the 95 animals with an active CL showed that although about two-thirds of these animals had no young CA, the remainder had one to five, suggesting that they had ovulated several times shortly before becoming pregnant. (The whale with one CL and 5 young CAs was No. 17-44 aged 16.5 years).

When Perrin *et al.* (1976) calculated ovulation rates for the spotted dolphin, *Stenella attenuata*, they corrected for individual differences in age at first ovulation and calculated ovulation rates in terms of reproductive age rather than absolute age. We decided not to do this because the sample size was rather small (Table 8) to make a meaningful correction.

The relationship between age and number of corpora obviously cannot be satisfactorily described by a single straight line (Fig. 25). Mizroch (1981) attempted to overcome this problem for a corresponding (but much larger) data set for fin whales by fitting a series of straight lines, each for an arbitrarily-selected, non-overlapping age range. We attempted this initially but ultimately rejected the approach because (1) the data are so variable that the results obtained were greatly affected by the way in which the age range was subdivided and (2) the corpus counts at a given age (especially for low ages) do not meet the assumptions of least-squares curve-fitting (see Zar, 1974), which makes the validity of comparing the slopes of a series of different lines rather dubious.

While acknowledging that the data do not meet all the assumptions, we decided to fit a single curve to the corpus count/age data of 243 females (including immature individuals) seven years old or older. (This sample was not corrected for bias; see Kasuya and Marsh, 1984). A single-phase, exponential curve of the form

$$Y = 13.39 - 19.65 (0.95)^x$$

where  $Y$  = number of corpora at age  $x$  had an  $r^2$  of 0.66, i.e. the curve explained about two-thirds of the variation

in corpus count with age. The residuals showed no systematic bias. The estimates of all the parameters were significantly different from zero at the 0.001 level. This curve suggests that the ovulation rate falls continuously throughout life from about 0.7 ovulations per year for seven-year-olds to about 0.14 per year for 39-year-olds (inset, Fig. 25). Obviously this curve is not completely satisfactory, as it does not model the complete cessation of ovulation after age forty, but the value it gives for the annual ovulation rate for animals older than 40 years is very low ( $< 0.13$ ).

An alternative approach is to fit an exponential curve to the corpora counts of animals between 7 and 40 years old. This curve is, however, less satisfactory, as it gives a higher ovulation rate at age 40 years (i.e. 0.22 ovulations per year).

### Changes in ovarian condition with age

As discussed by Kasuya and Marsh (1984) the percentage of pregnant females decreases with age. The oldest pregnant female in our sample was 34.5 years old, with a predicted age at parturition of 35.5 years. This was also the age of the oldest female which had recently given birth (the day before her death). Even though 12 whales older than 35 years (the ovaries of 10 of which were studied in detail) were still lactating, consideration of the ages of their probable calves suggests that these animals might also have given birth to their last calf when, at most, 37 years old (see Table 19, Kasuya and Marsh, 1984).

The ovaries of seven females aged between 36.5 and 39.5 years, inclusive, showed evidence of recent ovulation (including one with bilateral ovarian papilloma (K. Benirschke, pers. comm.)). Four of these whales had one 'CLO', two had one young CA, and one had two young CAs. However, we could find no evidence of recent ovulation in the ovaries of any of the 49 females 40 years old or older (Fig. 8). We therefore investigated the possibility that the infertility of these old females might be due to age-related changes in their ovaries.

Fig. 8 summarizes the reproductive status and ovarian condition of 245 mature females. Although the ovaries of no female over 40 years old contained both medium CAs and macroscopic follicles, 12 had medium CAs and no follicles that were not obviously atretic on macroscopic examination. As discussed above, although we have evidence that a CL can degenerate into an old CA within two years, we suspect that this rate of regression is greatly reduced in old animals and do not consider that the presence of medium CAs in the ovaries of animals older than 40 years is proof that they have ovulated within the preceding two years.

Six whales over 40 years old had at least one macroscopic follicle in their ovaries, even though all their CAs were classified as old. It is likely that most, if not all, of these follicles were atretic or destined to become so. The large follicles from older females that we investigated histologically were all atretic (e.g. No. 25-31 aged 34.5 years had three large follicles 18 mm, 10.3 mm and 7.9 mm in mean diameter; all were cystic (Fig. 5E)).

Thirty-one of the 49 females over 40 years old (63%) had only old CAs and no macroscopic follicles in their ovaries (Fig. 8). Six of the 18 animals (33.3%) aged between 36 and 40 years also had ovaries in this condition, as did five of the 40 animals (12.5%) aged

between 28 and 36 years. All animals younger than 29 years had a CL or medium or younger CA in their ovaries.

Semi-quantitative histological study of samples from the ovarian cortex of 30 whales spanning an age range of 4.5 to 62.5 years (Table 9), indicated that the number of follicles was significantly reduced in older females. All 12 females studied which were 41 years old or older had a follicle abundance rating of one (i.e. < 2 follicles per  $10 \times 2.7 \text{ mm}^2$  fields) as did two younger animals (Nos. 23-3 aged 29.5 years and 17-20 aged 38.5 years), both of which had only old CAs and no macroscopic follicles in their ovaries (No. 23-3 was the youngest animal with this ovarian status).

We tentatively suggest that all females older than 40 years and all with only old CAs and no macroscopic follicles in their ovaries are post-reproductive. According to these criteria, about one quarter of the 245 mature females whose ovaries were studied and for whom age estimates were available were post-reproductive.

Apart from the very low number or absence of follicles, the ovaries of old females showed evidence of other histological changes (Fig. 6B). The cortex was reduced in thickness and very fibrous and the tunica albuginea was very thick. The walls of blood vessels were thickened and sclerosed. Parts of the ovarian surface were calcified in four animals (9-127 aged 38.5 years; 11-19, 44.5 years; 26-12, 48.5 years; 24-7, 62.5 years). The germinal epithelium of the ovaries of two whales (9-123, 38.5 years; 12-3, 48.5 years) had extensively ingrown towards the medulla. No. 23-16 (34.5 years) had a pendulous cyst on one ovary, and No. 13-6 (34.5 years) had a granulosa cell tumour in one ovary. Some of this pathology has been detailed by Benirschke and Marsh (1984).

#### *Decline in the fertility of ovulating whales with increasing age*

Table 11 compares the incidence of 'CLOs' (excluding 'recent ruptures') and CLPs in whales 20 years old and younger with that in older whales. The proportion of the well-established corpora lutea which were classified as CLPs was significantly lower in the older whales ( $\chi^2$ ,  $P < 0.005$ ), indicating that ovulation is less likely to be followed by pregnancy in older females.

Table 11

Incidence of corpora lutea of 'ovulation' and pregnancy in both females twenty years old and younger and in older females. ('CLOs' classified as 'recent rupture' and animals which had recently aborted were not included)

|                            | Age (years) |      |
|----------------------------|-------------|------|
|                            | ≤ 20        | > 20 |
| No. of whales with a 'CLO' | 2           | 13   |
| No. of whales with a CLP   | 44          | 29   |

## DISCUSSION

The ovarian changes associated with varying reproductive status are not completely separable from those due to advancing age, as reproductive status is partially age-dependent. However, this macroscopic and histological study of the ovaries of a large sample of short-finned

pilot whales for which independent absolute age estimates were available does permit some distinction along these lines as discussed below.

### Changes in the ovary associated with reproductive status

#### *Follicular development and atresia*

Follicular development in *G. macrorhynchus* follows the usual mammalian pattern outlined in this volume (Appendix B of Perrin and Donovan, 1984).

The various products of follicular atresia, a particularly conspicuous feature of the ovaries studied, were also substantially similar to structures described in other mammals, including cetaceans (Figs 1 and 2, Appendix B of Perrin and Donovan, 1984). Best (1967) described and figured structures in sperm-whale ovaries similar to corpora fibrosa (Fig. 5A). Corpora atretica *b* (atretic luteinized follicles) (Fig. 5C) have been recorded from mature females of several cetacean species. Different terms have been used by different workers to describe different stages in the development and regression of these bodies (for references see Appendix B of Perrin and Donovan, 1984). In this study we have shown (1) that corpora atretica *b* are formed when the thecal cells of an unruptured antral follicle luteinize and subsequently go through a process of 'fatty' degeneration (Fig. 5C) while accumulating a 'ceroid-type' pigment and (2) that corpora atretica *b* do not persist (Fig. 13). These results should overcome previous confusion about the etiology and persistence of these bodies.

Corpora atretica *b* appear to be much more common in *G. macrorhynchus* than in the other odontocetes for which figures are available. We recorded 938 corpora atretica *b* in 235 pairs of mature ovaries (about 4 per pair) compared with means of 0.28 per pair (Perrin *et al.*, 1975) and 0.27 per pair (Kasuya *et al.*, 1974) in *Stenella attenuata*. We found one or more corpora atretica *b* in about 75% of mature ovary pairs; in contrast, Best (1967) recorded them in about 50% of sperm whale ovaries. Corpora atretica *b* were found in *G. macrorhynchus* at all stages of pregnancy (Fig. 14). This is not surprising. In our sample, although the presence of large (> 5 mm in diameter) follicles was limited to pilot whales with fetuses less than 50 cm long, there were whales with medium-sized follicles (1 < 5 mm in diameter) at all stages in the gestation period (Fig. 10). As Miller and Campbell (1978) point out (for cattle, *Bos taurus*), although the normal oestrus cycle does not occur during pregnancy, follicles can still undergo (limited) development and atresia.

At least one corpus atreticum *a* (Fig. 5B) was recorded in about 10% of mature ovaries (Table 4), a proportion similar to that in the sperm whale (Best, 1967). In both species, the recently ovulated females have the highest incidence of these bodies. The general morphology of corpus atreticum *a* in *G. macrorhynchus* (Figs. 5B, D, E) is very similar to the corresponding structures in other species as discussed in Appendix B of Perrin and Donovan (1984). However, pilot-whale corpora atretica *a* are, not unexpectedly, smaller than their counterparts in large whales, and we consider that they may not produce a persistent scar of connective tissue such as the corresponding structures do in the larger species (loc. cit.).

Accessory corpora lutea (*sensu loc. cit.*) (Fig. 11B) are



not common in *G. macrorhynchus*, occurring in about 6% of ovaries. A similar structure was described in *Globicephala melaena* by Harrison (1949). Cystic atresia similar to that recorded in *G. macrorhynchus* (Fig. 5E) has also been described in the long-finned pilot whale, *G. melaena*, by Sergeant (1962).

#### *The corpus luteum*

The general development of the corpus luteum (CL) of the short-finned pilot whale is similar to that in the Franciscana, *Pontoporia blainvillei*, as described by Harrison *et al.* (1981). When fully formed, the CL contained two distinct types of luteal cells (Fig. 11C), a feature reported for several cetaceans (van Lennep, 1950; Best, 1967; Hirose *et al.*, 1970; Mossman and Duke, 1973; Harrison *et al.*, 1981), including *G. melaena* (Harrison, 1949). Although the basic morphology of the CL is similar in the two species of pilot whale, CLs of *G. melaena* (Sergeant, 1962) are generally larger than those of *G. macrorhynchus*. The difference probably reflects the larger body size of *G. melaena*. About 10% of CLs had a jelly-filled centre in *G. melaena* (Sergeant, 1962), which is lower than the 15% incidence of this feature in *G. macrorhynchus* (Fig. 3D).

In both species, some of the 'CLOs' were within the size range of CLPs, while most were smaller (Fig. 16; Sergeant, 1962). Sergeant considered that 'CLOs' in the larger size group were probably corpora of early pregnancy or the result of early embryonic death. We agree with Benirschke, Johnson and Benirschke (1980) that the placental membranes of even very small monotocete embryos are of appreciable size and not easily overlooked and consider that probably only the youngest of the structures that we classified as 'CLOs' were associated with a pregnancy. This conclusion is supported by our observation that 'CLOs' were relatively more common in older whales. We were unable to reliably separate 'CLOs' from CLPs in *G. macrorhynchus* on morphological or histological grounds (see also Appendix B of Perrin and Donovan, 1984).

Sergeant (1962) reported that in *G. melaena* 'CLOs' were particularly noticeable in the young first-maturing males, implying that these animals were sub-fertile. In contrast, it appears that a high proportion of *G. macrorhynchus* in our sample became pregnant at their first ovulation (Table 8). This may be a result of the difference in the age of sexual maturation for the two populations studied rather than an inherent specific difference. Sergeant (1962) estimated that the long-finned pilot whales in the population he studied first conceived at 4.5 to 8 years; whereas our estimate of the pre-reproductive period for this population of *G. macrorhynchus* is 7 to 12 years (Kasuya and Marsh, 1984). Perrin *et al.* (1977) noticed a parallel difference in the fertility rates of very young mature females of *Delphinella attenuata* and *S. longirostris*. As they pointed out, such differences in the fertility of young females may reflect inherent differences or may reflect differential ovulation status with respect to exploitation. If females from a more heavily exploited population tend to become sexually mature at an earlier age, they could still be less fertile in terms of pregnancies per ovulation than if they matured when older.

Sergeant (1962) considered that the CLP of *G. melaena* reaches a maximum diameter in the early part of fetal life

and shrinks again thereafter. However, his evidence for a reduction in the size of a CLP during the gestation period is much less convincing than the evidence of a similar phenomenon in *S. attenuata* (Kasuya *et al.*, 1974; Perrin *et al.*, 1976) and *S. coeruleoalba* (Miyazaki, 1977). We have no evidence that the CLP of *G. macrorhynchus* changes size during the gestation period (Fig. 16), although the evidence for a change in the lipid metabolism of a CLP during this time is convincing (Fig. 11E, F). Histological evidence of a change in the activity of a CL during pregnancy has also been reported for blue and fin whales, *Balaenoptera musculus* and *B. physalus* (Mackintosh and Wheeler, 1929); the sperm whale (Chuzhakina, 1961); the harbour porpoise, *Phocoena phocoena* (Fisher and Harrison, 1970) and *S. graffmani* (= *S. attenuata*) (Harrison *et al.*, 1972).

Multiple CLPs are fairly rare in both *G. macrorhynchus* (Table 2) and *G. melaena* (Sergeant, 1962) as in most other cetaceans with the exception of the white whale, *Delphinapterus leucas* (Brodie, 1972; Sergeant, 1973) and the narwhal, *Monodon monoceros* (K. Hay, pers. comm., 1981) (see Appendix B of Perrin and Donovan, 1984).

#### *The corpus albicans*

The pattern of regression of CAs in *G. macrorhynchus* is very similar to that reported for many other cetaceans, including *G. melaena* (Sergeant, 1962). The connective-tissue elements in the walls of the arteries of the original CL are particularly resistant to change and make up the greater part of the structure of old CAs (Fig. 19D).

Several workers have separated the CAs observed in various cetaceans into two types (usually on histological grounds) and have suggested that one type might have developed from CLOs and the other from CLPs (e.g. Peters, 1939; Sleptsov, 1940; van Lennep, 1950; Robins, 1954; Zemskiy, 1956; Ivashin, 1958; 1984; Hirose *et al.*, 1970; Fisher and Harrison, 1970; Zimushko, 1970; Harrison and Brownell, 1971; Harrison *et al.*, 1969; Harrison *et al.*, 1972; Collet and Harrison, 1981). The essential distinguishing feature of the two types is usually held to be the amount of amorphous, relatively acellular, hyaline material present.

Several workers (e.g. Fisher and Harrison, 1970; Collet and Harrison, 1981) have suggested that CAs which consist of little more than coils of obliterated blood vessels with sparse hyaline material may be the end point in the regression of CLOs, while those with a considerable quantity of acellular material result from CLPs. However, as all 12 CAs of a lactating 48.5-year old female *G. macrorhynchus* (which almost certainly had not ovulated for at least nine years) consisted of little more than coiled blood vessels (e.g. Fig. 19D), we consider that previous workers have been confused by the stages in the regression of CLs to old CAs. We could make no histological distinction between young CAs derived from CLPs and those derived from presumed 'CLOs' (Table 7) and agree with most other cetologists who have studied the CAs from a large series of conspecifics (e.g. Laws, 1961; Sergeant, 1962; Best, 1967; Gambell, 1968, 1972; Kasuya, 1972; Kasuya *et al.*, 1974; Perrin *et al.*, 1976; Miyazaki, 1977; Harrison *et al.*, 1981), that it is not possible to separate CAs of pregnancy from those of ovulation.

Laws (1961) found that for cetaceans the diameter of the fully regressed CA tended to be a constant percentage

of the diameter of the CLP. He calculated that the regression ranged from between 82.6 and 84.1%. The mean diameter of old CAs in *G. macrorhynchus* was 6.4 mm, while the mean diameter of the CLPs was 37.6 mm. Thus the shrinkage was 83%, within the range suggested by Laws.

In *G. macrorhynchus*, CAs can regress to the 'old CA stage' within two years in at least young animals (Table 8). This time is comparable to that suggested for *G. melaena* by Sergeant (1962) who suggested that the rate of regression of a CA may be influenced by the hormonal status of the animal. Our results (Fig. 21) suggest that the rate of regression of young CAs is very slow during pregnancy. A similar result was obtained for sperm whale CAs by Best (1968).

We counted up to 18 CAs per pair of ovaries in *G. macrorhynchus*; Sergeant (1962) found a maximum of 16 per pair in *G. melaena*. Figs 20, 22, and 23 provide convincing evidence that CAs persist in the ovaries of *G. macrorhynchus* as a permanent record of ovulations. This conclusion is supported by our study of the CAs present in old post-reproductive females that had probably not ovulated for up to 20 or more years. In contrast, Harrison (1949) suggested that in *G. melaena*, the CAs may regress to such a degree that they cannot be detected without serial histological sectioning of the ovaries. Sergeant (1962) also considered that some of the small CAs of ovulation may be lost in this species. However, most cetologists working on species for which a large series is available (e.g. Mackintosh and Wheeler, 1929; Mackintosh, 1942; Chittleborough, 1954; Chuzhakina, 1961; Laws, 1961; Ohsumi, 1965; Best, 1967; Gambell, 1968, 1972; Kasuya, 1972; Kasuya *et al.*, 1974; Perrin *et al.*, 1976; Miyazaki, 1977) have considered that CAs persist.

#### *The pattern of ovarian activity*

Both Harrison (1949) and Sergeant (1962) agreed that ovulation could take place from any point on the surface of either ovary in *G. melaena*. *G. macrorhynchus* appears to be similar in this regard. In contrast, Best (1967) reported a definite polarity of ovulation sites in sperm whale ovaries. Both *G. melaena* and *G. macrorhynchus* show some bilateral difference in ovarian activity, more ovulations tending to occur from the left ovary in both species. However, the dominance of the left ovary is much less dramatic in *Globicephala* spp. than in many other odontocetes (for references see Appendix B of Perrin and Donovan, 1984).

There is no good evidence that either *G. melaena* or *G. macrorhynchus* can be polyovular. Multiple CLs of pregnancy have occasionally been observed in both species, but the supernumerary CLPs were probably derived from unruptured follicles. However, it seems likely that pilot whales can be polyoestrous. We have evidence of up to six ovulations occurring in fairly quick succession in *G. macrorhynchus* (Fig. 26). Harrison (1949) and Sergeant (1962) also report instances of two or more CAs of about the same size in *G. melaena* ovaries.

#### *Seasonality of oestrus*

Kasuya and Marsh (1984) concluded from their data on estimated parturition dates and the length of gestation that conceptions in this population of *G. macrorhynchus*

occurred in all months of the year, with a single peak in April/May. The ovarian data on the seasonality of oestrus fit this picture reasonably well. Follicle size seemed to be greatest in both immature and lactating females in the months May to July, while 'CLOs' were most commonly observed both during this period and in October. Some resting females also had large (> 8 mm in diameter) follicles in February, October and December, suggesting that oestrus activity may be prolonged in resting females that fail to conceive in the main part of the mating season.

### **Changes in the ovaries of *G. macrorhynchus* with age**

#### *Follicle abundance*

The age-related decline in follicle abundance in the ovaries of *G. macrorhynchus* is conspicuous both macroscopically and histologically (Fig. 6). Chuzhakina (1961) documented a similar decline in sperm whale ovaries and claimed that follicles were completely absent from whales with 13 or more corpora. These results are not surprising if we accept the evidence presented by Zuckerman (1956) (which albeit is based on very few species, none cetaceans), that there is no renewed proliferation of oocytes beyond fetal or early post-natal life in mammals. The decline in the population of female germ cells in the ovary begins prior to birth in the species that have been studied and continues until the cells are exhausted or the animal dies (Talbert, 1977). However, the relationship between exhaustion of developing oocytes and normal life span is extremely variable among the few mammalian species, and even strains of species, that have been studied (Talbert, 1977). We observed no CLs or young CAs in any of the 49 short-finned pilot whales aged over 40 years, suggesting that none of these animals had ovulated recently.

Although our assessment of the oocyte population in the ovaries of specimens of *G. macrorhynchus* of various ages (Table 9) can, at best, be regarded as semi-quantitative, it is obvious that the oocyte population of whales over 40 years old is severely depleted. (To obtain a numerical estimate of the oocyte stock of such animals is impracticable: about 6,000 histological sections would be needed per ovary).

Sergeant (1962) noted that there may be only a single enlarging follicle in older long-finned pilot whales 'approaching oestrus'. We have also observed older short-finned pilot whales with only one large follicle. Such animals are unlikely to conceive. Research on other mammals has shown that during the fertile part of the life span, antral follicle production is always in excess of the number ovulated. Those follicles which remain may, however, serve an important hormonal function and give rise to the oestrogen that seems essential for the continued growth of those that ovulate (Jones, 1970). According to Nalbandov (1964), if all but one or two of the developing follicles are destroyed during the follicular phase, none of the remainder ovulates normally. Thus the presence of a small number of macroscopic follicles in the ovaries of *G. macrorhynchus* females over 40 years of age is not inconsistent with our classification of these animals as post-reproductive. Although menopause can

occur in women before the stock of oocytes is completely exhausted, it is generally agreed that post-menopausal ovaries show little or no follicular response to exogenous gonadotrophins (Talbert, 1968).

#### *Follicular atresia*

Large follicles that do not ovulate, degenerate (Jones, 1970). The follicles that have been studied in short-finned pilot whales aged 40 or more years were all atretic. Cystic atresia of macroscopic follicles was common (Fig. 5E). Accessory corpora lutea were present in the ovaries of nine non-pregnant whales (Table 2). Similar bodies in the ovaries of old mice, *Mus musculus*, have been described as 'examples of total failure of the ovarian mechanism' (Jones, 1970).

Atresia accompanied by thecal luteinization to produce corpora atretica *b* is a conspicuous feature of mature *G. macrorhynchus* ovaries. Although the incidence of these bodies was significantly lower in females over 40 years old than in those aged between 20 and 40 years old, we consider that this reflects the much lower number of follicles in the ovaries of these older whales (Table 9) rather than a reduction in the tendency for follicles to become atretic.

The incidence of follicular atresia has also been shown to be age-related in other mammals. Best (1967) reported a sudden and rapid increase in the proportion of sperm whales exhibiting follicular atresia after 13 CAs had accumulated in the ovaries (unfortunately, absolute age estimates were not available). Ageing rodents show an increasing tendency for follicles to luteinize prematurely so that ovulation cannot take place (Talbert, 1968). Ceroid associated with regressing luteal bodies (corpora atretica *b*) was present in greater amounts in the ovaries of aged pigtail macaques, *Macaca nemestrina*, than in younger animals (Graham, Kling and Steiner, 1979).

Factors which control the rate of follicular atresia have the greatest influence on the rate of loss of oocytes (Talbert, 1977). In all mammals the loss of follicles by ovulation is insignificant compared to the 'devastating effects of atresia' (Jones, 1970). The rate of follicular atresia in mature *G. macrorhynchus* ovaries (which, as discussed above, seems to be unusually high) is probably an immediate cause of the high proportion of females in our sample which were post-reproductive. Follicular atresia accompanied by luteinization is also a conspicuous feature of the ovaries of mature pigtail macaques (Graham *et al.*, 1979), one of the few non-human primates in which a menopausal condition has been convincingly documented.

#### *Other changes*

In addition to the decline in follicular abundance and increase in atresia discussed above, other age-related changes occur in the histology of the ovaries of *G. macrorhynchus*. These are similar to those which have been documented in post-menopausal humans. Such changes include a general decrease in volume of the cortex and thickening and sclerosis of arterial walls (Graham *et al.*, 1979), ingrowth of the surface epithelium (Peters and McNatty, 1980) and increased pathology (Labhsetwar, 1970). Chuzhakina (1961) observed some of these

features in the ovaries of sperm whales with 13 to 16 CAs which also exhibited no evidence of recent ovulations or primordial follicles. However, as discussed below, the evidence for a climacteric in the sperm whale is far from conclusive.

We were surprised to find no generalized reduction in ovarian weight in the females which were classified as post-reproductive (Fig. 4). Ovaries have been reported to become atrophic in menopausal women, rats, mice and hamsters (for references see Labhsetwar, 1970), but actual data on the weight changes are scanty. As Labhsetwar (1970) points out, the accurate determination of weight changes is complicated by the tendency of old gonads to undergo pathological changes and by the positive correlation between body weight and ovarian weight. We have not attempted to separate these confounding influences on ovarian weight in *G. macrorhynchus*.

#### *Comparison with other odontocetes*

A small percentage of adult female spotted dolphins, *S. attenuata*, (Perrin *et al.*, 1976) and spinner dolphins, *S. longirostris*, (Perrin *et al.*, 1977) were classified as post-reproductive or senile by criteria including (1) being neither pregnant nor lactating; (2) having small withered ovaries; (3) having no macroscopic ovarian follicles and (4) having no young CAs. As most of the specimens of *G. macrorhynchus* that we have classified as post-reproductive do not satisfy these criteria (Figs 4 and 8), we cannot make a meaningful comparison between the incidence of post-reproductive females in these species.

Sergeant (1962) considered that about 5% of mature specimens of *G. melaena* were post-reproductive. Again it is difficult to make between-species comparisons as Sergeant apparently assumed that lactating females had not reached the climacteric. However, as the relative proportions of pregnant, lactating and resting females in Sergeant's sample of *G. melaena* are very different from the values we have obtained for *G. macrorhynchus* (Kasuya and Marsh, 1984), it seems likely that the proportion of post-reproductive females is lower in *G. melaena* than in *G. macrorhynchus*. (It is, of course, possible that the differences observed merely reflect population differences in age composition and/or history of exploitation).

As discussed above, anatomical studies of sperm whale ovaries reveal clear indications of a drop in fertility with age. Age-specific differences are also found in the proportions of the various reproductive classes in the catch. Age-specific pregnancy rates have been demonstrated by Ohsumi (1965), Best (1968; 1980) and Gambell (1972), but only Ohsumi's rates are expressed in terms of absolute age rather than relative age estimated from corpus counts. As the number of corpora present at a given age in the sperm whale is highly variable (see Ohsumi, 1965), the latter approach has obvious limitations. Ohsumi's data are also potentially misleading in that he has assumed that all females with a CL are pregnant. However, Ohsumi (1965) has aged a 'pregnant' sperm whale at 59 to 60 years, not much younger than the oldest animals he recorded, aged 63 to 64 years. We conclude that, at present, there is no evidence for an age-specific climacteric in the sperm whale, even though fertility seems to be significantly reduced in old animals.

*Other anatomical changes associated with reproductive senescence*

Age-related changes in pregnancy rate are not dependent solely on ovarian status but on the sum of the total age changes in the reproductive tract. Although primary ovarian failure is thought to contribute significantly to the age-related decline in reproductive capacity in women (Jones, 1970), in laboratory rodents reproductive decline has been shown experimentally to be primarily due to the uterus (Finn, 1970). Sergeant (1962) described degenerative changes in the uterus of 'senile' specimens of *G. melanaea*. We have not yet established whether there are parallel changes in the uterine morphology of *G. macrorhynchus*, but they cannot be ruled out as a possible cause of the decline in fertility observed in ovulating whales older than 20 years (Table 11). Partial uterine failure is suggested as one of the causes of the low incidence of pregnancy in pre-menopausal women (Finn, 1970).

**Concluding remarks**

Krohn (1964) claimed that there is 'no reasonable doubt that the likelihood of conceiving declines with increasing age in all species (of mammal) for which there is any information at all'. Unfortunately such information is difficult to obtain, especially for marine mammals with life spans similar to our own. One of the best ways to obtain this information is to use the carcass-salvage approach on a large sample of conspecifics obtained through a fishery, as has been done for a number of cetacean species. However, if age-specific reproductive rates are to be measured accurately, reliable estimates of absolute age are essential. It should also be remembered that data on the age-specific abundance of fetuses are (with suitable corrections) likely to be much more valuable to management than inferences based on the study of the ovaries alone. Unfortunately most studies of cetaceans do not meet these criteria.

As reviewed by Marsh and Kasuya (in press), a decline in pregnancy rate with advancing age seems likely for at least several odontocetes. However, at this stage there is no firm evidence that the post-reproductive phase occupies a major portion of the total life span of the females of any wild mammal other than *G. macrorhynchus* which (in this population) ceases to produce calves by the age of about 36 years when it still has a life expectancy of about 14 years (Kasuya and Marsh, 1984). Kasuya and Marsh also point out that the behaviour work of Bigg (1982) and his co-workers suggests that killer whales may be similar. These species certainly present the closest parallel to the situation in humans, where even in societies, such as India in the 1880's, which did not have the benefits of 'modern medicine', the life expectancy for women of menopausal age was about 15 years (Dublin, Lotka and Spiegelman, 1949).

**ACKNOWLEDGEMENTS**

We wish to thank the following people: the dolphin hunters at Futo and Taiji and the many people who assisted with field work; L. Winsor, P. Osmond, L. Reilly, S. Crosby and P. Moreton for assistance with histology;

Z. Florian for helping with the photomicroscopy; C. Mills for drawing the figures; the Photography Department at James Cook University; L. M. Marsh and M. Kahn, Mathematics Department, James Cook University for advice about the techniques of curve-fitting; and an anonymous referee for helpful suggestions.

**REFERENCES**

- Anon. 1975. *IMSL Library 3 Reference Manual*. International Mathematics and Statistics Library, Incorporated, Houston, Texas.
- Benirschke, K. and Marsh, H. 1984. Anatomic and pathologic observations of female reproductive organs in the short-finned pilot whale, *Globicephala macrorhynchus*. (Published in this volume.)
- Benirschke, K., Johnson, M. L. and Benirschke, R. J. 1980. Is ovulation in dolphins, *Stenella longirostris* and *Stenella attenuata*, always copulation induced? *Fish. Bull.*, U.S. 78: 507-28.
- Best, P. B. 1967. The sperm whale (*Physeter catodon*) off the west coast of South Africa. 1. Ovarian changes and their significance. *S. Afr. Div. Sea Fish. Invest. Rep.* 61: 1-27.
- Best, P. B. 1968. The sperm whale (*Physeter catodon*) off the west coast of South Africa. 2. Reproduction in the female. *S. Afr. Div. Sea Fish. Invest. Rep.* 66: 1-32.
- Best, P. B. 1980. Pregnancy rates in sperm whales off Durban. *Rep. int. Whal. Commn* (special issue 2): 93-7.
- Bigg, M. 1982. An assessment of killer whale (*Orcinus orca*) stocks off Vancouver Island, British Columbia. *Rep. int. Whal. Commn* 32: 655-70.
- Brodie, P. F. 1972. Significance of accessory corpora lutea in odontocetes with reference to *Delphinapterus leucas*. *J. Mammal.* 53: 614-16.
- Byskov, A. G. 1979. Atresia. pp. 41-58. In: A. R. Midgley and W. A. Sadler (eds). *Ovarian Follicular Development and Function*. Raven Press, New York, 414 pp.
- Chittleborough, R. G. 1954. Studies on the ovaries of the humpback whale, *Megaptera nodosa* (Bonnaterre), on the western Australian coast. *Aust. J. mar. Freshwat. Res.* 5: 35-63.
- Chuzhakina, E. S. 1961. [Morphological characterization of the ovaries of the female sperm whale (*Physeter catodon* L. 1778) in connection with a specific age]. *Trudy Inst. Morf. Zhivot.* 34: 33-53.
- Collet, A. and Harrison, R. J. 1981. Ovarian characteristics, corpora lutea and corpora albicantia in *Delphinus delphis* stranded on the Atlantic coast of France. *Aquat. Mamm.* 8: 69-76.
- Curtis, F. 1905. Nos méthodes de coloration élective du tissu conjonctif. *Archs. Med. exp. Anat. path.* 17: 603-36.
- Drury, R. A. B. and Wellington, E. A. 1967. *Carleton's Histological Technique*. 4th ed. Oxford University Press, Oxford, 432 pp.
- Dublin, L. I., Lotka, A. J. and Spiegelman, M. 1948. *Length of Life. A Study of the Life Table*. Rev. Ed., Ronald Press Co., New York, 379 pp.
- Finn, C. A. 1970. The ageing uterus and its influence on reproductive capacity. *J. Reprod. Fert., Suppl.* 12: 31-8.
- Fisher, H. D. and Harrison, R. J. 1970. Reproduction in the common porpoise (*Phocoena phocoena*) of the North Atlantic. *J. Zool., Lond.* 161: 471-86.
- Gambell, R. 1968. Seasonal cycles and reproduction in sei whales of the Southern Hemisphere. *Discovery Rep.* 35: 31-134.
- Gambell, R. 1972. Sperm whales off Durban. *Discovery Rep.* 35: 199-358.
- Gomori, G. 1950. A rapid one-step trichrome stain. *Am. J. Clin. Path.* 20: 662-4.
- Graham, F. E., Kling, O. R. and Steiner, R. A. 1979. Reproductive senescence in female nonhuman primates. pp. 183-202. In: D. M. Bowden (ed.). *Aging in Nonhuman Primates*. Van Nostrand Reinhold Company, New York.
- Harrison, R. J. 1949. Observations on the female reproductive organs of the ca'aining whale *Globicephala melanaea* Traill. *J. Anat.* 83: 238-53.
- Harrison, R. J. 1972. Reproduction and reproductive organs in *Platanista indi* and *Platanista gangetica*. *Invest. on Cetacea* 4: 71-82.
- Harrison, R. J., Boice, R. C. and Brownell, R. L., Jr. 1969. Reproduction in wild and captive dolphins. *Nature, Lond.* 222: 1143-7.
- Harrison, R. J. and Brownell, R. L., Jr. 1971. The gonads of the South American dolphins, *Inia geoffrensis*, *Pontoporia blainvillei* and *Sotalia fluviatilis*. *J. Mammal.* 52: 413-19.

- Harrison, R. J., Brownell, R. L., Jr and Boice, R. C. 1972. Reproduction and gonadal appearances in some odontocetes. pp. 361-429. In: R. J. Harrison (ed.). *Functional Anatomy of Marine Mammals*, Vol. 1, Academic Press, London and New York, 451 pp.
- Harrison, R. J., Bryden, M. M., McBrearty, D. A. and Brownell, R. L., Jr. 1981. The ovaries and reproduction in *Pontoporia blainvillei* (Cetacea, Platanistidae). *J. Zool., Lond.* 193: 563-80.
- Harrison, R. J. and McBrearty, D. A. 1973-4. Reproduction and gonads of the black finless porpoise, *Neophocaena phocaenoides*. *Invest. on Cetacea* 5: 225-30.
- Harrison, R. J. and McBrearty, D. A. 1977. Ovarian appearances in captive delphinids (*Tursiops* and *Lagenorhynchus*). *Aquat. Mamm.* 5: 57-66.
- Harrison, R. J. and Ridgway, S. H. 1971. Gonadal activity of some bottlenose dolphins (*Tursiops truncatus*). *J. Zool., Lond.* 165: 355-66.
- Hirose, K., Kasuya, T., Kazihara, T. and Nishiwaki, M. 1970. Biological study of the corpus luteum and the corpus albicans of blue white dolphin (*Stenella caeruleo-alba* [sic]). *J. Mammal. Soc. Japan* 5: 33-40.
- Ivashin, M. V. 1958. [A method to determine the corpora lutea traces of pregnancy and ovulation in humpback whales]. *Bull. Mosk. Obshtstiv. Isp. Prirody., Ord. Biol.* 63: 161-72.
- Ivashin, M. V. 1984. Characteristics of ovarian corpora in dolphins and whales as described by Soviet scientists. (Published in this volume.)
- Jones, E. C. 1970. The ageing ovary and its influence on reproductive capacity. *J. Reprod. Fert., Suppl.* 12: 17-30.
- Jubb, K. V. S. and Kennedy, P. C. 1970. *Pathology of Domestic Animals*. 2nd ed. Vol. 1. Academic Press, New York and London, 593 pp.
- Kasuya, T. 1972. Growth and reproduction of *Stenella coeruleoalba* based on the age determination by means of dentinal growth layers. *Sci. Rep. Whales Res. Inst., Tokyo* 24: 57-79.
- Kasuya, T. and Marsh, H. 1984. Life history and reproductive biology of the short-finned pilot whale, *Globicephala macrorhynchus*, off the Pacific coast of Japan. (Published in this volume.)
- Kasuya, T. and Matsui, S. 1983. Age determination and growth of the short-finned pilot whale off the Pacific Coast of Japan. *Sci. Rep. Whales Res. Inst., Tokyo*.
- Kasuya, T., Miyazaki, N. and Dawbin, W. H. 1974. Growth and reproduction of *Stenella attenuata* in the Pacific coast of Japan. *Sci. Rep. Whales Res. Inst., Tokyo* 26: 157-226.
- Krohn, P. L. 1964. The reproductive life span. *V. Congress Internazionale per la Riproduzione animale e la Fecondazione artificiale*: 23-55.
- Labbsetwar, A. P. 1970. Ageing changes in pituitary-ovarian relationships. *J. Reprod. Fert., Suppl.* 12: 99-117.
- Laws, R. M. 1958. Recent investigations on fin whale ovaries. *Norsk Hvalfangsttid.* 47(5): 225-54.
- Laws, R. M. 1959. The foetal growth rate of whales with special reference to the fin whale *Balaenoptera physalus* Linn. *Discovery Rep.* 29: 281-307.
- Laws, R. M. 1961. Reproduction, growth and age of southern fin whales. *Discovery Rep.* 31: 327-486.
- Lenep, E. W. van 1950. Histology of the corpora lutea in blue and fin whale ovaries. *Proc. K. ned. Akad. Wet.* 53: 593-9.
- Mackintosh, N. A. 1942. The southern stocks of whale bone whales. *Discovery Rep.* 22: 197-300.
- Mackintosh, N. A. and Wheeler, J. F. G. 1929. Southern blue and fin whales. *Discovery Rep.* 1: 257-540.
- Marsh, H., Heinsohn, G. E. and Spain, A. V. 1977. The stomach and duodenal diverticula of the dugong, *Dugong dugon*. pp. 271-295. In: R. J. Harrison (ed.). *Functional Anatomy of Marine Mammals*. Academic Press, New York, 428 pp.
- Marsh, H. and Kasuya, T. in press. Evidence for reproductive senescence in female cetaceans. *Rep. int. Whal. Commn* (special issue 8).
- Miller, R. J. and Campbell, R. S. F. 1978. Anatomy and pathology of the bovine ovary and oviduct. *Vet. Bull.* 48: 737-53.
- Miyazaki, M. 1977. Growth and reproduction of *Stenella coeruleoalba* off the Pacific coast of Japan. *Sci. Rep. Whales Res. Inst., Tokyo* 29: 21-48.
- Mizroch, S. A. 1981. Analysis of biological parameters of the Antarctic fin whale (*Balaenoptera physalus*). *Rep. int. Whal. Commn* 31: 425-34.
- Mossman, H. W. and Duke, K. L. 1973. *Comparative Morphology of the Mammalian Ovary*. University of Wisconsin Press, Wisconsin, 461 pp.
- Nalbandov, A. V. 1964. Follicular growth, ovulation, formation of corpora lutea. Chapter 5. In: *Reproductive Physiology*, 2nd ed. Freeman, San Francisco, 316 pp.
- Ohsumi, S. 1965. Reproduction in the sperm whale in the north-west Pacific. *Sci. Rep. Whales Res. Inst., Tokyo* 19: 1-35.
- Perrin, W., Coe, J. M. and Zweifel, J. R. 1976. Growth and reproduction in the spotted porpoise, *Stenella attenuata*, in the offshore eastern tropical Pacific. *Fish. Bull. U.S.* 74: 229-69.
- Perrin, W. F. and Donovan, G. P. 1984. Report of the Workshop. (Published in this volume.)
- Perrin, W. F., Holts, D. B. and Miller, R. B. 1977. Growth and reproduction of the eastern spinner dolphin, a geographical form of *Stenella longirostris* in the eastern tropical Pacific. *Fish. Bull. U.S.* 75: 725-50.
- Peters, N. 1939. Über Grösse, Wachstum und Alter des Blauwales (*Balaenoptera musculus* (L.)) und Finnwales (*Balaenoptera physalus* (L.)). *Zool. Anz.* 127: 193-204.
- Peters, H. and McNatty, K. P. 1980. *The Ovary. A Correlation of Structure and Function in Mammals*. Granada, London, 175 pp.
- Robins, J. P. 1954. Ovulation and pregnancy corpora lutea in the ovaries of the humpback whale. *Nature, Lond.* 173: 201-3.
- Sergeant, D. E. 1962. The biology of the pilot or pothead whale, *Globicephala melanea* (Traill) in Newfoundland waters. *Bull. Fish Res. Bd Can.* 132: 1-84.
- Sergeant, D. E. 1973. Biology of white whales (*Delphinapterus leucas*) in western Hudson Bay. *J. Fish. Res. Bd Can.* 30: 1065-90.
- Sleptsov, M. M. 1940. [On the biology of reproduction of the Black Sea Dolphin, *Delphinus*]. *Zool. Zh.* 20: 632-53.
- Talbert, G. B. 1968. Effect of maternal age on reproductive capacity. *Am. J. Obstet. Gynec.* 102: 451-77.
- Talbert, G. B. 1977. Aging of the reproductive system. pp. 318-56. In: C. E. Finch and L. Hayflick (eds). *Handbook of the Biology of Aging*. Van Nostrand Reinhold Co., New York, 771 pp.
- Weir, B. J. and Rowlands, I. W. 1977. Ovulation and atresia. pp. 265-301. In: Professor Lord Zuckerman and J. B. Weir (eds). *The Ovary*. Vol. 1. Academic Press, New York, 517 pp.
- Zar, J. H. 1974. *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, N.J. 620 pp.
- Zemskiy, V. A. 1956. [Methods of establishing traces of corpora lutea of pregnancy and ovulation on the ovaries of female whalebone whales]. *Bull. Mos. Soc. Nat. New ser., Biol. Sect.* 61: 5-13.
- Zimushko, V. V. 1970. [Materials on the determination of corpora lutea traces in the ovaries of the gray whale, *Eschrichtius gibbosus* (Cetacea, Eschrichtiidae)]. *Zool. Zh.* 69: 1073-80.
- Zuckerman, S. 1956. The regenerative capacity of ovarian tissue. pp. 31-58. In: G. E. Wolstenholme and E. C. F. Millar (eds). *Aging in Transient Tissues*, Vol. 2, Ciba Found. Colloq. Aging, Little Brown, Boston.