GAMETOGENESIS IN THE CAPELIN, *Mallotus villosus* (MÜLLER) FROM THE NORTHWEST ATLANTIC

SHAWN R. FLYNN

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Gametogenesis in the Capelin, *Mallotus villosus* (Müller) from the Northwest Atlantic.

by

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A thesis submitted in partial fulfillment of
the requirements for the degree of
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Abstract

Capelin, *Mallotus villosus* (Müller), from the Northwest Atlantic were examined to describe the anatomical structure of the gonads and their positioning in the body cavity in relation to that of other fish. The testes of immature fish were thread-like, elongate, smooth and clear without obvious internal divisions in histological cross section. Those of maturing fish were elongate and smooth but much more robust, flattened and white.

In maturing fish the final stages of spermatogenesis begin in the Fall of the year before spawning and are easily recognized as primary spermatogonia begin a process of division. This results in the division of the testes into lobules containing cysts. Within the cysts cells develop synchronously but the development of cells from different cysts is asynchronous. Sperm cells first appear in October and as development continues cysts filled with sperm break down and form larger pools of sperm starting in February. Fish examined immediately after spawning had gonads that were distended and contained only small amounts of residual sperm.

The ovarian anatomy is unusual in that ovarian wall covers only the medial half of the organ and the oocytes develop in lamellae that extend freely into the body cavity. This type of ovarian structure was previously described by Brock, 1878 and Huxley, 1883.

Four potential year classes of oocytes develop in the capelin ovary simultaneously and can be distinguished on the basis of size and cytological character. In immature fish all four are previtellogenic, but in maturing fish the fourth year class begins vitellogenic

growth during the fall (September - October) of the year prior to spawning. This year class will mature over the winter passing through several developmental phases until final ovulation in June - August. Mature eggs are ovulated directly into the body cavity and spawned through the gonopore located just posterior to the anus. The previtellogenic stages continue to develop during this time but at a slower rate. Their slight increase in size is accompanied by changes in the density of cytoplasm and the occurrence of such organelles as Balbiani bodies.

Post-spawned capelin were captured at Middle Cove and maintained in aquaria at the Ocean Science Centre in Logy Bay. All the male fish died within several weeks but some female fish survived until the following year. Samples of these fish were taken in the winter and spring and showed clear evidence of development of gonads for the upcoming spawning season. The ovaries of recovering fish, soon after spawning, are distended and histological cross sections reveal thickened areas of folded tissue in the ovarian wall as well as loosely packed previtellogenic oocytes and residual eggs. These features have been used to develop a method to distinguish recovering females from first time spawners.

The timing for the sequence of developmental steps of gametogenesis occurring in the last year before spawning, for both male and female capelin, was documented. It was shown that fish of different lengths have different rates of reproductive development.

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Allow your minds enough slack to think. The greatest discoveries come from thinking.

Go away, and have a think on it.

Introduction

The capelin, *Mallotus villosus* (Müller) is a small pelagic fish abundant in the Holarctic region (Scott and Scott, 1988). It is very important as prey for many marine species including cod, salmon, and marine mammals and birds (Carscadden, 1983). As such, our management of the capelin, to ensure its continued abundance, may play a vital role in the recovery of many overexploited commercial fish stocks. Management depends on a good understanding of all aspects of the biology of the species to be managed.

The life history and biological changes associated with the maturation of capelin have been well documented (Jeffers, 1931; Templeman, 1948, 1968; Winters, 1966; 1970; Winters and Campbell, 1974; Jangaard, 1974). Most capelin in the Northwest Atlantic migrate inshore to coastal beaches to deposit their eggs on or near shore. One stock spawns on the ocean floor at 25 fathoms on the Southeast Shoal off Newfoundland (Carscadden, 1978) similar to capelin stocks in the Barents Sea and Iceland (Jangaard, 1974). Eggs develop and larvae are released into the open ocean after 2-4 weeks (Frank and Leggett, 1981). By the beginning of the winter they are 4 cm in length or less (Templeman, 1948). Metamorphosis occurs at about 8 months of age and then growth is continuous until the third year when most fish spawn (Bailey *et al.*, 1977). Spawning may occur at age 2 and capelin as old as age 6 and 7 may be found in the spawning population (Templeman, 1948) however, over 80% of the spawning population consists of 3-4 year old fish (Carscadden *et al.*, 1994).

The study of the reproductive success of capelin has largely been limited to the

development of fertilized eggs and the degree of larval emergence. Frank and Leggett, (1981) predicted egg development and mortality rates from meteorological, hydrographic and biological data. Leggett *et al.*, (1984) reported a significant correlation between environmental factors and year class strength of capelin based on similar data. Research to understand further the relationships between environmental conditions, spawning patterns and development of capelin eggs and larvae are being pursued (Nakashima and Winters, Department of Fisheries and Oceans St. John's Newfoundland, pers. comm.).

Aspects of capelin reproduction dealing with the anatomy of the gonad and the cycle of gametogenesis leading up to spawning have received little attention, especially in males. Understanding gametogenesis is critical if we are to understand changes in spawning behaviour such as the delayed spawning in the 1990's (Carscadden *et al.*, 1996; Carscadden, et al., 1994; Nakashima, 1996). Brock (1878), Huxley (1883), and Goodrich (1958) have reported the anatomy of different types of fish ovaries and alluded to the type found in capelin. Gérard (1958) gave a general account of the ovarian structure of teleosts and the relationships between the ovary and the oviduct; but not the arrangement of the developing oocytes within the ovary.

Gametogenesis refers to the differentiation of primordial germ cells into gametes. The early stages of this process are not well understood for most fish species. Bowers and Holliday (1961) described the histological changes in the gonad associated with the reproductive cycle in herring (*Clupea harengus L.*). Khoo (1978) studied the histochemical and endocrine control of vitellogenesis in goldfish (*Carassius auratus*)

ovaries. More comprehensive references for this type of work include Harder (1975), Wallace and Selman (1981) and Guraya (1986). Two studies have been published on capelin gametogenesis; both in the Barents sea. Migalovsky's (1968) study of gametogenesis of female capelin from the Barents Sea provided a useful overview of ovary maturation during the spawning migration and included descriptive terminology to outline the main stages of gametogenesis. Forberg in his 1977 thesis and subsequent publications in 1978 and 1983 described the maturation cycle of male and female capelin from the Barents Sea. Forberg (1977) suggested the possibility of variation in egg size and maturation stage within the ovary and in reference to the fish length and seasonality. Forberg (1977) also examined variation in size between oocytes of different maturation stages.

While Forberg's (1977) account was more detailed, both he and Migalovsky (1968) attempted to create a maturation scale that was more representative than currently used macroscopic scales, such as the herring maturation scale (Burnett *et. al*, 1989). Maturation scales based on histological features are more accurate but applying them is time consuming and impractical for most laboratory and field situations. They can however be used to assess the utility of macroscopic scales. Both Migalovsky (1968) and Forberg (1977) gave good qualitative descriptions of the changes which occur throughout the final stages of gametogenesis and clearly identified the presence of females recovering from spawning. Although Migalovsky (1968) states that capelin spawn only once he also states that capelin can spawn more than once and the histological

descriptions in his paper clearly identify recovering females.

The purpose of this study was to examine the position of the gonad in relation to the internal body organs and the anatomy of the ovary in relation to other types found in the cod (*Gadus morhua L.)*, salmon (*Salmo salar L.)*, smelt (*Osmerus mordax* (Mitchel), and the primitive holostean *Amia calva (L.)*. The timing of the stages of gamete maturation (in capelin) leading up to spawning was studied in relation to fish length and time of year. The rate of maturation during a cold year and a warm year was considered, and a preliminary comparison of the developmental stages of local capelin with Icelandic capelin was made.

The topic of differential post-spawning mortality between males and females was examined and finally a histological method was developed to better estimate the proportion of repeat spawners in capelin stocks (Shelton *et al.*, 1993; Shackel *et al.* 1994).

Materials and Methods

During 1993-95 a total of 310 (145 male and 165 female) capelin *Mallotus villosus* (Müller), were collected by the Department of Fisheries and Oceans predominantly from the Northwest Atlantic divisions of 3K and 3L (Table 1; Fig. 1) and frozen or preserved directly in Bouin's fixative (N.B. Fixation with Bouin's destroys otoliths, thus the ages of the fish could not be determined). Frozen samples were also obtained from Iceland for 1994-95. Sub-samples of 5-20 fish of each sex were randomly selected from each sample location. In addition samples of live capelin (59 males, June 30, 1993; 29 females, July 12, 1993; 22 males, July 25, 1995) were captured at Middle Cove during spawning and maintained in 800 L tanks with circulating sea water at ambient inshore temperatures at the Ocean Sciences Centre in Logy Bay. These fish were kept to elucidate the survival rate of post-spawning males and females, and to identify characteristics unique to recovering gonads. No attempt was made to control environmental conditions. Fish were fed by adding ≤ 50 ml of thawed *Artemia* to the tank water several times per week.

Frozen and Bouin's preserved fish were dissected and both gonads were removed. All samples were fixed in Bouin's fixative for a minimum of 24 hours and then preserved in 70% ethanol. Portions of the gonads were dehydrated through a standard alcohol series to 100% ethanol and cleared in xylene (Appendix 1). Dehydration times increased with increasing size of the tissue segment. For excised gonads each wash was approximately 45 minutes. Ovaries with yolky oocytes or segments of whole fish (ie. the muscular body wall with associated internal structures) required longer washing times. Large pieces were bathed in several washes of 20 mls of the same concentration of

alcohol until, the fluid interphase formed between the specimen and the alcohol could no longer be seen, then transferred to the next step in the series. This procedure was repeated when samples were being cleared in xylene. Smaller tissue samples were transferred directly to pure paraffin wax at 60° C for two 45 minute washes before embedding. Larger portions were transferred into pure wax after several washes of xylene/wax mixtures. The proportion of wax was increased gradually with each wash. The length of time at each wash varied from 1 hour to several hours and yolky ovaries were left in xylene/wax (1 xylene :3 wax) mixtures over night followed by two washes for several hours in pure wax at 60°C to ensure proper infiltration. A disadvantage with the above procedure is that tissue samples became very hard and difficult to section after prolonged exposure to alcohol and xylene. To correct for this problem, and in particular for yolky ovaries, trimmed blocks were soaked in a 9:1 mixture of 60% alcohol and glycerine for easier sectioning (Baker, 1950: *fide* Bowers and Holliday 1961). Serial sections were cut at 7 µm and stained with aqueous Ehrlich's haematoxylin and 1% eosin in 95% ethanol (Appendix 1).

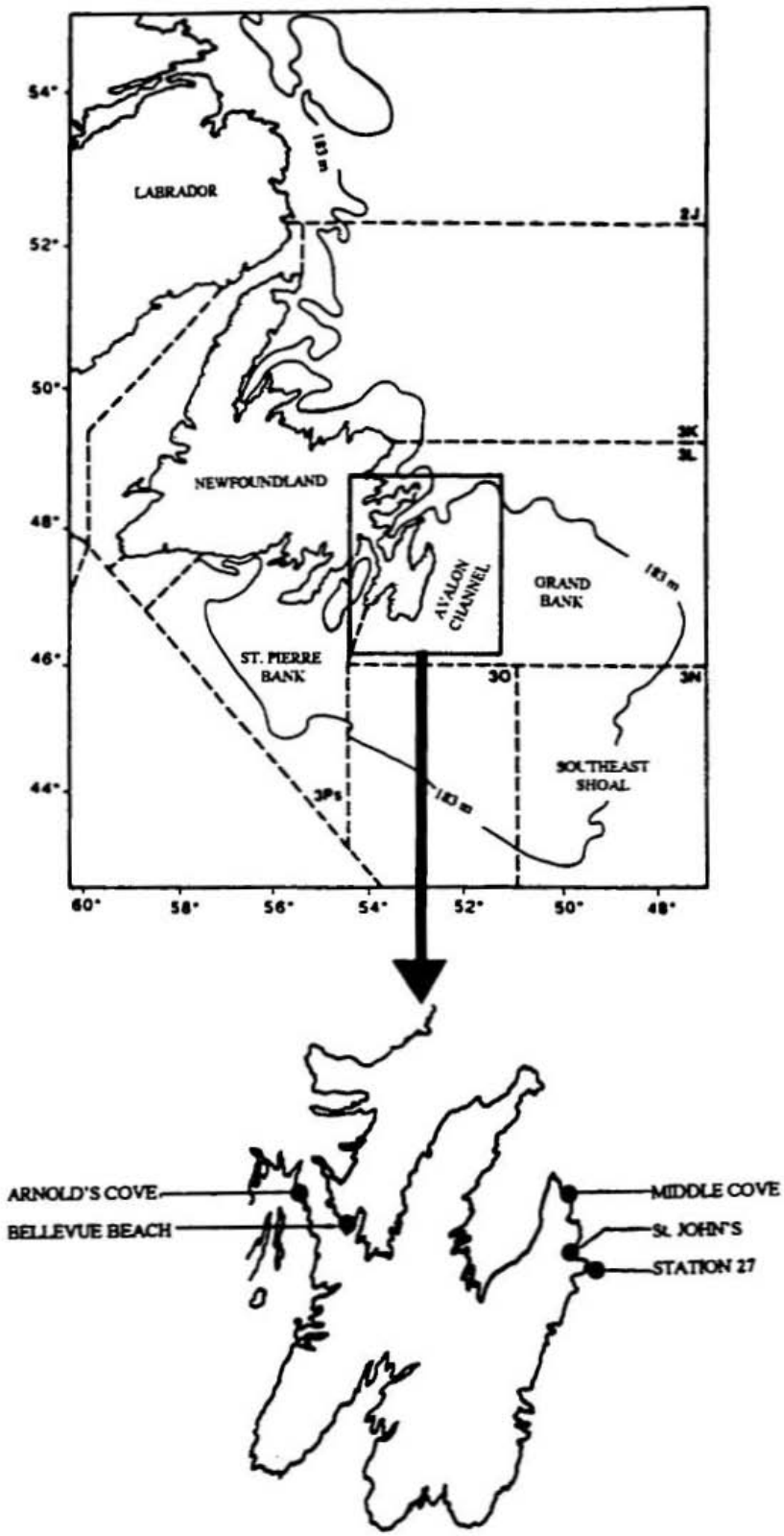
All measurements of oocyte diameter were made using an ocular micrometer at a magnification of 100 (ie. 10X ocular and 10X objective). The arithmetic mean total length and standard deviation was calculated for samples of male and female fish. The arithmetic mean and standard deviation for the diameter of oocytes was calculated by measuring the two main axes of five oocytes, of each stage, for each of five fish.

Table 1: Sample dates and locations including the number of male (♂) and female (♀) fish and the total number of fish per sample. Vessel codes: G= Gadus Atlantica, WT= Wilfred Templeman, T= Teleost. *Note that the sample 8-16/10/93 is comprised of samples taken over this time period from the area between 46°32.7N 48°59.0W and 46°32.7N 48°08.0W. All samples were fixed fresh in Bouin's fixative except 16/11/93 which was frozen before fixation in Bouin's.

Date D/M/Y	Vessel Trip/Set	Number of ♂	Number of ♀	Total number	Latitude	Longitude
23/6/93	Bellevue Beach	5	5	10	47°38.6′	53°47.3′
*8-16/10/93	G234	4	6	10	47°55.0′	48°59.0′*
16/11/93	G237/?	5	5	10	51°34.5′	51°21.9′
16/5/94	WT153/15	5	5	10	44°22.6′	50°06.7′
29/5/94	WT154/25	5	5	10	48°56.9′	51°32.3′
7/7/94	Arnolds Cove	5	5	10	47°45.2′	53°59.3′
29/9/94	G248/28	5	5	10	50°21.4′	55°34.0′
8/10/94	WT159/137	4	9	13	48°26.6′	51°16.6′
2/12/94	WT162/76	10	10	20	48°11.3′	49°01.2′
3/12/94	G251/98	11	14	25	51°03.7′	52°36.7′
17/12/94	G252/65	8	7	15	49°55.3′	51°04.7′
6/1/95	WT164/10	15	11	26	49°16.7′	51°47.8′
3/2/95	T5/6	10	20	30	48°54.6′	50°05.6′
12/2/95	T?/16	10	-	10	49°05.8′	50°11.1′
22/2/95	T?/92	10	-	10	44°34.3′	53°38.8′
25/2/95	T6/123	6	9	15	45°04.6′	55°21.5′
12/3/95	G255/114	-	11	11	46°30.3′	54°57.5′
11/5/95	WT168/76	10	10	20	43°42.3′	52°02.5′
1/6/95	WT169/82	2	13	15	46°40.3′	52°07.6′
8/6/95	WT170/27	10	10	20	49°04.2′	50°43.4′
20/7/95	Bellevue Beach	5	5	10	47°38.6′	53°47.3′
Total		145	165	310		

N. B. Samples not listed above: Middle Cove/ Oceans Sciences Center, 1993/94; Icelandic samples were taken from January to March for 1993 and 1994 (see Methods and Materials).

Figure 1: The study area in the Northwest Atlantic where *M. villosus* samples were collected for histological examination of gonads.


54°
52°
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48°
46°
44°
60°
58°
56°
54°
52°
50°
48°
LABRADOR
NEWFOUNDLAND
ST. PIERRE BANK
AVALON CHANNEL
GRAND BANK
SOUTHEAST SHOAL
183 m
2J
3K
3L
3N
3O
3Ps
ARNOLD'S COVE
BELLEVUE BEACH
MIDDLE COVE
St. JOHN'S
STATION 27

Results

The gonads of capelin are asymmetrical with the left being larger than the right (Winters, 1971). My histological examination of different regions of the gonads throughout the year prior to spawning indicate that both mature at the same rate during gametogenesis. In both sexes the gonads develop in close association to the kidney and are suspended by mesenteries from this region in a position dorsal to the swim bladder as is common among teleost fishes (Harder, 1975).

The Testes

Testes of capelin preserved *in situ* in whole fish are similar to those of other teleosts in that they are slender elongate structures which are smooth externally, thin and flattened. Viewed in transverse section the testes of capelin may be compressed, oval or slightly triangular (Fig. 2). The capelin testes are of the lobular type according to the descriptions of Nagahama (1983) and in maturing adults are composed of many lobules which are further divided into cysts filled with cells. The cells in each cyst presumably arise as a result of divisions of a single spermatogonium (Nagahama, 1983). In this report the testes are discussed in terms of lobules (the largest division within the testes), cysts (smaller divisions within the lobules), and the cells contained in these cysts referred to as spermatocytes which develop into spermatids and eventually form sperm (Fig. 3).

Spermatogenesis.

Spermatogenesis refers to the entire process of cell division which eventually results in the formation of sperm. It starts during embryogenesis when primordial germ cells migrate into the testis and undergo a sequence of mitotic divisions and cytoplasmic and nuclear reduction to produce a series of cell types which include primary and

secondary spermatogonia, spermatocytes and spermatids to finally result in the production of sperm (Alberts *et al.*, 1983). The final part of spermatogenesis, spermiogenesis, specifically refers to the cytoplasmic reduction and nuclear condensation of the spermatids which have formed via meiosis from spermatocytes (Wolfe, 1993). In maturing capelin the final stages of spermatogenesis, from the spermatocyte stage onward, is clearly observed in the testes by the presence of lobules and cysts of different developmental stages during the year preceding spawning. Within the cysts all spermatocytes develop synchronously but individual cysts start developing at different times or different rates and as such they appear as isolated groups (Fig. 4).

The cells in the gonads of immature fish may be arranged into groups but all the pregametic cells are still spermatogonia. There are primary and secondary spermatogonia appearing as relatively large cells consisting of light nuclei (approximately 6.5μm for primary and about half this for secondary cells each with a dark central nucleolous) surrounded by a high volume of lightly staining or clear cytoplasm. Except for this difference in size, all pregametic cells in sections taken through the testes are at the same level of development (Fig. 5).

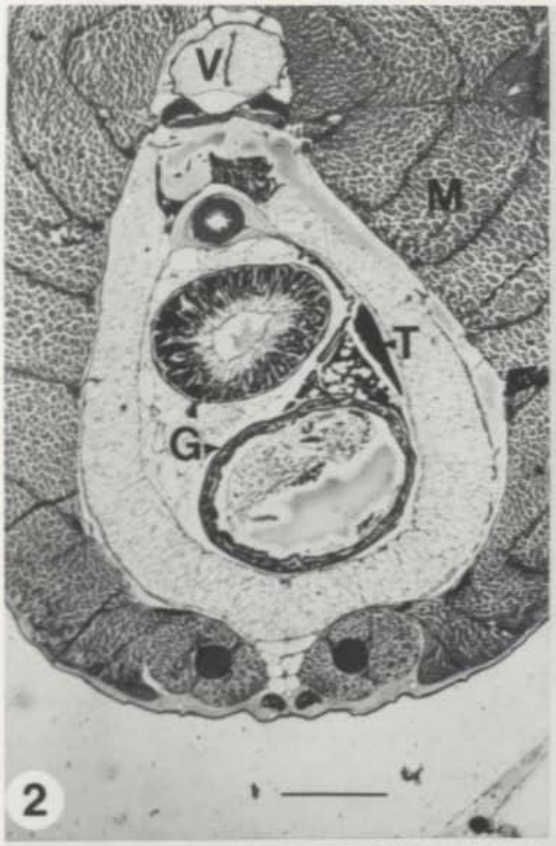
There is considerable variation in the length at which male fish begin to mature (Table 2 and 3). In this study immature male fish ranged up to 143 mm in total length but individual fish found to be maturing ranged from 128 mm upwards. Increased numbers of cysts containing developing spermatids indicate a more advanced stage of development. Within each sample taken at any time in the year longer males are more advanced reproductively than shorter males and fish can be arranged into relative length

Figure 2: A transverse section through a whole male *M. villosus* showing the position of the testis in the body cavity. Only the left testis is showing because the section was taken anterior to the right testis (G = gut, M = muscle, T = testes, V = vertebra). Scale bar = 1000 μm.

Figure 3: A transverse section through a testis of *M. villosus* showing two adjacent lobules containing cysts of various developmental stages (L = lobules, SC = a cyst containing spermatocytes, SD = a cyst containing spermatids). Scale bar = 100 μm.

Figure 4: A transverse section through a testis of *M. villosus* which is just beginning to mature showing synchronous development of cells within cysts and asynchronous development of cysts relative to each other (C = cyst). Scale bar = 50 μm.

Figure 5: A transverse section through a testis of an immature *M. villosus* showing uniform cellular development throughout (SG1 = primary spermatogonium, SG2 = secondary spermatogonium). Scale bar = 50 μm.



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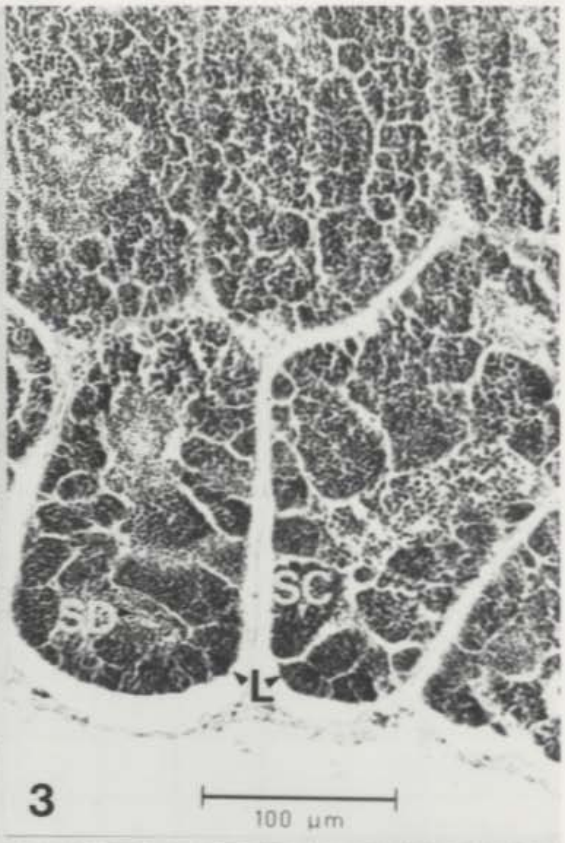

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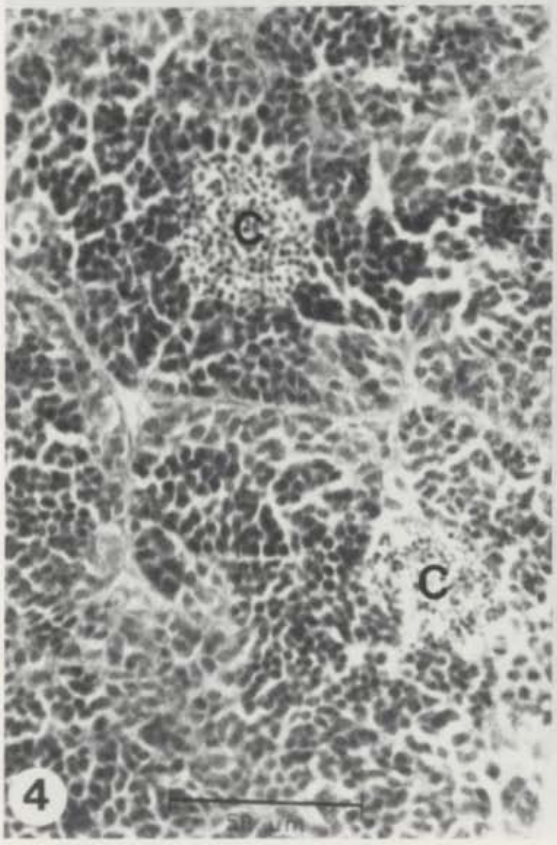

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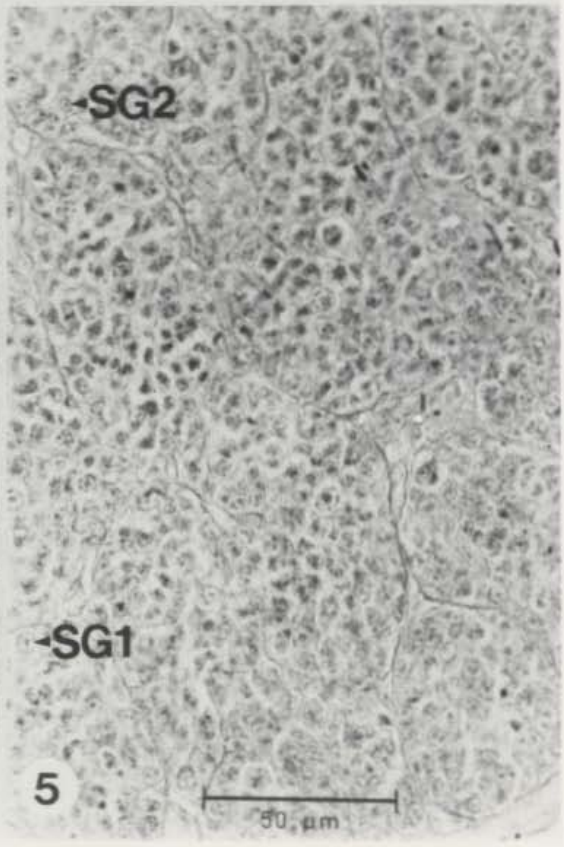

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Table 2: Length frequency distribution for all male fish sampled in the Northwest Atlantic in 1993-1995. The numbers represent the number of fish/mm interval in each sample and the average length (X) and standard deviation (S) for each sample.

Date D/M/Y	Number of Male Fish Per mm Interval									X/S
	<90	100	110	120	130	140	150	160	170>	
23/6/93								3	2	169/6.2
8-16/10/93		1		1	2					124.3/14.4
16/11/93							4	1		156.8/3.3
16/5/94							1	1	3	169.0/6.3
29/5/94				1	1	1	1	1		146.0/16.2
7/7/94							1	1	3	170.4/10.5
29/9/94					1	3	1			143.8/7.0
8/10/94				1	1	2				136.0/10.3
2/12/94					1	3	4	1	1	153.1/11.0
3/12/94				1		4	4	2		150.5/10.8
17/12/94					4	2	2			140.8/8.6
6/1/95				1	2	6	5	1		147.7/11.2
3/2/95				1	2	4	3			143.5/10.4
12/2/95					4	4	2			142.9/6.6
22/2/95				1	9					132.6/3.0
25/2/95		3	2		1					112.7/11.1
12/3/95										No Males
11/5/95					2		5	3		153.6/9.7
1/6/95					2					135.5/0.7
8/6/95					1	2	4	3		152.9/8.4
20/7/95						1	2	1	1	158.4/10.1

Table 3: The number of males (♂) and females (♀) sampled in each of three reproductive categories: Immature; fish which have not spawned and are not developing for the upcoming spawning season, Maturing; fish which are developing for the upcoming spawning season, Recovering; fish which are maturing for the next spawning season and have spawned previously. Parentheses indicate the length (mm) of individual fish.

Date D/M/Y	Immature		Maturing		Recovering	
	♂	♀	♂	♀	♂	♀
23/6/93			5	5		
8-16/10/93			4	6		
16/11/93			5	5		
16/5/94			5	5		
29/5/94	(122,139)	0	3	5		
7/7/94			5	5		
29/9/94			5	5		
8/10/94	(131,124)	0	2	6	0	(143,129,162)
2/12/94	(139)	0	9	10		
3/12/94	(143)	0	10	13	0	(150)
17/12/94			8	7		
6/1/95	(136,135)	0	13	11		
3/2/95	(124)	0	9	20		
12/2/95			10	0		
22/2/95			10	0		
25/2/95	(107,119,100,104)	0	2	9		
12/3/95			0	9	0	(130,133)
11/5/95			10	9	0	(166)
1/6/95	0	(75,82,85)	2	10		
8/6/95			10	10		
20/7/95			5	5		

categories on the basis of the degree of gamete development in the transverse sections of the testes (Figs. 6-9). The difference in development relative to length may explain why larger fish spawn earlier in the season than smaller fish (Templeman, 1948; Nakashima, 1983).

Sperm appeared first in early October, 1994 in small isolated groups. Slightly more reproductively advanced fish with greater quantity of sperm were found in November, 1993 indicating that spermiogenesis starts in the early fall. Development continues gradually with more groups of sperm occurring in samples taken from later in the reproductive cycle (Figs 10-13).

As development of adjacent cysts continues, the barriers between them break down and smaller pools of sperm join to form larger pools. The initial stages of cyst breakdown first occur in early February of 1994 in advanced fish and is quite prevalent in May of both 1994 and 1995 samples (Fig. 12). By the end of May of 1994 and early June of 1995 the lobules, which are filled with free sperm, no longer contained in cysts, begin to break down. This results in the formation of still larger pools of sperm. Not all of the lobules break down synchronously, even in spawning males (Fig.13). Since these fish make several runs onto the beach during spawning it may be that sperm remain divided into portions so that only some are released at each spawning run.

Water temperature data collected by the Department of Fisheries and Oceans from station 27 (a hydrographic monitoring center established in 1946; located 8 km off St. John's) indicate that the surface (0-20M) water temperature from 1991-1993 was colder than average (Colbourne *et al.*, 1995; Colbourne, 1995; Carscadden *et al.*, 1996).

Figure 6: A transverse section through a testis of an immature *M. villosus* of length 136 mm captured January 6, 1995. All cells are at the spermatogonium stage. Scale bar = 100 µm.

Figure 7: A transverse section through a testis of a maturing *M. villosus* of length 140 mm captured January 6, 1995. Most cells are at the spermatocyte stage with some spermatids appearing as smaller cells. There are no obvious spermatogonia. Scale bar =100µm.

Figure 8: A transverse section through a testis of a maturing *M. villosus* of length 146 mm captured January 6, 1995. More cells are at the spermatid stage, but the spermatocyte stage is still prevalent. Scale bar = 100µm.

Figure 9: A transverse section through a testis of a maturing *M. villosus* of length 161 mm captured January 6, 1995. The spermatocyte stage is not as prevalent as in Fig. 8 and the most common cell types are spermatids and sperm with tails (ST = sperm tails). Scale bar = 100µm.

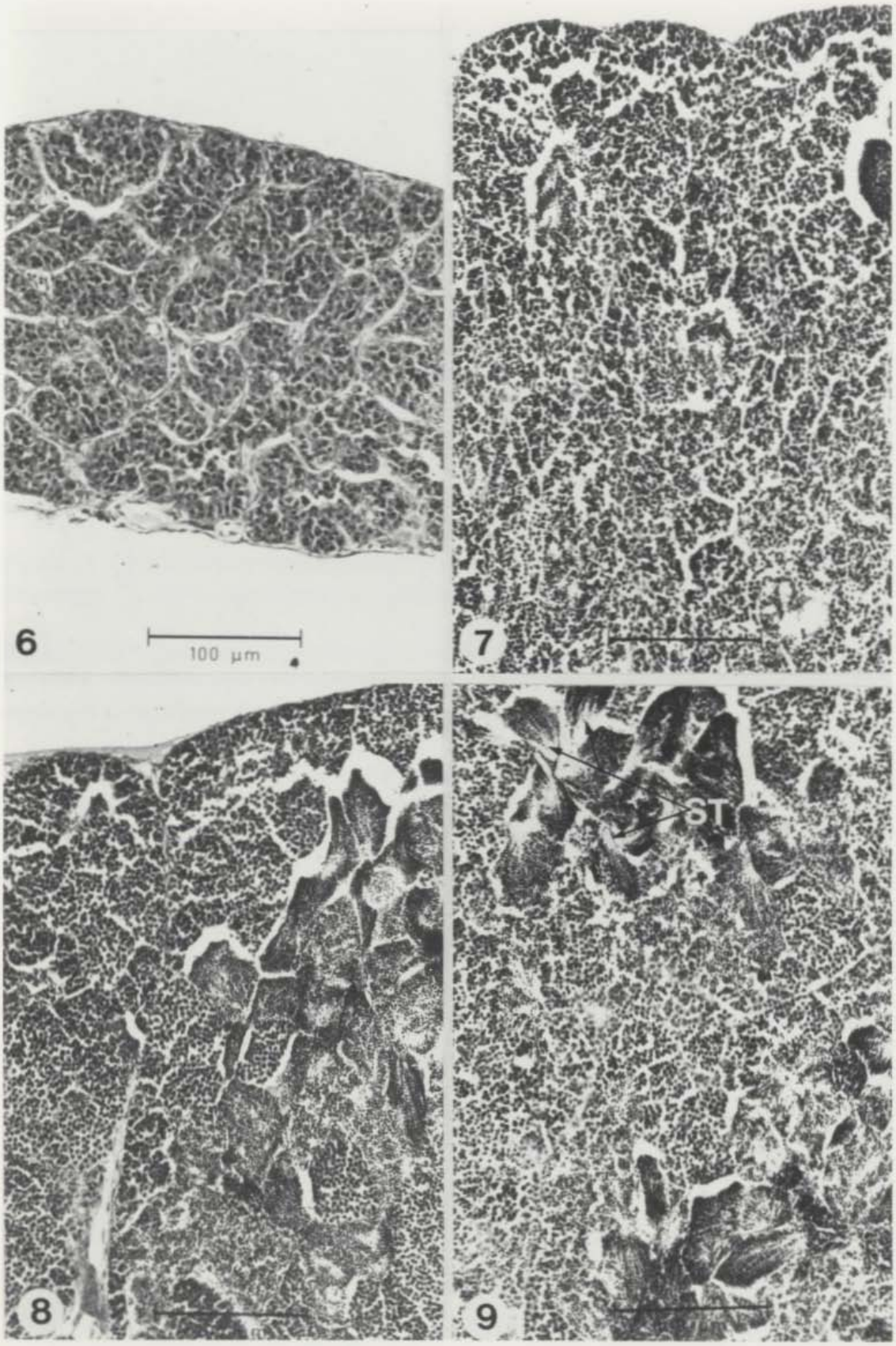
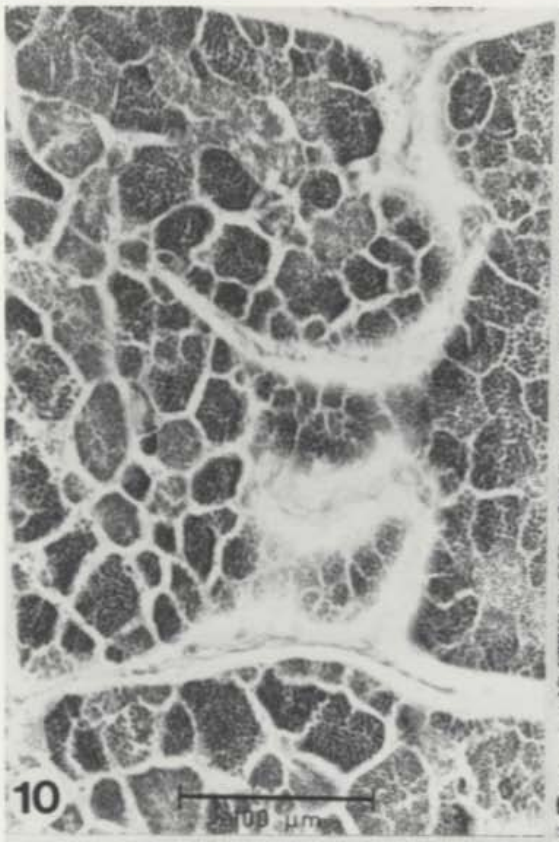

6
100 µm
7
8
9
ST

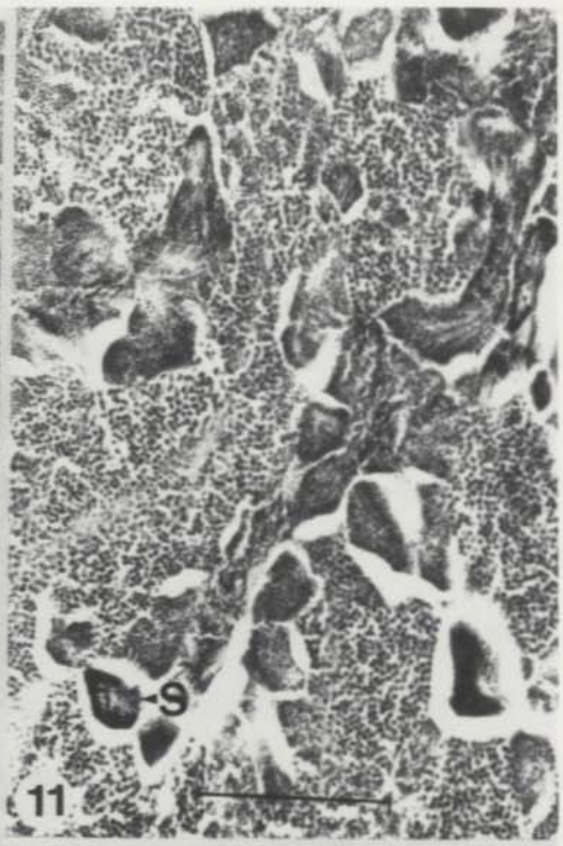
Figure 10: A transverse section through a testis of a maturing *M. villosus* captured September 29, 1994. Lobules and cysts are clearly developed and the most abundant cell types are at the spermatocyte and spermatid stages.

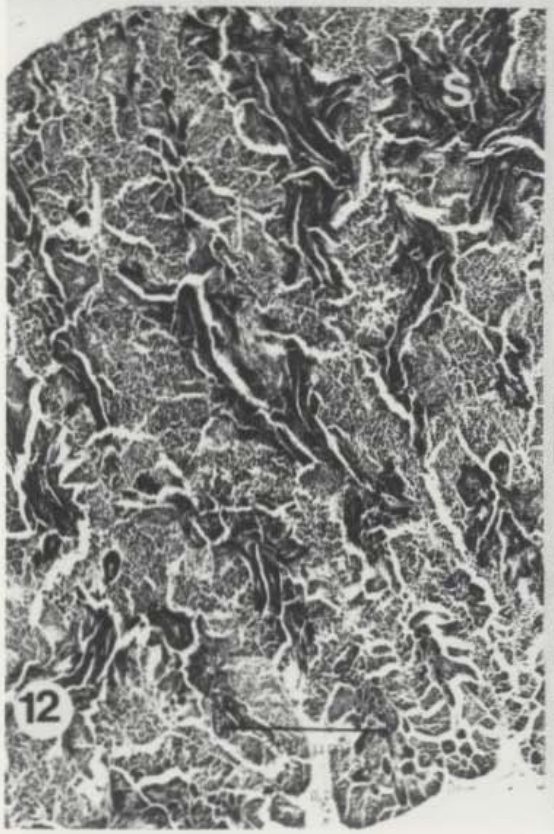
Figure 11: A transverse section through a testis of a maturing *M. villosus* captured December 17, 1994. Spermatocytes, spermatids and sperm are present (S = sperm).

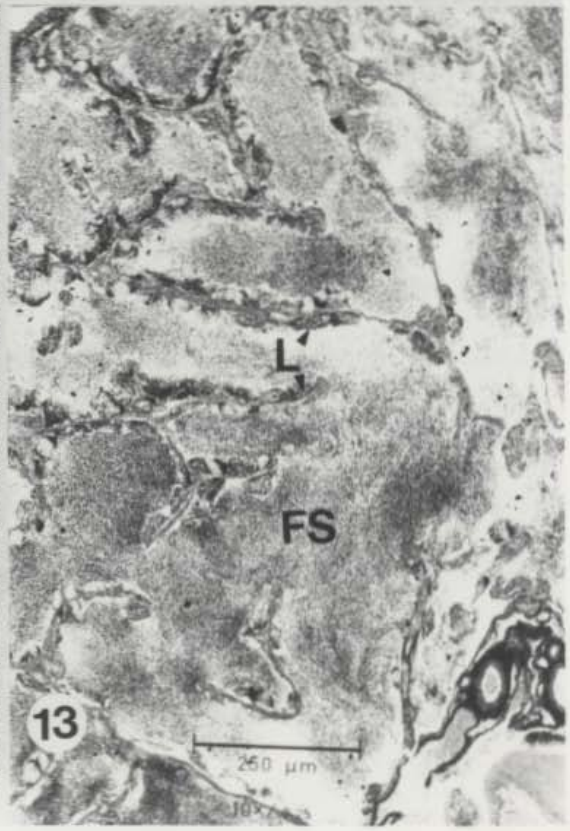
Figure 12: A transverse section through a testis of a maturing *M. villosus* captured May 11, 1995. The dominant cell types are spermatids and sperm and the dark regions represent areas where the cysts of sperm have broken down (S = sperm).

Figure 13: A transverse section through a testis of a mature *M. villosus* July 20, 1995. Advanced lobular break down has resulted in the formation of large pools of free sperm (L = broken lobules; FS = pools of free sperm).


10
100 µm


9
11


S
12

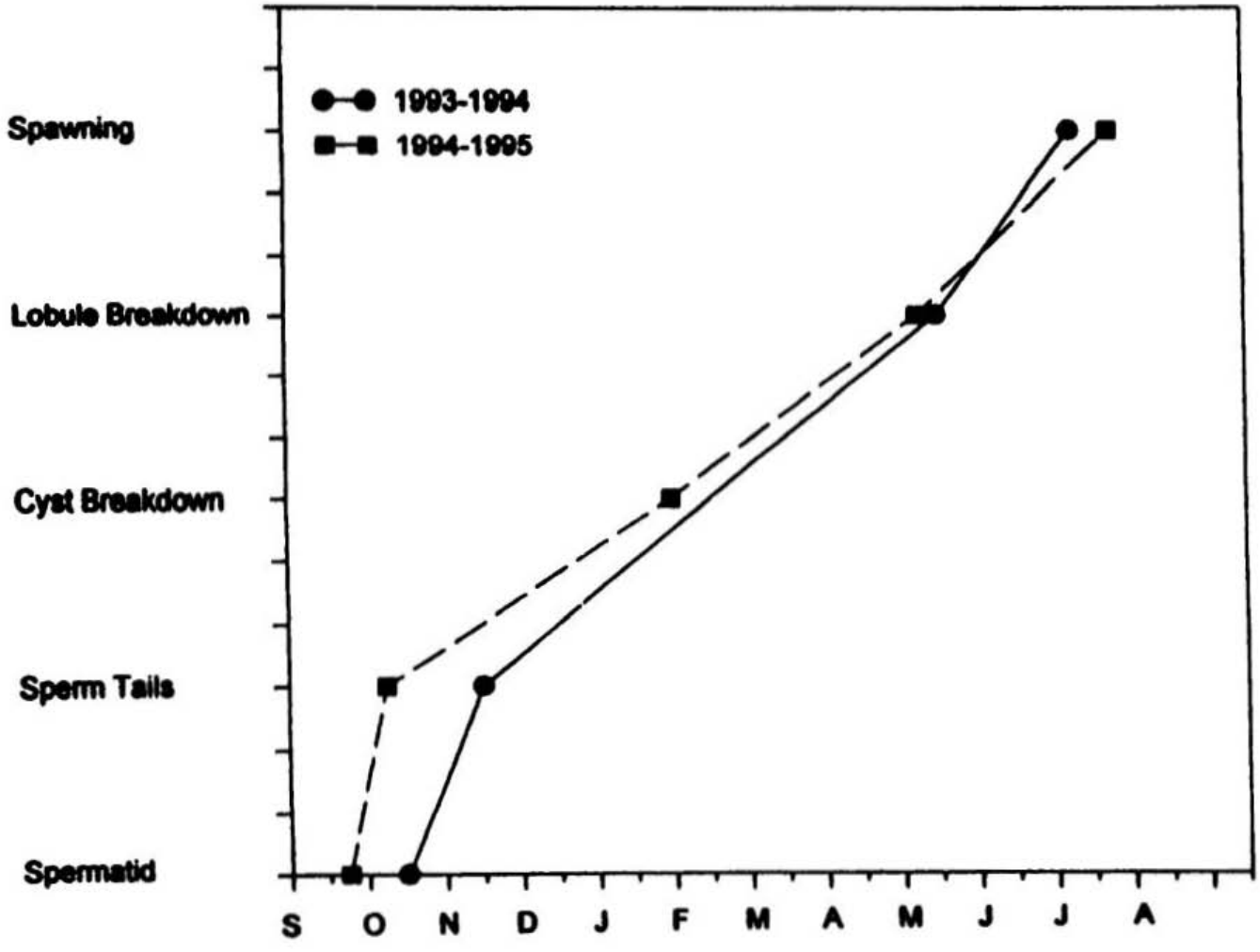

L
FS
13
250 µm

The summer (April-August) of 1994 marked a change in this trend and surface water was warmer during this time than in the previous three years (Colbourne, 1995). No major difference could be seen in the reproductive development of male capelin in the spring or fall sampling periods during 1993 and 1994 (Fig. 14). There are, however, limitations to the sample size and the information gathered during sampling periods. Five to twenty fish are enough to elucidate the timing of the sequence of spermiogenesis but because development varies between individual fish and may be affected by the length, age and environmental conditions to which these fish are exposed, these sample sizes may be inadequate to detect between year differences. Furthermore, the ages of the fish used in this study could not be determined because the otoliths were destroyed during the fixation with Bouin's fixative. It is possible that any of the above factors which affect the level of reproductive development of fish may differ between the different samples of capelin (Fig. 1). It is known that fish spawning in cold years such as 1991-1994 were smaller and spawned later than fish spawning in warm years (Carscadden *et al.*, 1996).

Few valid comparisons could be made between the development of Northwest Atlantic capelin and Icelandic capelin. The Icelandic fish were frozen before fixation in Bouin's fixative. It was hoped that these fish could be compared to frozen samples of local fish from equivalent times during the spawning cycle but the damage caused by freezing was enough to prevent accurate preservation of subtle changes in the gonads as development proceeded. This was particularly true for the sperm which are much smaller than the female oocytes and lack a protective structure analogous to the oocyte membrane. Nonetheless the final stages of gametogenesis in Icelandic fish appears to be

Fig. 14 Timing of Spermiogenesis in M. villosus M.

(Data points indicate the first observation of a new stage.)



the same as in the Newfoundland fish, but according to Forberg (1977), Icelandic fish spawn in March and April. The samples for this study corroborate this.

The Ovary.

Macroscopically the capelin ovary is smooth medially and striated laterally. A transverse section of the whole fish revealed the true nature of these striations and clearly shows the structure of the ovary (Fig 15). In the capelin there is a medial ovarian wall extending ventrally from the dorsal mesentery. Thin-walled lamellae extend laterally and eggs are ovulated directly into the body cavity. Examination of local samples of *Osmerus mordax* and the Holostean, *Amia calva* (distributed throughout North America from the Mississippi to the Great Lakes), revealed the same basic structure (Fig. 16 and 17). Observations of capelin that are ready to spawn reveal clearly that the body cavity is filled with a mass of loose eggs and the ovarian wall is located medially and dorsally behind these eggs which leave the body cavity through the gonopore located just anterior to the anus.

Oogenesis

Oogenesis refers to the developmental process occurring in the ovary that results in the production of the female gamete: the egg (Campbell, 1987). Before development is complete the cells are referred to as the oocytes (Wolfe, 1993).

In capelin there are four groups of oocyte represented within the ovary of mature fish (Fig. 18; Table 4 and 5). The cells within each group develop synchronously but the groups themselves are asynchronous and appear to represent four distinct year classes or stages labeled OC1 -OC4 (Fig. 18; least advanced to most advanced) that are present at

any time during the life of the fish. It is believed that these represent different year classes because at spawning the mature stage 4 cells are released and the stage three cells now appear to be the same as stage 4 cells had been one year previously. The same appears to be true of the other stages (Table 4). A year prior to spawning each immature stage of oocyte will have one year of development remaining before reaching the next stage. While the exact moment of transformation to the next stage is unknown I have arbitrarily placed it at spawning. Once the large yolky stage 4 cells are released examination of the distended ovary reveals that their are still 4 stages of cells remaining. The smallest of these stages is believed to have been recently recruited from oogonia. At no point does there appear to be asynchronous development whereby individual cells of lower stages "catch up" with cells from the next most advanced stage. These stages may be distinguished fundamentally on the bases of size. It is better however to differentiate on the bases of cytological characteristics, as there are variations in size and measurements of cells from one stage may overlap with those from the next. The mean size and cytological character of each stage will change as the reproductive cycle continues but at any point in time four basic stages can be distinguished.

At several times in the development of oocytes (cells of all four stages) the cytoplasm contains Balbiani bodies which occur as basophilic structures arranged in a ring in the cytoplasm surrounding the nucleus (Fig 19). The occurance of this structure at several different stages within cell development has not been previously reported. They are believed to consist of a basophilic yolk nucleus, mitochondria, lipid bodies and Golgi bodies and are involved in the formation of other organelles and inclusions which are

Table 4: The average diameter (Xμm) and standard deviation (S) of the four year classes of oocytes at different times throughout the year. At the spawning event the stage 4 cells are released and each immature stage advances.

Date D/M/Y	Stage 4 X/S	Stage 3 X/S	Stage 2 X/S	Stage 1 X/S
8/10/94	139.6/7.7	73.8/6.6	35.4/5.0	17.7/1.7
2/12/94	184.4/9.0	74.0/6.4	28.9/7.0	12.4/2.4
25/2/95	206.4/11.6	92.6/13.2	53.5/12.7	21.3/3.5
12/3/95	220.8/22.4	88.9/7.6	51.7/7.4	22.3/2.8
11/5/95	359.6/18.2	92.1/12.2	58.3/4.4	32.0/6.4
1/6/95	323.7/52.2	90.4/7.7	53.5/2.5	28.1/5.5
20/7/95	Spawning event	102.0/6.1	52.9/6.1	24.7/2.6
New cycle starts	102.0/6.1	52.9/6.1	24.7/2.6	10.2/4.0

Table 5: The average diameter (Xμm) and standard deviation (S) of the four year classes of oocyte from six fish of different length (mm) captured on June 1, 1995.

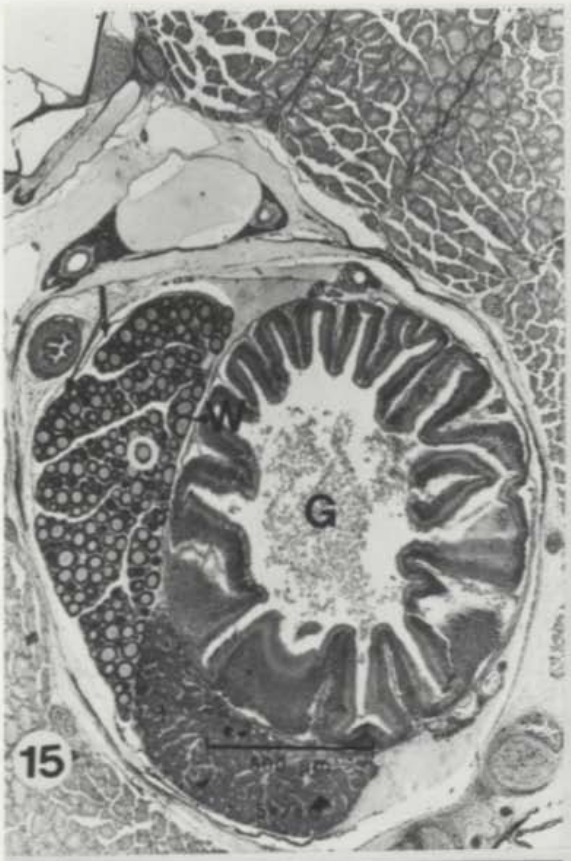
Fish length	Stage 4 X/S	Stage 3 X/S	Stage 2 X/S	Stage 1 X/S
111	240.3/14.7	80.3/14.3	54.8/6.3	31.8/6.5
125	305.6/17.3	94.8/9.4	56.3/10.2	30.3/10.4
129	299.6/7.5	95.0/8.2	54.5/10.5	34.0/4.3
133	355.0/12.8	80.8/10.1	54.5/5.4	22.8/5.1
135	358.0/16.3	93.5/15.5	53.3/8.8	29.8/5.8
147	383.7/28.5	97.8/7.2	49.0/9.1	20.0/6.7

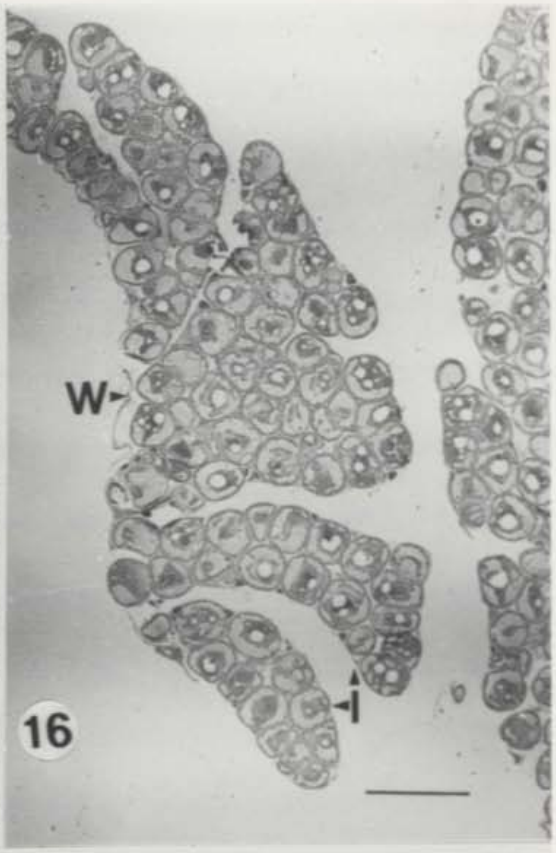
Figure 15: A transverse section through a whole immature female capelin *M. villosus* showing the positioning of the ovary in the body cavity and the lamellae extending laterally from the ovarian wall (G = gut, l = lamellae, O = ovary, W = ovarian wall). Scale bar = 500μm.

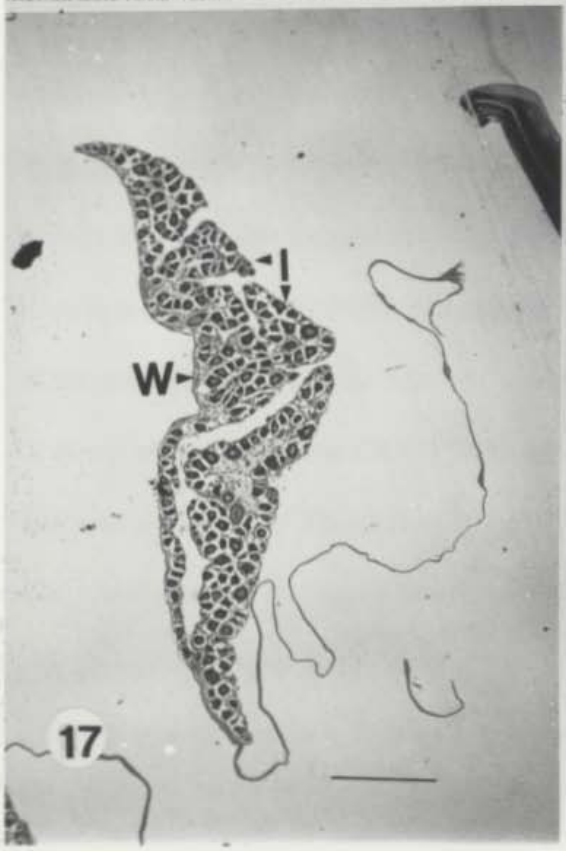
Figure 16: A transverse section through an ovary of the smelt *O. mordax* showing the ovarian wall and laterally extending lamellae (l = lamellae, W = ovarian wall). Scale bar = 1000 μm.

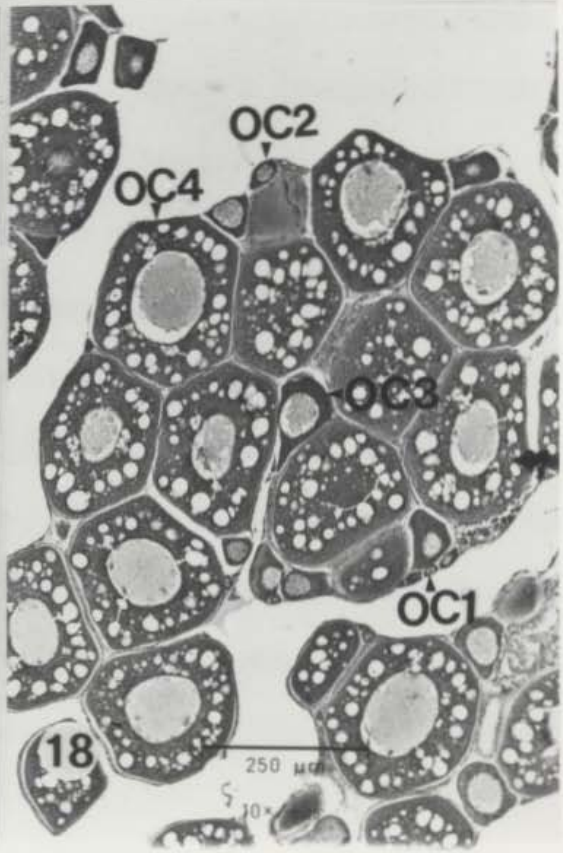
Figure 17: A transverse section through an ovary of the bowfin *A. calva* showing the ovarian wall and laterally extending lamellae (l = lamellae, W = ovarian wall). Scale bar = 1000 μm.

Figure 18: A transverse section through an ovary of *M. villosus* showing four stages of oocytes (OC1 = stage 1, OC2 = stage 2, OC3 = stage 3, OC4 = stage 4). Scale bar = 250 μm.


W
G
15


W
I
16


I
W
17


OC2
OC4
OC3
OC1
18
250 µm

are necessary for the formation of yolk (Guraya, 1986). In capelin these occur in all stages of oocytes which are not maturing for the next spawning season throughout the fall and winter. By March this stage seems to be much less prevalent and persists mainly in the stage 4 oocytes of immature fish and stage 3 of maturing fish where the stage 4 cells have become yolky. Because this time corresponds closely to the end of the winter fast in March (Winters, 1970) it seems likely that they are involved in some sort of synthesis which leads to oocyte growth. Throughout this entire period the cells seem to be changing in size and appearance, and it is difficult to clearly differentiate the immature stages 1 and 2.

In the late summer or early fall of the year before spawning the most advanced stage of oocyte (stage 4) is approximately 140 μm in diameter. At this point in the development of stage 4 the Balbiani bodies, which were the predominant cytoplasmic structure throughout the previous spring and summer, disappear. The cortical alveoli soon appear as clear holes, ie. areas without stain in the cytoplasm (Fig. 20). The volume of cytoplasm increases at this stage so that the diameter of the nucleus comprises roughly half the total diameter of the cell. The stage 3 oocytes are approximately 70 μm in diameter (Table 5) at this time and may have Balbiani bodies at this time. The volume of cytoplasm is much less than in stage 4 with the nucleus comprising approximately 2/3 of the total diameter. The colour is slightly darker and more solid due partly to the lack of cortical alveoli. In stage 2 oocytes the diameter is approximately 35 μm at this time and the nucleus is the most dominant structure of the cell surrounded by a thin dark layer of cytoplasm which has a very solid appearance when it is not interrupted by Balbiani

bodies. Finally stage 1, the most infrequently observed is approximately 20 µm or less. These are light in colour, ranging from white to mauve, when sectioned through the nucleus. The layer of cytoplasm is barely visible and appears as a darkly stained line surrounding the nucleus (Fig. 18). Their appearance is similar to that of oogonia but the oogonia are only about 5-6 µm.

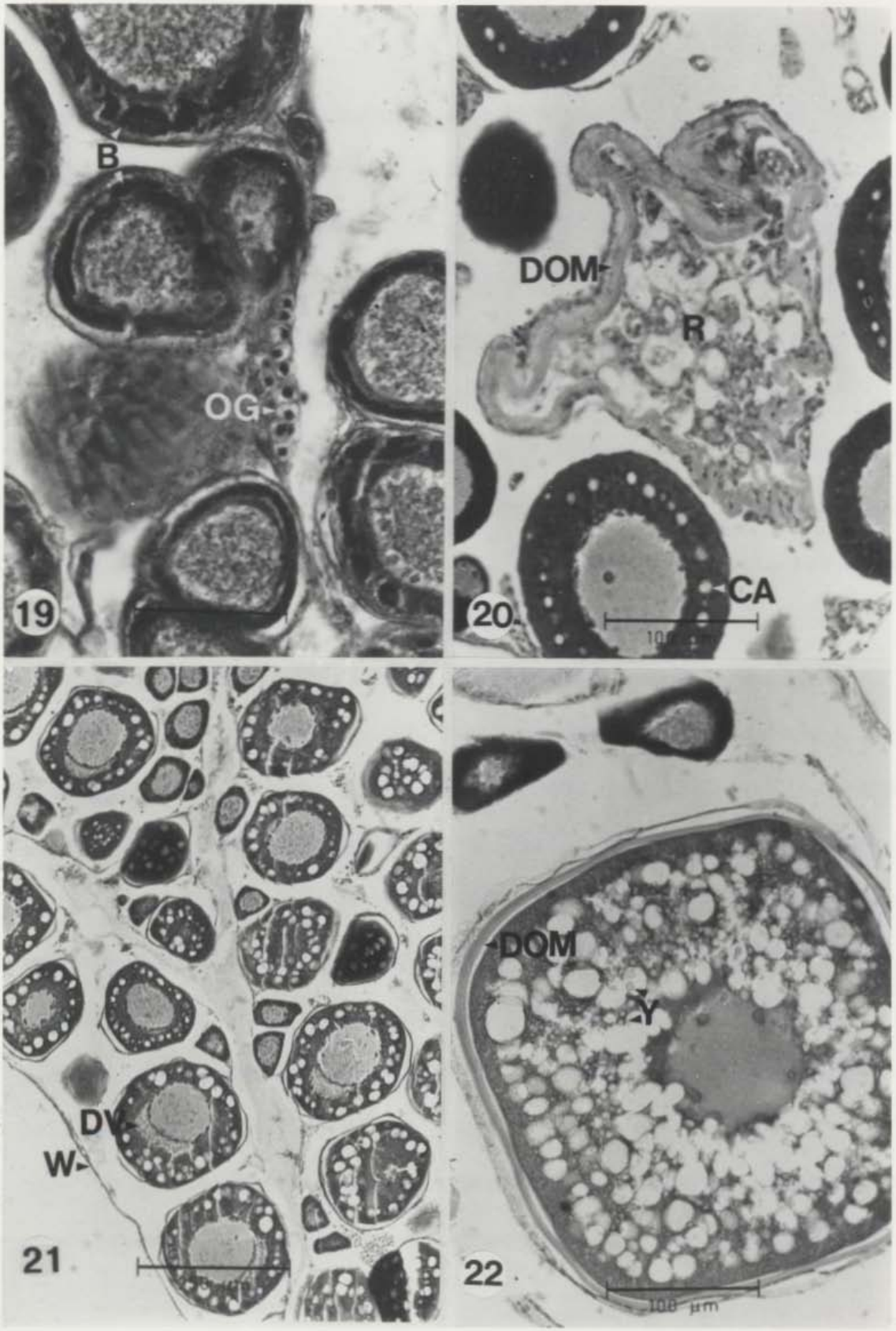
After the appearance of the cortical alveoli in the fall the cytoplasm becomes more vacuolated as yolk production continues. In early December sections clearly reveal a very highly vacuolated region occurring on one side of the nucleus, separating it from the cytoplasm (Fig. 21). These vacuoles are much smaller than the cortical alveoli and more densely packed. They are frequently accompanied by irregularly shaped nucleoli which may be vacuolated or appear to be contained within a vacuole and are arranged peripherally around the nucleus in close association with the nuclear membrane. The appearance of these vacuoles suggests that this is a time of intense activity for synthesis or transfer of material from the nucleus to the cytoplasm. Areas of densely stained material in the nucleus suggests the presence of condensed chromatin which may be lampbrush chromosomes (Fig. 21). According to Guraya (1986), the presence of lampbrush chromosomes indicates a high rate of RNA synthesis. This could explain why most of the oocyte growth occurs between December and spawning. While stage 4 oocytes in the fall are approximately 140 µm the same stage in December is approximately 180 µm, in May/June 300 - 350 µm and at spawning, approximately 500 µm. Fertilized eggs freshly deposited on a beach are approximately 1 mm in diameter, but this latter increase in size is largely due to hydration and changes which occur after

Figure 19: A transverse section through an ovary of *M. villosus* showing oocytes with Balbiani Bodies and a nest of oogonia (B = Balbiani Bodies, OG = oogonium). Scale bar = 50 μm.

Figure 20: A transverse section through an ovary of *M. villosus* recovering from spawning showing a residual egg and a stage 4 oocyte with cortical alveoli (CA = cortical alveolus, DOM = double oocyte membrane, R = resorbing egg). Scale bar = 100 μm.

Figure 21: A transverse section through an ovary of *M. villosus* showing dense vacuoles between the cytoplasm and the nucleus. Within several nuclei there are dark regions which could be lampbrush chromosomes. The ovarian wall is visible (DV = dense vacuoles, W = ovarian wall). Scale bar = 250 μm.

Figure 22: A transverse section through an advanced oocyte of *M. villosus* showing the double oocyte membrane and yolk granules in the cytoplasm (DOM = double oocyte membrane, Y = yolk granules).
Scale bar = 100 μm.


B
OG
19
DOM
R
CA
20
DV
W
21
DOM
Y
22
10.0 µm

fertilization.

The next major change is the development of exogenous yolk (Fig. 22). This was present (perhaps at an advanced stage) in the May samples from 1994 and 1995 and appears as bright, translucent eosinophilic granules. No clear evidence for the presence of exogenous yolk could be seen in fish up to March 12, 1995, the last samples of fish taken before May. Samples were unobtainable in April, 1995, but the beginning of the exogenous yolk formation is suspected to occur during this time.

Also in May the oocyte membrane develops and changes structurally and chemically. In January and February the membrane was thin, approximately 2 μm wide and appeared as a single layer. By May it was approximately 7 μm thick in the thickest area (Fig. 22) and this region was clearly divided into an inner light orange layer that surrounded the cell and lacked striations, and a bright pink, heavily striated outer layer that was present on only half of the cell. In early June the membrane was about 12 μm thick at the thickest area and a micropyle had developed on the opposite side of the cell in the thin single layer. Finally in mid July the double region of the membrane is about 25 μm thick, the entire volume of the cell is filled with yolk and the nucleus, which may have broken down (germinal vesicle breakdown) is not clearly visible (Fig. 23). These last measurements were taken from residual oocytes in the ovary of fish which had spawned. This is the most developed stage that the cells reach before they are hydrated and subsequently spawned.

At spawning the most mature year class of oocytes is released and the next most mature year class continues developing to be spawned during the next year, if the fish

survives. Supporting evidence for this can be found in histological sections taken throughout the year which show representative cells of each potential year class are present at all times even in immature fish (Fig. 15, Fig. 18 - 23).

Only three immature female fish, captured on June 1, 1995, were examined. Their average length was 81 mm (Table 3) and the average diameter of the most mature oocytes was approximately 66 μm (Fig. 15). Immature female fish can be distinguished histologically on the basis of their small darkly staining basophilic oocytes. It is interesting that the most mature oocyte of the immature fish appear to be intermediate in size and structurally the same as stage 3 oocytes from spent fish from July 20, 1995 and fish from October 8, 1994 which were preparing to spawn during the next summer. If the stages represent different year classes of oocytes then the immature female fish found in this study were two years away from spawning.

Female fish of different length (Table 6) can be separated into length categories on the basis of oocyte development only near spawning. It was most evident after the formation of exogenous yolk was advanced and the oocyte membrane had undergone considerable change to become very eosinophilic and divided into two layers.

The smallest maturing female captured on February 25, 1995 was 99 mm long, and its most mature oocytes were on average 195 μm in diameter. This value was not unlike the average oocyte diameter of approximately 206 μm for the sample (Table 4). The largest oocytes at approximately 217 μm were only marginally larger and under microscopic examination all these cells appear to stain in the same manner, have the same level of development of the double oocyte membrane and have the same structures

Table 6: Length frequency distribution for female capelin sampled in the Northwest Atlantic in 1993-1995. The numbers represent the number of fish/mm interval for each sample and the average length (X) and standard deviation (S) for each sample.

Date D/M/Y	Number of Female Fish Per mm Interval									X/S
	<90	100	110	120	130	140	150	160	170 >	
23/6/93							3	2		156.0/7.3
8-16/10/93	1		1		3		1			127.4/21.2
16/11/93						1	3	1		155.0/8.6
16/5/94				1		1		2	1	152.4/17.8
29/5/94				1	1	2		1		141.4/14.0
7/7/94						1	2	1	1	161.4/13.5
29/9/94				3	1	1				132.0/10.1
8/10/94				4	3	1		1		134.5/11.4
2/12/94				1	2	7				141.0/5.9
3/12/94			1	1	4	5	2		1	141.6/12.1
17/12/94				1	3	2	1			138.3/9.2
6/1/95				1	5	4	1			139.0/7.5
3/2/95				1	11	8				138.1/6.2
12/2/95										No Females
22/2/95										No Females
25/2/95	1	2	3	3						114.3/96
12/3/95		1		5	3	2				128.9/9.7
11/5/95					1	3	2	3	1	151.4/12.0
1/6/95	3		1	4	4	1				118.2/23.0
8/6/95			1	2	2	3	2			139.0/13.3
20/7/95					1	2		2		146.6/9.8

in the cytoplasm (n.b. all fish from each sample were batch processed and stained in exactly the same way). The sample taken on March 12, 1995 showed a similar relationship although the smallest fish at 109 mm did have an average stage 4 oocyte diameter of 189 μm; small compared to 221 μm, the average oocyte diameter for the sample, but again in this sample from fish to fish the level of development was not notably different and the oocyte diameter did not consistently increase with fish length.

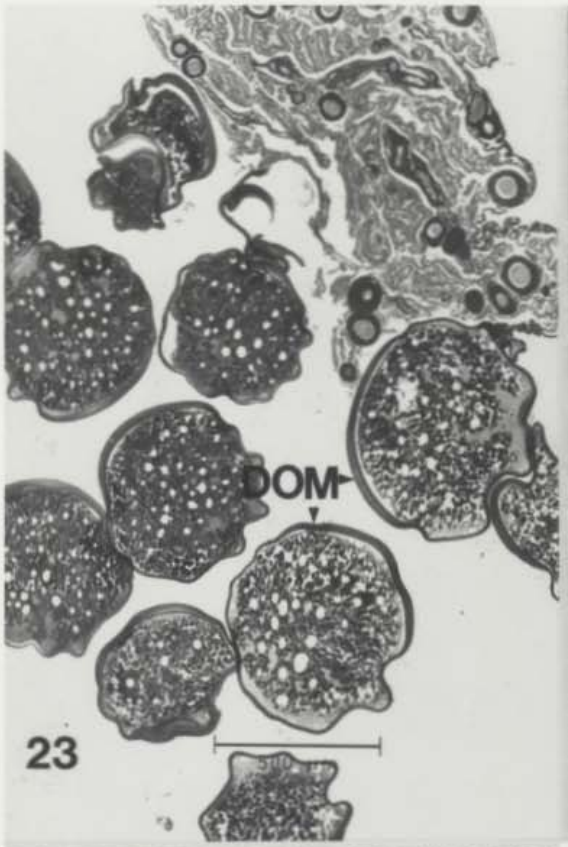
In the sample from June 1, 1995 the smallest maturing fish was 111 mm long and its most mature oocytes were approximately 240 μm; very small compared to the average of approximately 323 for the sample of 6 fish (Table 5). In this sample the small cells of shorter fish (Fig. 24) were also less developed than the those of larger fish (Fig. 25) in that they were less yolky, the double oocyte membrane was thinner and less eosinophilic, and the micropyle had not developed. Generally the average diameter and the level of development of the stage 4 oocytes increased with fish length. This was true only for the stage 4 oocytes. The stage 3 cells showed no apparent trend and the stage 2 and 1 cells appeared to get smaller (Table 5). This latter trend in size could have been the result of recruitment of new cells from the oogonia stage which were expected to be less developed and smaller. As more new cells become noticeable in the ovary their measurements may lead to a decrease in the mean diameter of this stage.

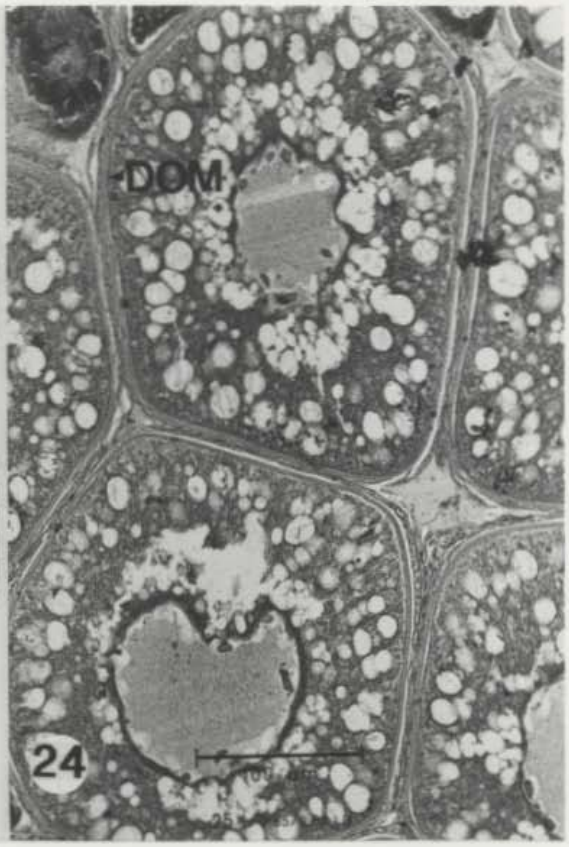
The trend towards increased development of stage 4 oocytes with increased length of the females was not apparent until the late spring or early summer in these samples. This may be significant in terms of differential rates of development, but apparently would affect only the latter stages of development. Historically smaller

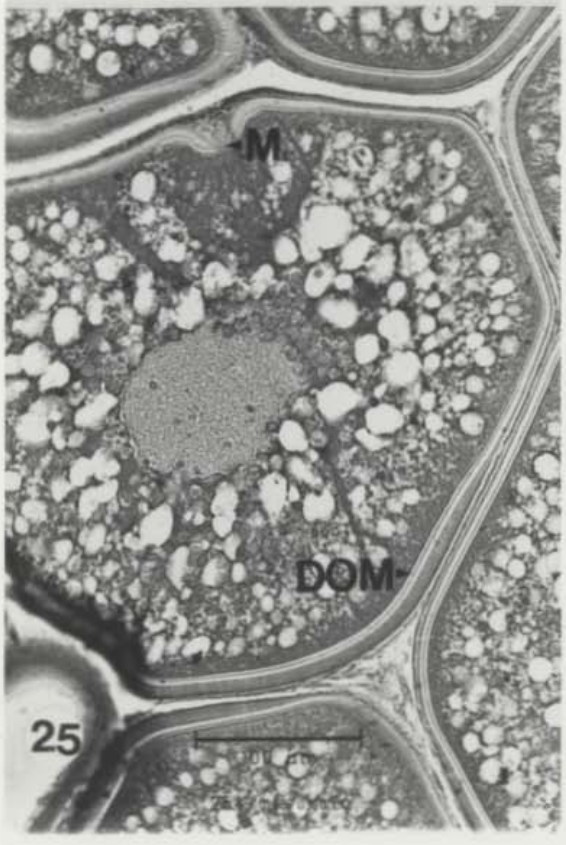
Figure 23: A transverse section through ovulated eggs of *M. villosus* with double membranes next to a section of spent ovary containing immature oocytes (DOM = double oocyte membrane). Scale bar = 500 µm.

Figure 24: A transverse section through an ovary of *M. villosus* (111 mm in length) captured June 1, 1995, showing yolky oocytes with a thin membrane and lacking a micropyle. Scale bar = 100 µm.

Figure 25: A transverse section through an ovary of *M. villosus* (147 mm in length) captured June 1, 1995, showing yolky oocytes with a thick membrane and a micropyle (M = micropyle). Scale bar = 100 µm.


DOM
23


DOM
24


M
DOM
25

capelin, usually two year old fish, spawn several weeks after the main spawning of three year old fish in middle to late June (Templeman, 1948). If size is the limiting factor then the prolonged periods of colder than average temperature experienced in the early 1990s and which were correlated with shorter fish (Carscadden *et al.*, 1996) may also result in a considerable delay of the spawning cycle.

The results of this study are limited by small sample sizes and it is difficult to clearly demonstrate factors which affect the rate of gonad maturation especially since the length of mature female capelin is variable (Tables 3 and 6) and the ages of these fish were not determined. It is difficult to delineate a very clear point in development from which we may predict the timing of the eventual spawning. If the appearance or disappearance of a particular developmental stage can be precisely timed then it may be used as a marker. Arrival at this marker early could indicate early spawning, while late arrival at this marker could indicate late spawning.

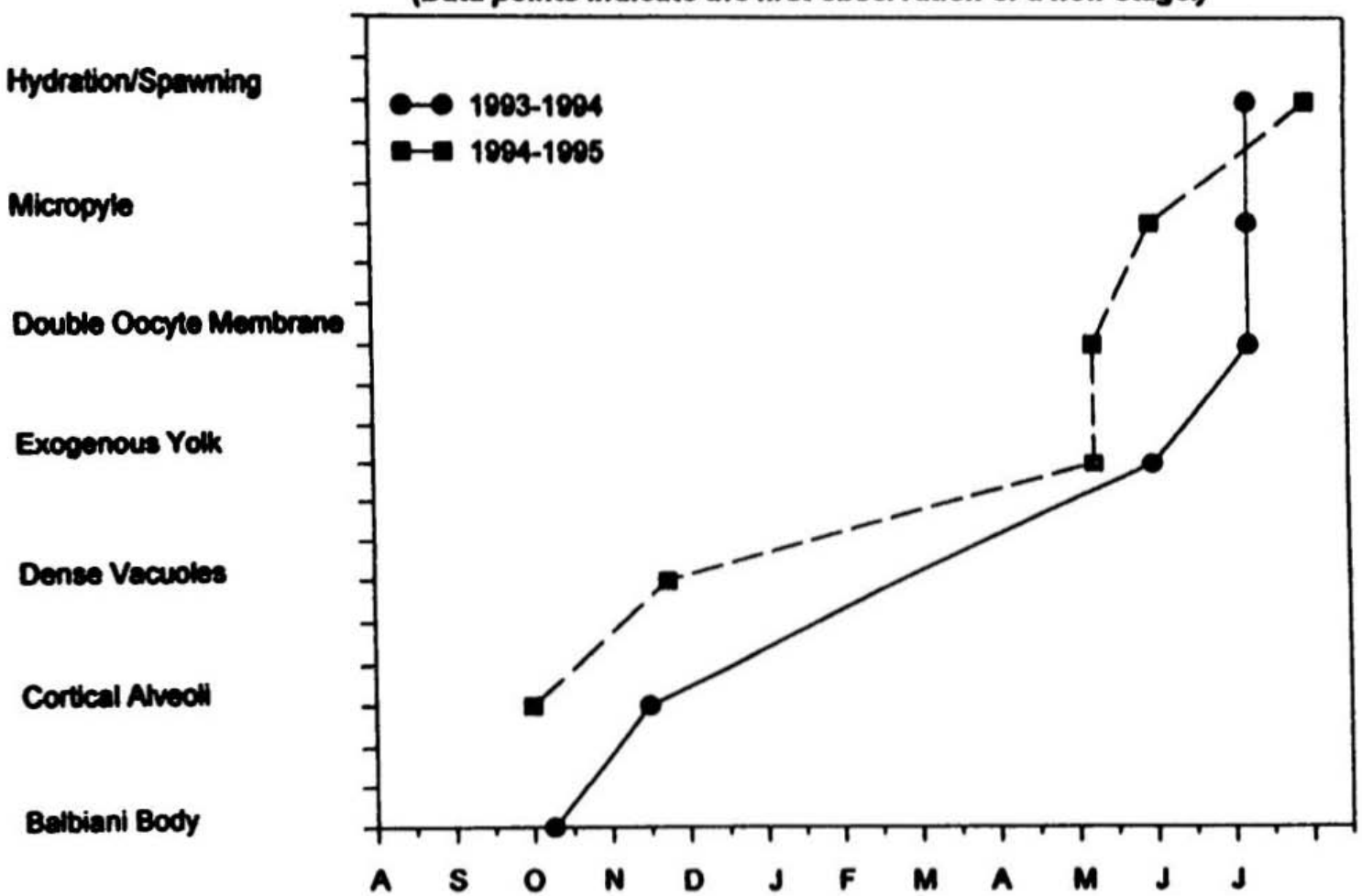
It is important in the development of gametes to recognize a point of commitment to final development. Once the gametes reach this stage their development will proceed to completion. However before this stage is reached factors such as temperature or poor nutrition will have their most pronounced affect on development and in some species, such as flounder, are known to cause a period of stasis (Burton, 1991).

This "commitment stage" is more easily recognized in fish that have long lives and can spawn for many seasons. For these fish a spawning season is often followed by some recovery period. A very healthy fish might spawn again during the next season but it is not uncommon even for fish to spawn biannually.

For the relatively short lived capelin there does not seem to be a recovery period during which time the gamete development is suspended while condition improves. Certainly for the female, the oocytes needed for the next year develop along with those that are developing for the coming spawning season. For the oocytes themselves a possible marker occurs when the Balbiani stage disappears and the cortical alveolar stage begins some time in September or October (Fig. 26). Cortical alveoli appear in the cells as circular areas without stain. The presence of these vacuoles indicates the beginning of endogenous yolk formation and development proceeds continuously till spawning during the next summer. In 1993 the Balbiani body stage persisted in the stage 4 oocytes until the middle of October when it was replaced by the cortical alveoli stage. This same transition had already occurred by the end of September in 1994 (Fig. 26). If we assume that the remainder of the cycle proceeds at approximately the same rate this estimate places the 1994 fish two weeks ahead of the 1993 fish at the same time in the Fall. Spawning may occur in isolated regions throughout the summer but usually one massive spawning period can be identified. In 1994 that period was July 10-16, as opposed to 1995 when it was July 21-27 (Nakashima and Winters, pers. Comm.). This corresponds to the findings of this study (Fig. 26) and contradicts the prediction that entering the Balbiani body stage early in the fall is reflected in earlier spawning the subsequent year. However, different stocks (Fig.1) will be exposed to different environmental conditions at least for the latter part of their spawning migration and possibly throughout the year if stocks remain separate offshore. Increased sampling during the fall from several areas that cover the full extent of the capelin's range may differentiate the off-shore stock

Fig. 26 Timing of oogenesis in M. villosus M.

(Data points indicate the first observation of a new stage.)



structure on the basis the degree of gonad development. However if temperature is a limiting factor, gonad development will be most rapid outside the influence of the Labrador current.

Post Spawning Survival

Laboratory observations on fish captured at Middle Cove in 1993 and maintained in captivity revealed a difference in the rate of post-spawning survival for male and female capelin. All the male fish maintained a schooling pattern in the tank and often collided with the tank wall. Within only a matter of weeks all the male fish had died and many had damaged snouts from hitting the tank.

Female fish did not maintain a schooling pattern and did not collide with the wall of the tank. Ten of these survived and were killed and examined in December (5 fish), May (3 fish) and June (2 fish). The development of the next stage of oocytes proceeded as normal but appeared to be slower than that of wild females (Figs. 27-30; the oocytes were smaller and slow to complete developmental stages). In this case it is likely that reduced food availability and the difference between natural and laboratory conditions (eg. temp, light and no migration) was responsible for the slow rate of development. There was no difference between the developmental rate of identifiable post-spawners captured in the wild and other fish from the same samples. Examination of captive fish also revealed residual eggs which could be seen macroscopically in December, and a thickening of the ovarian wall.

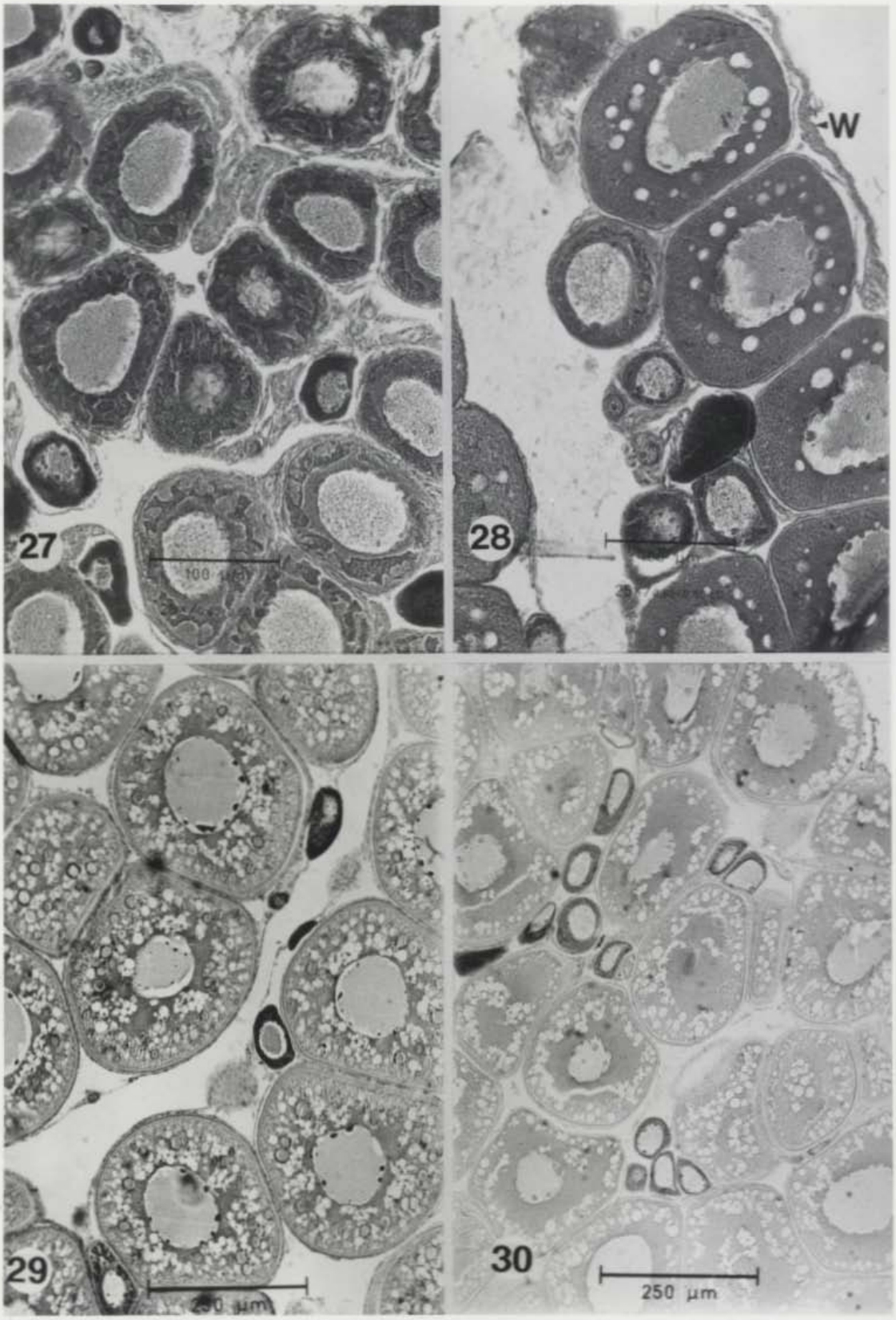
At first it was suspected that the difference in behaviour during captivity was due to the stage in the spawning cycle that the fish had reached at the time of capture. It is

Figure 27: A transverse section through an ovary of a recovering *M. villosus* maintained in an aquarium at the Ocean Sciences Centre and killed December 1, 1993. All the maturing oocytes are at the Balbiani Body stage.

Figure 28: A transverse section through an ovary of a recovering *M. villosus* maintained in an aquarium at the Ocean Sciences Centre and killed December 1, 1993 showing the more advanced cortical alveoli stage and a thick ovarian wall (W = ovarian wall).

Figure 29: A transverse section through an ovary of a recovering *M. villosus* maintained in an aquarium at the Ocean Sciences Centre and killed April 28, 1994 showing advanced yolk development.

Figure 30:A transverse section through an ovary of a recovering *M. villosus* maintained in an aquarium at the Ocean Sciences Centre and killed June 16, 1994 showing oocytes of a more advanced state than the April 28, 1994 fish.


27
100 µm
28
W
29
250 µm
30
250 µm

known that female fish make only one run up the beach to spawn, whereas males make several and patrol the beach in shallow water waiting for groups of females to move up the beach (Templeman, 1948). The 1993 sample of male fish had been collected very early in the spawning season at Middle Cove and were not spent. They maintained a fast moving school and often collided with the tank wall. The females were selected by hand after they had spawned and displayed no schooling behaviour and swam very slowly.

In the summer of 1995 the experiment was repeated using only male fish that were taken from much later in the spawning period at Middle Cove and which were more likely to be spent. These fish did not display schooling behaviour during captivity and did not collide with the tank walls, but nonetheless all died within only a few weeks of capture.

After spawning most capelin die, especially male fish, which by virtue of their behaviour (ie. multiple spawning runs) expose themselves to greater risk of predation and damage due to contact with the substrate (Templeman, 1948; Jangaard, 1974). The captive males from either year (free from predation) did not appear to be damaged upon capture. The captive males from 1995 did not display schooling tendencies and did not damage themselves on the tank walls and yet none of the males survived for more than several weeks. These results support the previous observations that males have high spawning mortality (Templeman, 1948; Shackell *et al.*, 1994) and most die after spawning. Both laboratory experiments and samples of wild fish suggest that some females survive and may contribute to the next years spawning. These results indicate that more females survive than males.

Miglovsky (1968), and Forberg (1977) revealed residual eggs in post-spawned female fish although they did not recognize the true nature of these structures and assumed that the presence of these large cells may have been due to the advanced premature development of few individual cells. My study has followed the sequence of the types of oocytes which develop and the various stages they must go through before development is complete. Most of the thickening of the double oocyte membrane occurs only in the last months of development from May to June/July. Also known is that each year class or group of oocytes is developing synchronously. There is no evidence to suggest that oocytes of any year class undertake independent development. Moreover close examination reveals that the structure of the double oocyte membrane (with the radiated external layer and the smooth internal layer; Fig. 20) matches that of the residual eggs found in the ovary or body cavity of some female fish ≥ 129 mm throughout the year (Table 3).

There is another feature of spent ovaries which has potential use in identifying recovering female fish. During the development of the ovary the dramatic increase in size of the organ stretches the ovarian wall. Upon ovulation the stretched tissue either attempts to regenerate damaged areas or simply folds up onto itself. This results in a thickening of the material. The normal thickness of the ovary wall is approximately 10 µm (Fig. 21). In female fish captured at Middle Cove the ovarian wall has a high proportion of thickened regions measuring approximately 20 µm (Fig. 28). This is twice the normal thickness. Another obvious difference is the very loose arrangement of the developing oocytes, which had been stretched some distance apart by the mature oocytes

that developed and were ovulated in the last season.

A final possibility referred to by Winters, (1971) is that the spawning activity shows up as a check or interruption of the normal ring pattern on the otoliths. In this case a problem is that any check on the otoliths may be obscured by the natural variation of the ring patterns and so the judgement as to whether or not a particular mark is a spawning check is subjective.

All the previous markers for recovering spawners (eg. thick walls, residual eggs etc.) are also limited in their use in that after some time the development of successive generations of oocytes and the natural resorption of residual eggs will erase all evidence of previous spawning. Because residual egg membranes are very durable their presence in developing ovaries persists until well into the next year (as late as May 11, 1995). Examination of the ovaries of fish greater than 125 mm captured during the regularly scheduled fall capelin survey will accurately reveal the percentage of repeat spawning fish in the spawning population. If the fish are frozen only the residual egg membranes will be preserved and other more subtle histological changes will be destroyed. Macroscopic examination of the body cavity and ovary combined with stereoscope examination and light microscopy of histological sections will reduce the possibility of not observing residual eggs which are present to an acceptable level.

Discussion

Structure of the Ovary

The shape of the capelin ovary is unlike that found in many other local species of fish. Commonly ovaries are cylindrical, consisting of an outer thick ovarian wall with multiple lamellae pointing centrally towards a duct, as is the case for cod (Morrison, 1990) or flounder (Burton and Idler, 1987). Histological examination of salmonids reveals a similar structure but there is no central oviduct and the outer wall, through which the eggs are ovulated into the body cavity, is very thin (Burton, pers. comm.). The capelin appears to have characteristics of both these ovarian types. Like the cod there is a distinct ovarian membrane although it is much thinner in the capelin and is only on the medial side of the ovary: as if the cod ovary type had been cut longitudinally and opened with the wall on one side and the lamellae protruding laterally. Like the salmon ovary there is no oviduct and the mature eggs are released into the body cavity.

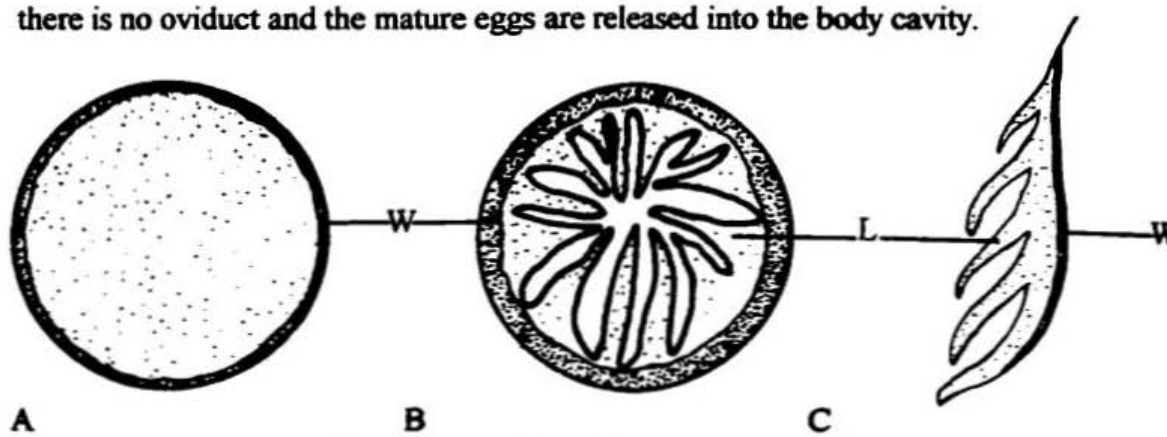


Figure 31: A diagrammatic representation of transverse sections through the ovaries of A. *Salmo salar*, B. *G. morhua* and C. *M. villosus* showing the arrangement of lamellae. Note that the ovary of *S. salar* does not have clear lamellae or an oviduct while the ovary of *G. morhua* has lamellae pointing centrally to an oviduct and the ovary of *M. villosus* has laterally extending lamellae without an oviduct. W = ovarian wall, L = lamellae.

The physical structure of fish ovaries has received little attention in the literature but Brock (1878), in reference to a description of the ovary of *Cobitis fossilis* by Hyrtl (1850) alludes to a type of lamellate ovary as found in *Osmerus eperlans*. Unfortunately, the reference is not clear and lacks mention of *M. villosus*. Huxley (1883), in reference to *Osmerus eperlans*, states that right and left peritoneal folds open into the body cavity via ostia and, comparable to fallopian tubes, lead eggs out of the body. These are homologous to the oviducts of ganoids (eg. *Acipenser*, *Polyodon*, *Polypterus*, and *Amia*, but excluding *Lepidosteus* which has an advanced ovary that is closed or sac like) and some other salmonidae (Huxley, 1883). Goodrich (1958) commented that *Mallotus*, *Coregonus*, and *Argentina* show stages of oviduct development which is intermediate between the well developed ducts of *Osmerus* and *Amia* and the short funnel type found behind the ovaries in *Salmo*. I have seen no evidence that this is true for *Mallotus* which appears to lack any form of duct leading to the gonopore.

The evolutionary significance of the occurrence of this ovary structure is not understood. Smelt and capelin are in the family Osmeridae, order Osmeriformes (Nelson, 1994). How the primitive *Amia*, attained the same structure is a mystery. Harder (1975) suggested that similarities in ovarian structure are likely due to environmental factors or spawning behaviour than phylogenetic relationship. A phylogenetic relationship in this case is difficult to support, however it is equally difficult to imagine similarities in the habitat and behaviour of the freshwater *Amia*, the anadromous smelt and the marine capelin. Huxley (1883), believed that the trend

towards the reduction of oviducts began in *Osmerus* and was completed in *Salmo*; he concluded that the ovarian structure could not be used as a taxonomic character to distinguish the ganoids from the teleosts. Goodrich (1958), concluded that the closed ovarian structure with its complete sac like wall and oviduct was normal for most teleostei. The occurrence of the free ovary (as in capelin) in unrelated teleost families suggests that it is secondarily evolved and marks a return to the primitive structure from the advanced closed structure found in the more primitive *Lepidosteus* (Goodrich, 1958).

Layers of the Oocyte Membrane

Another peculiarity in the capelin is the layering of tissues surrounding the developing oocyte. Migalovsky (1968) and Forberg (1977) described this structure, but there is considerable variation in the terminology used in the literature to describe the follicular layers and the chorion surrounding the ooplasm. The typical arrangement of layers surrounding a developing oocyte includes a chorion or oocyte membrane immediately surrounding the ooplasm and separating it from two follicular layers; the theca interna (granulosa) and the theca externa. Forberg (1977) describes four layers surrounding the developing oocytes of capelin; a theca externa, a granulosa (together forming the follicular layer), and what he refers to as a double cortex radiata which I presume is a double chorion. Davenport *et al.* (1986) has also referred to a double chorion in the eggs of capelin and used scanning electron micrographs to show a clear double layered region. He used Fridgeirsson (1976) as a supporting reference for the presence of a double layered chorion but Fridgeirsson (1976) does not specifically refer

to a double chorion, simply two outer membranes.

The chorion is analogous to an egg shell and forms as an acellular layer between the surface of the oocyte and the follicular epithelium and is commonly referred to as the vitelline envelope (Hyllner and Haux, 1995), vitelline membrane, zona pellucida, zona radiata or cortex radiata (Guraya, 1986). In this thesis it is referred to as the double oocyte membrane and described in the results as consisting of a smooth internal layer and a radiated external layer which becomes very thick at one pole near spawning. External to this there appears to be a very thin follicular layer which is not readily divisible into two layers under light microscopy. This description accounts for only three of Forberg's (1977) layers. Close examination of the layers immediately surrounding the ooplasm reveals that inside the radiated layer there may be two layers rather than just one (Fig. 25). Forberg (1977) described both layers of the cortex radiata as being radiated but I have seen no evidence (in the material for this study) to suggest that it is. These layers also remain thin throughout development and the external layer does not meet Forberg's description of the cortex radiata externa (eg. "Much thickened at ca. 5-6 μm"). It is possible that the inside layer is artifactual and may be a space which formed between the chorion and the ooplasm as a result of shrinkage after processing. This leaves only three layers surrounding the developing oocyte, and if the thick radiated layer is the external chorion (which fits Forberg's description) then the follicle has only one visible layer.

Fridgeirsson (1976) also noted the double oocyte membrane and refered to the outter layer as being made of an adhesive material which is " very thin except on the

vegetative pole. There it forms a thick layer that covers about 1/3 of the inner membrane." This arrangement could be advantageous in that the asymmetry of the layer may cause the eggs to be heavier at the vegetal pole so they settle on the substrate with micropyle in the animal pole exposed for penetration by sperm. My study revealed a similar arrangement in the smelt *O. mordax* where again there are two major layers (a smooth internal layer and a radiated external layer) inside the follicular layer. The deposited eggs attach to the substrate by way of a stemmed external layer (Scott and Scott, 1988). Without further work at higher magnification the arrangement of layers around the capelin oocyte cannot be ascertained.

Identification of Recovering Females.

Whatever the arrangement of the membranes surrounding the capelin eggs, together they form a very resilient structure (Davenport *et al.* 1986) which, according to my observations, takes months to be resorbed by post-spawned fish. Histologically the structure is unmistakable, and is the single most important feature of the recovering ovary in terms of identifying fish which have previously spawned. More reliable estimates of the percentage of repeat spawning females will permit more accurate estimates of the spawning biomass. In depth study of gametogenesis will give additional information on the reproductive biology of capelin, a species that plays a vital role in the future of our fisheries.

Migalovsky's (1968) work on gametogenesis of female capelin contained brief descriptions of characteristics of capelin oocytes which occur throughout their

development. He attempted to describe maturation stages of female capelin, although the characteristics of these stages were unclear. He concluded that capelin spawn only once but also that histological studies indicate they can survive spawning and probably spawn for the second time. It is likely that a translational error is responsible for the apparent conflict between these two conclusions. Perhaps the intended meaning was that capelin usually spawn only once but they are physiologically capable of spawning more than once. Finally he concluded that spawning grounds can be identified on the basis of the maturity stage of the gonads of fish captured in these areas.

Forberg's (1977) work was much more detailed and included a 6 stage maturation scale for male capelin and a 14 stage maturation scale for females based on histological examination. This allowed him to determine the duration of maturation stages and the growth curve for the oocytes for female fish in 1974. He developed a 10 stage maturation scale based on descriptions of whole oocytes viewed with transmitted light and a 12 stage scale based on oocyte diameter. These he believed might be more useful in the field, than his previous 14 stage scale based on complicated and time consuming histological procedures. It may also be more accurate than the "herring scale" which, he proved, gave an underestimate for the population of maturing female capelin.

Forberg (1977) further examined maturation in relation to coefficients of maturation and condition factor of the fish and tried to distinguish between winter and summer spawning stocks. Although recovering male spawners could not be identified he did identify recovering female spawners. He also examined variation in the diameter of

oocytes from different parts of the ovary, between years, from fish of different length, and between the various oocyte stages.

Predicting Spawning Time

Both these accounts offer much useful information for researchers who are interested in the minute details of gametogenesis. However, the attempt to develop maturation scales that are dependent on the microscopic analysis seems impractical. For fisheries science it is important to know the condition and size of the spawning biomass and to accurately predict when spawning will occur. This type of information should be based on intense study of the reproductive biology of the species which must include a histological account of the gamete development. However once this framework has been outlined, it may be possible to accurately predict spawning times on the basis of climate, food availability, length and age of fish or other factors which might influence the reproductive cycle. Knowledge of the reproductive cycle allows interpretation of events leading up to spawning.

The process of gametogenesis may be gradual, starting early in the development of the fish and continuing throughout its life time, so it is possible that it will change in response to prolonged changes in the environment. The longer the process the less likely it is to be dramatically affected by acute changes in the environment but for capelin, which usually spawn at age 3 years, even short anomalous periods might alter the timing of the sequence of gametogenesis. Similarly, because the capelin is a short lived species the effects of these events on reproductive potential should also be acute, lasting for only

few spawning year classes of fish.

The prolonged period of cold water temperatures in the early 1990s was accompanied by dramatic changes in spawning behaviour (Carscadden *et al.*, 1996). The direct cause for these anomalies is unknown and without a more complete database no histological explanation can be offered. This study attempted to document the histological changes which occurred from 1993 to 1995 and elucidates the most rudimentary sequence of steps in capelin gametogenesis. It cannot predict the elasticity of the cycle, although one basic trend is that longer fish complete development before shorter fish and this may reflect different stock structure. Longer fish may have greater accumulated energy reserves, which may in turn relate to the conditions they were subjected to during their migration. Fish from warmer water may have had access to greater food supplies or might simply have been able to assimilate more food energy and convert it to body mass. It may also be a function of age. Data collected by the Department of Fisheries and Oceans indicates that longer capelin are older in which case they would have had longer to accumulate food reserves and may be more experienced at catching food. Also if their physical growth slows as they get larger then they may be better able to shunt food energy reserves into growth and development of gonads.

Comparison with Forberg's Stages

Like Forberg my study found no correlation between the diameter of oocytes and the part of the ovary from which they were taken, indicating that all cells develop at the same rate. Similarly no major difference was observed between different years of

development from 1993-94 season to 1994-95.

Unlike Forberg I made no attempt in the current study to devise elaborate histological maturation scales. As Forberg (1983) noted, any maturation scale we derive to explain this process is artificial and subject to interpretation by different workers. The first three of Forberg's stages seem to correspond to the first three potential year classes of oocytes described in this study. All three are previtellogenic (often referred to in the literature as the slow growth phase) which means that they have not yet started yolk development and will not be spawned during the next spawning season. None of these stages appear to be static in that all undergo histological changes throughout the year as evidenced by the appearance of Balbiani bodies and an apparently continuous change in size.

The remaining potential year class identified in this study, stage 4, accounts for the final 11 stages of Forberg's histological maturation scale. These 11 stages provide an accurate account of the cytological changes that occur throughout the final year of development (the vitellogenic stage or period of fast growth) before the oocytes are spawned. This is the time during which the most dramatic changes in the growth of the cells occurs. Forberg (1977) had so many stages because he designated a new stage each time cells reached the next phase of development. His stage 14 cells (vitellogenesis VXI) at spawning however were the same year class of cells which he described as stage 4 (vitellogenesis I) a year previously and which I refer to as stage 4 throughout the developmental process.

The beginning of the stage is marked by the appearance of a ring of cortical alveoli in the cytoplasm and encompassing the nucleus of the cell. According to Khoo (1978) these are yolk vesicles and are composed of mucopolysaccharides which form under the regulation of estrogens. This kind of yolk is commonly referred to as endogenous yolk and is believed to form from materials already in the oocyte. The second type of yolk recognized by Khoo (1978) are the yolk granules composed of proteins, phospholipids and neutral lipids and formed under the regulation of pregnenolone. This is referred to by Wallace and Selman (1981) as true vitellogenesis and is often called exogenous yolk in the literature. It is referred to as exogenous because the yolk protein which develops during this stage is derived from vitellogenin which is formed in the liver and released into the blood stream to be sequestered into the oocytes of the ovary (Wallace and Sellman, 1981).

All of my stages can be distinguished on the basis of size and clearly different cytological character at most times throughout the year, while distinguishing Forberg's (1977) stages requires close attention to more subtle cytological changes and less dramatic changes in cell size.

Post Spawning Survival

The occurrence of multiple year classes of oocytes is physiological proof that capelin have the potential to undergo multiple spawnings. The survival of the spawned females captured at Middle Cove in 1993 throughout the winter until the summer of 1994 suggests that a certain percentage of the females survive and spawn again the following

season. Evidence for repeat spawning in the Northwest Atlantic was suggested by Winters (1971) who examined the otoliths of fish for the presence of spawning checks. Shackel *et al.*, (1994), estimated survival rates by linear least squares models using catch record data from acoustic/trawl research surveys conducted from 1982-1990. Such estimates of the percentage of repeat spawning females are inferred and can not be related to direct evidence of previous spawning. Nakashima (1992) recovered tagged mature fish one year after release but the likelihood of recovering such tags is remote.

The examination of female capelin for the presence of residual eggs during the Fall after spawning is a method that allows direct assessment of the number of females that have previously spawned. While gross examination for residual eggs may underestimate the number of recovering females, microscopic examination is less likely to overlook residual eggs and may also rely upon other characteristics of recovering ovaries such as the arrangement of the developing oocytes and the condition of the ovarian wall. Presumably, if the fish have survived and show evidence of recovering (e.g. development of oocytes for the next spawning season) they have a good chance of surviving until the next spawning period. Thus the estimate of the number of recovering females in the Fall can be used as a more precise estimate of the number of repeat spawners during the spawning season than the indirect methods currently used.

Conclusions

The testes of capelin are lobular. During development spermatogonia divide to form cysts of spermatocytes within larger divisions called lobules. Individual cysts develop asynchronously within lobules so cells of many different stages (eg. Spermatocyte, spermatid, sperm) occur simultaneously until development is complete. Lobular breakdown occurs gradually so all sperm is not released at once.

The wall of the capelin ovary is restricted to the medial aspect of the organ and lamellae extend from this laterally into the body cavity. Eggs are ovulated into the body cavity and the ovary retracts, appearing as a baggy sac behind the egg mass. Eggs are spawned through the gonopore just posterior to the anus.

The rate of gametogenesis is positively correlated with the length of the fish. For males this relationship is valid throughout the year of development before spawning from October until just before spawning when all cells are fully developed. For females the relationship was first observed in June when maturing oocytes of longer fish become more yolky and have a thicker more eosinophilic double oocyte membrane than shorter fish.

Male capelin could not be maintained in aquaria for more than several weeks after spawning. Males at spawning show clear lobules which have thick eosinophilic walls. Recovering males should show large lobular spaces containing only residual amounts of sperm, and developing spermatocytes. No recovering males were found in the samples for this thesis suggesting that their occurrence in the wild population is very infrequent.

Female capelin are physiologically capable of repeat spawning as evidenced by the following:

- There are four potential year classes (stages 1-4) of oocytes. The most advanced (stage 4) enter into endogenous yolk development during the Fall and mature for the following spawning. Stages 1-3 develop slowly over the year and gradually enter into the next most advanced stage at the time of spawning. Oogonia advance to form the next years stage 1 cells.

- Post-spawned female fish were maintained in aquaria over-winter and showed clear signs of development for the next spawning season.

- Recovering females can be identified in the wild by the presence of residual eggs, loosely arranged developing oocytes and thickening of the ovarian wall.

There is no resting stage of oocyte. All cells change in size or cytological character (ie. the appearance of Balbiani bodies or the number and distribution of nucleoli). All stages can be differentiated on the basis of size and cytological character at all times during the year.

Oocytes have a double external layer.

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Appendix 1

Fixation, Staining, Embedding, and Sectioning Procedures

Fixation: All tissues were fixed for a minimum of 24-48 hrs in Bouin's fluid (Humason,1967):

Picric acid, Saturated aqueous	75.0 ml
Formalin, concentrated	25.0 ml
Glacial acetic acid	5.0 ml

Preservation: Samples were stored in 70 % ethanol until the time of embedding.

Processing: Ethanol dehydration of:

small or non yolky samples:		large or yolky samples:
50%	1 hr	two washes 50% 1 hr each
70%	1 hr	70%
95%	1 hr	95%
100%	1 hr	100%
100%	1 hr	100%
Xylene	1 hr	Xylene
		3 Xylene: 1 Wax; 1 hr @ 60° C
		2 Xylene: 2 Wax; 1 hr @ 60°C
		1 Xylene: 3 Wax; 1 hr @ 60° C
		or over night
Wax 1	1 hr	1hr
Wax 2	1 hr	1 hr
Embed		

Sectioning: Sample blocks were trimmed using a razor blade then mounted on an American Optical microtome and sectioned till a full transverse section of the tissue sample was exposed. If serial sections of 7 µm thickness could be obtained in this manner no further processing was needed and sections could be mounted on albumin coated slides. The lengthy dehydration and infiltration procedure often left samples hard and prone to static build up (static is also common on an inadequately greased microtome). To avoid this the trimmed blocks were soaked in a mixture of nine parts 60% ethanol and one part glycerine (Baker,1950: *fide* Bowers and Holliday), for several hours or left over night. This allows some moisture back into the tissue and facilitates serial sectioning. It should be noted that the tissue immediately in contact with the glycerine-alcohol will swell and become puckered. An excess of sections were made and the first were discarded.

Staining:

Xylene 1	5 min
Xylene 2	"
100 % Ethanol	"
95 %	"
70 %	"
50 %	"
Distilled water	"
Ehrlich's Haematoxylin	3-5 min
Tap water	rinse
Scotts blueing solution	3-5 min
50 % Ethanol	5 min
70 %	5 min
95 %	5 min
Eosin Y (1% in 95% Ethanol)	30 sec
100 % Ethanol	5 min
100 %	"
Xylene	"
Xylene	"