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Assessment of gonad staging systems and other methods used in the study of the reproductive biology of narrow-barred Spanish mackerel, *Scomberomorus commerson*, in Western Australia

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Fish for the future

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Assessment of gonad staging systems and other methods used in the study of the reproductive biology of narrow-barred Spanish mackerel, *Scomberomorus commerson,* in Western Australia.

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Abstract

Recent research by the Department of Fisheries in Western Australia into the reproductive biology of narrow-barred Spanish mackerel has required the use of various field and laboratory techniques. This report documents the procedures and adaptations of some of these techniques. Assessment and development of illustrated, relevant macroscopic and microscopic staging systems were of particular focus because both have been essential components of the research. The simplified and more reliable macroscopic staging system we developed allowed full use of all gonad samples and a more complete (albeit less distinct) overview of the seasonal pattern of gonad reproductive development. This macroscopic staging system will be useful for ongoing, low budget monitoring of Spanish mackerel stocks in Western Australia. More detailed and accurate (but less complete) data were provided by the microscopic staging system, allowing specific information about spawning and other reproductive characteristics of this species. Methods used in the collection and processing of samples and in the estimation of batch fecundity of females are also documented along with conversion factors between weights of fresh, frozen and formalin-fixed gonads.

1.0 Introduction

The narrow-barred Spanish mackerel (S*comberomorus commerson*) is a pelagic, top level predator found throughout tropical marine waters of the Indo-West Pacific. Juveniles inhabit shallow inshore areas whereas adults are found in coastal waters out to the continental shelf, and may reach 240 cm fork length, 70 kg and over 15 years of age. Adults are usually found in small schools but often aggregate at particular locations on reefs and shoals to feed and spawn. Whilst some individuals may reside in the same area throughout the year, most mackerel appear to undertake lengthy migrations (Luna 2000, Collette and Nauen 1983).

S. commerson is of major fisheries importance and targeted throughout its range by commercial, artisanal and recreational fishers. The main methods of capture are drift nets and trolling lines. Estimated global catches of this species between 1993 and 1998 ranged from 124 570 to 158 735 t, with most catches obtained from the central western Pacific and western Indian Ocean regions (FAO 2000).

Commercial catches of *S. commerson* in Australian waters are minor compared to other regions, varying from 1 191 to 1 635 t between 1993 and 1998 (FAO 2000). Approximately one third of this catch was taken along the Western Australian (WA) coast between Geraldton and the Northern Territory border, making *S. commerson* one of the most valuable finfish species in Western Australia. Although 75 commercial fishing vessels reported catches of Spanish mackerel in 1999, only about ten of these specifically targeted mackerel. A significant number of *S. commerson* are also caught by recreational anglers as far south as Geographe Bay (Crowe *et al.* 1999, Sumner *et al.* in press).

The main method used to capture *S. commerson* in WA is trolling, using either lures or baited hooks. In the commercial fishery up to seven lines are trolled at a time, with braided nylon or rope ‘sash’ cord attached to heavy wire trace traditionally used as the main line. Sash cord is still used in the north of WA but has been replaced by lighter monofilament line elsewhere in the state. Due to concerns over increased catches and anecdotal evidence to suggest that the species is under the threat of overfishing, an Interim Management Plan (IMP) is currently under review. The capture of *S. commerson* by commercial and recreational fishers is also subject to a minimum legal size of 90 cm total length and a recreational bag limit of four fish per day per angler.

1.1 Research on *S. commerson* in Western Australia

A joint Commonwealth Department of Primary Industry and WA Department of Fisheries and Wildlife research program was undertaken between July and October 1981 to gather data on the distribution and abundance of *S. commerson* along the north west coast of WA (Donohue *et al.* 1982). Preliminary biological, catch per unit effort and economic data were also gathered to assess the viability of commercially fishing for mackerel in the north. More recent concern over the continued exploitation of *S. commerson* has led to the initiation of two new research projects. The first of these, a joint WA-NT-QLD project funded by the Fisheries Research and Development Corporation (FRDC; Project # 98/159), was commenced in 1998 to determine the stock structure of *S. commerson* in Australian waters using genetic markers, otolith stable isotope ratios, and parasitic fauna. The second project, also funded by the FRDC (Project # 99/151), began in 1999 and will assess the status of *S.*

commerson stocks within WA waters. Integral to this second project is the gathering of biological information which, along with commercial catch and effort data, will be used in the development and interpretation of stock assessment models and in determining how the WA Spanish mackerel fishery may be sustainably managed.

Information on the reproductive biology of *S. commerson* is an essential component of the biological research. The data required to provide such information is typically gathered through the examination and classification of gonads into developmental stages so that parameters such as reproductive period, spawning frequency, size at sexual maturity and sex ratios can be determined. The most accurate and detailed means of staging gonads is by microscopic examination of histologically prepared sections of each specimen, although this method is costly and time consuming. Macroscopic staging of gonads based on their colour and general appearance is a cheaper and faster method, and may be more appropriate if the samples are not fresh enough to be fixed in preservative for histological examination. Consequently gonads are routinely staged this way during reproductive studies, although the information so gained is limited in detail and often unreliable (West 1990, McPherson 1992, Garcia-Diaz *et al.* 1997).

1.2 Aims of this report

Because of the uncertainty associated with macroscopically staging gonads, the data is often not utilised. However, few attempts are made to assess and improve the accuracy of this technique so that it might be used in situations where histological methods are not practical - for instance, when funds are limited and a cheap, rapid technique for ongoing biological monitoring is required, when time and space do not permit sampling of the whole catch amid the chaos of a busy fishing deck, or when the samples are frozen or have been on ice for several days after capture. These were considerations in the study of *S. commerson* biology, thereby prompting the development and validation of an unambiguous and accurate macroscopic staging system. The aims of this report were, therefore, to:

- Develop an illustrated, relevant macroscopic staging system for *S. commerson* gonads, which can be reliably used by personnel in the field.
- Develop an illustrated, relevant microscopic staging system for *S. commerson* gonads that enables maximum information about gonad development and spawning.
- Further aims of this report were to detail other methods required in the study of *S. commerson* reproductive biology, including:
- Determine conversion factors between the weights of fresh, frozen, and formalin-fixed whole gonads, thus allowing for standardisation of methods such as gonado-somatic ratios.
- Detail the methods used in the collection and processing of samples used in the estimation of batch fecundity in females.

The study focuses on ovaries since the developmental stages of these are easier to distinguish than in testes, and because ovarian development usually defines the spawning season and number of offspring produced during spawning (De Martini and Fountain 1981). It is important to note that *S. commerson* has a prolonged spawning season during which

eggs are spawned by females in multiple batches (McPherson 1993), and oocytes of varying developmental stages are present within the ovary at the same time (*pers. obs.*). This *asynchronous* development of the ovary (Wallace and Selman 1981) is common in other exploited species of fish that inhabit tropical marine waters, and the staging systems described in this report may also be relevant to these. However, for species of fish with ovaries in which oocytes develop and are spawned as one single group (*synchronous* development) or as two or more homogenous groups (*group synchronous* development), the staging systems for *S. commerson* may be inappropriate.

2.0 Methods

2.1 Collection of samples

Fresh gonad samples were collected by Fisheries WA personnel onboard commercial and recreational vessels and from recreational fishing competitions. Frozen samples were also obtained from commercial and recreational fishers. Most of these samples came from the vicinity of Broome, Port Hedland, Dampier and Carnarvon, with others collected less frequently from Exmouth, Denham, Geraldton and Cervantes (Figure 1, Table 1).

Fresh samples

Length and weight (kg) were obtained for each fish. Fork length (FL) of all fish and, where possible, total length (TL) were measured (all lengths in this study are mm). Total lengths were measured to the tip of the dorsal fork of the tail, with the tail laid flat in the normal swimming position and the forks compressed slightly towards each other to remove 'play' in the tail. If the dorsal tip was damaged then TL was taken to the ventral tip, although this is usually shorter and required appropriate adjustment to the equivalent dorsal fork measurement. Note that TL is not as precise a measure as FL in *S. commerson*. Measurements of the head (tip of the mouth to the firm edge of the operculum) and of the jaw (tip of the mouth to the posterior edge of the upper jaw (premaxilla)) were also taken. Where possible, the whole weight, clean weight (viscera and gonads removed), and head weight (including gills) of each fish were also obtained (body weight to 0.1 kg, head weight to 0.1 gm). Refer to the Appendix A for detail of the data sheet used to record information whilst in the field.

Fresh gonads were usually removed from the fish within a few hours of capture, and their sex and stage of reproductive maturity determined using a macroscopic staging system (Appendix B). Gonads obtained from recreational fishers could usually be weighed fresh (0.01 gm). Two or three transverse cuts were then made through each gonad to ensure proper fixation before placing them into a perforated cellophane bag and then into plastic drums containing 10% formalin in seawater. If space permitted, the whole gonad was preserved, otherwise an 8 cm long mid-section of one lobe was saved.

Frozen samples

Frozen samples obtained from commercial fishers comprised the head, gut and gonad of each fish, whereas frames (fillets removed) were obtained from recreational fishers. Samples

were thawed in freshwater prior to examination. Gonads were weighed (0.01 gm), sexed and staged macroscopically (Appendix B). Some frozen gonads were preserved in formalin for histological processing although these were of poor quality due to the rupture of cells and deterioration of tissue when frozen.

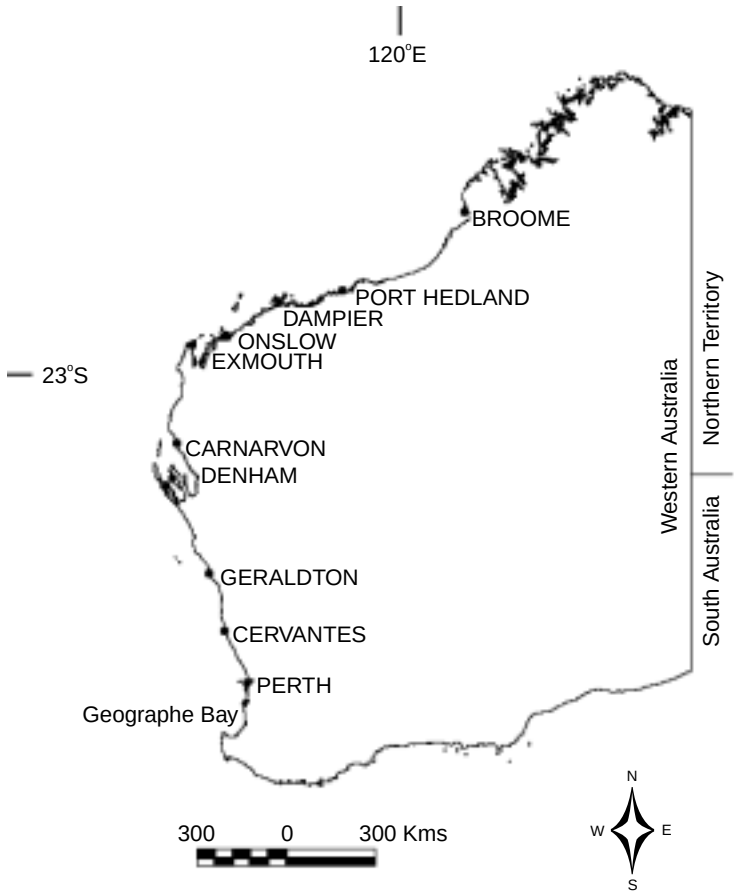


Figure 1. Sampling locations used in the study of *S. commerson* biology.

Table 1. The number of fresh and frozen samples of *S. commerson* ovaries obtained from each region between 1998 and 2000. Kimberley; east of 1200 E. Pilbara; north of 230 S to the Kimberley border. West Coast; south of 230 S. Macro; macroscopically staged ovaries. Hist; histologically staged ovaries. Note that all histologically staged ovaries were also staged macroscopically.

Region	1998				1999				2000			
	Fresh		Frozen		Fresh		Frozen		Fresh		Frozen	
	Macro	Hist	Macro	Hist	Macro	Hist	Macro	Hist	Macro	Hist	Macro	Hist
Kimberley	-	-	55	-	336	325	10	4	1136	421	1	-
Pilbara	48	-	9	2	100	96	60	30	331	258	238	10
W Coast	14	-	26	5	49	24	46	-	27	22	72	3

2.2 Processing of samples

Preserved gonad samples were removed from the formalin solution after several weeks. Whole samples were blotted dry and re-weighed (0.01 gm). A transverse portion of each gonad was then removed and processed using standard histological techniques to provide 5 – 7 μm sections that were stained using Harris's haematoxylin and eosin (H&E) for microscopic examination. The transverse portion was removed from the middle region of one lobe following an investigation to determine whether development of gametes was uniform throughout the gonad (described below). A sample data sheet used in the analysis of the histologically prepared gonad specimens is provided in Appendix C.

2.2.1 Comparison of gamete development throughout the gonad

The investigation of gamete development was made using histologically prepared material, with the circumference of 25 latest-stage oocytes measured from the anterior, middle, and posterior regions of either the left or right ovary of 7 females (Yoshida, 1964). The reproductive stage of these ovaries was either mature resting, developing, or developed (refer to Results, Table 7). Oocytes sectioned through the nucleus were randomly selected for measurement by moving the microscope field of view (x4 or x10 objective magnification) horizontally back and forth across the section so that new oocytes could be measured. Using the same technique, comparison was made of oocyte development within the middle region of the left and right ovary (n = 9), using ovaries from mature-resting to pre-spawning stages. These data were compared using a one-way Analysis of Variance in order to determine whether samples taken from mid-section of the gonad of either lobe were representative of gamete development throughout the ovaries. Development of sperm tissue throughout the testes was compared visually by microscopic examination of sections cut from anterior, middle and posterior sections of each lobe (n = 5).

2.2.2 Estimates of batch fecundity and comparison of oocyte hydration throughout the ovary

To determine whether hydration of oocytes occurred at a similar rate throughout the ovary, batch fecundity estimates of 6 pre-spawning (Stage 5a) ovaries taken from anterior, middle and posterior regions of both lobes were compared. Prior to estimating batch fecundity, the fixed ovaries were removed from the formalin solution and cellophane bag and placed in a sieve (mesh size 0.5 mm). The bag and ovary were rinsed with fresh water in order to remove loose oocytes and to rinse the sample of excess formalin. Both ovarian lobes were slit to allow excess liquid to drain, and the outer membrane dried using paper towel before the ovary was weighed to 0.1 mg. Tissue samples were taken from the middle region and one-fifth of the distance from each end of one lobe. These samples were cut to include a 3 x 3 mm square of outer membrane plus the connected ovarian tissue (since membrane weight is also included in the whole gonad weight), and weighed between 130 - 200 mg so that 150 - 200 hydrated oocytes could be counted.

A transverse section of ovary approximately 3 mm thick was also removed from the central region of the lobe in which the above tissue samples were obtained. This section was processed using histological techniques to confirm whether the ovary was suitable for fecundity estimates. For instance, if not all oocytes from the batch to be spawned were fully hydrated (eg still in the migratory nucleus stage) or if some had already been released from

the lamellae the estimate of batch fecundity is likely to be incorrect. Each tissue sample was weighed to 0.1 mg, and as evaporation caused a steady decrease in tissue weight all three samples from a particular lobe were weighed in quick succession. Each sample was then placed on a glass slide and covered with several drops of glycerin. After 10-15 minutes the oocytes were loosened by gently teasing apart the tissue with forceps, 3-4 more drops of glycerin were added, and the sample spread over the slide. Hydrated oocytes were then counted using a dissecting microscope (x 10). These were easily distinguishable from other oocytes by their large size (usually greater than 0.8 mm along the major axis), wrinkled appearance compared to other non-hydrated oocytes when preserved in formalin, and by their translucence (non-hydrated oocytes are relatively opaque). In the case of damaged hydrated oocytes, only fragments judged to be a major portion of the oocyte were counted. Batch fecundity for each female was subsequently calculated from the product of the number of hydrated oocytes per unit weight in the tissue sample and the ovary weight (both lobes combined). Note that batch fecundity is an estimate of the potential number of eggs released during one spawning event and not an estimate of the total number spawned throughout the spawning season. Refer to Appendix D for details of the data sheet used to record fecundity.

2.2.3 Comparison of fresh, frozen and preserved ovarian weights

The affects of freezing and preservation on ovary weight were assessed using samples obtained from recreational fishing competitions. The two lobes of each gonad were separated and individually weighed while fresh (0.01 gm). One lobe was then frozen and the other fixed in 10% formalin solution. After several weeks, the frozen lobe was thawed in freshwater, blotted dry, and again weighed and macroscopically staged whilst the fixed lobe was blotted dry, weighed and processed using histological techniques. These weights were used to determine conversion ratios between fresh gonad weight and both fixed and frozen weights. The stages were used to compare the accuracy of macroscopically staging fresh and frozen gonads with the microscopically staged fixed gonads (the latter regarded as the true stage).

2.2.4 Microscopic staging system

Nomenclature for stages of oogenesis followed that of Wallace and Sellman (1981) and West (1990), as described below (H&E stain):

1. Pre-vitellogenic Growth

Chromatin-Nucleolus Stage: 10 - 35µm in diameter (mean ≈ 18µm). Cytoplasm strongly basophilic (dark staining). The nucleus is about half the size of the oocyte and clear staining, with conspicuous chromatin strands and a single large nucleolus.

Perinucleolus Stage: 15-120µm in diameter (mean ≈ 80µm). Thin follicular layer and irregular shape (spherical to elongate and often angular). Cytoplasm strongly basophilic with a large nucleus about a third of the area of the oocyte. Chromatin strands are conspicuous throughout the clear staining nucleus and nucleoli are prominent around the periphery.

Cortical Alveoli (Yolk Vesicle) Stage: 110-320µm in diameter (mean ≈ 225µm). Distinct thecal layer and zona radiata. The nucleus is about half the size of the oocyte and the cytoplasm is less basophilic (lighter staining) and grainier than in previous stages. Small clear staining yolk vesicles appear throughout the mid and outer regions of the cytoplasm,

eventually forming a narrow row (the cortical alveoli) near the periphery of the cytoplasm. Clear staining oil droplets also appear within the inner region of the cytoplasm, increasing in size and number around the central nucleus. The cortical alveoli stage marks the commencement of gonadotropin-dependent oocyte growth when vitellogenesis occurs (Wallace and Sellman 1981), and was therefore used to identify the developing ovarian (F3) stage.

2.Vitellogenic Growth

Yolk Globule Stage 215-640μm in diameter (mean ≈ 450μm). Development into this stage is marked by the appearance of small pinkish-red (acidophilic) yolk globules in outer regions of the cytoplasm. These can only be distinguished under high magnification to begin with but increase in size and number to fill the cytoplasm as the oocyte increases in size. The zona radiata is well developed and striated.

Ripe Stage: 500-800μm diameter (mean ≈ 690μm) during migratory nucleus stage; 560-1,140μm diameter (mean ≈ 870μm) when hydrated. Maturation into this stage is marked by the migration of the nucleus to the periphery of the oocyte and coalescence of the oil droplets. The nucleus breaks down when it reaches the periphery, the yolk globules coalesce and hydration occurs as the oocyte takes on a uniform pale pink appearance and rapidly expands in size. The zona radiata becomes reduced in thickness.

Classification of gonads into developmental stages was based on the staging system used by McPherson (1992) for tuna, with modifications made to the names and order of the stages. This system was used to microscopically stage preserved gonads that had been sectioned transversely at 5 μm using standard histological techniques. The same system was also used to macroscopically stage fresh and frozen gonads prior to review of its accuracy for this purpose and subsequent simplification.

2.2.5 Staging of post-ovulatory follicles

Prior to ovulation, each oocyte is encased in a follicle comprised of an inner epithelial layer of granulosa cells and an outer connective tissue layer of thecal cells (Hunter and Macewicz 1985). At ovulation, the oocyte is released into the lumen whilst the ruptured follicle (post-ovulatory follicle) remains within the lamellae. Post-ovulatory follicles (POFs) are short-lived but readily distinguishable, particularly because they are usually quite common when present. In fish inhabiting tropical waters, they may remain up to 24 hrs in the ovaries before being reabsorbed (West 1990, Samoilys and Roelofs 2000), with evidence to suggest this is the case with *S. commerson* in Queensland waters (McPherson 1993). Post ovulatory follicles present in the ovaries of Spanish mackerel were categorised as either ‘new’ or ‘old’ based on their appearance (Table 2), in order to distinguish between groups of POFs and provide more detail of spawning history.

Table 2. Descriptions used to categorise post-ovulatory follicles in *S. commerson* ovaries according to relative age.

Category	Description
New	(Plate 23) The POF is relatively large and the granulosa cells form a loose, convoluted layer inside the thecal cell layer. Nuclei of the granulosa cells are large and orderly arranged. The central lumen is distinct.
Old	(Plate 18). The POF is small and compact, and the outer thecal cell layer is angular in shape. The granulosa cells are difficult to define and may no longer form an unbroken layer. Eventually the POF is difficult to identify although the presence of other similar structures is confirmation.

2.2.6 Oocyte atresia

Ovaries were categorised according to the degree of oocyte atresia in order to provide additional information on the cycling of gametes (Table 3). This was particularly important for determining the spent (F6) stage of ovary development, for which the main criteria was >50% of atresia of yolk globule stage oocytes (Category 3; Hunter and Macewicz (1985) found that the probability of spawning in anchovy was very low when >50% of the advanced oocytes were atretic).

Table 3. Definitions and criteria used to categorise *S. commerson* ovaries according to the amount of oocyte atresia. Generally the criteria refer to the percentage of the latest stage oocytes that are atretic. However, if the latest stage comprises only a few oocytes then the definition becomes more important.

Category	Definition	Criteria
0	minor atresia	0 – 5%
1	notable atresia	6 – 15%
2	significant atresia	16 – 50%
3	major atresia	> 50%

3.0 Results

The gonads of male and female *S. commerson* were bi-lobed, elongate, and joined posteriorly to form a short gonoduct leading to the urogenital pore (Plate 10). The germ tissue was bound by a muscular wall and tunica, and suspended from the dorsal posterior wall of the body cavity by mesenteries. In ovaries, the oocytes developed within lamellae that were attached to the gonad wall. Ovulated eggs were shed into a lumen extending the length of the ovary and during spawning were released into the surrounding water via the gonoduct. Sperm developed in crypts and were released into peripheral sperm sinuses that opened into a muscular central sperm sinus. From there the sperm were released into the gonoduct during spawning.

Ovarian weight was reduced by 6.3% when preserved in 10 % formalin solution. The affects of freezing were negligible, resulting in a slight increase of 0.14 % in weight (Figures 2 and 3). Relationships between fresh, preserved and frozen gonads were:

Fresh weight (g) = 1.0452 x formalin preserved weight (g) (r^2 = 0.9985, n = 144)

Fresh weight (g) = 0.9986 x frozen weight (g) (r^2 = 0.9973, n = 45)

Comparison of the diameter of oocytes measured from anterior, middle and posterior regions of the lobe (Table 4), and between left and right lobes (Table 5), show that ovarian development was generally similar throughout the gonad. In most cases, the size of the most mature stage oocytes did not differ between region of the lobe, and in all cases the maturity stage of the most advanced oocyte was the same in each region. Estimates of batch fecundity for samples taken from anterior, middle and posterior regions of the lobe, and between left and right lobes, also showed that final maturation of oocytes was similar throughout the gonad (Table 6).

Table 4. Results of t-tests for dependent samples to compare the circumferences of 25 of the most mature stage oocytes within three locations of one ovarian lobe. Ant; anterior portion of the lobe. Mid; middle portion of the lobe. Post; posterior portion of the lobe. CAS; Cortical alveoli stage. PNS; Perinucleolus stage. YGS; Yolk globule stage. Refer to Methods for sampling details.

Ovary	Sample Source	Most Mature Oocyte	df	t	P
1	Ant v Mid	Ant = CAS	24	1.1357	0.2673
	Ant v Post	Mid = CAS	24	-0.7343	0.4699
	Post v Mid	Post = CAS	24	-1.3873	0.1781
2	Ant v Mid	Ant = PNS	24	2.7691	0.0106*
	Ant v Post	Mid = PNS	24	-2.2551	0.0335*
	Post v Mid	Post = PNS	24	-5.9050	0.0000*
3	Ant v Mid	Ant = CAS	24	1.1094	0.2783
	Ant v Post	Mid = CAS	24	-0.4997	0.6219
	Post v Mid	Post = CAS	24	-1.2231	0.2332
4	Ant v Mid	Ant = YGS	24	-1.4135	0.1704
	Ant v Post	Mid = YGS	24	-2.4945	0.0199*
	Post v Mid	Post = YGS	24	-1.4531	0.1591
5	Ant v Mid	Ant = CAS	24	-1.4581	0.1578
	Ant v Post	Mid = CAS	24	-0.8407	0.4088
	Post v Mid	Post = CAS	24	0.7835	0.4410
6	Ant v Mid	Ant = CAS	24	1.1426	0.2645
	Ant v Post	Mid = CAS	24	0.4368	0.6662
	Post v Mid	Post = CAS	24	-0.7749	0.4460
7	Ant v Mid	Ant = YGS	24	-1.3066	0.2037
	Ant v Post	Mid = YGS	24	-0.1306	0.8972
	Post v Mid	Post = YGS	24	0.7338	0.4702

Table 5. Results of t-tests for dependent samples to compare the circumferences of 25 of the most mature stage oocytes within the mid-region of each ovarian lobe. CAS; Cortical alveoli stage. MNS; Migratory nucleus stage. PNS; Perinucleolus stage. YGS; Yolk globule stage. Refer to Methods for sampling details.

Ovary	Most Mature Oocyte		df	t	P
	Left Lobe	Right Lobe			
1	YGS	YGS	24	2.5984	0.0158*
2	CAS	CAS	24	-0.9837	0.3351
3	CAS	CAS	24	0.6883	0.4979
4	CAS	CAS	24	0.1623	0.8724
5	CAS	CAS	24	-1.8283	0.0800
6	PNS	PNS	24	2.4577	0.0216*
7	MNS	MNS	24	3.083	0.0051*
8	MNS	MNS	24	1.7420	0.0943

Table 6. Results of t-tests for dependent samples to compare estimates of batch fecundity for *S. commerson* ovaries. Samples used in comparisons were taken from three regions along the ovarian lobe (anterior, middle and posterior), and from right and left lobes. Refer to Methods for sampling details.

Sample Source	df	t	P
Anterior v Middle	17	-0.3217	0.7516
Anterior v Posterior	17	-0.2780	0.7844
Middle v Posterior	17	0.1539	0.8795
Middle Left v Middle Right	7	-0.5498	0.5995

3.1 Staging system for microscopic analysis of formalin preserved, histologically prepared gonad sections

Descriptions of the developmental stages used in analysis of histologically prepared gonad samples are provided in Table 7. Photographs of the ovarian stages are also provided in Plates 1 to 24. The stages in this system follow the development of the gonad from the undifferentiated juvenile state to the immature virgin and reproductively mature gonad, and then through the annual reproductive cycle of the mature gonad.

3.1.1 Ovaries

A conceptual diagram of the reproductive status for each ovarian stage in Table 7 is given in Figure 4. In this diagram, the juvenile (J) and virgin (F1) gonads have relatively low reproductive status. This is also the case for the mature ovary when in the resting (F2) stage, until early signs of reproductive activity during the developing (F3) stage mark a rise in the reproductive status of the gonad. Within the period of reproductive activity, as marked by the presence of developed (F4) ovaries, the reproductive status is relatively high, with short-term peaks occurring during the brief periods when final maturation of the oocytes takes place (F5a) and spawning (F5b) occurs. This is followed by a drop in reproductive status during the brief post-spawning (F5c) stage back to the developed stage. However, if repeat spawning occurs over a short period of time (e.g. on consecutive days), then several peaks in

reproductive status will be overlayed. This will be indicated by the presence of migratory nucleus stage oocytes, hydrated oocytes and early/late stage POFs within the same gonad (the number of peaks depending on how many of these stages are present). Finally, at the end of the spawning season, the ovary enters the spent (F6) stage when residual vitellogenic oocytes are resorbed, marking a decrease in reproductive status back to that of the resting ovary. Note that *S. commerson* ovaries in the spent (F6) stage also occur at other times during the annual cycle, indicating ovarian regression due to environmental, social or biological factors.

Further, note the shaded area in Figure 4, which indicates the period prior to reproductive activity when immature (F1) and mature resting (F2) ovaries may look quite similar and can therefore be confused. In contrast, these two stages are quite distinct soon after the reproductive period whilst the mature ovary still retains evidence of previous spawning. This evidence may include a loose, relatively thin tunica, misshapen lamellae with loose stroma and few previtellogenic oocytes, large amounts of vascular and muscular tissue, atretic vitellogenic oocytes, and yellow-brown bodies. As time since last spawning increases, this evidence is lost as the ovary tightens up and fills with previtellogenic oocytes. The best evidence to distinguish a mature resting from an immature ovary immediately prior to the start of the spawning season is the presence of yellow-brown bodies (melanomacrophage centres). These are distinctive, yellow-brown coloured masses that are repositories for the end products of cell breakdown (Ferguson 1989), and provide evidence that a particular ovary has undergone oocyte atresia and cell breakdown associated with spawning. Although reduced in size from those present in the ovary soon after spawning, the yellow-brown bodies present in mature ovaries prior to the spawning season are generally quite common. Immature ovaries may also contain yellow-brown bodies, but these are usually small and uncommon. The lamellae of mature ovaries also tend to be more branched than those in the immature ovary.

The F1a stage was used to identify ovaries that were probably immature but contained cortical alveoli stage oocytes. In some of these ovaries, the oocytes continue development as the fish becomes sexually mature and spawns. However, given the number of small females well below the estimated size at sexual maturity that had developing (but not developed) ovaries, it is likely that in some cases the cortical alveoli stage oocytes eventually atrophy because the fish is not physiologically ready to spawn. Classifying these ovaries as F3 would falsely inflate the number of mature fish in the samples as ovaries in this stage are generally regarded as moving from the mature resting to the mature developed stage. Ovaries classified as F1a were considered immature (F1) for analysis of size at maturity since they are either still in this stage or are at the end of it. This stage also enables comparison of early ovarian reproductive activity (as indicated by the appearance of cortical alveoli oocytes) in immature and mature fish.

3.1.2 Testes

S. commerson testes are difficult to categorise into stages because maturation of sperm tissue does not occur in distinct steps, but as a gradual change in the relative proportion of spermatocytes, spermatids and spermatozoa. There can also be considerable variation in the appearance of the sperm tissue for each staging category. For instance, in some ripe testes the tissue was dominated by late stage sperm in the peripheral sperm sinuses and outer regions of the gonad, whilst in others the late stage sperm dominated the inner regions and central sperm sinus. Staging of testes is therefore more prone to error than is the staging of ovaries. As with the ovarian cycle depicted in Figure 4, the immature (M1) and immature developing (M1a) stages have lowest reproductive status. A developing stage is not recognised in males because there is no clear demarcation in the transition from the mature resting (M2) to the mature ripe (M3) stage. For similar reasons a spent stage is not recognised. The ripe stage is the background state of the testis during the reproductive period, with peaks in reproductive status during spawning (M4), as shown for females in Figure 4. However, the testis holds no evidence to identify whether a particular male is just about to or has recently spawned – only that it is in the process of doing so.

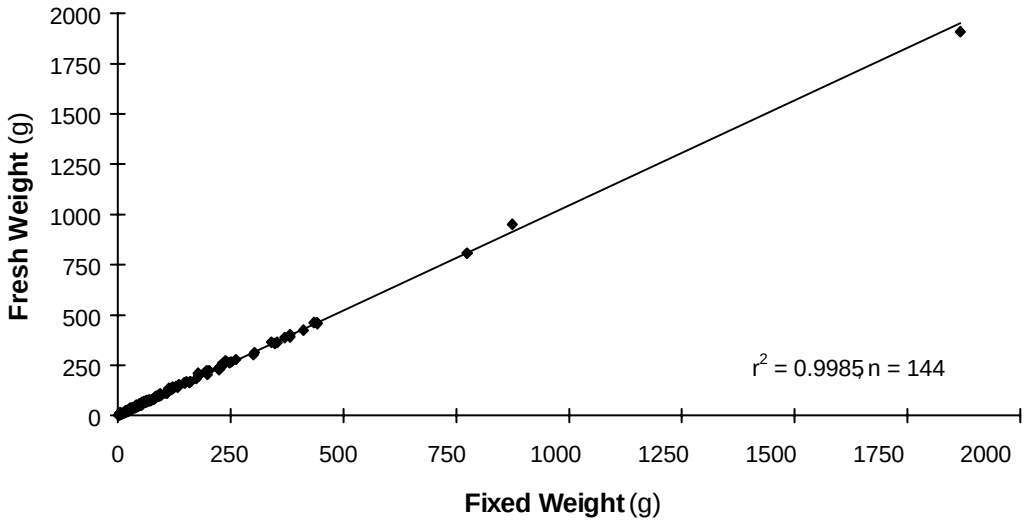


Figure 2. Comparison of fresh and formalin preserved ovary weights of *S. commerson.* Note that weights are of only one lobe of each ovary.

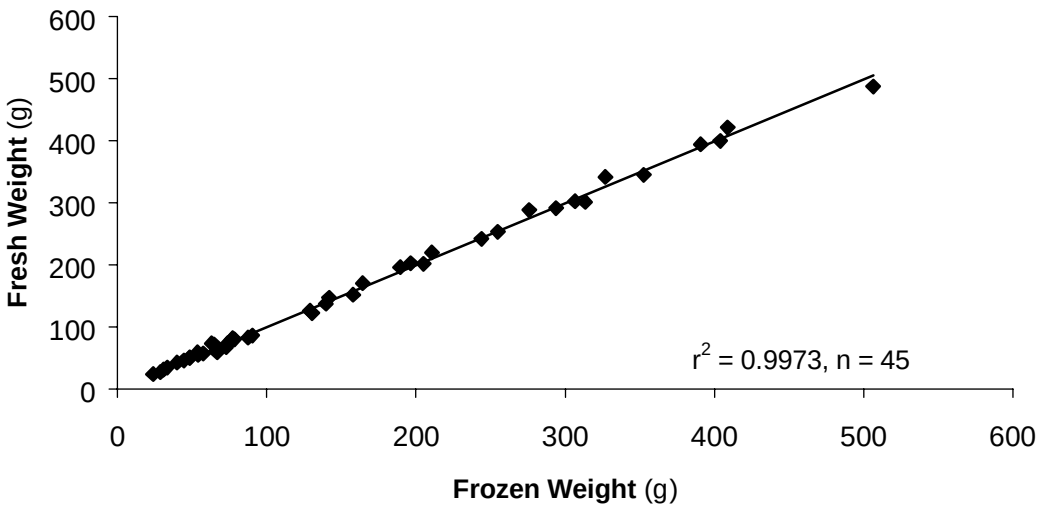


Figure 3. Comparison of fresh and frozen ovary weights of *S. commerson.* Note that weights are of only one lobe of each ovary.

Table 7. Microscopic staging system used in the histological analysis of *S. commerson* gonads (stained using Haematoxylin and Eosin). F = female, M = male.

J (Juvenile)	Gonad is tiny. Germ tissue is rudimentary and comprised of undifferentiated gonia (may become either a testis or an ovary).
F1 (Virgin/immature)	The newly formed ovary contains little ovarian tissue. Only chromatin nucleolus stage (CNS) oocytes line the lumen and the lamellae are barely evident. As the ovary develops, the chromatin nucleolus and perinucleolus stage (PNS) oocytes increase in number and fill the lamellae, which lengthen but remain relatively narrow and less branched than in the mature ovary. Yellow-brown bodies are occasionally present whilst the tunica is relatively thin (although it may appear quite thick because of the small diameter of the ovary). During or prior to the reproductive season some oocytes may develop into the cortical alveoli stage (CAS). In this case, the ovary is classified in the F1a stage. Refer to Plate 2.
F1a (Immature developing)	Used to stage ovaries prior to the reproductive season that had features of the virgin ovary as well as CAS oocytes. It is likely that in some ovaries these CAS atrophy, whilst in others they develop further as the fish becomes reproductively mature. F1a ovaries were considered immature (F1) for analysis of size at maturity since they may still be or are at the end of this stage. However, for analysis of spawning season these fish were considered developing (F3), since they also indicate the commencement of reproductive activity. If >50% of the CAS oocytes within these ovaries were atretic it was classified as F1. Refer to Plate 4.
F2 (Mature resting)	Soon after the preceding spent (F6) stage, the tunica (gonad wall) of the resting ovary may be relatively thin and loose-fitting. CAS oocytes may still be present but these soon disappear and chromatin nucleolus and perinucleolus stage oocytes dominate the ovary. Yellow-brown bodies and vascular tissue may also be prominent soon after the spawning season. The former generally remain for some time and are the main indicators of prior spawning when all other evidence has vanished. As time since spawning increases, the early stage previtellogenic oocytes increase in number and fill the lamellae, the tunica contracts and thickens, and vascular tissue is reduced. Refer to Plate 5.
F3 (Developing)	This stage commences with the appearance of CAS oocytes and ends with the appearance of early yolk globule stage (YGS) oocytes. Note that CAS oocytes may also be the latest stage oocyte in spent and newly resting ovaries, although these ovaries will also contain evidence of recent spawning (disorganised tissue, yellow-brown bodies etc). Refer to Plates 7 and 9.
F4 (Developed)	The 'background' state of ovaries during the reproductive season, which commences with development of oocytes into the early YGS and ends when the ovary enters the spent (F6) stage. Early in this stage, the ovary is dominated by early yolk globule and previtellogenic stage oocytes. The latter become less evident as the YGS oocytes mature and grow, causing the lamellae to expand, the lumen to decrease, and the tunica to stretch and thin. As the reproductive season progresses and oocytes are spawned, the ovary becomes emptier and fewer vitellogenic oocytes are present within in the lamellae whilst yellow-brown bodies and vascular tissue become more common. Refer to Plates 10, 12 and 13.
F5a (Pre-spawn)	A short stage that commences with the appearance of migratory nucleus stage (MNS) oocytes and ends when the hydrated eggs are released from the lamellae into the lumen. Early in the reproductive season, the ovary is very large and packed with late yolk globule and migratory nucleus stage oocytes. Few other features may be evident, however, towards the end of the season, there will be decreased supplies of vitellogenic oocytes and the

	ovary will begin to appear disorganised. Yellow-brown bodies and vascular tissue will become more prominent at this time, and post-ovulatory follicles (POFs) may be present if the fish has previously spawned. Refer to Plates 17, 18 and 19.
F5b (Spawning/ Running Ripe)	A short (and rarely observed) stage at the time of spawning when ovulated eggs are found in the ovarian lumen and new POFs are present in the periphery of the lamellae. The occasional unspawned hydrated oocyte may still be present in the lamellae, as may be MNS oocytes if the fish is preparing for further spawning and older POFs if the fish has recently spawned. General appearance of the ovary through the reproductive season will change in a similar manner to the pre-spawning (F5a) ovary. Note that ovulated eggs may not be evident in the lumen after histological processing. Refer to Plates 22 and 23.
F5c (Post-spawn)	A short stage defined by the presence of old and/or new POFs. Occasionally, a hydrated oocyte not released during the recent spawning may still be present within the stroma. The general appearance of the ovary through the reproductive season will change in a similar manner to the pre-spawning (F5a) ovary. Refer to Plates 18 and 23 for views of new and old POFs (although the ovary shown in Plate 18 has POFs it is considered pre-spawning due to the presence of hydrated oocytes).
F6 (Spent)	A short stage between the developed and resting stages. The main criteria is > 50% atresia of the late YGS oocytes. Late in this stage, only previtellogenic oocytes (including CAS oocytes) and remnants of atrophied YGS oocytes remain. Once the latter are gone, the fish is classified as resting (F2). The gonad tissue is disorganised with yellow-brown bodies, muscle, and vascular tissue usually prominent. The lamellae are thin, the lumen is large, and the tunica thickens as the gonad enters the resting state.
M1 (Virgin/immature)	The newly differentiated testis contains spermatogonia and isolated pockets of spermatocrypts. These mainly contain spermatocytes although crypts of later stage sperm soon appear. Closer to maturity, the testis is similar to the mature resting testis. Peripheral sperm sinuses may contain spermatazoa although generally the testis is dominated by connective tissue and little sperm tissue is present. The central sperm sinus is small and empty.
M1a (Immature developing)	Used to stage testis that had features of both the virgin and mature testes.
M2 (Mature resting)	Soon after spawning, the peripheral sperm sinuses are present but contain little sperm. Yellow-brown bodies, connective and muscle tissue are prominent but sperm tissue is uncommon. Spermatocytes are the dominant sperm tissue.
M3 (Developed)	Appearance of the ripe testis varies, with the main criteria being abundance of spermatozoa and/or spermatids in the outer portions of the gonad. In some testes (notably prior to or at the start of the reproductive season), the central sperm sinus may be small with a thick muscular wall and contain little or no sperm. However the peripheral sperm sinuses are conspicuous and filled with spermatozoa. Crypts of spermatozoa and spermatids are confined to the outer portion of the testis, and in some cases may be uncommon (although spermatogonia are common). In other testes, early stage spermatic tissue (particularly 1 and 2 spermatocytes) are abundant, although peripheral sperm sinuses are well developed, and spermatozoa and spermatids dominate the inner regions of the testes (occupying more than half of the gametic tissue mass). The central sperm sinus may contain sperm.
M4 (Spawning)	Running ripe. Testis is large in size and dominated by large peripheral and central sperm sinuses that are filled with spermatozoa. Crypts of spermatocytes are uncommon and confined to the most outer region of each lobe.

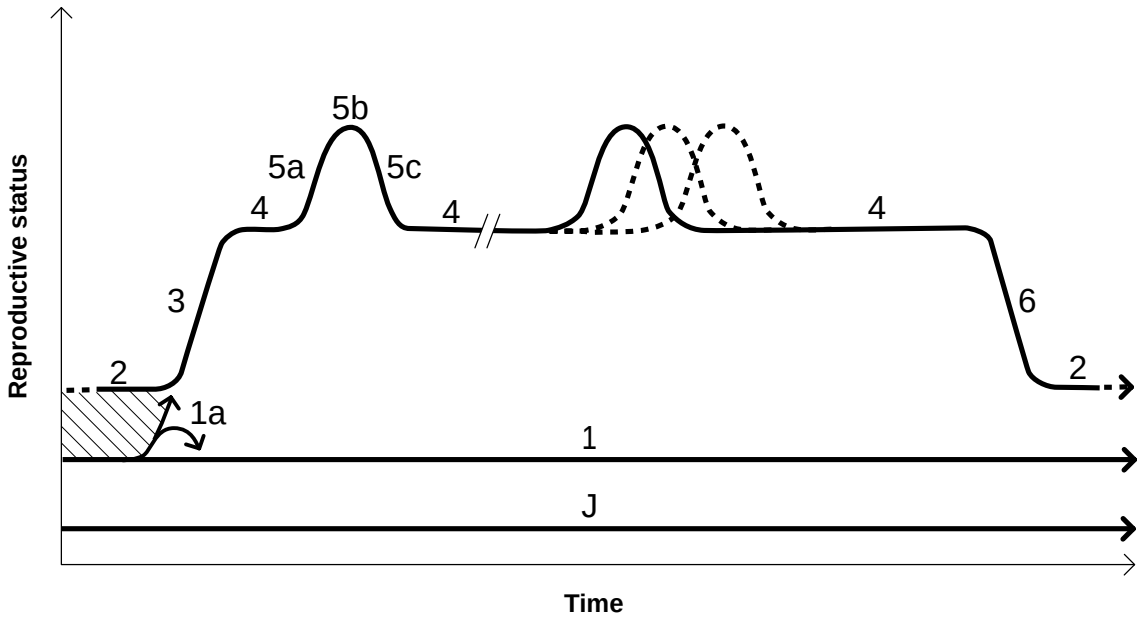


Figure 4. Developmental and maturation cycle of *S. commerson* ovaries showing the relative reproductive status of each ovarian stage through time. J; juvenile stage. 1 to 6; ovarian stages as detailed in Table 7 (without the 'F'). The cross-hatched area indicates the period when immature (stage 1) and mature resting (stage 2) ovaries are most difficult to tell apart. Note that the duration of the non-spawning period (as indicated by stage 2 ovaries) will be longer than depicted here.

3.2 Staging system for macroscopic analysis of whole gonads

Description of the initial macroscopic staging system used in the analysis of whole gonads (ovaries and testes) is provided in Appendix B. Photographs of these stages are provided in Plates 1 to 24.

3.2.1 Ovaries

3.2.1.1 Assessment of the accuracy of macroscopic staging

To determine the accuracy of the initial system for staging *S. commerson* ovaries, the macroscopic stage assigned to each ovary was compared with the histological stage given to that same ovary (Figures 5). These data show that in most cases, the accuracy of macroscopic staging improved as personnel became more experienced (data for 1999 compared to that for 2000), although the error rates were still greater than 40% for many stages. Breakdown of the data obtained during 2000 into the proportion of macroscopically staged ovaries in each histological stage identified where the errors were made (Figure 6). Fourteen percent of immature (F1) ovaries were wrongly classified as mature (F2 or 3) using macroscopic criteria. This could affect estimates of size at sexual maturity. A further 7% of F1 ovaries were classified as immature developing (F1a) although the consequences of this error are minor because these ovaries are still considered immature. Accuracy of macroscopic staging of F1a ovaries was low (30%). Seventeen percent of these ovaries were

wrongly called F1, mainly because cortical alveoli stage oocytes were too small to be seen by eye. This would be of little consequence, however the erroneous classification 52% of F1a ovaries as either F2 or F3 could affect estimates of size at sexual maturity.

Mature resting (F2) ovaries were uncommon during the periods selected for comparison of staging systems and were correctly identified macroscopically, although some ovaries in other stages of development were incorrectly staged as F2. Examination of data obtained during other periods when F2 ovaries were more common suggests a 29% error rate for this stage. Most of this error occurs when the ovaries are wrongly classed as F3, indicating that late perinucleolus stage oocytes are sometimes mistaken for early cortical alveoli stage oocytes. Errors in classification of mature developing (F3) ovaries may also be due to difficulties in identification of cortical alveoli oocytes – either because they were too small to detect or because they were wrongly considered to be in the yolk globule stage. These mistakes are always likely to occur but should have minor affect on conclusions drawn from the data, except for those wrongly classified as F1a (9%) as these could influence estimates of size at sexual maturity.

The accuracy of macroscopically classifying F4 ovaries was reasonable (81%). Most error was again due to misidentifying yolk globule as cortical alveoli stage oocytes, leading to 7% of the F4 ovaries being called F3. As before, this is likely to occur regularly in a small number of cases but should have minimal effect on general conclusions. Five percent of F4 ovaries were designated as F1a, perhaps because they were reaching sexual maturity for the first time and the yolk globule stage oocytes were wrongly thought to be in the cortical alveoli stage. Again, this could affect estimates of size at sexual maturity. Most (91%) F5a ovaries were properly identified. Difficulty in macroscopically distinguishing oocytes in the migratory nucleus stage of development will always lead to some error in identifying these from F4 ovaries (4% in this case), whilst a further 4% were classified as F5c. These errors should be of minor consequence for general description of spawning patterns.

Spawning (F5b) ovaries were rare but unmistakable when present. Post-spawning (F5c) ovaries were difficult to reliably identify macroscopically because POFs cannot usually be detected by eye. As a consequence, 18, 26 and 11% of F5c ovaries were called F4, F5a and F6, respectively. Identification of F6 ovaries is very unreliable because at the end of the spawning season many gonads are flaccid and bloody even if they are still reproductively active. Only in a few cases can mass atresia of the yolk globule stage oocytes be detected macroscopically.

Data concerning the accuracy of macroscopically staging ovaries that have been frozen or on ice for several days is limited (n = 80 for all stages combined), and confounded by the fact that histological analysis of frozen ovaries is also prone to error. Generally though, this data indicates that breakdown of oocytes leads to confusion between stages 1a, 2 and 3, and between stages 4, 5a-c and 6.

3.2.1.2 Development of a more accurate macroscopic staging system

Given the above assessment of error sources, accuracy of the macroscopic staging system was improved by pooling stages 1a, 2 and 3 together (as F2-3), stages 4, 5c and 6 together (as F4), and stages 5a and b together (as F5; Figure 7). Stage 1 was retained as previous (as F1). This resulted in a simpler and more reliable macroscopic staging system that provides a quick and cheap means of determining sex and general maturation cycle of the ovary (Table 8).

Because stage 1 has not been pooled with other stages, the accuracy of macroscopically detecting it remains at 79% (Figures 8). Stage 2-3 now defines the non-reproductive period, with the accuracy of macroscopic staging improved to 86%. Some error is inevitable in classification of ovaries into this stage because late cortical alveoli stage oocytes will sometimes be identified as yolk globule stage (and the ovary wrongly classed F4). With the inclusion of F1a ovaries, some stage 2-3 ovaries that look immature will also be considered F1. However these errors will have little affect on general analyses such as reproductive cycle. Note that this stage could simply be called F2, but leaving out the '3' may cause confusion since there is a jump to F4 in the new macroscopic staging to retain compatibility with the microscopic staging system. The combined stages of 4, 5c and 6 (as F4) now define the period when the ovary is reproductively developed, with an accuracy of 81% using macroscopic criteria. Again, some error is inevitable if yolk globule stage oocytes are incorrectly called cortical alveoli stage. Finally, stages 5a and b indicate spawning peaks within the reproductive period (as F5), with the macroscopic criteria accurate 88% of the time. Some detail about spawning is lost with the exclusion of post-spawning (F5c) ovaries, again highlighting the requirement for histological sampling if more complete information on spawning is required. The main source of error in macroscopically identifying the new stage 5 lies in mistakenly calling them F4 because oocytes in the migratory nucleus stage cannot be identified by eye.

3.2.2 Testes

Accuracy of the macroscopic staging system for *S. commerson* testes was not assessed due to the focus on ovarian development as the more reliable and relevant descriptor of spawning. Nevertheless, during the course of this study many male testes were examined macroscopically (n = 1906) and a number of these were processed histologically (n = 236). These histological samples were used to confirm the appearance of the macroscopic stages given to testes so that they could be described (Table 8). Note that freezing of testes is likely to create more errors in macroscopic staging than freezing of ovaries, because rupture of spermatic tissue usually produces a milt-like appearance regardless of the true gonad stage.

Table 8. Simplified macroscopic staging system for *S. commerson* gonads. F = female, M = male.

J (Juvenile)	Gonad is a small, translucent pink ribbon lying imperceptibly along the dorsal wall of the peritoneal cavity. Sex of the fish cannot be determined. Refer to Plate 1A.
F1 (Virgin)	Ovaries are small and usually translucent pink, apricot or ivory in colour (more opaque and red in unbled fish). In smaller females, the ovaries are flattened, flaccid, and relatively inconspicuous, but they become rounded and firmer with a distinct lumen as the fish approaches maturity. The oocytes are microscopic resulting in a smooth, uniform appearance to the ovarian tissue. Yellow-brown bodies are uncommon. Refer to Plates 1B, 2 and 3.
F2-3 (Mature resting)	Soon after completion of spawning activity, the resting ovaries appear flaccid with prominent exterior blood vessels. Internally, the lumen is large. Few, if any, oocytes can be seen, whilst yellow-brown bodies are distinct (sometimes very common) and blood clots may also be present. As time since spawning increases, the ovaries become progressively rounder and firmer as the gonad wall contracts and thickens and the ovarian tissue develops. Yellow-brown bodies may be evident for sometime and are the main feature used to distinguish mature resting from virgin ovaries. Colour is typically semi-translucent rose, pink or ivory, although in unbled fish the ovaries are often red. Refer to Plates 4 – 9.
F4 (Developed)	Early in this stage, the ovaries appear semi-translucent and speckled because of the many pre-vitellogenic oocytes. As more oocytes develop and turn opaque, the ovaries become large, rotund and opaque with prominent blood vessels. The opaque oocytes are visible through the thin gonad wall and the colour is typically pale yellow or apricot. Towards the end of the reproductive period, the ovaries become more bloodied and flaccid as oocyte reserves are depleted during spawning, and yellow-brown bodies may become more common and the lumen larger. Refer to Plates 10 – 16A.
F5 (Spawning)	Ovaries are very large and swollen, although towards the end of the reproductive season they may become somewhat flaccid. Colour is apricot to peach with a prominent network of external blood vessels. The presence of translucent hydrated oocytes gives the ovaries a distinctive speckled or granular appearance through the thin gonad wall. Eggs may also be released from the gonoduct when pressure is applied to the abdomen and may be present within the ovarian lumen. Refer to Plates 16B, 17, 20 – 22, 24.
M1 (Virgin)	Testes are small and straplike with a smooth appearance and opaque, ivory or bone colour (red if unbled). No milt is present in the transverse section. It is difficult to distinguish testes early in this stage from juvenile gonads, and testes late in this stage from mature resting (M2) testes.
M2 (Mature resting)	Testes are small, opaque and straplike. Little or no milt is extruded from the transverse section when squeezed (unless the sample has been frozen). The section is quite angular in shape, with the central tissue often browner than the bone or ivory coloured peripheral tissue. Sometimes the testes may also be tinged in red.
M3 (Developed)	Testes are large, opaque, and ivory or bone in colour. The exterior dorsal blood vessel is large and small blood vessels are usually present. Internally, white sperm (milt) can usually be squeezed from the central sperm sinus. In some cases this may not be possible, although milt should be visible in the outer areas of the transverse section.
M4 (Spawning)	Running ripe. Similar to the ripe testis but more swollen and with larger exterior blood vessels. Milt is released with little or no pressure on the abdomen or when the testis is cut.

3.3 Pictorial guide to staging *S. commerson* ovaries

An insert booklet designed as a convenient field guide provides photographs (Plates 1 to 24) of *S. commerson* ovaries to complement the descriptive macroscopic and microscopic staging systems given above. These photographs show the range in shape and colour of ovaries within each stage, as well as the close-up macroscopic appearance of the ovarian tissue and histological features of the ovaries.

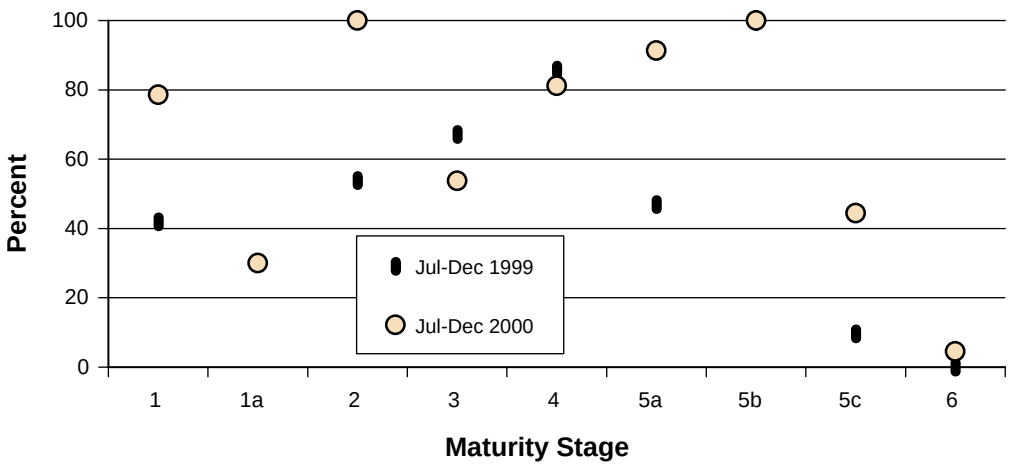


Figure 5. Proportion of *S. commerson* ovaries that were given the same macroscopic and histological stage. Note that the 1a stage was not used in 1999.

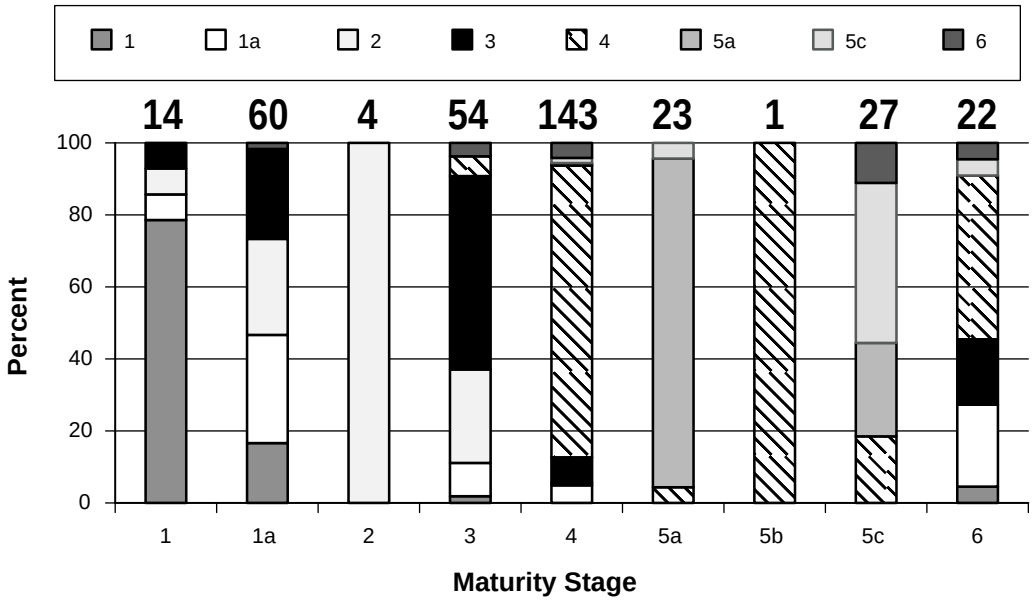


Figure 6. Macroscopic stage composition of each histological stage for *S. commerson* ovaries collected between July and December 2000. Sample sizes are given above each column.

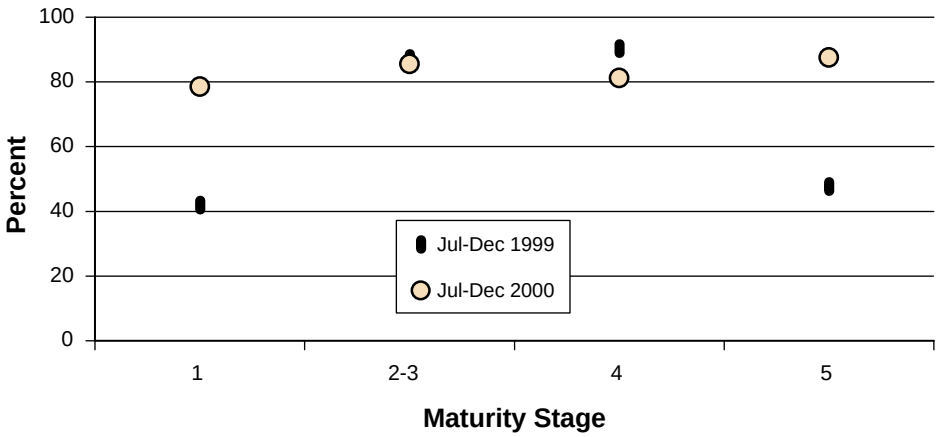


Figure 7. Proportion of *S. commerson* ovaries that were given the same macroscopic and histological stage using the simplified staging system. Data for the last six months of 1999 and 2000 are compared.

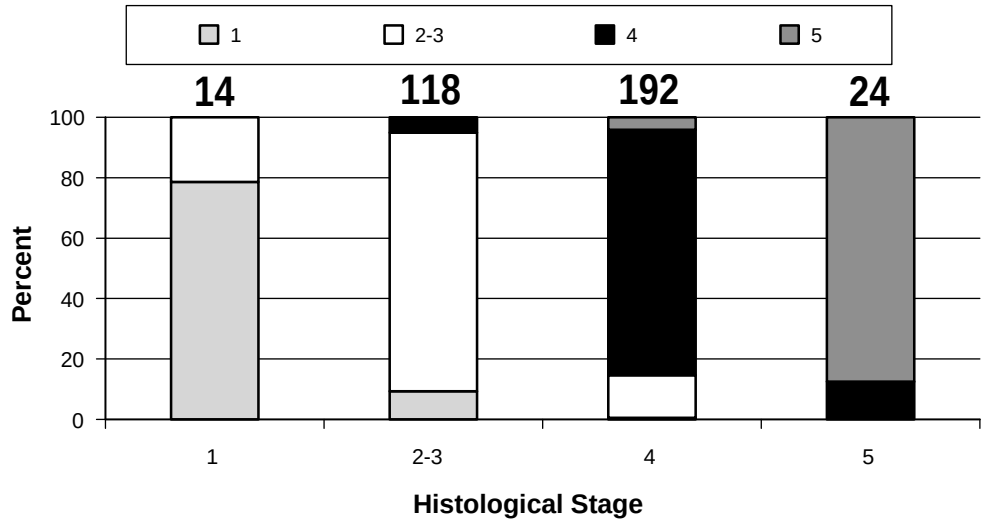


Figure 8. Macroscopic stage composition of each histological stage for *S. commerson* ovaries collected between July and December 2000. Sample sizes are given above each column.

4.0 Discussion

4.1 Staging systems

Studies of fish reproduction are typically based on the microscopic examination of histologically prepared gonad sections because of the accuracy and detail this method provides. In contrast, data obtained using macroscopic staging is less frequently used because it is less reliable and only appropriate for analyses of group statistics such as sex ratios and general patterns of gonad development through the season. Macroscopic staging does, however, have the advantage of speed and low cost, and is therefore ideal for routine monitoring of exploited fish stocks. In some circumstances, such as when samples are frozen, macroscopic staging may also be the most appropriate method to use.

Depending on the nature of the study, advantages of the macro- and microscopic staging systems can be exploited to provide the best possible use of the available resources and samples. This was pertinent in the present study of *S. commerson*, where the sampling area was large (> 1200 km of coastline), personnel and funding were limited, and a variety of fresh, iced and frozen samples were the best that could be practically obtained. Using the staging systems detailed in this study, all of these samples were staged macroscopically to provide a general picture of *S. commerson* reproduction, whilst as many fresh samples as time, space and budget allowed were histologically processed in order to validate the macroscopic method and to gather information requiring detailed microscopic scrutiny of individual gonads. This combination of data subsequently led to a more complete picture of reproduction than would have otherwise been possible.

In the present study, accuracies of 86, 81 and 88% for the resting, developed and ripe stages, respectively, were obtained for the macroscopic staging system. When compared with

similar data for the tropical snapper, *Lutjanus vittus*, in which accuracies for comparable stages of 92, 93 and 61% were recorded (West, 1990), there is clearly some room for improvement. Nevertheless, some errors with the use of the macroscopic staging system are inevitable because of the difficulty at times in distinguishing between oocyte maturation stages, especially between the late cortical alveoli and early yolk globule stage of development. Mistakes will also occur in distinguishing between immature and mature resting females, particularly prior to the onset of reproductive activity when mature ovaries have lost evidence of spawning. This is why size at sexual maturity should ideally be determined for fish obtained during the spawning season. Further, information about spawning at the individual level will always be incomplete because post-ovulatory follicles and migratory nucleus stage oocytes cannot generally be identified by eye (McPherson 1992). Such problems can be minimised with the use of a magnifying glass, appropriate lighting and experience. The latter only comes with time, but the comprehensive written and pictorial description presented here should speed the learning process and improve the accuracy of all personnel involved with staging gonads. As long as accuracy is optimised in this way the data obtained from macroscopic staging will be a valid and useful adjunct to reproductive studies.

Whether the staging systems presented here are appropriate for other species of fish should be critically assessed before usage because there are many such systems and few may be generally applicable among species (Hay and Outram 1981). Macroscopic systems require particular assessment as they often include too many poorly defined stages (Hilge 1977). In contrast, microscopic staging systems are sometimes too simplistic and do not allow all the information available in histological slides to be gathered. We believe that the staging systems developed here for Spanish mackerel adequately address these points and will be appropriate with minor modification to a wide variety of other species that are gonochoristic and have asynchronous ovarian development.

4.2 Other assessments

The 6% decrease in weight of formalin preserved ovaries is greater than that for preserved albacore ovaries (1%; Ramon and Bartoo, 1997), and should be considered in analyses using both fresh and preserved ovary weights. For instance, description of *S. commerson* gonadosomatic indices may require adjustment of the preserved ovary weights to make them comparable with fresh ovary weights when both data sets are used. In contrast, the affect of freezing on *S. commerson* ovaries was negligible (slight increase) whereas the weight of albacore ovaries was decreased by 6% after freezing (Ramon and Bartoo, 1997). Given the rupture of cells and flaccid appearance of ovaries that have been frozen, there is little doubt that freezing does result in fluid and hence weight loss as found in albacore. However, this appears to be negated in the case of *S. commerson* by uptake of the water used to thaw the gonads, even though they are drained prior to being weighed. Thus under normal procedures used in this mackerel study, the weight of frozen ovaries is a close indication of their fresh weight.

The occasional difference in the mean size of the most mature stage oocytes measured in different areas of the gonad is likely to have negligible influence on the stage given to the ovary, since the most mature oocyte stage did not differ between areas. Nevertheless, the difference in mean size suggests that at times the oocytes in one region of the gonad may

mature into the next stage before the oocytes in other regions. Therefore the sampling location within the ovaries should be standardised. Comparison of fecundity estimates indicates that final oocyte maturation is uniform throughout the gonad. The procedure used in determining the fecundity of female *S. commerson* is therefore appropriate.

4.3 Problems associated with analysis of histologically prepared gonad sections

The microscopic staging of gonads inevitably involves a degree of subjectivity when choosing between stages. Experience plays a key role in limiting uncertainty associated with this technique, particularly in being aware of the following sources of error:

- The presence of foreign gamete material on the histology slide: This can occur when the thinly cut sections are floated in the water bath before placement on the slide. Tiny portions of the sections sometimes break away in the bath and may be picked up on another slide. It can be difficult to identify this foreign material, although it usually contrasts with the rest of the section.
- The use of a blunt blade when cutting the thin sections: This may drag gonad material and can sometimes give a false impression of migratory nuclei in yolk globule stage oocytes. This problem is usually easy to identify because all the migratory nuclei will be travelling in the same direction and parts of the tissue will have a torn appearance.
- Inadequate preservation: If the gonad samples are not placed in preservative soon enough, if not enough preservative is used, or if the samples are frozen before preservation the resulting histological sections can be easily be misinterpreted. For instance, yolk globule stage oocytes in frozen samples may look hydrated or atretic due to rupture and coalescence of the yolk globules and presence of vacuoles.
- Categorising ovaries based on latest stage oocyte: This is the accepted criteria for assigning stages and is usually appropriate. However, on occasion the latest stage is not representative of the true ovarian state. An example of this is the presence of a hydrated oocyte that for some reason was not ovulated and remains embedded in the tissue despite the fact that the fish is no longer spawning.
- Yellow-brown bodies as an indicator of sexual maturity: These structures are generally more common in the gonads of mature fish due to atresia and breakdown of tissue during and at the end of the reproductive season. However, they can also occur in the gonads of immature fish as a result of tissue breakdown for reasons other than spawning (eg disease and stress).

5.0 Acknowledgements

The assistance of numerous commercial and recreational fishers who assisted in the collection of samples is greatly appreciated. In particular we wish to acknowledge the support of Hayden and Karen Webb, John and Doreen Higgins, Andy Gilchrist, Ron Goodlad, Jeff and Tony Westerberg, Ian Lew and Pam Canney, The KAI Fresh Fish Co crew, Peter, Phil and Shane Moore, Dion Hipper, Barry Paxman, Eric Mustoe, the Mackerel Islands crew, Ian Turner and the King Bay Game Fishing Club, Jamie Waite, and Snooky and the Exmouth Game Fishing Club. We would also like to thank Department of Fisheries staff who came on field trips, including Rod Lenanton, Dan Gaughan, Steve Newman, Justin King, Graeme Baudains, Tim Leary, Ron Mitchell, Craig Skepper, Fiona Webster and Jeff Norriss. The methods included in this Report will be utilised in research on Spanish mackerel in WA waters that is funded by the Fisheries Research and Development Corporation. Thanks to Rod Lenanton, Dan Gaughan, Gary Jackson, Glen Hynde and Glen Young for reviewing the draft manuscript and providing many useful comments.

6.0 References

Collette, B. B. and Nauen, C. E. (1983) FAO Species catalogue. Vol 2: Scombrids of the world. *FAO Fisheries Synopsis* **125,** 137pp.

Crowe, F., Lehre, W., and Lenanton, R. (1999) A study into Western Australia's open access and wetline fisheries. *Fisheries Research Report* **118,** 139pp.

DeMartini, E. E. and Fountain, R. (1981) Ovarian cycling frequency and batch fecundity in the queenfish, *Seriphus politus:* Attributes representative of serial spawning fishes. *Fisheries Bulletin* **79** (3), 547-559.

Donohue, K., Edsall, P., Robins, J., and Tregonning, R. (1982) Exploratory fishing for Spanish mackerel in waters off Western Australia during the period June 16 to October 16, 1981. *Fisheries Research Report* **57,** 46pp.

Ferguson, H.W. (1989). Systemic pathology of fish. Iowa State University Press, Ames. 263 pp.

Food and Agriculture Organisation (2000) *FAO Yearbook 1998 Fishery Statistics. Capture production*. 713pp FAO, Rome.

Garcia-Diaz, M. M., Tuset, V. M., Gonzalez, J. A., and Socorro, J. (1997) Sex and reproductive aspects in *Serranus cabrilla* (Osteichthyes: Serranidae): Macroscopic and histological approaches. *Marine Biology* **127**, 379-386.

Hay, D. E. and Outram, D. N. (1981) Assessing and monitoring maturity and gonad development in Pacific herring. *Canadian Technical Report of Fisheries and Aquatic Sciences* **998**, 31p.

Hilge, V. (1977) On the determination of the stages of gonad ripeness in female bony fishes. *Meeresforsch* **25**, 149-155.

Hunter, J. R and Macewicz, B. J. (1985) Measurement of spawning frequency in multiple spawning fishes. *In*: Lasker, R. (Eds) An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*. 79-94, U.S. Dep. Commer.

Luna, S.M. (2000) Species information sheet-*Scomberomorus*. *commerson*. *In* Froese, R. and Pauly, D. (eds) Fish Base (online) http://www.fishbase.org/Summary/SpeciesSummary.cfm?ID=121&genusname=Scomberomorus&speciesname=commerson. [Date accessed 22/05/2001]

McPherson, G. R. (1992) Assessing macroscopic and histological stageing of yellowfin tuna ovaries in the north-western Coral Sea. *Information Series of the Department of Primary Industries, Queensland* Q192021,19 pp.

McPherson, G. R. (1993) Reproductive biology of the narrow-barred Spanish mackerel (*Scomberomorus commerson* Lacepede, 1800) in Queensland waters. *Asian Fisheries Science* **6**, 169-182.

Ramon, D. and Bartoo, N. (1997) The effects of formalin and freezing on ovaries of albacore, *Thunnus alalunga*. *Fisheries Bulletin* **95**, 869-872.

Samoilys, M. A. and Roelofs, A. (2000) Defining the reproductive biology of a large serranid, *Plectropomus leopardus*. *CRC Reef Research Technical Manual* **31,** 36pp.

Sumner, N. R., Williamson, P. C., and Malseed, B. E. In press. A 12 month survey of coastal recreational fishing in the Gascoyne region of Western Australia during 1998/99. *Fisheries Research Report, Fisheries WA.*

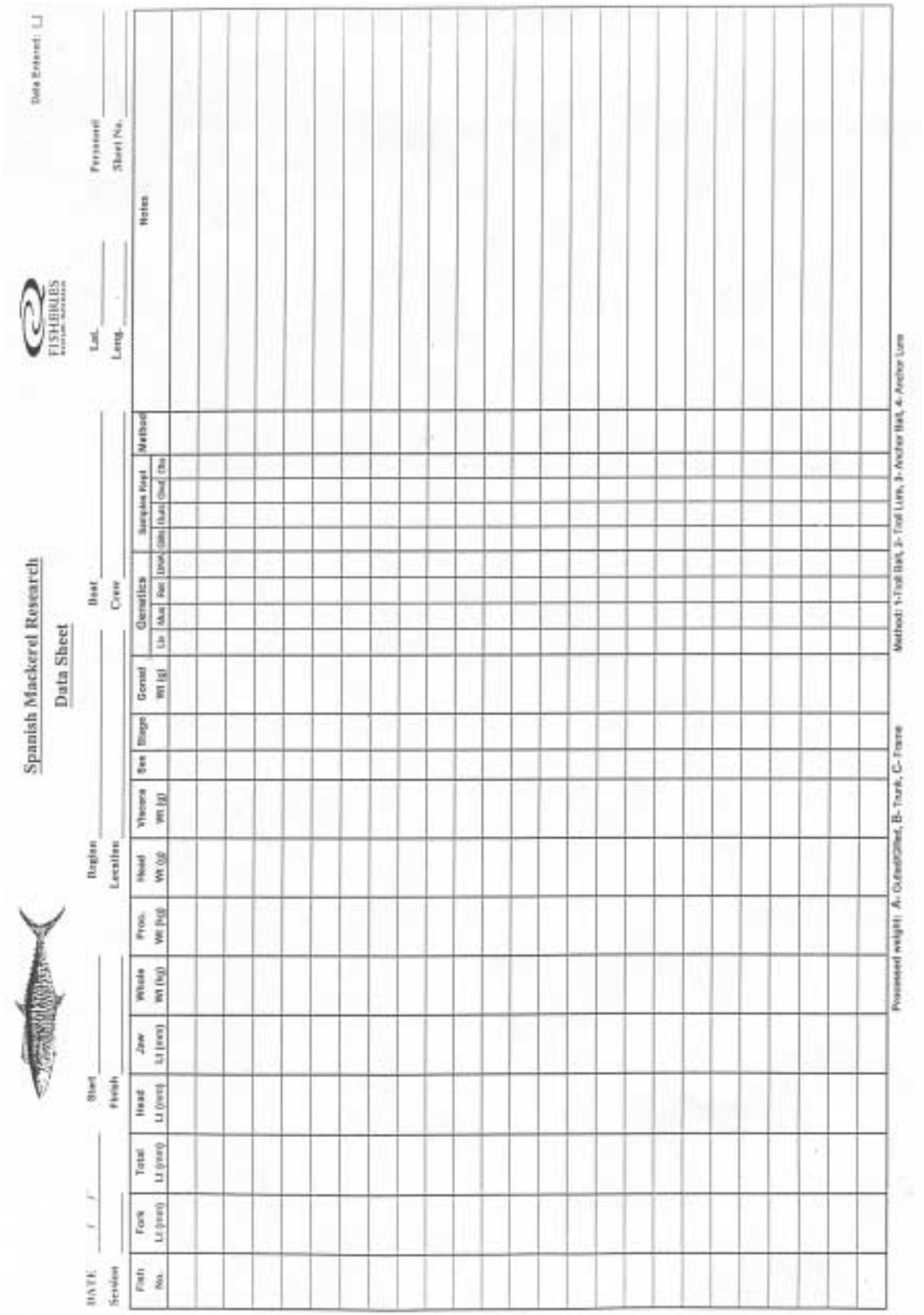
Wallace, R. A. and Selman, K. (1981) Cellular and dynamic aspects of oocyte growth in teleosts. *American Zoology* **21**, 325-343.

West, G. (1990) Methods of assessing ovarian development in fishes: A review. *Australian Journal of Marine and Freshwater Research.* **41**, 192-222.

Yoshida, H. O. (1964) Skipjack tuna spawning in the Marquesas Islands and Tuamotu Archipelago. *Fisheries Bulletin* **65** (2), 479-488.

7.0 Appendices

APPENDIX A. Field sampling sheet used in the study of *S. commerson* biology.



APPENDIX B. Initial macroscopic staging system used in the analysis of *S. commerson* gonads. F = female, M = male.

J (Juvenile)	Gonad is a small, translucent pink ribbon lying imperceptibly along the dorsal wall of the peritoneal cavity. Sex of the fish cannot be determined.
F1 (Virgin/immature)	Ovaries are small and usually translucent pink, apricot or ivory in colour (more opaque and red in unbled fish). In smaller females the ovaries are flattened, flaccid, and relatively inconspicuous, but they become rounded and firmer with a distinct lumen as the fish approaches maturity. The oocytes are microscopic resulting in a smooth, uniform appearance to the ovarian tissue. Yellow-brown bodies are uncommon.
F1a (Immature Developing)	Used to stage ovaries immediately prior to the spawning period which have the features of a virgin (F1) ovary but which also contain cortical alveoli stage oocytes.
F2 (Mature resting)	Soon after completion of spawning activity the resting ovaries appear flaccid with prominent exterior blood vessels. Internally the lumen is large. Few if any oocytes can be seen whilst yellow-brown bodies are distinct (sometimes very common) and blood clots may also be present. As time since spawning increases, the ovaries become progressively rounder and firmer as the gonad wall contracts and thickens and the ovarian tissue develops. Yellow-brown bodies may be evident for sometime and are the main feature used to distinguish mature resting from virgin ovaries. Colour is typically semi-translucent rose, pink or ivory, although in unbled fish the ovaries are often red.
F3 (Developing)	Ovaries may still have a semi-translucent, rose or pinkish colour (red if unbled), but become larger and lose translucency as the oocytes become opaque. This gives the ovarian tissue a slightly speckled or granular appearance. Exterior blood vessels become more prominent during this stage. It is often difficult to distinguish late developing from early developed ovaries.
F4 (Developed)	Early in this stage the ovaries appear semi-translucent and speckled because there are still many translucent immature oocytes. As more oocytes develop and turn opaque the ovaries become large, rotund and opaque with prominent blood vessels. The opaque oocytes are visible through the thin gonad wall and the colour is typically pale yellow or apricot. Towards the end of the reproductive period the ovaries become more bloodied and flaccid as oocyte reserves are depleted during spawning, and yellow brown bodies may become more common and the lumen larger.
F5a (Pre-spawn)	Ovaries are very large and swollen, although towards the end of the reproductive season they may become somewhat flaccid. Colour is apricot to peach with a prominent network of external blood vessels. The presence of translucent hydrated oocytes gives the ovaries a distinctive speckled or granular appearance through the thin gonad wall. However, if the oocytes are still in the migratory nucleus stage of development the ovary may be difficult to distinguish from the developed stage. This stage is distinguished from the next (5b) by the lack of hydrated oocytes within the lumen.

F5b (Spawning)	Running ripe. External appearance is similar to that of the pre-spawning (F5a) ovary, although eggs may be released from the gonoduct when pressure is applied to the abdomen of the fish. This may not be possible if spawning was not imminent, but eggs should still be present within the lumen.
F5c (Post-spawn)	Usually difficult to distinguish from developed ovaries except for a more flaccid appearance. May also be confused with pre-spawning ovaries if unspawned hydrated oocytes are still present.
F6 (Spent)	Difficult to distinguish from developed ovaries late in the reproductive season that are also flaccid and bloodied, with a large lumen and relatively few opaque oocytes present within the ovarian tissue. Yellow-brown bodies may also be common and the external blood vessels prominent. This stage may also be difficult to identify from ovaries that have recently entered the non-spawning period.
M1 (Virgin)	Testes are small and straplike with a smooth appearance and opaque, ivory or bone colour (red if unbled). No milt is present in the transverse section. It is difficult to distinguish testes early in this stage from juvenile gonads, and testes late in this stage from mature resting (M2) testes.
M1a (Immature developing)	Used to stage testes that are small with features of the virgin (M1) testes but which also produce milt when squeezed.
M2 (Mature resting)	Testes are small, opaque and straplike. Little or no milt is extruded from the transverse section when squeezed (unless the sample has been frozen). The section is quite angular in shape, with the central tissue often browner than the bone or ivory coloured peripheral tissue. Sometimes the testes may also be tinged in red.
M3 (Developed)	Testes large, opaque, and ivory or bone in colour. The exterior dorsal blood vessel is large and small blood vessels are usually present. Internally, white sperm (milt) can usually be squeezed from the central sperm sinus. In some cases this may not be possible, although milt should be visible in the outer areas of the transverse section. In the case of the latter, the testes may be misidentified as a resting or virgin stage.
M4 (Spawning)	Running ripe. Similar to the ripe testis but more swollen and with larger exterior blood vessels. Milt is released with little or no pressure on the abdomen or when the testis is cut.

APPENDIX C. Reproductive analysis worksheet used in the study of *S. commerson* biology.

Spanish Mackerel
Preserved Gonad Data Sheet

Date Processed / /

Personnel: ____________

Data Entered: ☐

Histo. No.	Sample	Fish No.	Sex	Histol Stage	Fixed Wt (g)	Hydrat. Eggs Early	Hydrat. Eggs Late	Migrat. Nuc. Early	Migrat. Nuc. Late	P.O.Fs New	P.O.Fs Old	Atretic	Fecund Est?	Fresh Wt (g)	Notes

APPENDIX D. Fecundity analysis worksheet used in the study of *S. commerson* biology.

Spanish Mackerel
Fecundity Data Sheet

Date Processed / /

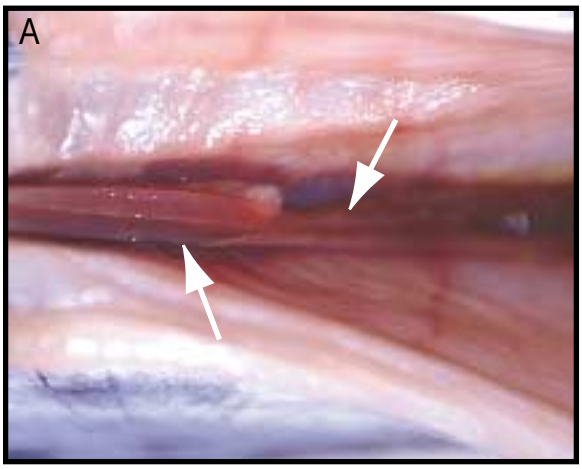
Personnel:

Data Entered: ☐

Sample	Fish	Whole Fixed	Histo	Lobe	Section A (Anterior)		Section B (Middle)		Section C (Posterior)		Notes
	No.	Wt (g)	No.		Wt (g)	Egg Count	Wt (g)	Egg Count	Wt (g)	Egg Count	

Note: Leave gonad drying in sieve for approx. 3 mins then weigh with fume hood turned off

List of Plates



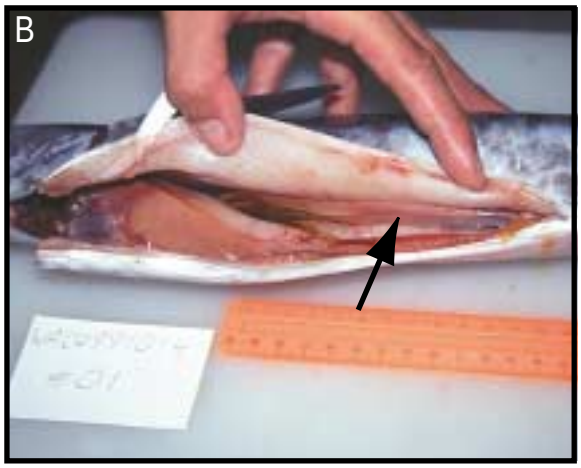


Plate 1. In situ views of a juvenile gonad (A) and an immature ovary (B) (both arrowed), showing the position of the gonad along the dorsal posterior wall of the visceral cavity. Note that viscera have been removed so the gonad can be viewed. Fork lengths of fish: 400 and 580 mm (respectively).

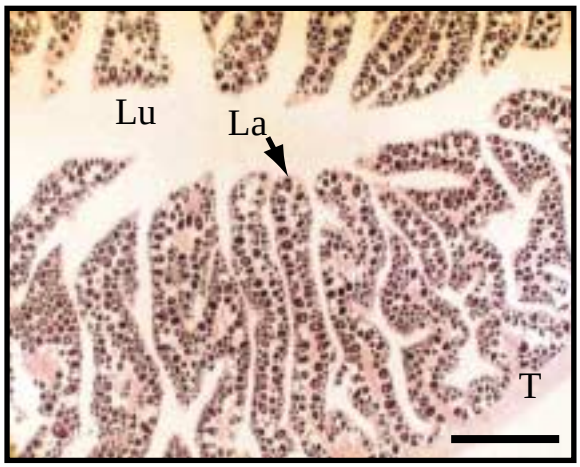


Plate 2. Whole and sectioned views of an immature (F1) ovary. La; lamellae. Lu; lumen. T; tunica. Scale bar = 1 mm. Fork length of fish: 669 mm.

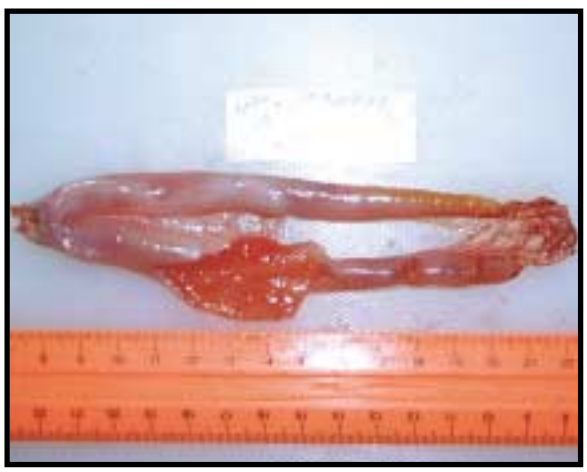


Plate 3. Whole immature (F1) ovaries. Note that the first ovary is the same as shown in Plate 1(B). Fork length of fish: 580 and 826 mm, respectively.

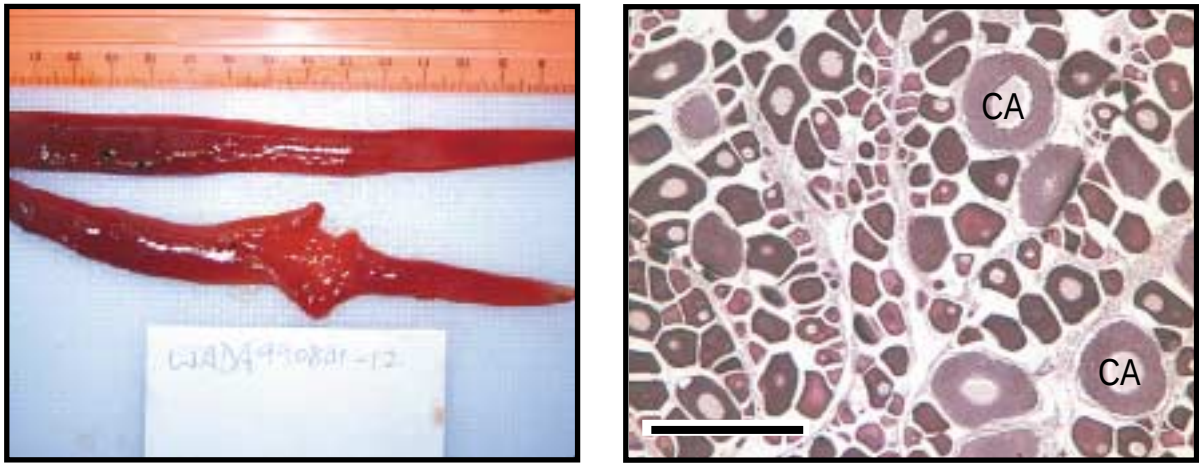


Plate 4. Whole and sectioned views of an ovary classified as F1a because it is probably an immature (F1) ovary in which oocytes have developed into the cortical alveoli stage (CA). The CA stage precedes vitellogenesis and is used to identify ovaries that are showing early signs of reproductive development (F3). It is likely that some F1a ovaries will continue developing and become reproductive whilst others will regress back to the immature (F1) state. Note: this ovary is classified as F2-3 using the simplified macroscopic staging system. Scale bar = 300 mm. Fork length of fish: 884 mm.

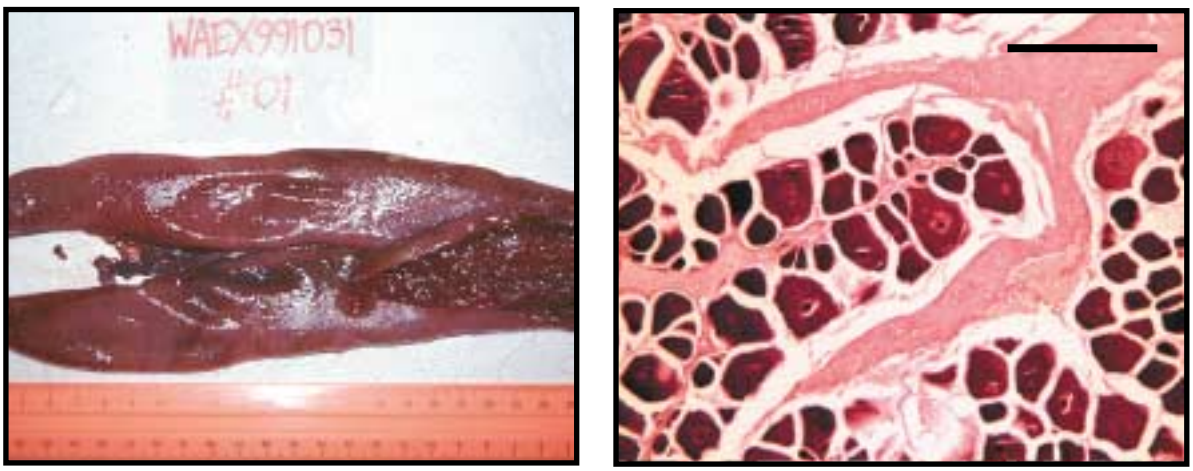


Plate 5. Whole and sectioned views of a mature resting (F2) ovary (F2-3 using the simplified macroscopic staging system). Note the red colour of the whole ovary, which is probably due to the fish not being bled after capture. Scale bar = 300 mm. Fork length of fish: 1500 mm.

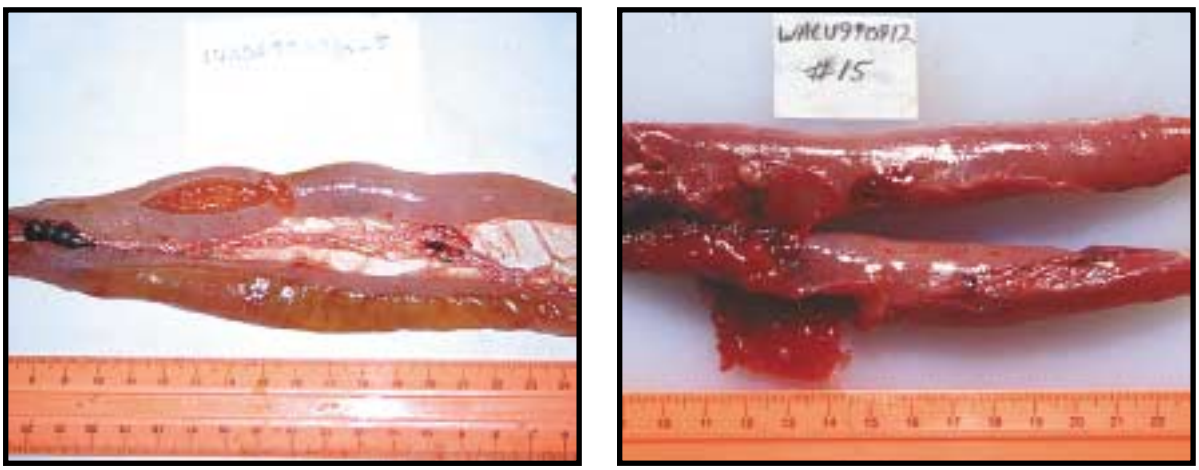


Plate 6. Whole mature resting (F2) ovaries (F2-3 using the simplified macroscopic staging system). Fork length of fish: 1021 and 1077 mm, respectively.

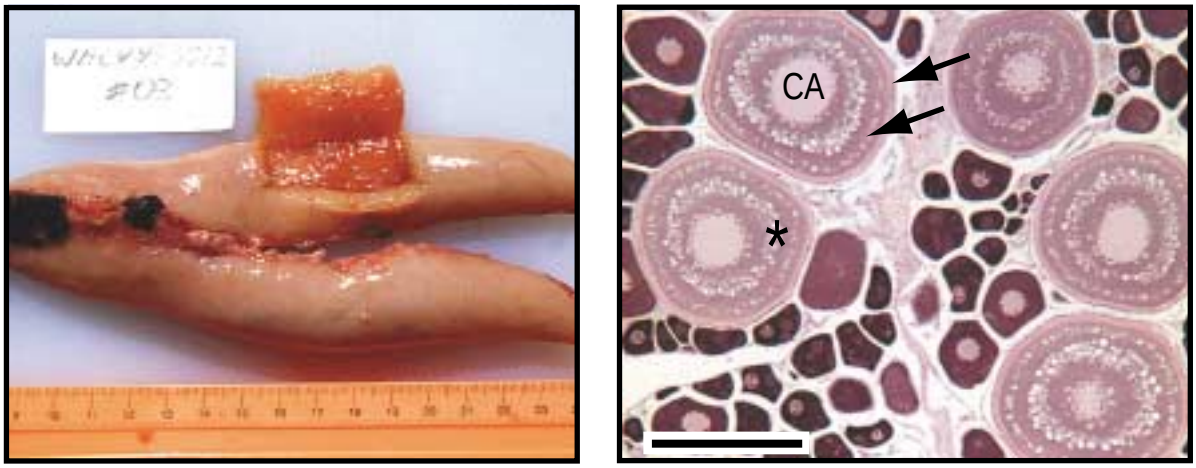


Plate 7. Whole and sectioned views of a late developing (F3) ovary (F2-3 using simplified macroscopic staging system). The cortical alveoli (bottom arrow) and oil droplets (*) are distinct in the cortical alveoli stage oocytes (CA). The zona radiata (top arrow) is also well formed, indicating that these oocytes were soon to develop into the yolk globule stage of development. Once this occurs the ovary is classified as reproductively developed (F4). Scale bar = 300 mm. Fork length of fish: 1012 mm.

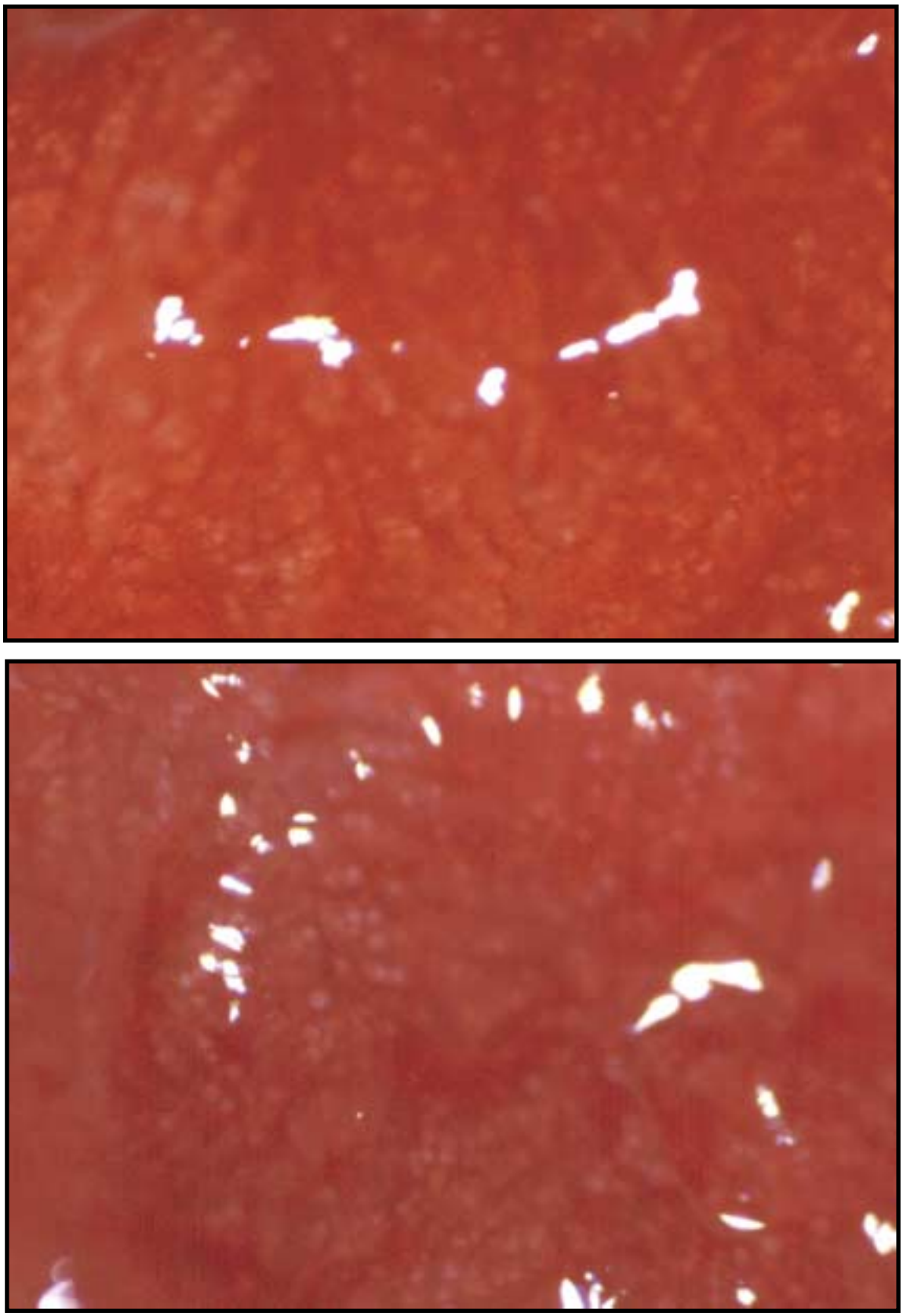


Plate 8. Close-up macroscopic views of developing (F3) ovarian tissue (F2-3 using simplified macroscopic staging system). Note the grainy texture of the tissue due to the presence of partially opaque cortical alveoli stage oocytes within the lamellae. Photographs taken of fresh tissue; in frozen ovaries the appearance of the gonad tissue may be altered due to the rupture of cells, making it difficult to reliably stage some gonads.

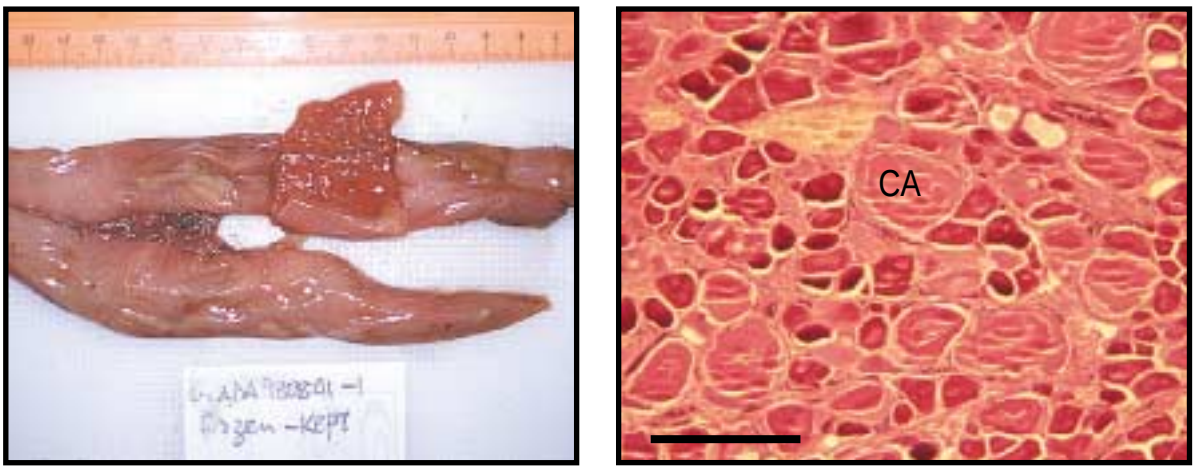


Plate 9. Whole and sectioned views of a developing (F3) ovary that was frozen after capture (F2-3 using the simplified macroscopic staging system). CA; cortical alveoli stage oocyte. Scale bar = 300 mm. Fork length of fish: 1180 mm.

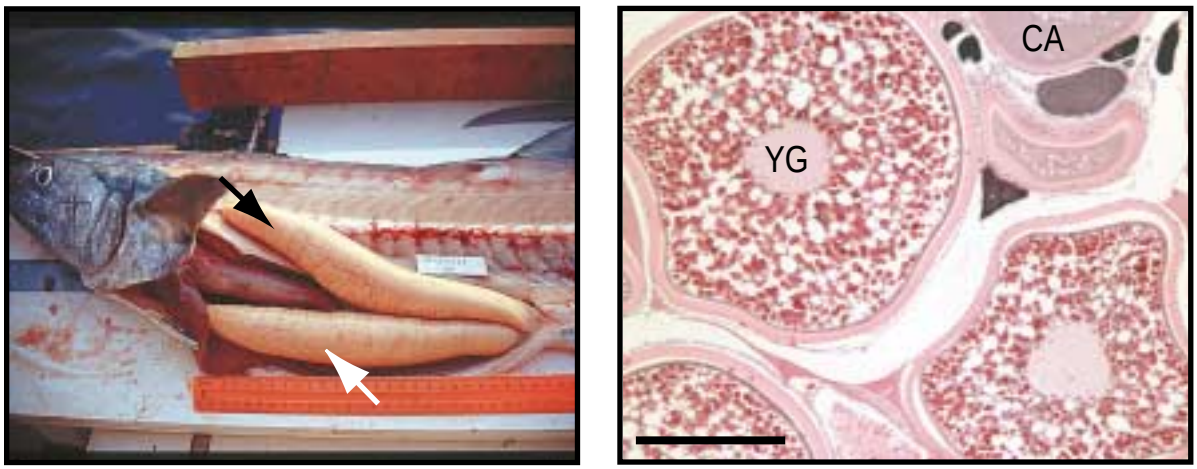


Plate 10. Whole and sectioned views of a fully developed (F4) ovary. The whole ovary is shown in situ (arrows) and fills most of the visceral cavity of the fish (note that the fish has been filleted). The sectioned view shows larger yolk globule stage oocytes (YG) alongside cortical alveoli stage oocytes (CA). Scale bar = 300 mm. Fork length of fish: 1026 mm.

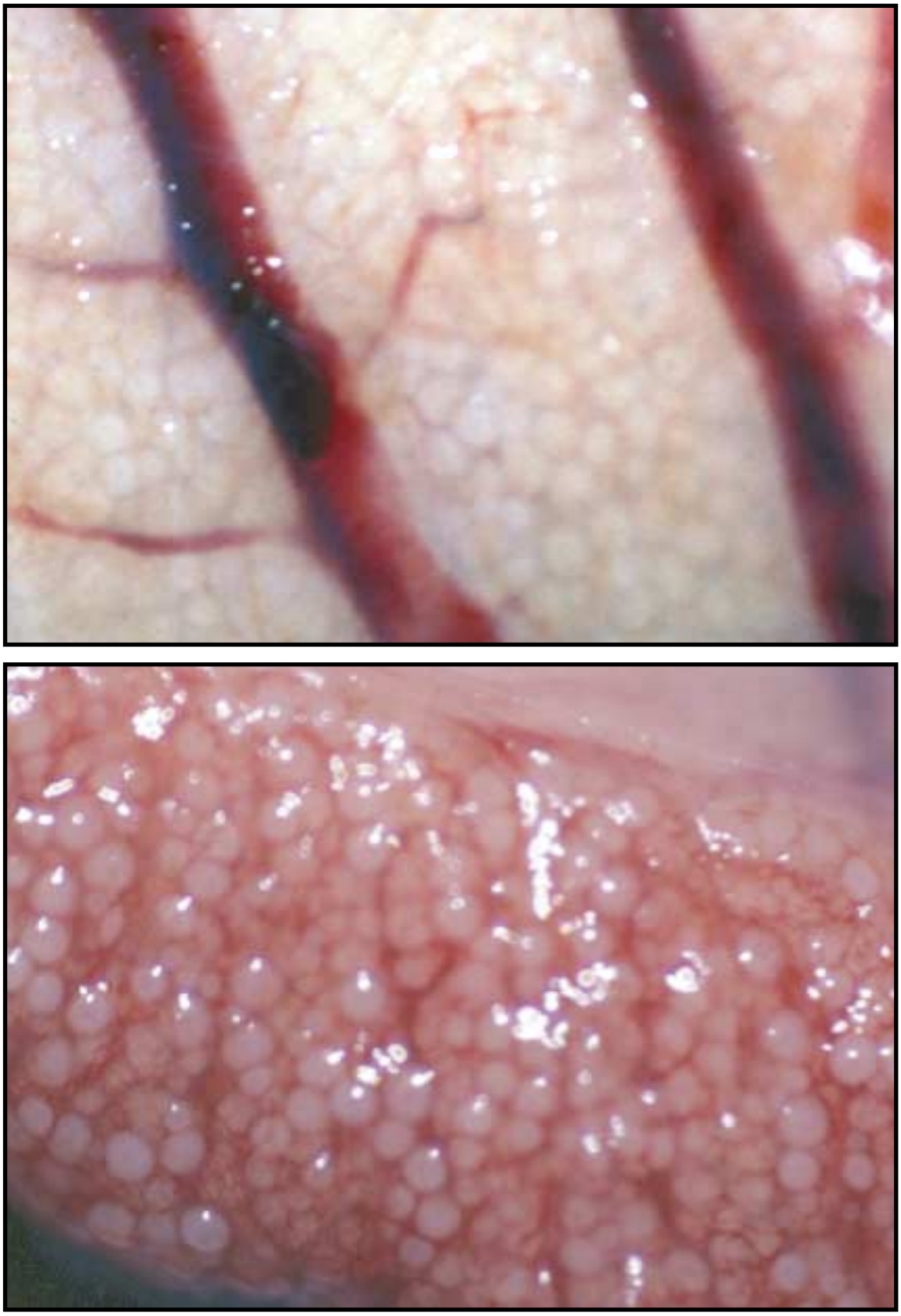


Plate 11. Close-up macroscopic views of developed (F4) ovarian tissue. The top photograph shows blood vessels and opaque yolk globule stage oocytes viewed through the ovarian wall. In the bottom photograph the ovary has been cut to expose the tightly packed yolk globule stage oocytes within the lamellae.

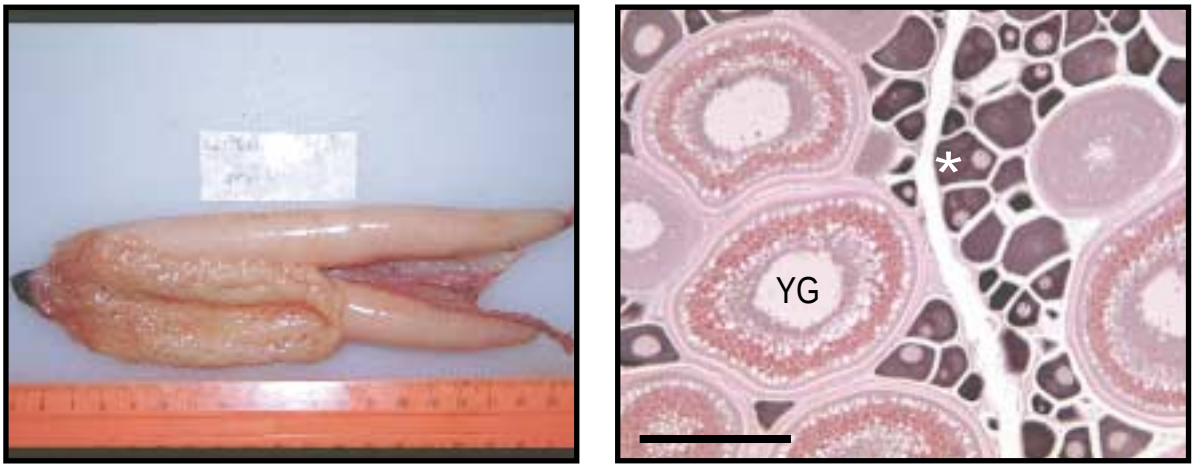


Plate 12. Whole and sectioned views of an early developed (F4) ovary. In the sectioned view the yolk globule stage oocytes (YG) are not fully developed, with relatively small yolk globules that do not yet fill the oocyte. Previtellogenic oocytes such as the one marked with an asterix are also still prominent. Compare this ovary with the fully developed ovary in Plate 10. Scale bar = 300 mm. Fork length of fish: 926 mm.

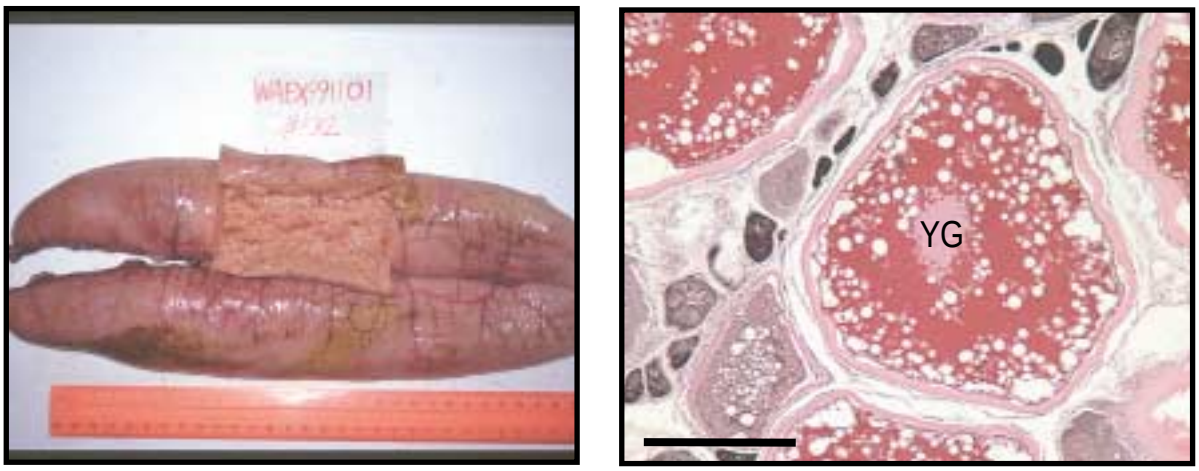


Plate 13. Whole and sectioned views of a developed (F4) ovary that was frozen after capture, causing the yolk globules to rupture and coalesce in the yolk globule stage oocytes (YG). Note that it is often difficult to distinguish the effects of freezing from real processes such as oocyte atresia and final oocyte maturation. Scale bar = 300 mm. Fork length of fish: 1390 mm.

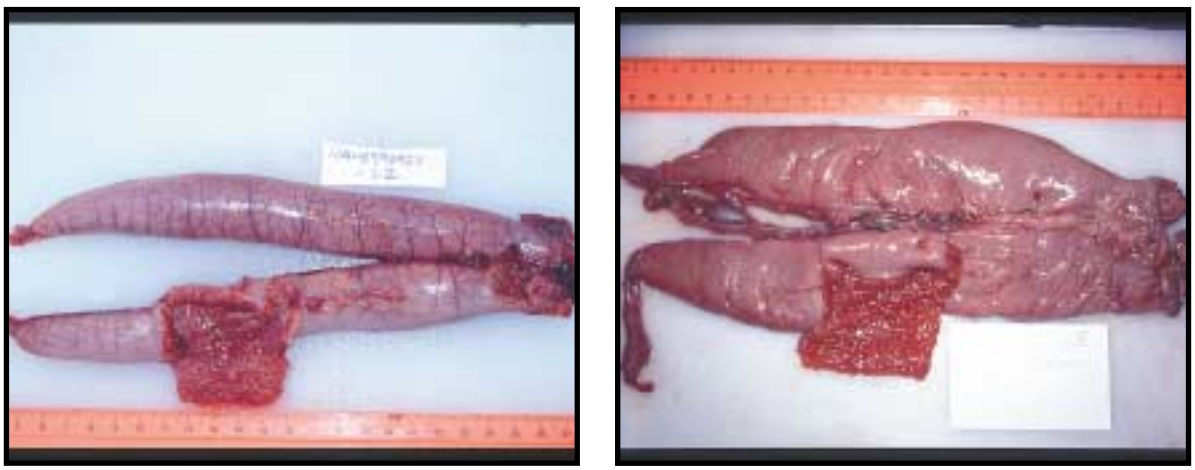


Plate 14. Whole developed (F4) ovaries. The ovary on the left is fresh whilst that on the right has been frozen, giving it a flaccid appearance due to rupture of cells and release of their contents within the tissue. Fork length of fish: 990 and 1500 mm, respectively.

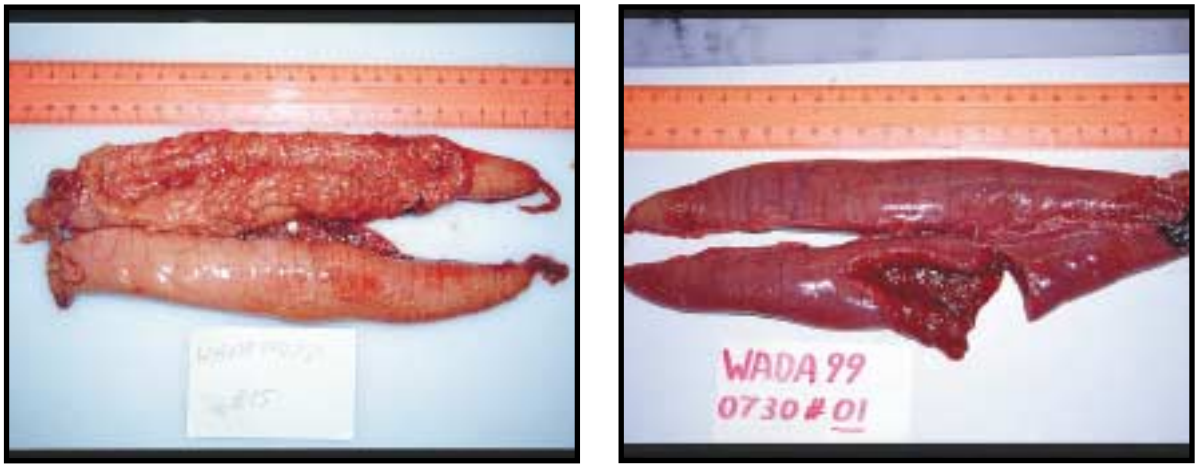


Plate 15. Whole developed (F4) ovaries (both fresh). Note the difference in colour. The ovary on the right is beginning to look somewhat flaccid, indicating that this fish had spawned previously and the ovary was becoming deplete of oocytes. Fork length of fish: 1181and 1213 mm, respectively.

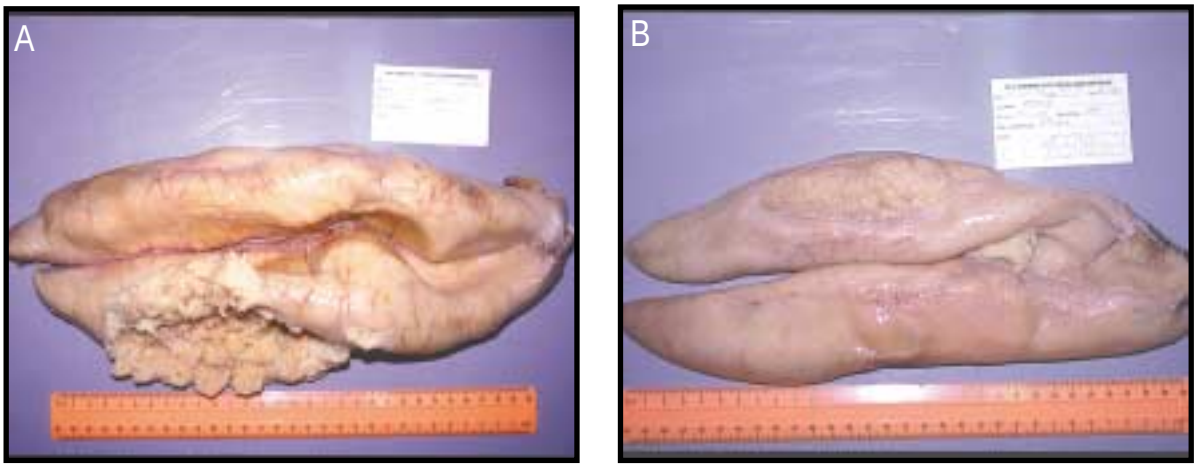


Plate 16. Whole views of (A) a developed (F4) and (B) a pre-spawning (F5a) ovary (F5 using the simplified macroscopic staging system). Both ovaries were frozen after capture.

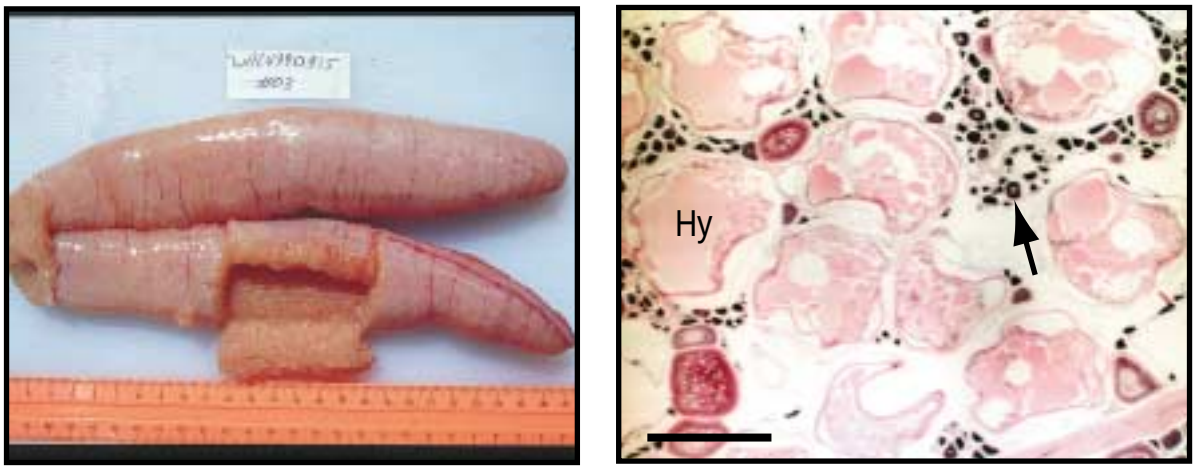


Plate 17. Whole and sectioned views of a pre-spawning (F5a) ovary in which the oocytes have become hydrated (Hy) prior to ovulation and spawning (F5 using the simplified macroscopic staging system). Note the size of the hydrated oocyte in comparison to the previtellogenic oocytes (arrow). Scale bar = 1mm. Fork length of fish: 1063 mm.

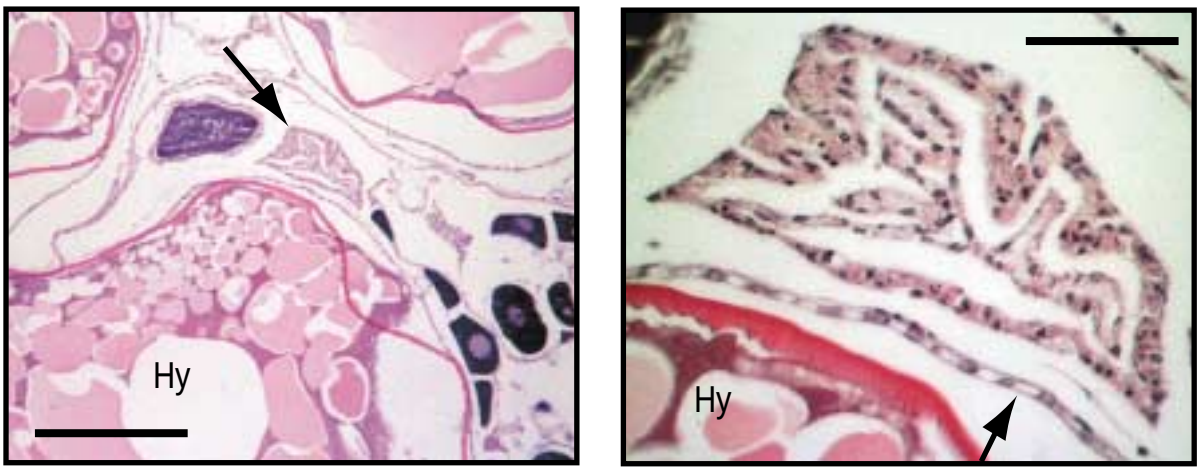


Plate 18. Sectioned views of the ovary in Plate 17. The left photograph shows a hydrated oocyte (Hy) with a large clear staining oil droplet. The yolk globules have not fully coalesced to produce a uniform yolk mass. A 'late' stage post-ovulatory follicle is arrowed, and is shown in magnified view in the right photograph. Note the granulosa cell layer (bottom arrow) that has become detached from the hydrated egg in the bottom left hand corner of the right photograph. When the oocyte is released into the lumen at ovulation this follicle (comprising the granulosa and thecal cell layers) will be left behind as the post- ovulatory follicle. Scale bars: left = 300 mm, right = 60 mm.

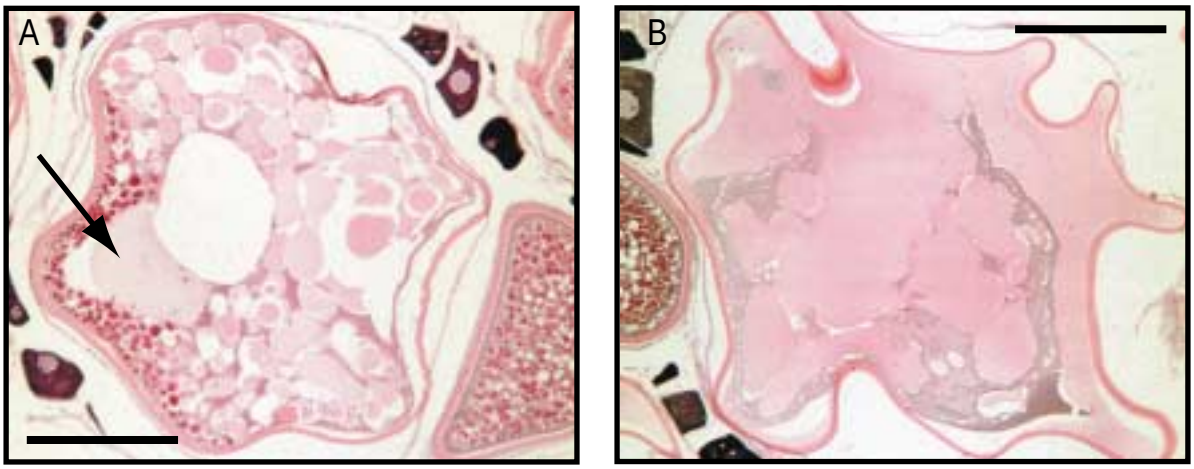


Plate 19. Final maturation of the oocyte prior to spawning. (A) Migratory nucleus stage in which the nucleus (arrowed) has moved to the periphery of the oocyte prior to it breaking down. The large oil droplet is evident and some yolk globules are still intact. (B) Hydrated stage showing the fairly uniform yolk mass which stains pale pink but is translucent when viewed macroscopically. Scale bars in both photographs = 300 mm.

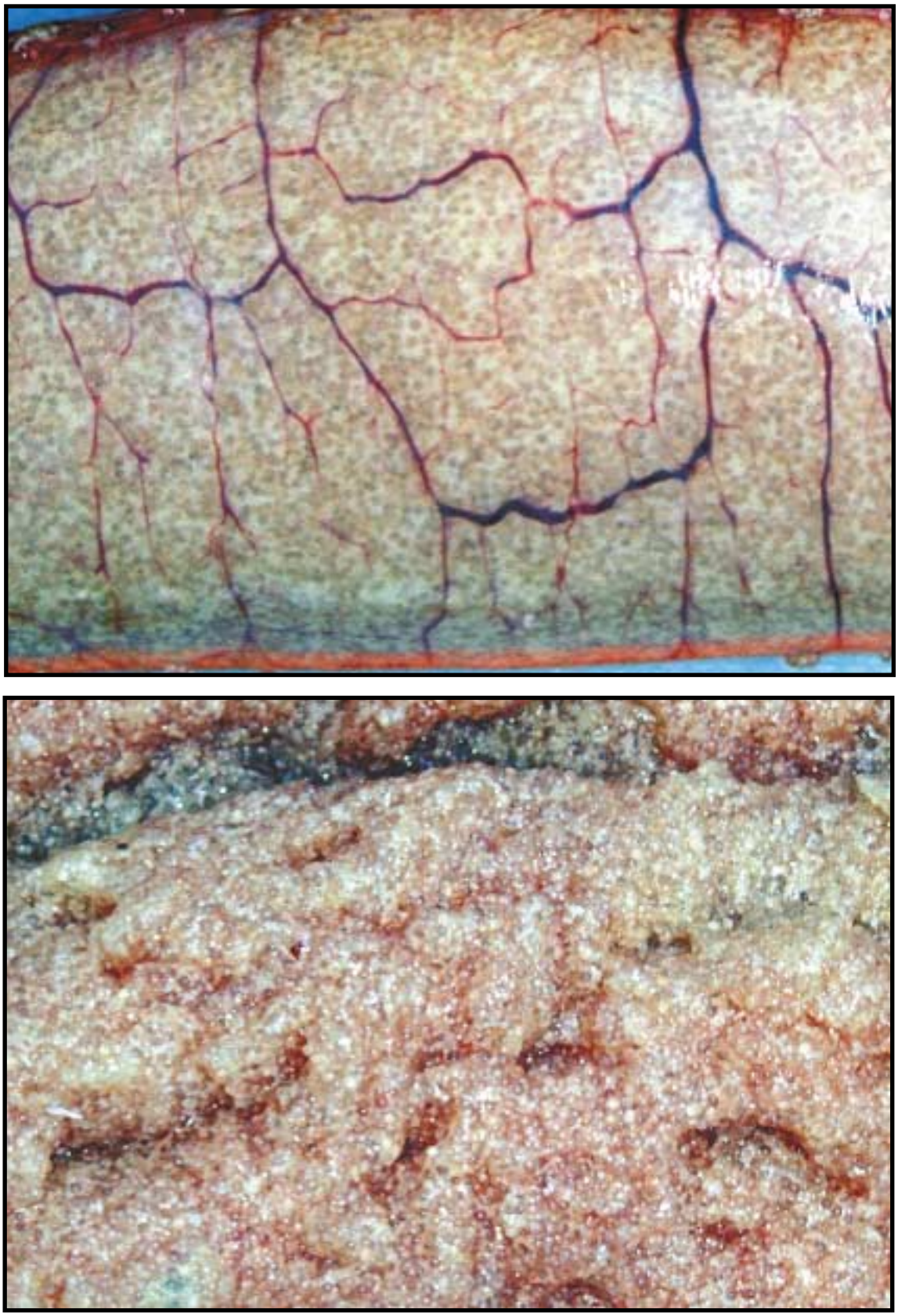


Plate 20. Close-up macroscopic views of a pre-spawning (F5a) ovary (F5 using the simplified macroscopic staging system). Blood vessels and translucent hydrated oocytes are clearly visible through the gonad wall in the top photograph. The ovarian tissue has been exposed in the bottom photograph to show the irregular texture of the tissue due to the mix of translucent hydrated and opaque yolk globule stage oocytes.

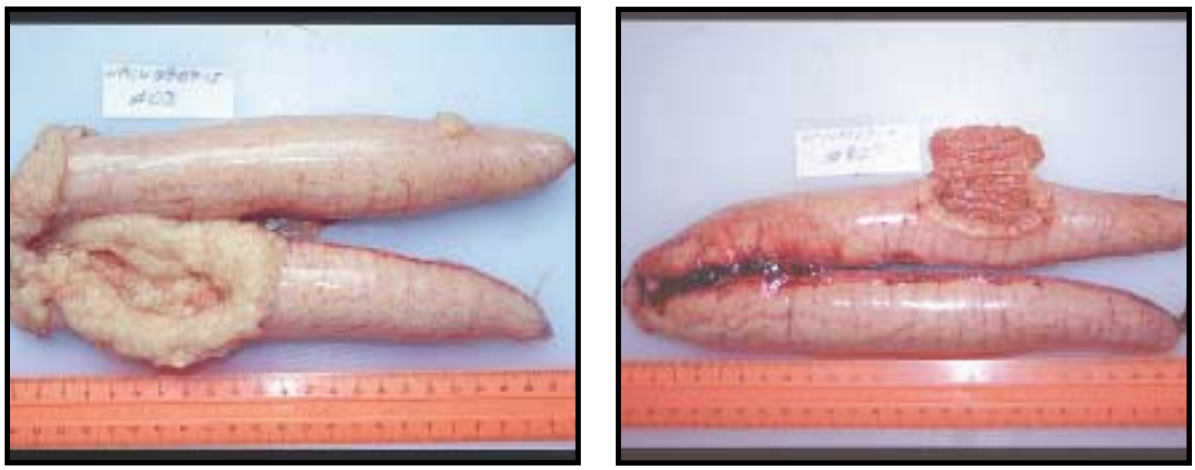


Plate 21. Whole views of pre-spawning (F5a) ovaries (F5 using the simplified macroscopic staging system). Fork length of fish: 1027 and 1112 mm, respectively.

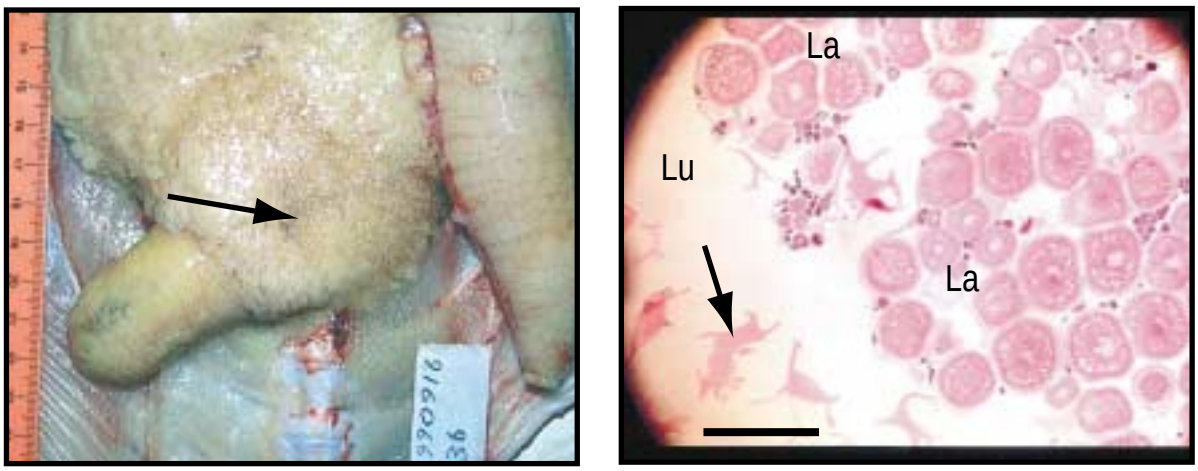


Plate 22. Macroscopic and sectioned views of a spawning (F5b) ovary (F5 using the simplified macroscopic staging system). Hydrated oocytes fill the ovarian lumen (arrows) and readily flow out of the ovary when cut (more easily seen in Plate 24). The sectioned view shows the hydrated oocytes within the lumen (Lu) whilst yolk globule and earlier stage oocytes remain within the lamellae (La). The ovulated oocyte is termed an egg. Scale bar = 1 mm. Fork length of fish: 1011 mm.

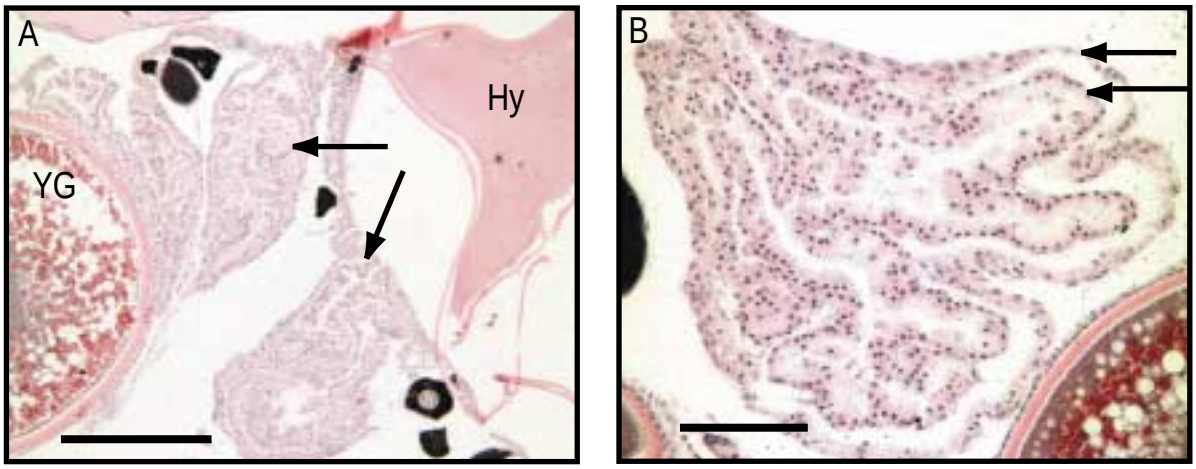


Plate 23. Section views of the spawning (F5b) ovary shown in Plate 22. (A) A hydrated oocyte (Hy) in the lumen of the ovary adjacent to 'new' post-ovulatory follicles (arrows) that remain embedded in the tissue at the edge of the lamella. A yolk globule stage oocyte (YG) is also present in the tissue. Scale bar = 300 mm. (B) A 'new' post-ovulatory follicle showing the outer thecal (top arrow) and inner granulosa (bottom arrow) cell layers. Note the linear arrangement of cells and convoluted appearance of the post-ovulatory follicle soon after spawning (compare with the 'old' post-ovulatory follicle shown in Plate 17). Scale bar = 100 mm.

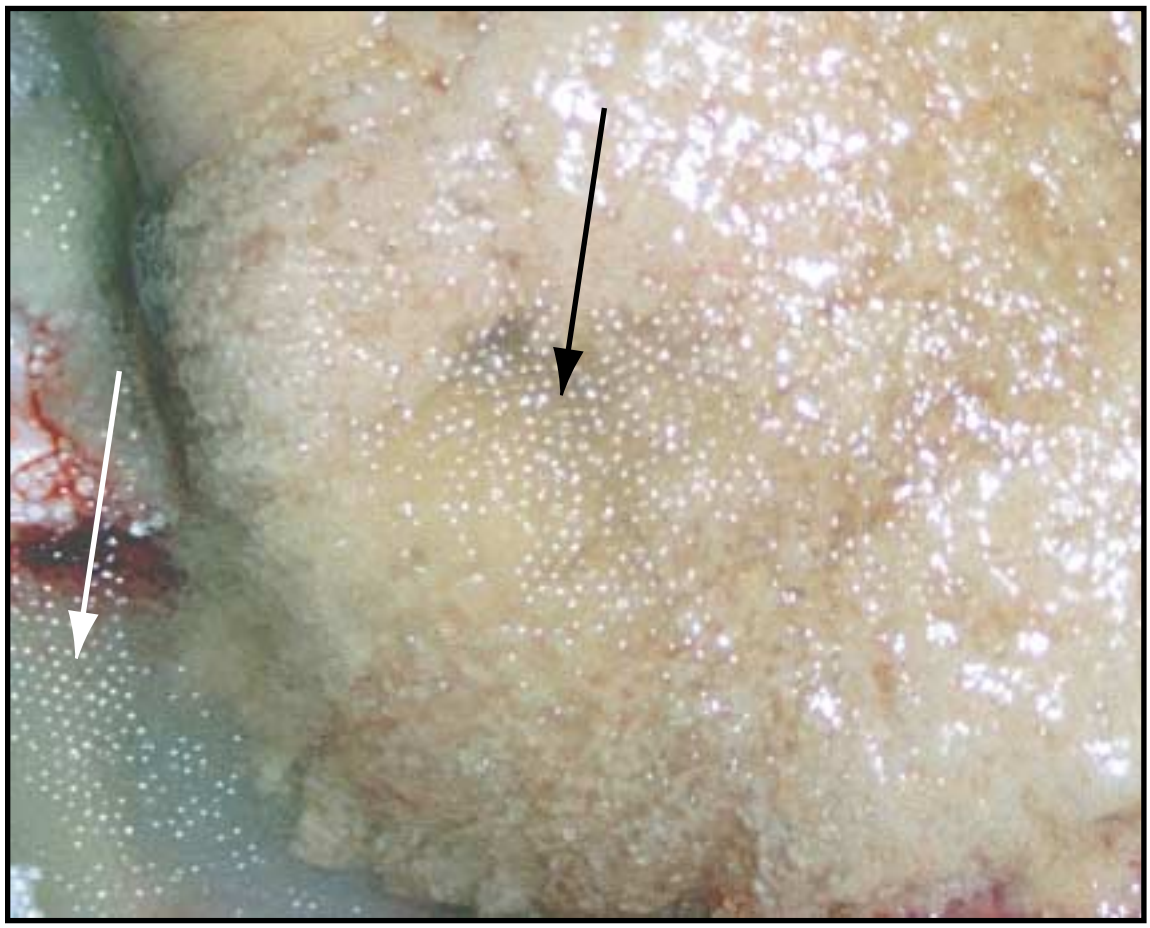


Plate 24. Close-up macroscopic view of the spawning (F5b) ovary shown in Plate 22 (F5 using the simplified macroscopic staging system). Arrows point to hydrated oocytes flowing out of the lumen.