Fisheries Research Report No. 184, 2009

Macroscopic and microscopic description of snapper (*Pagrus auratus*) gonads from Shark Bay, Western Australia

M. Mackie, G. Jackson, N. Tapp, J. Norriss, and A. Thomson

Government of **Western Australia**
Department of **Fisheries**

Fisheries Research Division
Western Australian Fisheries and Marine Research Laboratories
PO Box 20 NORTH BEACH, Western Australia 6920

Fish for the future

Fisheries Research Reports

Titles in the Fisheries Research Report series present technical and scientific information for use in management processes. Research Reports are subject to full internal refereeing by senior scientists of the Fisheries Research Division, and in many cases, elements of the work are published in international scientific literature.

Correct citation:

Mackie, M., Jackson, G., Tapp, N., Norriss, J. and Thomson, A. 2009. Macroscopic and microscopic description of snapper (*Pagrus auratus*) gonads from Shark Bay, Western Australia. Fisheries Research Report No. 184. Department of Fisheries, Western Australia. 35 p.

Enquiries:

WA Fisheries and Marine Research Laboratories, PO Box 20, North Beach, WA 6920
Tel: +61 8 9203 0111
Email: library@fish.wa.gov.au
Website: www.fish.wa.gov.au
ABN: 55 689 794 771

A complete list of Fisheries Research Reports is available online at **www.fish.wa.gov.au**

ISSN: 1035 - 4549 ISBN: 1 921258 38 1

Contents

Macroscopic and microscopic description of snapper (*Pagrus auratus*) gonads from Shark Bay, Western Australia

Michael Mackie, Gary Jackson, Nadia Tapp, Jeff Norriss and Adrian Thomson

Western Australian Fisheries and Marine Research Laboratories
Hillarys Boat Harbour
PO Box 20
North Beach WA 6920

Abstract

Since 1997, research on snapper (*Pagrus auratus*, Sparidae) in the inner gulfs of Shark Bay has focussed on providing biological information required for the management of the regionally-important recreational fishery. A major component of this research has been a comprehensive investigation of snapper reproductive biology to determine size and age at maturity, the timing of the spawning season and to estimate batch fecundity. This research has necessitated a review of the macroscopic staging system used for *P. auratus* in Western Australian waters and the development of a more detailed microscopic system to allow accurate analyses of histological material. Based on *P. auratus* gonads collected from the Eastern Gulf, Denham Sound and Freycinet Estuary areas of Shark Bay between 1998 and 2003, the objectives here were to (i), produce an illustrated, relevant macroscopic staging system for *P. auratus* gonads that can be used in the future by personnel who have minimal experience in this area of research (ii), develop a microscopic (histological) staging system that will allow more detailed analysis of spawning activity by female *P. auratus* and (iii), use the understanding of cycles in spawning activity and results of histological analysis to improve the accuracy of batch fecundity estimates.

1.0 Introduction

Snapper, *Pagrus auratus* (Sparidae), is a key target species for commercial and recreational fishers throughout its distribution in temperate coastal waters of Western Australia (WA). The species is particularly abundant in the Shark Bay region (Figure 1), where an 'oceanic' stock found in continental shelf waters outside the Bay supports a major commercial fishery and three separate stocks in the inner gulfs ('Eastern Gulf', 'Denham Sound', 'Freycinet Estuary') are the basis of an important boat-based recreational fishery (Jackson *et al.* 2007). Snapper in oceanic waters were comprehensively studied during the 1980s and 1990s (Moran *et al.* 2005), whereas research on snapper in the inner gulfs commenced more recently (1997), in response to concerns of over-exploitation of spawning stocks (Jackson *et al.* 2005).

Research in the inner gulfs has focussed on providing biological information required for the management of the recreational *P. auratus* fishery including reproductive data to determine size and age at maturity, timing of the spawning season, and estimates of batch fecundity used to estimate spawning biomass based on the daily egg production method (DEPM, Jackson and Cheng 2001, Jackson 2007). The DEPM was originally developed for schooling pelagic species as a means of estimating spawning biomass from daily egg production (eggs produced each day per unit area) and the average batch fecundity of spawning females (Lasker 1985, Alheit 1993).

This research has required a renewed focus on the reproductive biology of *P. auratus* in Shark Bay. More recent research on the species elsewhere along the WA coast has also focussed on reproductive biology (Wakefield 2006, Lenanton *et al*. 2008). These studies have involved a considerable number of personnel who typically learn 'on the job' the fundamental skills of staging *P. auratus* gonads, either macroscopically ('by eye') whilst in the field, or by microscope (using histological techniques) in the laboratory. This is typical in fisheries biological research because there are few dedicated university courses and/or limited literature on such topics. As a consequence the reliability of gonad staging is often questionable. This is particularly the case for macroscopic staging (West 1990), a frequently used technique that is rarely validated for accuracy. Nevertheless it can be important for sampling purposes and analyses – for instance in determining peak spawning times when DEPM egg surveys should be conducted, and in identification of spawning ovaries to be used for fecundity estimates. However, because the biological evidence of gonad maturation can be minute and the general appearance misleading, the only way to ensure the accuracy of macroscopic stages is by using microscopic examination of histologically prepared gonad samples. The macroscopic staging system that has been used in the reproductive analyses of *P. auratus* in WA up to now has been based on that of Crossland (1977a, Appendix 1). Although this staging system is appropriate for the description of reproductive season and size/age at maturity, it lacks the level of detail required to distinguish between ovaries that are suitable for estimating batch fecundity and those which are not. Nor does it permit more detailed analyses of reproductive activity to investigate for example, the frequency and timing of spawning. This information is also important for determining the timing of sampling for the DEPM, enables more detailed description of the reproductive biology and, ultimately, leads to better scientific advice to fishery managers.

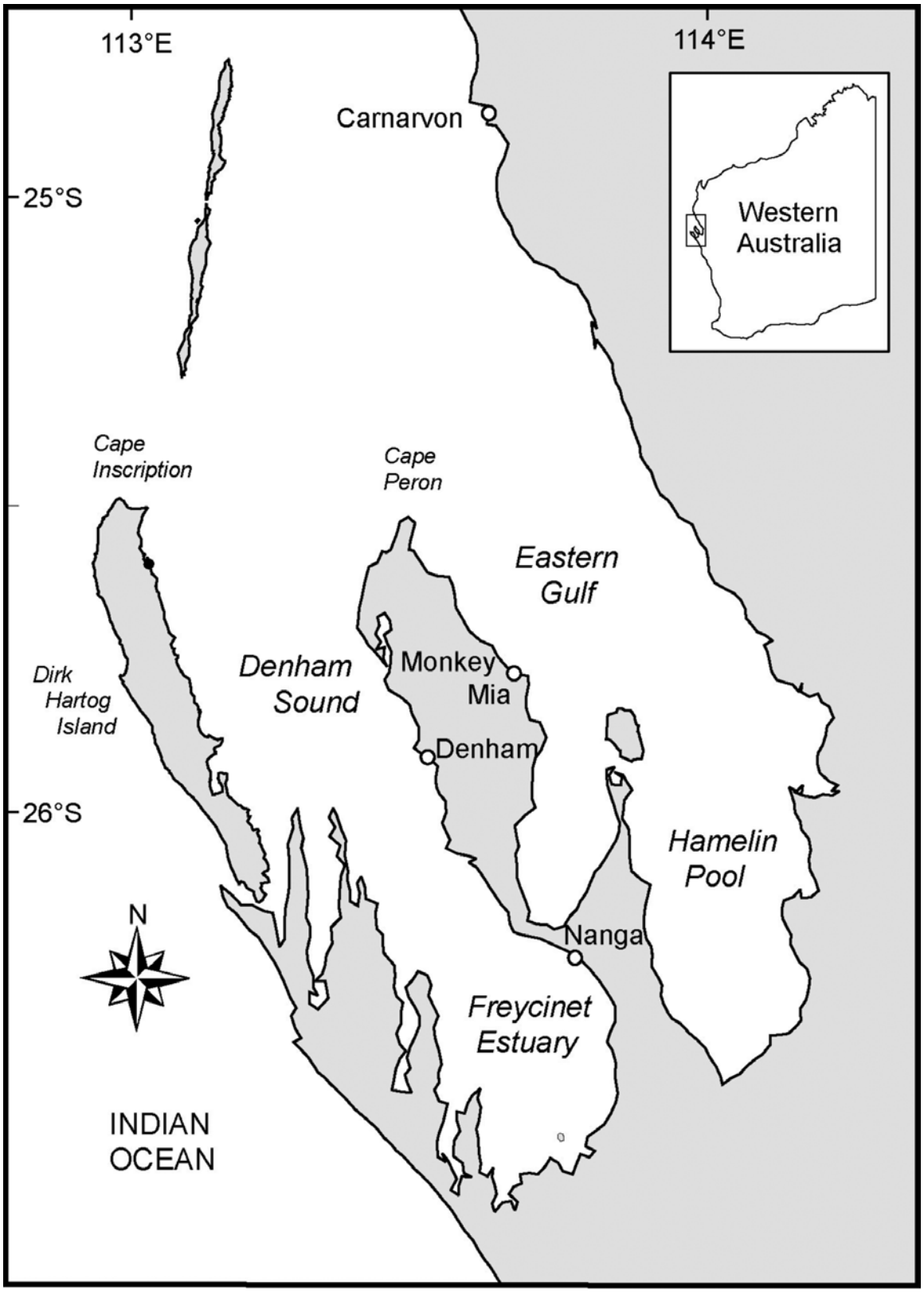


Figure 1. Location of Shark Bay, Western Australia, showing the inner gulf waters where *Pagrus auratus* were collected and gonad samples obtained between 1998-2003. Denham Sound and the Freycinet Estuary are collectively referred to as the Western Gulf. Hamelin Pool is a Class A Marine Reserve where fishing is not permitted.

The DEPM-based assessments of *P. auratus* in the inner gulfs necessitated modification and improvement of the macroscopic and microscopic gonad staging systems that had previously been used by the Department of Fisheries, Western Australia, (DoFWA) for this species. The aims of this report are to:

(i) Provide an illustrated, relevant macroscopic staging system for *P. auratus* gonads that can be reliably used by personnel who have minimal experience in this field of research;

(ii) Provide a microscopic (histological) staging system that will allow more detailed analysis of spawning activity by female *P. auratus* and thereby improve the accuracy of batch fecundity estimates.

In both cases, the modified staging systems needed to remain consistent with each other and with that used previously for *P. auratus* in the Shark Bay region. The study focussed on ovaries since these are more reliably staged than are testes and usually define the spawning season and number of offspring that are produced during spawning (DeMartini and Fountain 1981).

Although individual *P. auratus* undergo prematurational protandrous (male to female) sex-change (Francis and Pankhurst 1988), they are considered gonochoristic (non-sex changing) for the purpose of fisheries assessments. Testicular stages of development are also provided in this report, although the accuracy of these is not assessed here.

In addition the report aims to:

(iii) Detail the methods used in the collection and processing of batch fecundity samples and assess improvements to the batch fecundity – body length relationship that resulted from using the more detailed microscopic (histological) staging system compared with the macroscopic staging system alone.

2.0 Methods

2.1 Collection of samples

Samples of *P. auratus* were collected from various locations within the Eastern Gulf, Denham Sound and Freycinet Estuary areas of inner Shark Bay between 1998 and 2003 (Figure 1, Table 1). These samples contained a disproportionate number of fish in spawning condition because fish were mostly obtained during DEPM surveys conducted between May and September. These fish were mostly caught by hook and line from small recreational vessels (6 – 8 m), bled and placed in an ice-slurry onboard. Filleted carcases (frames) of recreationally caught snapper were also obtained from fishers at the main boat ramps (Monkey Mia, Denham and Nanga). At the end of each fishing day the fork length (FL, mm) of each fish was measured. The gonads were removed, weighed (0.01 g), sexed and staged using macroscopic criteria. A selection of ovaries were then photographed and fixed in 10% formalin and seawater for subsequent histological examination.

Table 1. The number of *P. auratus* ovaries used in this study, obtained from the inner gulfs of Shark Bay between 1998 and 2003. Macro = macroscopically-staged ovaries. Histo = histologically-staged ovaries.

	1998		1999		2000		2001		2002		2003	
	Macro	Histo	Macro	Histo	Macro	Histo	Macro	Histo	Macro	Histo	Macro	Histo
Eastern Gulf	199	139	120	113	187	95	153	102	72	67	94	92
Denham Sound	206	40	125	56	177	65	126	72	7	4	74	71
Freycinet Estuary	122	69	167	85	123	62	137	111	78	78	–	–

2.2 Processing of samples

In the laboratory, a portion from the mid-region (approximately 3 mm thick) of one ovarian lobe was embedded in paraffin wax, cut into 7-8 µm transverse sections and stained using Harris's haematoxylin and eosin (H&E).

2.2.1 Gonad staging systems

The staging systems used to describe sexual development of *P. auratus* from Shark Bay were based on the macroscopic staging system described by Crossland (1977a, Appendix 1). This system has historically been used to stage *P. auratus* gonads from WA waters (M. Moran, DoFWA, pers. comm.). Both macro and microscopic staging systems were subsequently refined to allow more detailed analysis of spawning activity (Appendix 2). This revised staging system has been used to stage *P. auratus* gonads from inner Shark Bay since 1998. Microscopic staging of ovaries is based on the developmental stage of oocytes, as described below for *P. auratus* using the nomenclature of Wallace and Sellman (1981) and West (1990) (refer to Plates 12, 13, 19, 21 for examples):

I. Pre-vitellogenic oocyte growth

Chromatin-Nucleolus Stage: oocyte diameter range 10 – 60 µm (mean = 35 µm). The cytoplasm is 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: oocyte diameter range 40 – 110 µm (mean = 75 µm). Thin follicular layer and irregular shape (spherical to elongate and often angular). The cytoplasm is 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: oocyte diameter range 55 – 295 µm (mean = 175 µm). 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 were therefore used to identify the developing ovarian stage.

II. Vitellogenic oocyte growth

Yolk Globule Stage: oocyte diameter range 215 – 475 µm (mean = 345 µm). 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: oocyte diameter range 285 – 500 µm (mean 390 µm) during the migratory nucleus stage, 370 µm - 740 µm (mean 555 µm) 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.

2.2.2 Batch fecundity

Batch fecundity was estimated gravimetrically using the hydrated-oocyte method (Hunter *et al.* 1985). Formalin-preserved, macroscopically staged ovaries from spawning females were drained in a sieve (mesh size 0.5 mm) and weighed to the nearest 0.1 g. Zeldis and Francis (1998) have shown that estimates of batch fecundity for *P. auratus* are not dependent upon which part of the ovary the tissue sample is taken from. In this study therefore, three transverse sections (typically 30-70 mg each) were randomly taken from the mid-region of each ovary, *i.e.* two from one lobe and one from the other. These sections were weighed (nearest 0.1 mg), placed on a microscope slide and covered in glycerine. Oocytes were loosened after 10-15 minutes by gently tapping the tissue section with the blunt tip of a pair of forceps, after which 3-4 more drops of glycerine were added, and the sample spread over the slide. Hydrated oocytes in each section were identifiable by their relatively large size and transparent, wrinkled appearance. Those that were either whole or, if damaged, more than 50% present, were counted using a dissecting microscope (x 10 magnification). Batch fecundity for each individual female was then calculated by multiplying the mean number of hydrated oocytes per gram of ovary section by the total weight of both ovaries.

A transverse section of approximately 3 mm thickness was also removed from the central region of the same ovaries used for estimating batch fecundity. This section was processed using histological techniques to confirm whether the ovary was in the 'pre-spawning', 'spawning' or 'post-spawning' stage (see Table 5). Ovaries in 'pre-spawning' condition with fully hydrated oocytes were deemed suitable for batch fecundity estimation. However, 'pre-spawning' ovaries

with migratory nucleus stage oocytes may provide biased estimates of batch fecundity because of similarities between these and yolk-globule stage oocytes. Similarly, estimates of batch fecundity from 'spawning' and 'post-spawning' ovaries may also be biased if eggs have already been ovulated (Mackie *et al.* 2005).

Crossland (1977b) showed the relationship between female body length and fecundity in *P. auratus* from New Zealand to be positive and non-linear. The relationships between fork length and batch fecundity for females from the Eastern Gulf, Denham Sound and Freycinet Estuary were described by fitting non-linear regression models to these data. The relationship between batch fecundity and fork length for both macroscopically and microscopically staged ovaries were compared amongst sampling areas and sampling years (Jackson 2007).

2.2.3 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 distinguishable structures, especially since they are usually quite common when present. At moderate temperatures (13-19° C), POFs may be distinguishable for up to two days after spawning (Hunter and Macewicz 1985). POFs present in the ovaries of *P. auratus* 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 recent spawning history.

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

Category	Description
New	(Plate 25) 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 26) 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 helps with confirmation.

2.2.4 Oocyte atresia

Ovaries were categorised according to the amount 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 (Table 3 Category 3). Hunter and Macewicz (1985) found that the probability of spawning in anchovy was very low when more than 50% of the advanced oocytes were atretic.

Table 3. Definitions used to categorise *P. auratus* ovaries according to the relative percentage of atresia among yolk globule stage (YGS) oocytes.

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

3.0 Results and Discussion

The gonads of male and female *P. auratus* are typically bi-lobed and elongate. Lobes are joined posteriorly to form a short gonoduct leading to the urogenital pore. Germ tissue is bound by a muscular wall and tunica, and suspended from the dorsal posterior wall of the body cavity by mesenteries. In ovaries, the oocytes develop within lamellae attached to the gonad wall. Ovulated eggs are shed into a lumen extending the length of the ovary and during spawning are released externally via the gonoduct. Sperm develop in crypts and are released into peripheral sperm sinuses that open into a central sperm sinus. From there the sperm are released into the gonoduct during spawning.

3.1 Macroscopic staging system

3.1.1 Accuracy of macroscopic stages

Using microscopic stages as the 'true' reproductive stage, the accuracy of macroscopic stages assigned to *P. auratus* ovary samples from 1998 to 2003 (pooled) varied from 18.2% for F6 (spent) ovaries to 96.0% for F5 (spawning) ovaries (Figure 2). A breakdown of the macroscopic staging data (Figure 3) shows that:

- 70.4% of **immature** (F1) ovaries (Plates 1 and 2) were correctly identified macroscopically. The remaining 29.6% of ovaries classified into this stage were actually mature resting (F2) or developing (F3) ovaries. Errors occur when distinguishing between these three stages because of similarities in the external appearance of ovaries that have never spawned and ovaries that have spawned, but not for sometime, and do not yet show macroscopic evidence of development towards the next spawning season. During this period the ovaries of each stage are typically small, compact and translucent, with minimal vascularisation. As described below, developing ovaries should soon be distinguishable from immature and mature resting stages, as should resting ovaries when sampled soon after the spawning period has finished. No other developmental stages were wrongly classified as immature.
- 85.7% of **mature resting** ovaries (Plates 3 and 4) were correctly identified macroscopically. This is a relatively high success rate, with errors due to misidentification of immature (10.7%) and developing (3.6%) ovaries. Such errors are inevitable, as discussed above and shown by comparison of immature, mature resting and developing ovaries in Plates 1 - 4 and 7.
- Only 45.6% of **developing** ovaries (Plate 7) were correctly identified macroscopically, the rest actually being immature (17.5%), mature resting (30.7%) or reproductive (6.2% for developed, spawning and spent stages combined). This high error rate is unexpected because the cortical alveoli stage (CAS) oocytes defining this stage lose the transparency of earlier oocyte stages and become visible macroscopically (as indicated in the close-up of a developing Spanish mackerel (*Scomberomorus commerson*) ovary in Appendix 3). It is likely, therefore, that this error rate can be reduced through improved understanding of the macroscopic features described in this report. Nevertheless, mistakes will still be made when identifying ovaries at the start of this stage, when the CAS oocytes are still relatively small and translucent (and the gonad compact), and at the end when the CAS oocytes are large and can be confused with yolk globule stage oocytes.
- Only 33.8% of **developed** (F4) ovaries (Plates 8 – 10) were correctly identified macroscopically. This low success rate is due to the misidentification of post-spawning ovaries (F5c), which made up 48.6% of this stage. Whilst artificially inflated in this

assessment due to the high proportion of spawning ovaries in the samples (as a result of targeted sampling for DEPM analyses), it is inevitable that this error will occur because POFs cannot be identified by eye. Some errors (5.1%) also occurred in misidentifying pre-spawning ovaries as developed because of difficulties in distinguishing migratory nucleus stage oocytes (Plate 19) by eye.

- 96% of **spawning** (F5) ovaries (combining microscopic stages F5a,b,c; Plates 15 – 18) were correctly identified macroscopically. This high success rate is due to the distinct appearance of hydrated oocytes, with only a minor percentage of ovaries in other stages erroneously classified as spawning – perhaps, for example, because the tissue of developed ovaries sampled at the end of the spawning season can be translucent and hence appear to contain hydrated oocytes. This error should therefore be reduced through improved understanding of the macroscopic features of ovaries described in this report. As indicated above, the biggest error with staging spawning ovaries lies in misidentifying post- (and to a lesser extent pre-) spawning ovaries as developed ovaries because, whilst hydrated oocytes can be readily identified macroscopically, migratory nucleus oocytes and POFs cannot.
- Only 18.2% of **spent** (F6) ovaries (Plate 27) were correctly identified macroscopically. As indicated in Figure 3, this is because ovaries in other stages of development are readily misidentified as spent since the main identifying characteristics of a spent ovary – bloody and flaccid appearance – are not unique to spent ovaries. Freezing also makes ovaries flaccid, and if a fish is not bled the blood can drain to the ovary and give it a bloody appearance (Mackie and Lewis 2001). Furthermore, as shown in Plate 27, a spent ovary can also appear unbloodied and similar to a developed ovary.

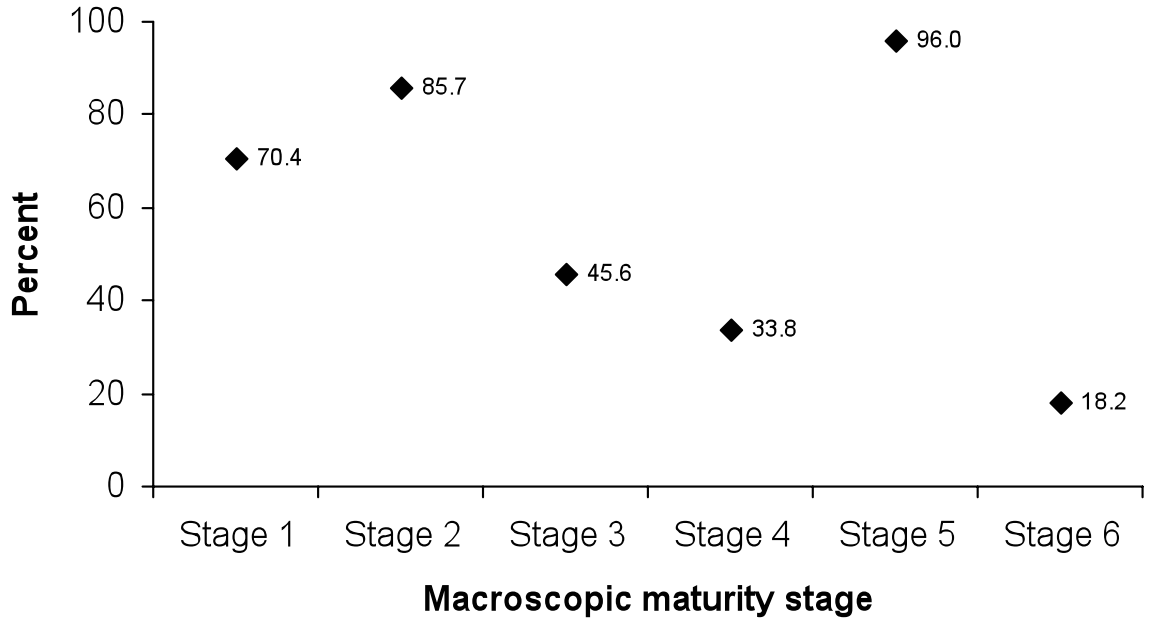


Figure 2. Percentage of macroscopically staged *P. auratus* ovaries that were given the same microscopic stage. Note that macroscopic stage 5 includes stages F5a, F5b and F5c of the microscopic staging system.

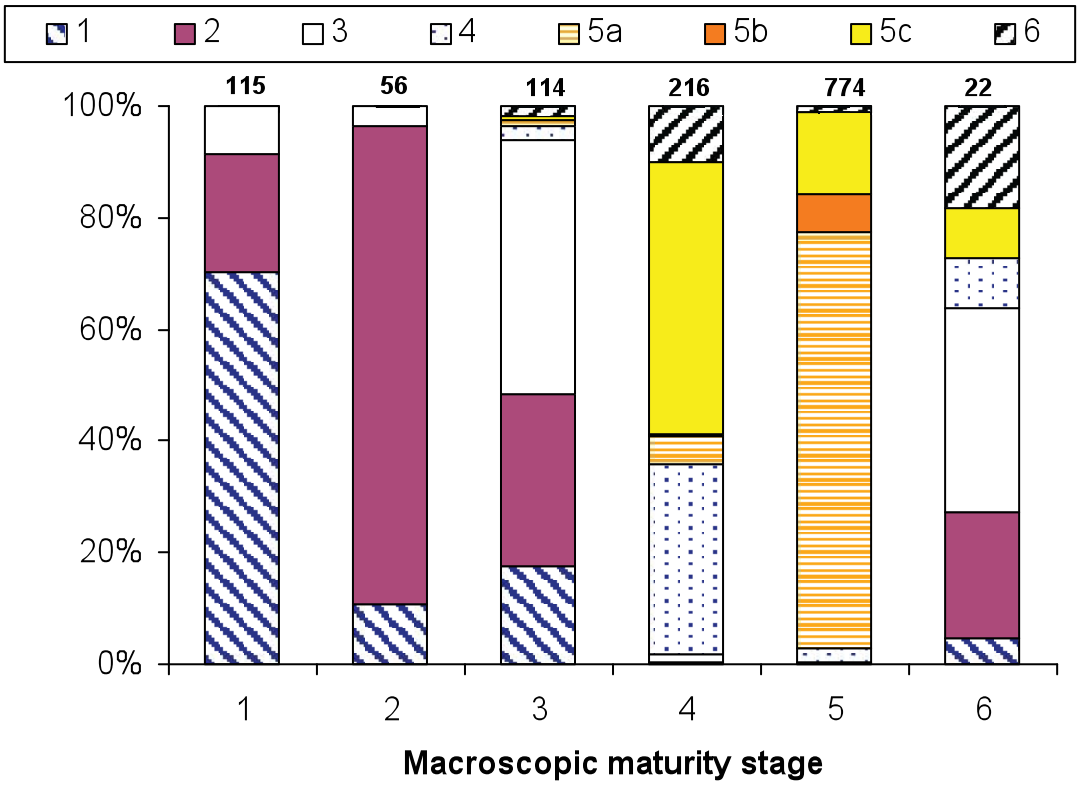


Figure 3. Comparison between macroscopic stages assigned to whole ovaries of *P. auratus* and microscopic stages subsequently assigned to the same histologically processed ovaries. Sample sizes are shown above each column. The legend at the top of the graph depicts microscopic stages.

3.1.2 Development of a more reliable macroscopic staging system

3.1.2.1 Ovaries

It is clear from the above analysis that some ovarian stages can be correctly identified macroscopically with reasonable accuracy, whereas others cannot. Observations made in the field confirm that some errors made in assigning an ovary to a particular stage are often due to inexperience and therefore can be improved upon. For instance, in classifying spent ovaries as immature or developing ovaries as resting. To date, this error is likely to have been common in research on *P. auratus*, because it has by necessity involved a large number of field personnel of varying skill and experience. In future, this type of error can be minimised using the information presented in this report.

However, the inconspicuous nature of some criteria will always result in errors when macroscopically staging ovaries, regardless of experience and training. A more reliable macroscopic staging system will therefore require pooling of those stages that are difficult to correctly identify by eye with other stages. At the same time the simplified staging system needs to remain biologically appropriate (*i.e.* the stages need to describe a particular level of reproductive activity), and be consistent with the microscopic staging system described below. This is achieved by pooling stages F1,2,3 and 6 to describe 'non-developed' ovaries, stages F4 and F5c to describe 'developed' ovaries and stages F5a,b to describe 'spawning' ovaries (Table 4), to produce a system that is less detailed but more reliable. Using this approach, there was 97.1, 82.4, and 81.4% agreement, respectively, between the microscopic and macroscopic

staging (Figure 4). In future, an even higher level of percent agreement should be possible using this staging system because of enhanced training of field personnel. Using the new system along with the illustrations provided below, researchers may classify *P. auratus* ovaries to describe general reproductive patterns and to identify gonads that can be used for more detailed histological assessment of spawning activity and batch fecundity.

3.1.2.2 Testes

Accuracy of the macroscopic staging system for *P. auratus* testes was not formally assessed. Nevertheless, many testes have been examined during the research program within Shark Bay (n = 2,196), and the simplified staging system for testes described in Table 4 is considered reliable and relevant.

Table 4. Simplified, macroscopic staging system used for describing *P. auratus* gonads from inner Shark Bay, Western Australia. Main features are highlighted in bold. The names of each stage are compatible with the microscopic staging system.

J (Juvenile)	**Small translucent ribbons. Sex indistinguishable.**
I. Females	
F1-3 (Non-developed)	Variable in size (small to large), colour (translucent pink to bloody), texture (smooth to irregular) and firmness (taut to flaccid). Main characteristics are a lack of both discernable oocytes and the colouration of stages described below.
F4 (Developed)	Large, varying from taut to flaccid and generally **an opaque, apricot-pink colour**. Blood vessels prominent on surface, and tissue may be quite bloody towards the end of the season. **Opaque oocytes** obvious (but may be reduced in number; Appendix 4).
F5 (Spawning)	Large, similar in character to Developed ovaries but with **large translucent oocytes** obvious throughout the tissue and, if spawning is imminent or occurring, in the lumen, duct or external (Plate 22).
II Males	
M1-2 (Non-developed)	Size: small and ribbon-like to large. Colour: translucent white, greyish, creamy white, bloody. Firmness: taut to flaccid.
M3 (Developed)	Large, opaque, creamy white. May be bloody and flaccid towards end of spawning season. Small amounts of milt may be squeezed out of tissue when pressure is applied to the testis.
M4 (Spawning)	As for Developed testis. Milt freely released through duct with or without pressure.

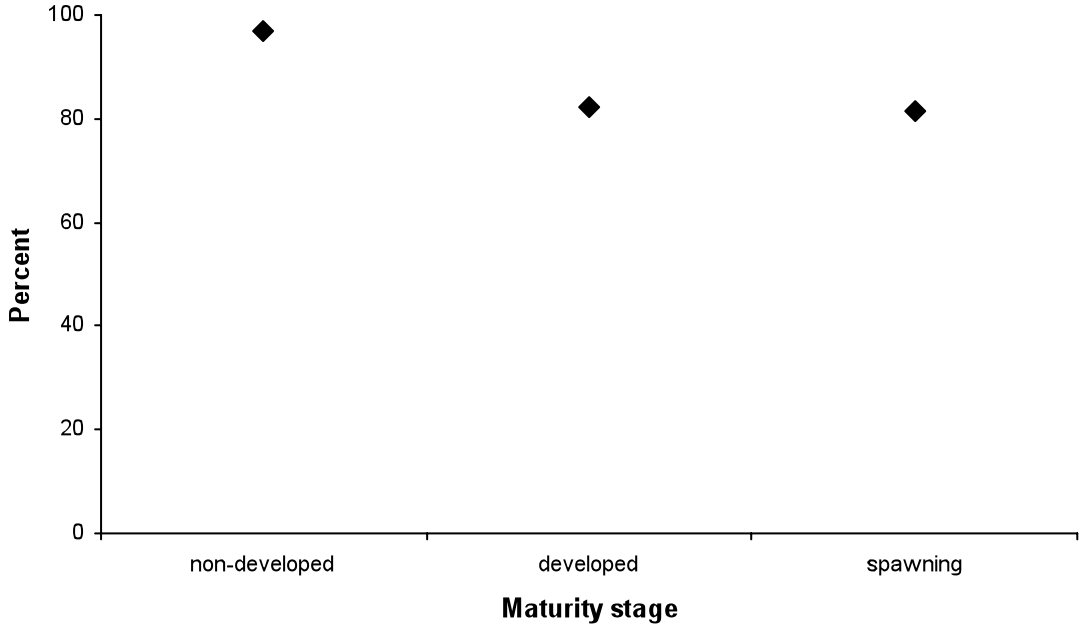


Figure 4. Percentage agreement between microscopic and pooled macroscopic stages for *P. auratus* ovaries. 'Non-developed' includes stages F1,2, 3 and 6; 'developed' includes stages F4 and F5c; 'spawning' includes stages F5a and b.

3.2 Microscopic staging system

In contrast to the pooled, simplified macroscopic staging system described above, more information can be gathered about spawning in *P. auratus* by expanding the microscopic staging system. This has been done by splitting the spawning stage (F5) into three biologically relevant sub-stages based on histological characteristics - pre-spawning (F5a), spawning (F5b) and post-spawning (F5c). As shown in Table 5, the characteristics defining these stages include (main features in bold):

Pre-spawning (spawning 'imminent'): Early or late hydrated oocytes **in lamellae**, early or late migratory nucleus stage oocytes in lamellae (Plates 19 – 21). **No 'new' post-ovulatory follicles** (POFs). 'Old' POFs may/may not be present. The fish has commenced final maturation of a batch of oocytes in preparation for spawning.

Spawning ('running ripe'): Hydrated oocytes **in lumen** (Plate 23) or being extruded from body, and '**new' POFs** (Plate 24) in lamellae. 'Old' POFs may/may not be present. Final maturation has finished and the oocytes (now 'eggs') have been ovulated into the lumen ready for spawning.

Post-spawning (recently spawned): 'New' and/or 'old' POFs **only** in the lamellae. The batch of eggs has been spawned and few if any un-ovulated hydrated oocytes remain in the ovary. The presence of new POFs indicate that spawning took place less than approximately twelve hours ago, whereas old POFs indicate that it occurred before this time.

Note: if spawning occurs on consecutive days an overlap in these spawning stages will occur, resulting in a combination of characteristics in the ovary. For example, it is not unusual to find migratory nucleus stage oocytes and new POFs together in the lamellae (Plate 19), indicating that the fish has recently spawned and is about to do so again (*i.e.* concurrent stages F5a and

F5c). When this occurs, the ovary is categorised according to the *next* stage that will occur (F5a in the example presented).

A conceptual diagram of the reproductive status for each stage is provided in Figure 5. Note that fish reproduction is a dynamic and variable process and some stages can occur at different times from that shown in the diagram. This is particularly the case for developing (F3) and spent (F6) ovaries, which can occur (usually in small numbers) throughout the spawning period when ovaries atrophy due to reasons other than completion of spawning (*e.g.* due to stress, injury or sickness).

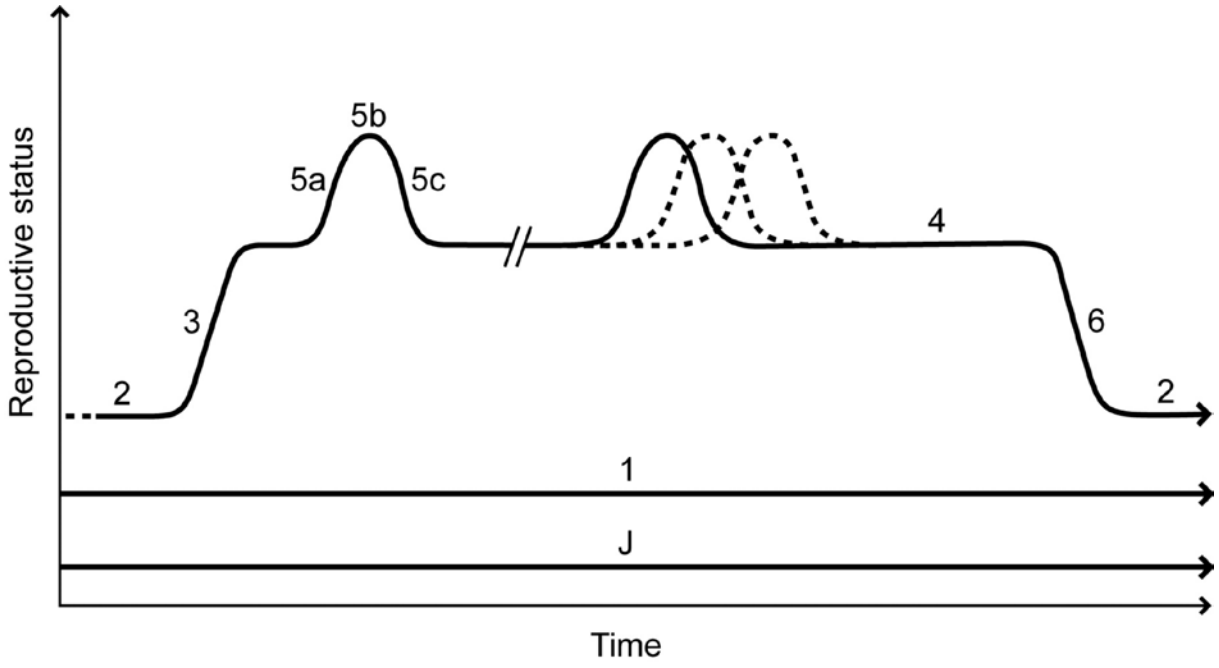


Figure 5. Diagrammatic representation of the ontogenetic development and seasonal maturation of *P. auratus* ovaries. Numbers indicate the occurrence of ovaries in each reproductive stage, as described in Table 5 (note that the prefix 'F' has been omitted).

Table 5. Microscopic staging system used for describing *P. auratus* gonads from Shark Bay, Western Australia.

J (Juvenile)	**Gonad very small. Germ tissue is rudimentary and comprised of undifferentiated gonia. Sex not identifiable.**
I. Females	
F1 (Immature)	(Plate 5) Ovary compact, with relatively orderly lamellae, a tight, thick gonad wall and few, if any, yellow-brown bodies. Perinucleolus (PN) and chromatin nucleolus stage (CN) oocytes present.
F2 (Mature resting)	(Plate 6) Evidence of prior spawning is most evident soon after the spawning season (eg loose, thin gonad wall and kinked, disorganised lamellae that contain low numbers of oocytes and considerable amounts of somatic tissue. Atretic vitellogenic oocytes may also be present). This evidence decreases and it becomes increasingly difficult to differentiate F1 and F2 ovaries. Only PN and CN oocytes are present.
F3 (Developing)	(Plate 11) Gonad wall is usually quite thick and contracted, and the lamellae are packed with previtellogenic oocytes. These include PN and CN oocytes as well as cortical alveoli (CA) stage oocytes that are indicative of the onset of reproductive activity.
F4 (Developed)	(Plate 12) The ovary is reproductively active and has enlarged considerably. The tunica becomes expanded and thin, and yolk globule stage (YGS) oocytes dominate the lamellae.
F5a (Pre-spawning)	(Plates 19 – 21) A brief stage just prior to ovulation when the ovary reaches maximum size and weight. Migratory nucleus stage (MNS) or hydrated oocytes are present within the lamellae (once the latter have been ovulated the ovary is staged F5b).
F5b (Spawning)	(Plate 23) A brief stage marking the time when the fish is ‘running ripe’ and ovulated oocytes (eggs) are present within the lumen ready to be spawned. New POFs are present on the periphery of the lamellae.
F5c (Post-spawning)	(Plate 24) A brief stage after spawning during which only POFs are present within the ovary (note that if migratory nucleus or hydrated stage oocytes are present the ovary is staged either 5a or b). POFs are broken down into two stages (“new” and “old”) as detailed in Table 2.
F6 (Spent)	(Plates 28 – 30) A brief stage occurring between the F4 and F2 stages. The ovary is flaccid and bloody with a loose, thin tunica. Lamellae are disorganised with relatively few oocytes and a lot of empty space, somatic tissue and yellow-brown bodies. At least 50% of the remaining oocytes are atretic.

Table 5 (cont.). Microscopic staging system used for describing *P. auratus* gonads from Shark Bay, Western Australia.

II Males	
M1 (Immature)	Gonad small and compact, and contain relatively little germ tissue. Spermatogonia and spermatocytes dominate, although spermatids may also be present in small quantities. A central sperm sinus (longitudinal collecting duct) is present but this contains little sperm. Radial sperm sinuses (tubular duct system) are poorly developed.
M2 (Mature resting)	Soon after the end of the spawning season the testis is small and contains little germ tissue (mainly spermatogonia and spermatocytes). The central sperm sinus is large. Closer to the spawning season crypts of spermatids become more common and the radial sperm sinuses more prominent (but contain relatively few spermatozoa).
M3 (Developed)	Spermatocytes and spermatids dominate. Many crypts of spermatids have broken down, mixing their contents. Many spermatozoa are found within the radial sperm sinuses. (Milt can be squeezed from the cut testis but is not free flowing).
M4 (Spawning)	Running ripe. The testis is large in size and dominated by large peripheral and central sperm sinuses that are filled with spermatozoa. (Milt flows from the gonoduct with little or no external pressure applied).

3.3 Pictorial guide to staging *P. auratus* ovaries

It is difficult to adequately describe or conceptualise gonad stages using written descriptions. Photographs of ovaries at all stages of development are therefore provided in Plates 1 – 30 to accompany the information provide in Tables 4 and 5.

3.4 Improvement to estimates of batch fecundity using the more detailed microscopic staging system

Batch fecundity estimates ranged from 1,510 to 530,752 oocytes in the Eastern Gulf, 2,358 to 473,465 oocytes in Denham Sound, and 4,230 to 653,261 oocytes in the Freycinet Estuary (Table 6). Batch fecundity was positively related to fork length in all three areas (Figure 6). The non-linear curves fitted the data well with between 78% and 95% of the variation in batch fecundity explained by the relationship with fork length. In all cases, there was improvement in the fecundity-fork length relationship (as measured by difference in the coefficients of determination, 'r2') when ovaries that were identified by microscopic examination as unsuitable for fecundity estimates were excluded from analyses (*e.g.* stage 5b ovaries that had lost oocytes). For example, the coefficient of determination improved by 0.14 for Freycinet Estuary females in 1998 (0.73 to 0.87) and Denham Sound females in 2000 (0.64 to 0.78) and by 0.06 for Eastern Gulf females in 2000 (0.88 to 0.94) (Figure 6). Ideally, therefore, only ovaries that are confirmed by microscopic examination as being stage 5a should be used for estimating batch fecundity. As there was no significant difference in the relationship between batch fecundity and fork length for fish from each area and year, it is appropriate to pool batch fecundity data from all areas/years (Figure 7).

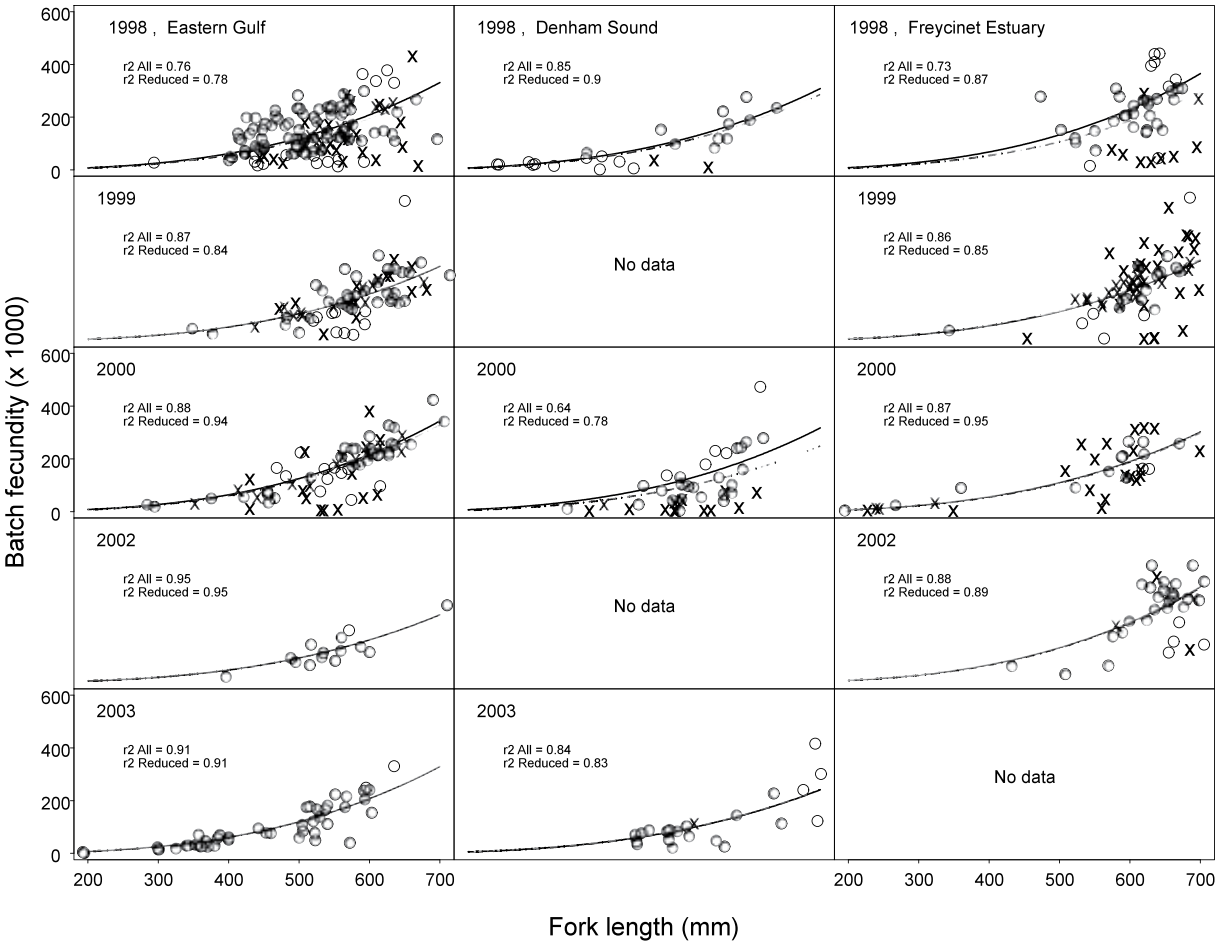


Figure 6. Relationships between batch fecundity and fork length of female *P. auratus* from the Eastern Gulf, Denham Sound and Freycinet Estuary areas of Shark Bay. Data is shown for ovaries obtained between 1998 and 2003. In each graph the solid line and associated coefficient of determination ('r2 Reduced') represents the non-linear curve of best fit for samples of ovaries in 'pre-spawning condition' (stage 5a) without migratory nucleus stage oocytes (o). The hatched lines and associated coefficient of determination ('r2 All') represents the curve fitted to all ovaries including those deemed not suitable for batch fecundity estimation. These latter ovaries are marked by a cross. Note that in some cases no unsuitable ovaries were found.

Table 6. Batch fecundity (BF) for *P. auratus* collected from the three areas within inner Shark Bay between 1998 and 2003. FL = fork length; *n* = sample size.

Area	Year	*n*	FL range (mm)	BF range (eggs x 10^3)
Eastern Gulf	1998	91	172 - 696	3 - 429
	1999	45	348 - 714	23 - 531
	2000	41	284 - 706	18 - 424
	2001	-	-	-
	2002	14	396 - 710	22 - 293
	2003	45	193 - 636	2 - 331
Denham Sound	1998	22	241 - 638	2 - 277
	1999	-	-	-
	2000	27	341 - 619	4 - 474
	2001	-	-	-
	2002	-	-	-
	2003	22	437 - 701	21 - 416
Freycinet Estuary	1998	37	473 - 741	15 - 653
	1999	18	343 - 685	8 - 543
	2000	14	195 - 670	4 - 265
	2001	-	-	-
	2002	32	432 - 725	32 - 660
	2003	-	-	-

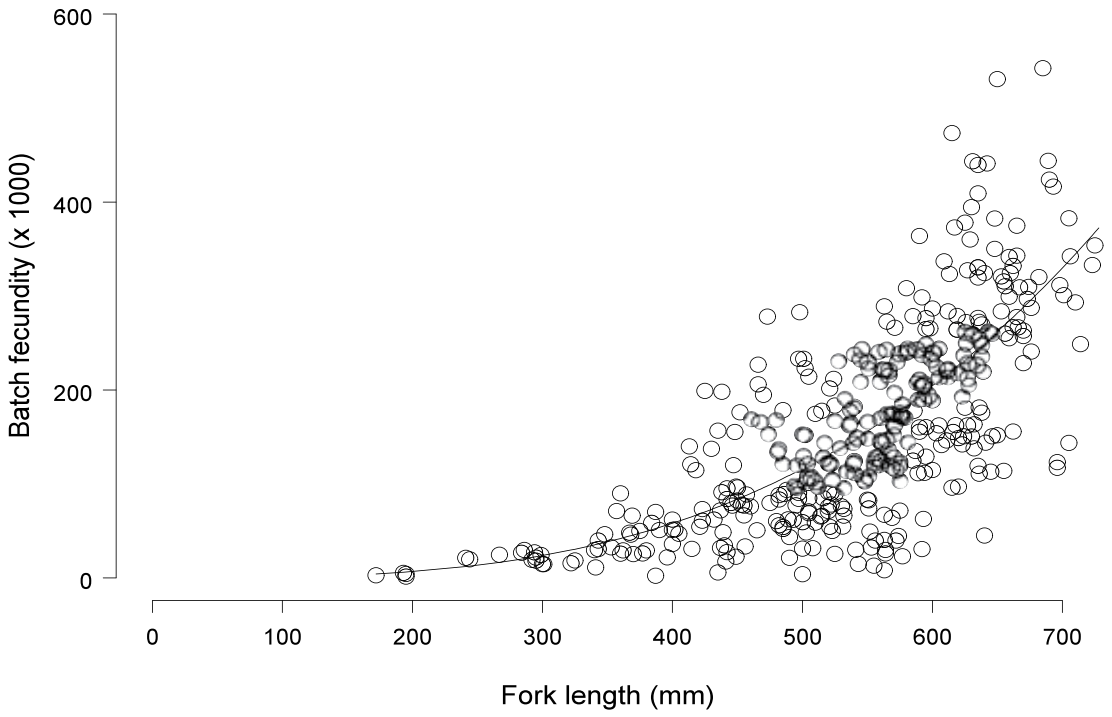


Figure 7. Batch fecundity against fork length for female *P. auratus* from the Eastern Gulf, Denham Sound and Freycinet Estuary pooled for years 1998-2003. The solid line is the common non-linear curve of best fit (can be used to estimate BF from FL).

4.0 Conclusions

Information about reproductive biology is critically important in the management of exploited scalefish stocks. This is particularly the case for *P. auratus* in Western Australia, where the daily egg production method has been and will continue to be used for estimating spawning biomass in Shark Bay and Cockburn Sound. However, this report highlights the errors that frequently occur at the outset of such biological studies, when staging gonads based on their macroscopic appearance alone. Such errors are often due to inexperience, lack of training, and unrealistic expectation of macroscopic staging systems. Nevertheless, the errors and biases that result are rarely given adequate consideration, even though they can significantly influence the conclusions drawn from the data and are easily reduced using relevant and adequately-described gonad staging systems.

Assessment of the errors associated with staging *P. auratus* gonads showed that the most relevant macroscopic staging system for the ovaries of this species is relatively simple because some staging criteria are often too difficult to identify 'by eye' only. However, in using this simplified system the question is raised over whether to apply it directly or by pooling stages prior to analyses. Here at the DoFWA the latter is preferred because it increases future analytical opportunities. In other words, we macroscopically stage all ovaries according to the traditional six-stage system but subsequently pool these into the three-stage system.

Fundamental errors can also occur when staging histologically processed gonad samples using a microscope. Again, these errors are mainly due to inexperience and inadequate training or description of microscopic staging criteria. However, in contrast to the simplified macroscopic staging system, a more detailed microscopic system can provide additional information about reproductive strategies and enable more accurate estimation of batch fecundity. For *P. auratus*, this was achieved by dividing the spawning event into three ovarian development stages.

A key reason for errors in staging gonads is the difficulty in interpreting written descriptions, particularly since they are often obtained from other studies focussed on other species. The detailed pictorial and written guide provided in this report will assist personnel to properly determine the reproductive stage of *P. auratus* ovaries, from all locations throughout the species range in Western Australia, both in the field and laboratory. Whilst care should be taken when applying staging systems to other species, it is expected that this guide will also have relevance to other scalefish species.

5.0 Acknowledgements

We thank the many Shark Bay recreational fishers who helped collect *P. auratus* samples between 1998-2003, particularly Errol Bartlett-Torr, Dexter Fowler, Jack Dekker, Paul Low, Robin Beauclarke, Les Oakley, Noel and Wende Smith, and the many Nanga crew including Geoff and Dave Franz, Bill Aird, JJ, and Leo Massey. We also acknowledge the efforts of the many DoFWA research staff who took part in the field work and laboratory processing over the period of the study. Histological preparation of the gonad material was undertaken initially by Gordon Thompson at Murdoch University and later by Ian Russel-Brown at WA Department of Agriculture. Our thanks to John McKinlay and Monty Craine for various statistical analyses associated with earlier stages of this study. Funding for this research was provided by the Natural Heritage Trust (Fisheries Action Program, Projects 973720 and 973340, Commonwealth Government) and the WA Department of Fisheries. Finally the authors thank Drs Steve Newman, Kim Smith and Ross Marriott for providing useful comments on earlier drafts of this report.

6.0 References

Alheit, J. (1993) Use of the daily egg production method for estimating biomass of Clupeoid fishes: a review and evaluation. Bulletin of Marine Science 53:750-767.

Crossland, J. (1977a) Seasonal reproductive cycle of Snapper *Chrysophrys auratus* (Forster) in the Hauraki Gulf. New Zealand Journal of Marine and Freshwater Research 11:37-60.

Crossland, J. (1977b) Fecundity of the Snapper *Chrysophrys auratus* (Pisces: Sparidae) from Hauraki Gulf. New Zealand Journal of Marine and Freshwater Research 11:767-775.

DeMartini, E.E and Fountain, R.K. (1981) Ovarian cycling frequency and batch fecundity in the Queenfish, *Serpihus politus*: attributes representative of serial spawning fishes. Fishery Bulletin 79:547-560.

Francis, M. P. and Pankhurst, N. W. (1988) Juvenile sex inversion in the New Zealand snapper *Chrysophrys auratus* (Sparidae). Australian Journal of Freshwater Research 39:625-31.

Hunter, J.R. and Macewicz, B.J. (1985) Rates of atresia in the ovary of captive and wild northern anchovy, *Engraulis mordax*. Fishery Bulletin 83:119-136.

Hunter, J.R., Lo, N.C.H and Leong, R.J.H. (1985). Batch fecundity in multiple spawning fishes. In Lasker R. (Ed.) An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy, *Engraulis mordax.* NOAA Technical Report NMFS 36, pp 67-77.

Jackson, G. (2007) Fisheries biology and management of pink snapper, *Pagrus auratus*, in the inner gulfs of Shark Bay, Western Australia. PhD Thesis, Murdoch University, Perth.

Jackson, G. and Cheng, Y.W. (2001) Parameter estimation with egg production surveys to estimate pink snapper, *Pargus auratus*, biomass in Shark Bay, Western Australia. Journal of Agricultural, Biological and Environmental Statistics 6: 243-257.

Jackson, G., Sumner N., Cribb, A. and Norriss, J. (2005) Comparing conventional 'social-based' and alternative output-based management models for recreational finfish fisheries using Shark Bay pink snapper as case study. FRDC Project 2003/066 Final Report, Department of Fisheries, Western Australia, Perth.

Jackson, G., Marriot, R. and Lai, E. (2007) Gascoyne Demersal Scalefish Fishery Status Report. In Fletcher W.J. and Santoro, K. (Eds) State of the Fisheries Report 2006/07. Department of Fisheries, Western Australia.

Lasker, R. (1985) Introduction: an egg production method for anchovy biomass assessment. In Lasker R (Ed.) An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy, *Engraulis mordax.* NOAA Technical Report NMFS 36, pp 1-3.

Mackie, M. and Lewis, P. (2001) 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. Fisheries Research Report No. 136, Department of Fisheries, Western Australia, Perth.

Mackie, M.C., Lewis P. D., Gaughan, D.J. and Newman, S.J. (2005) Variability in spawning frequency and reproductive development of the narrow-barred Spanish Mackerel (*Scomberomorus commerson*) along the west coast of Australia. Fishery Bulletin 103: 344-354.

Moran, M., Stephenson, P., Gaughan, D., Tapp, N. and Moore, J. (2005) Minimising the cost of future stock monitoring, and assessment of the potential for increased yields, from the oceanic Snapper, *Pagrus auratus*, stock of Shark Bay. FRDC Project 2000/138 Final Report, Department of Fisheries, Western Australia, Perth.

Scott, S. G. and Pankhurst, N. W. (1992) Interannual variation in the reproductive cycle of the New Zealand snapper *Pagrus auratus* (Bloch & Schneider) (Sparidae). Journal of Fish Biology 41: 685-696.

Lenanaton, R., StJohn, J., Keay, I., Wakefield, C., Jackson, G., Wise, B. and Gaughan, D. (Eds) (2008) Spatial scales of exploitation among populations of demersal scalefish: implications for management. Part 2: Stock structure and biology of two indicator species, Western Australian dhufish (*Glaucosoma hebraicum*) and pink snapper (*Pagrus auratus*), in the West Coast Bioregion. FRDC Project 2003/052 Final Report. Fisheries Research Report No 174, Department of Fisheries, Western Australia, Perth.

Wakefield, C.B. (2006) Latitudinal and temporal comparisons of reproductive biology and growth of snapper, *Pagrus auratus* (Sparidae), in Western Australia. PhD thesis, Murdoch University, Perth.

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

West, G. (1990) Methods of assessing ovarian development in fishes: a review. Australian Journal of Marine and Freshwater Research 41:199-222.

Zeldis, J. and Francis, R.I.C.C. (1998) A daily egg production method estimate of snapper biomass in Hauraki Gulf, New Zealand. ICES Journal of Marine Science 55:522–534.

7.0 List of Plates

Plates 1 and 2: Whole immature ovaries (F1) - small, translucent and rose in colour. The bloody appearance of the ovary in Plate 2 is probably due to the draining of blood after death.

Plates 3 and 4: Whole mature resting ovaries (F2). Soon after the spawning season these ovaries usually appear flaccid and may be dark reddish in colour. However as time progresses resting ovaries become increasingly similar in appearance to immature ovaries.

Plate 5: General microscopic view of an immature ovary (F1). Perinucleolus and chromatin nucleolus stage oocytes are present. The ovary is compact with relatively orderly lamellae and little vascular tissue.

Plate 6: General microscopic view of a resting ovary (F2). Perinucleolus (PN) and chromatin nucleolus stage oocytes are present (see Plate 12 for detail of these). Disorderly lamellae, low numbers of oocytes, and much somatic and vascular tissue (*) are indicative of previous spawning activity, showing that this ovary was collected soon after the end of the spawning season.

Plate 7: Whole developing ovary (F3). Enlarged from previous stages and may have a less translucent appearance as oocytes become opaque (see Appendix 3).

Plates 8, 9 and 10: Whole developed ovaries (F4) that are enlarged, vascularised and apricot in colour. They are also opaque due to the presence of opaque yolk globule stage (YGS) oocytes (see Appendix 4). They will become increasingly flaccid as the spawning season progresses and oocyte stocks become depleted.

Plate 11: General microscopic view of a developing ovary (F3). Perinucleolus (PN), chromatin nucleolus and cortical alveoli (CA) stage oocytes are present. Although usually considered a short stage marking transition from the resting to developed stages, developing ovaries can also occur at other times in the ovarian cycle, such as between the spent (F6) and resting stages. This is probably the case with the ovary shown in Plate 11 given the large amount of vascular tissue that is present.

Plate 12: General microscopic view of a developed ovary (F4) showing early (eYGS) and late (IYGS) yolk globule stage oocytes. Perinucleolus (PN), chromatin nucleolus (CN) and cortical alveoli (CA) stage oocytes are also present.

Plate 13: Detailed microscopic view of a developed ovary (F4) showing an early yolk globule stage oocyte. Note the red-stained yolk globules, clear oil droplets (OD), central nucleus (N), zona radiata (ZR) and follicular layer (FL). Detail of perinucleolus (PN) and chromatin nucleolus (CN) stage oocytes also shown. Note the multiple nucleoli in the PN oocytes.

Plate 14: Detailed microscopic view of a developed ovary (F4) showing a late, yolk globule stage oocyte. Note the red-stained yolk globules (YG), white oil droplets (OD), central nucleus (N), relatively thick zona radiata (ZR) and follicular layer.

Plate 15: Whole spawning ovary (F5) in the gut cavity of a large female *P. auratus*.

Plates 16, 17 and 18: Whole spawning ovaries (F5). These highlight the large size and extensive vascularisation of this stage. The ovaries have a speckled appearance due to the presence of translucent hydrated oocytes within the lamellae (see Appendix 5).

Plate 19: Detailed microscopic view of an ovary that has recently become pre-spawning (F5a),

with migratory nucleus (MN) stage oocytes in which the nucleus is migrating towards the periphery of the oocyte and the oil droplets (OD) are coalescing. A new (small arrow) and old (large arrow) post-ovulatory follicle can also be seen, providing evidence of repeated spawning over a short time period.

Plate 20: Detailed microscopic view of a pre-spawning ovary (F5a), with migratory nucleus stage oocytes. The nucleus (N) has reached the periphery of the oocyte and most of the yolk globules have ruptured and coalesced.

Plate 21: Detailed microscopic view of a pre-spawning ovary (F5a), showing a fully hydrated oocyte (H). The follicle that surrounds the oocyte (arrowed) is composed of two cell layers; the outer protective thecal layer and the inner granulosa layer. At ovulation the oocyte (now an egg) is shed into the lumen leaving the follicle behind (post-ovulatory follicle – see Plates 24-26). This marks the spawning stage (F5b) of development.

Plate 22: Hydrated oocytes spill over the processing table from a spawning ovary (F5).

Plate 23: General microscopic view of a spawning ovary (F5b) showing hydrated eggs (H) in the lumen (LU). Hydrated eggs commonly appear distorted due to the fixing and histological process. Note earlier stage oocytes still within the lamellae.

Plate 24: General microscopic view of a post-spawned ovary (F5c) showing a new post-ovulatory follicle (small arrow), a late post-ovulatory follicle (large arrow) and an atretic oocyte (A).

Plate 25: Detailed microscopic view of a new post-ovulatory follicle. The inner layer of cells is the granulosa layer (small arrow) and the outer layer of cells is called the thecal layer (large arrow).

Plate 26: Detailed microscopic view of an old post-ovulatory follicle (POF). Old POFs are more angular and compressed than new POFS. The granulosa cell layer (small arrow) is less convoluted and the thecal layer (large arrow) thicker and more compact than seen in new POFs.

Plate 27: Whole spent ovary (F6). Spent ovaries are typically flaccid and bloody. However, as this photo demonstrates this is not always the case.

Plate 28: General microscopic view of a spent ovary (F6) showing a mix of atretic (A) and healthy oocytes.

Plate 29: Detailed microscopic view of a spent ovary (F6) showing a yolk globule stage oocyte in an early stage of atresia, as indicated by the eroded zona radiata. Also shown is a late stage atretic oocyte (large arrow) and a new post-ovulatory follicle (small arrow).

Plate 30: Detailed microscopic view of a spent ovary (F6) showing three atretic oocytes (small arrows). In each the zona radiata is eroded and incomplete (large arrow), few yolk globules remain and the nucleus is gone.

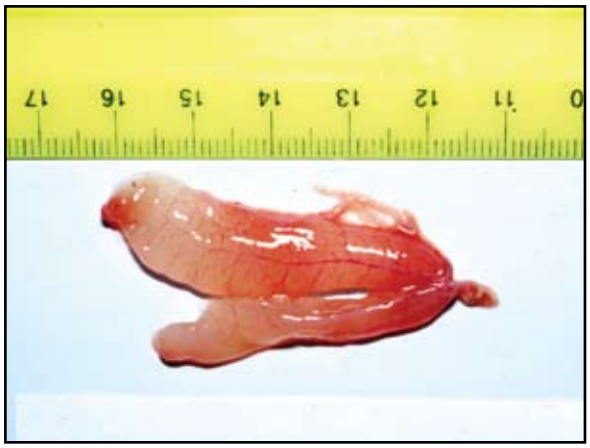


Plate 1

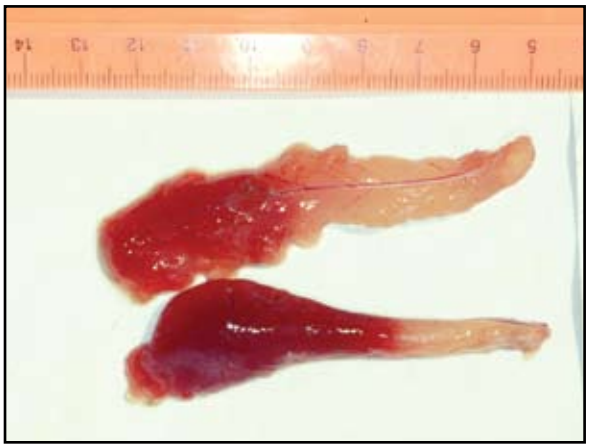


Plate 2

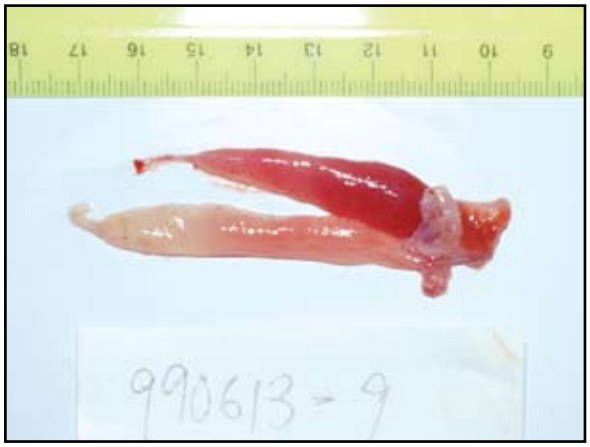


Plate 3

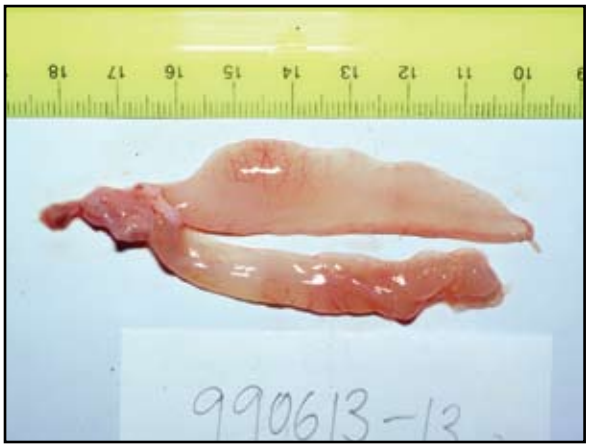


Plate 4

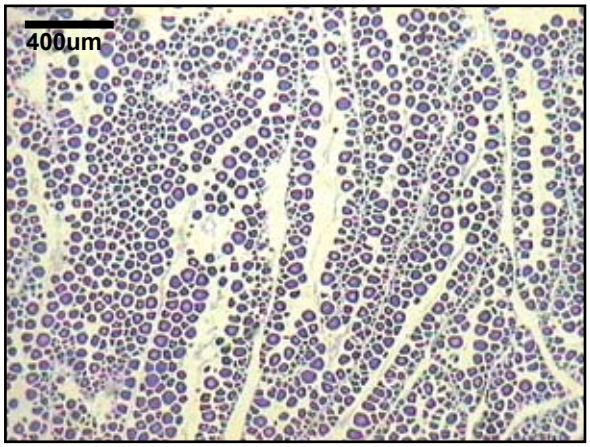


Plate 5

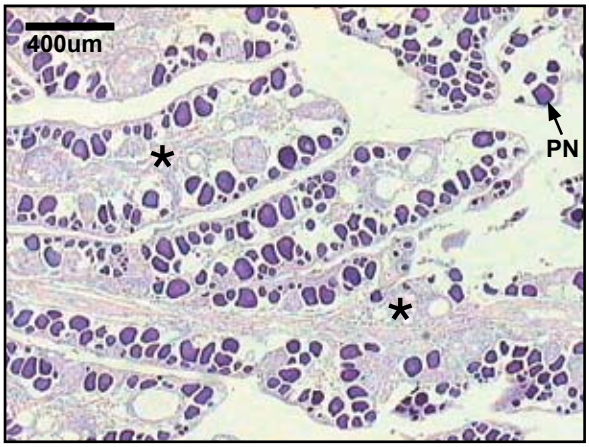


Plate 6

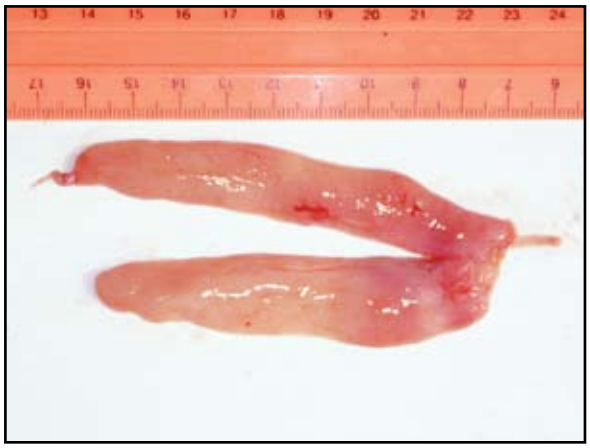


Plate 7

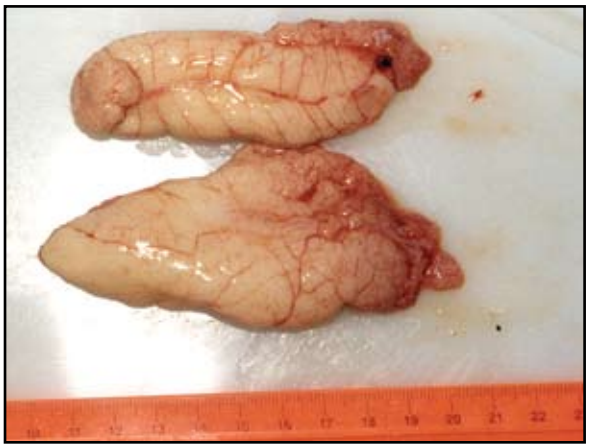


Plate 8

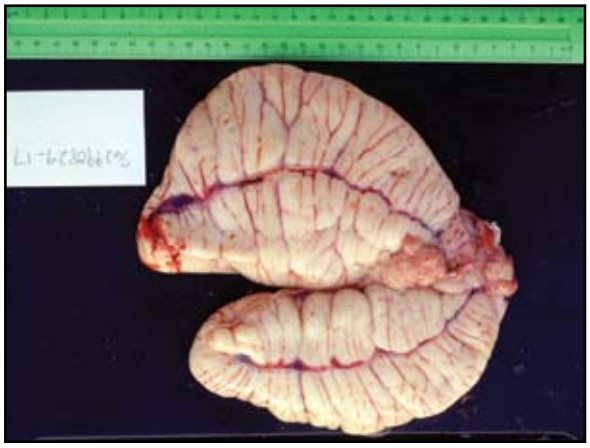


Plate 9

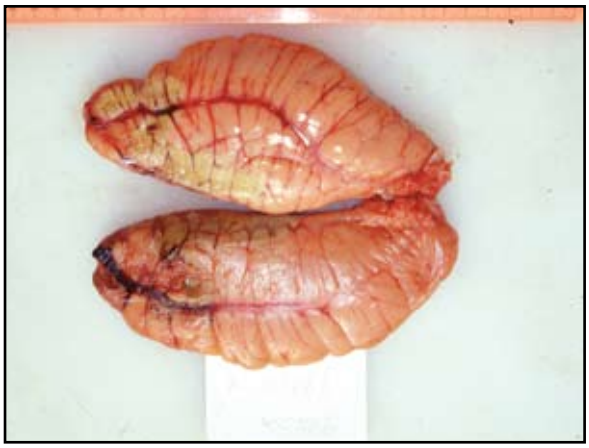


Plate 10

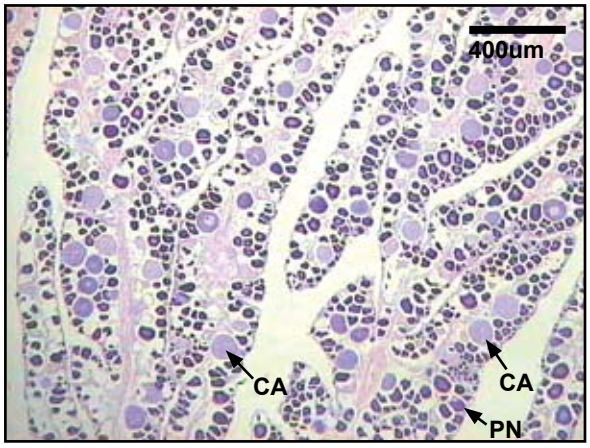


Plate 11

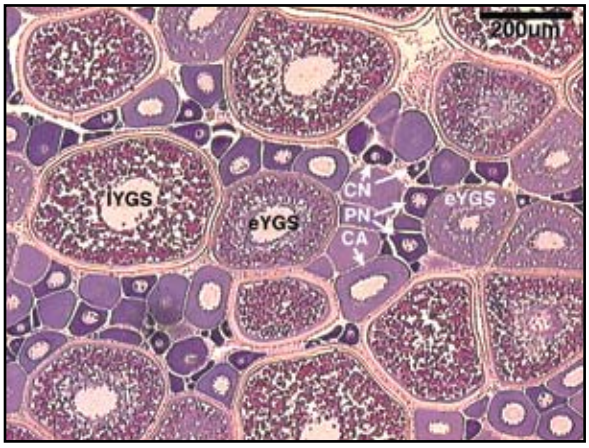


Plate 12

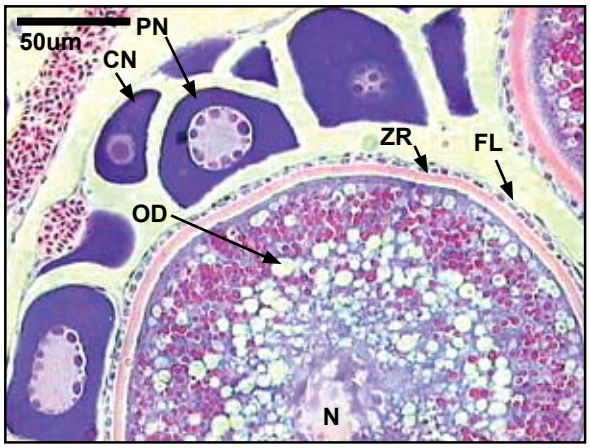


Plate 13

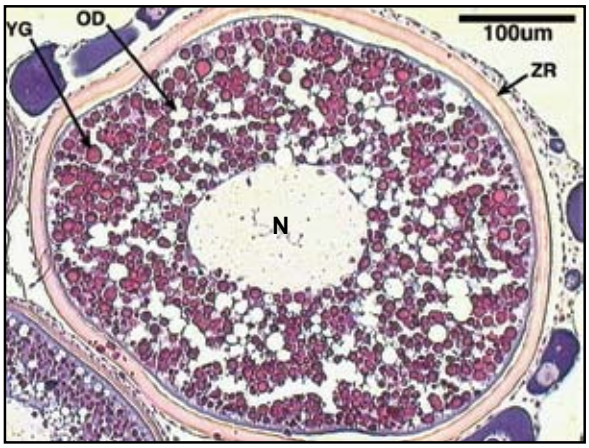


Plate 14

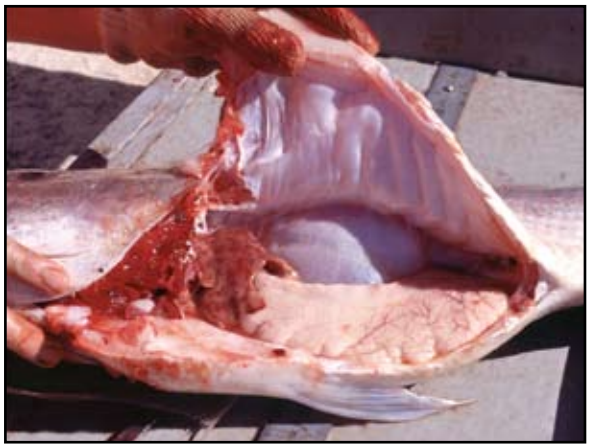


Plate 15

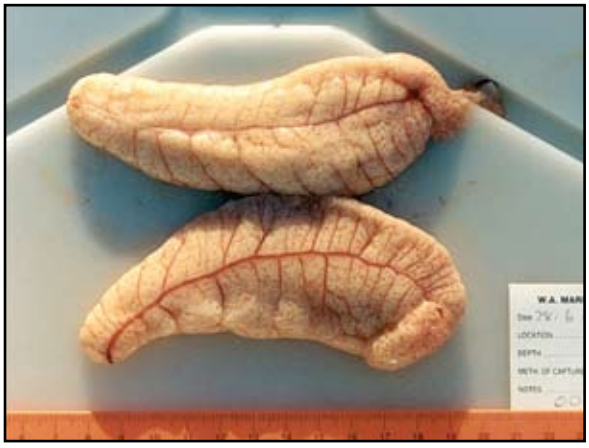


Plate 16

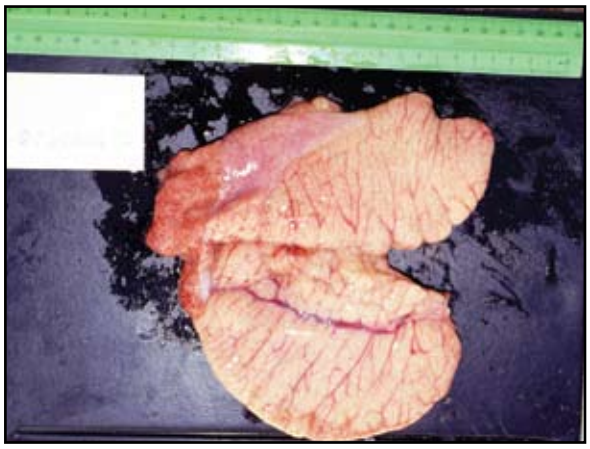


Plate 17

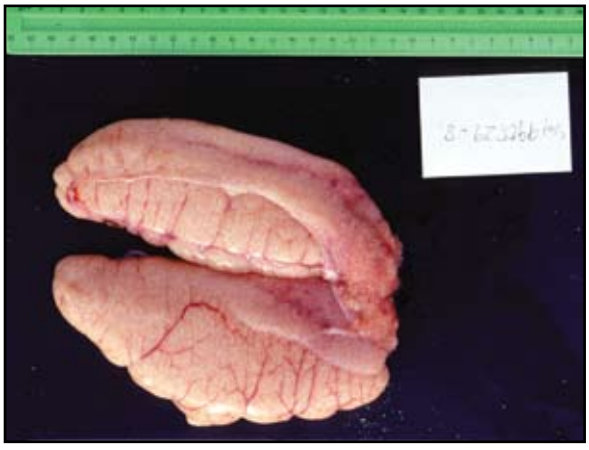


Plate 18

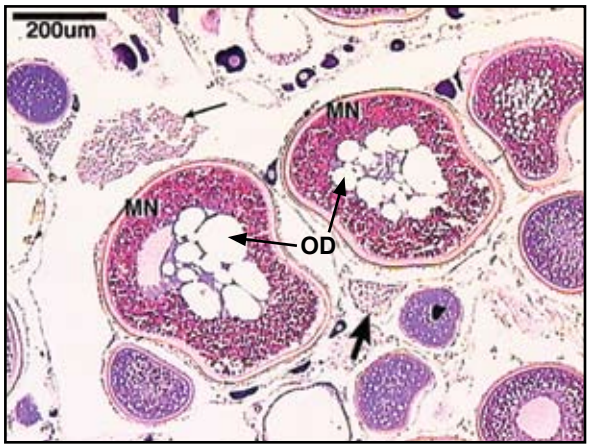


Plate 19

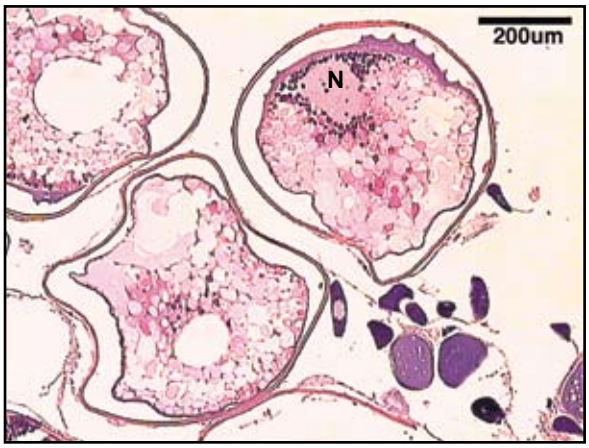


Plate 20

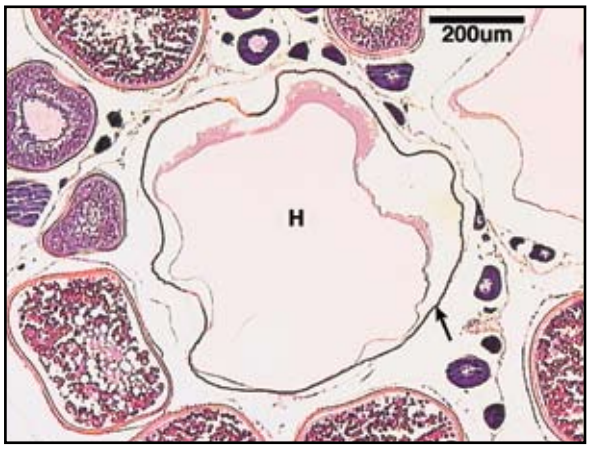


Plate 21

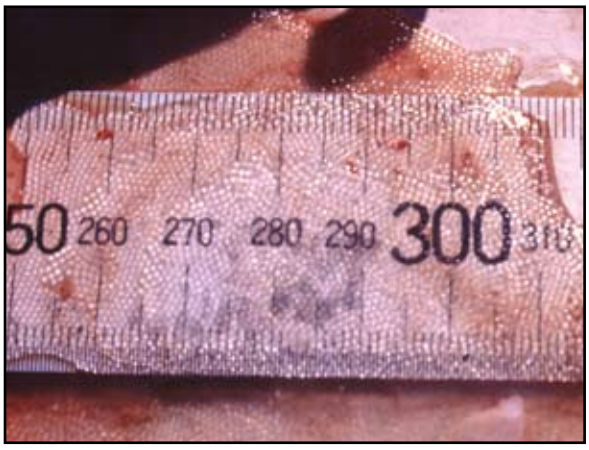


Plate 22

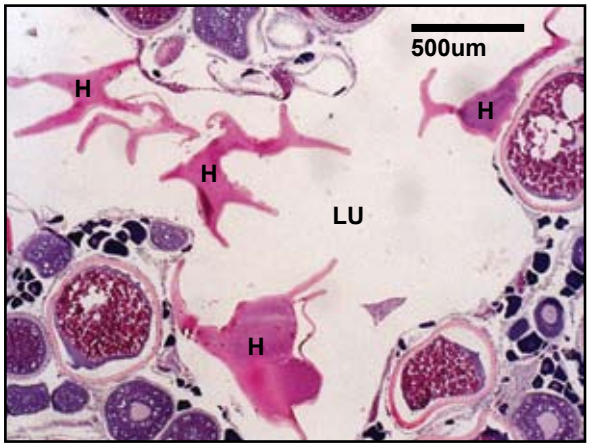


Plate 23

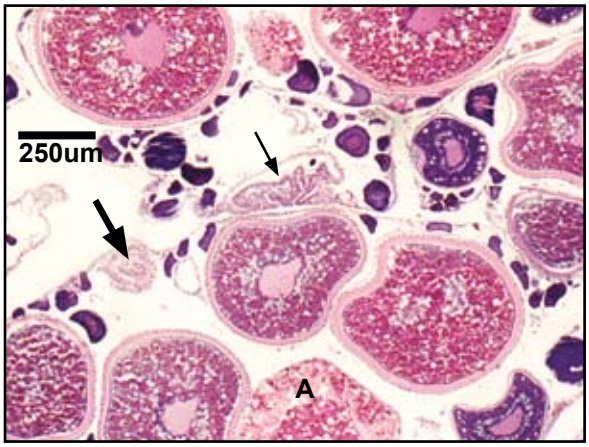


Plate 24

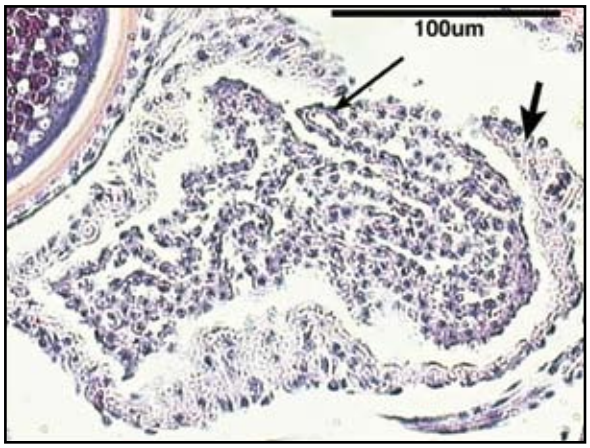


Plate 25

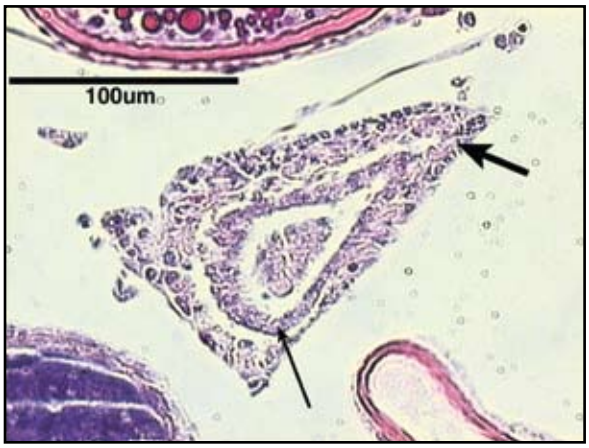


Plate 26

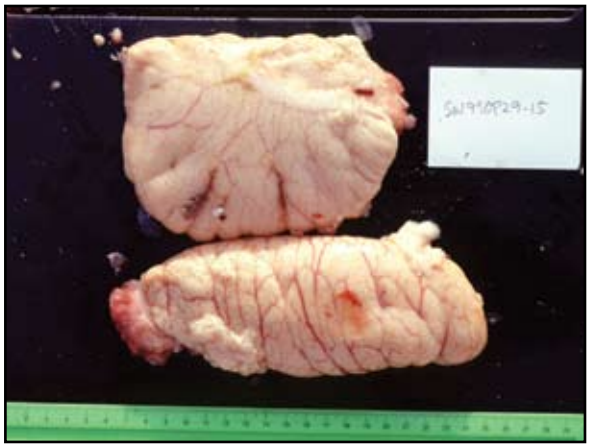


Plate 27

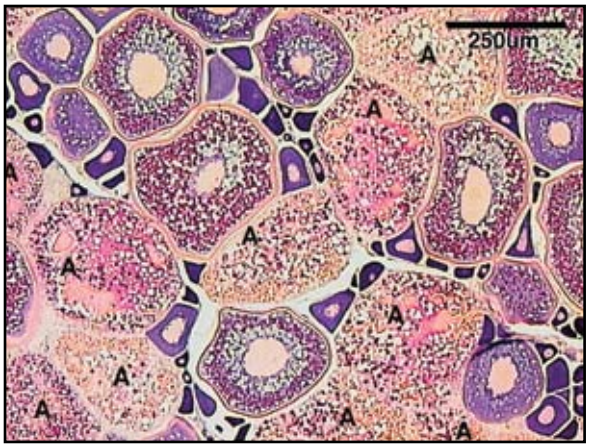


Plate 28

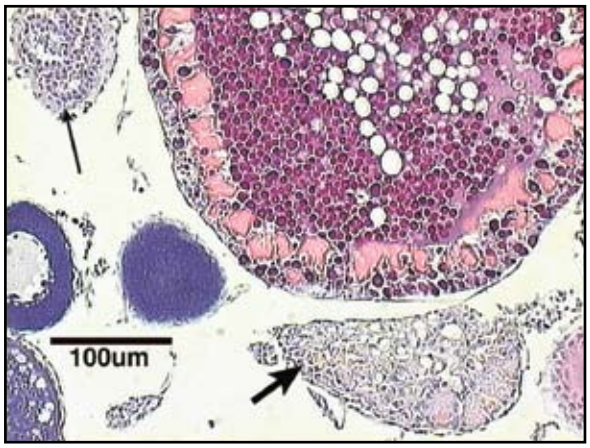


Plate 29

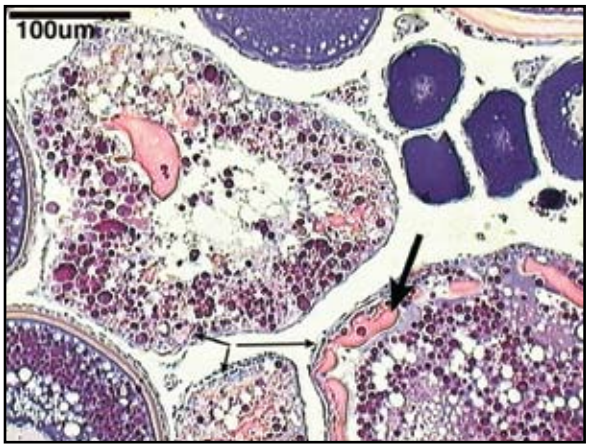


Plate 30

8.0 Appendices

Appendix 1

Macroscopic staging system developed by Crossland (1977a) for *P. auratus* gonads.

0. Immature	Opaque, small, grey/pink. Two ribbon like strings, lying immediately below the posterior 1/4 of the swim bladder. No eggs visible to the naked eye. Sexes not easily distinguished.
1. Resting/recovering	Males are translucent grey, females are light pink/orange. Have slightly scalloped edges. Approx. 1/4 – 1/3 length of body cavity.
2. Developing	Males: white-grey, 1/2 – 1/3 length of body cavity. No sperm released with pressure. Females: Translucent orange to reddish orange with blood capillaries noticeable on the surface. Approx. 1/3 – 2/3 length of body cavity.
3. Developed/mature	Males: white, firm with scalloped edges. When cut, sperm discharged with pressure. Females: Orange to yellowish and occupy 1/2 – 2/3 of body cavity. Small and larger yellow oocytes can be seen.
4. Ripe/running	Males: enlarged, very white sperm easily released with slight pressure. Not as firm. Females: Orange/yellow/red, enlarged. Speckled apprearance due to the yellow and translucent (hyaline) oocytes, the latter being released with slight pressure when running ripe.
5. Spent	Males: whitish grey with reddish tinges. Flaccid and reduced in size. Females: reddish orange, flaccid with prominent blood vessels. No hyaline oocytes present but some opaque oocytes can be seen.

Appendix 2

Staging system used to describe *P. auratus* gonads from the Shark Bay region since 1998 (similar to system used by Scott and Pankhurst 1992).

A) Ovaries

Stage	Macroscopic features	Histological features
1. Immature	Ovary thin & firm, pale or translucent pink. No oocytes visible. Difficult to distinguish Stage 1 ovaries from Stage 2.	Ovary compact, lamellae ordered, tunica tight. Previtellogenic oocytes. No evidence of previous reproductive activity.
2. Resting	Ovary more rounded, pale pink or red. No oocytes visible. Approx. 1/4 – 1/3 length of body cavity.	Previtellogenic oocytes dominate. Soon after spawning the ovary has empty lamellae, much vascular tissue, yellow- brown bodies, loose tunica. This evidence of prior spawning gradually diminishes until ovary similar in appearance to late Stage 1.
3. Developing	Ovary enlarged, pale orange or pink, blood vessels noticable. Oocytes visible, small. Approx. 1/3 – 2/3 length of body cavity.	Previtellogenic oocytes dominate early before the growth of cortical alveoli stage oocytes, which mark the start of this stage.
4. Developed	Ovary enlarged, orange or yellow but not speckled. Oocytes large, clearly visible.	Tunica expanded, thin. Lumen reduced. Lamellae contain yolk globule stage oocytes.
5. Spawning	Ovary much enlarged, translucent pale orange. Hydrated, clear oocytes visible giving speckled appearance. Eggs may be extruded with pressure to abdomen. Blood vessels prominent.	Oocytes in yolk globule and hydrated stages of development. Post ovulatory follicles present if spawning has recently occurred.
6. Spent	Ovary bloody and flaccid.	Tunica loose, lamellae disordered, much vascular tissue. > 50 % of vitellogenic oocytes are atretic.

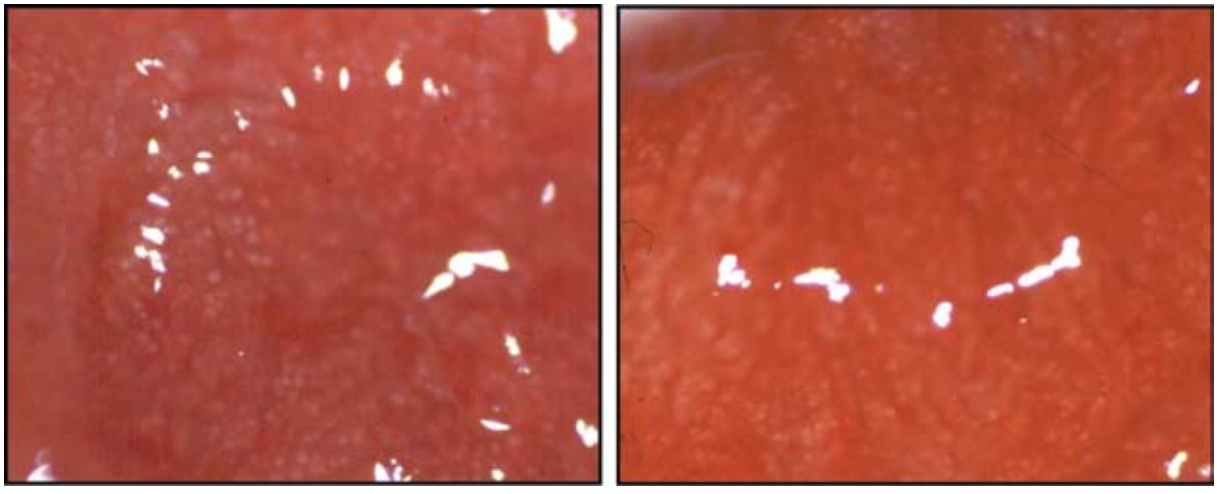
B) Testes

Stage	Macroscopic features	Histological features
1. Immature	Testis small and ribbon-like, translucent white. Sex not easily distinguished.	Testis composed mainly of connective tissue. Radial sinuses poorly developed. Spermatogonia and spermatocytes dominate. Previtellogenic oocytes may be present*
2. Resting	Testis greyish.	Central sinus small compared to later stages. Spermatogonia, spermatocytes and connective tissue dominate. Radial sperm sinuses poorly developed.
3. Developing	Testis enlarged, creamy white. 1/2 – 2/3 length of body cavity.	Radial sperm sinuses more prominent but contain few spermatozoa. Spermiogenesis underway, spermatocytes dominate.
4. Developed	Testis large, opaque, creamy white.	Spermatocytes and spermatids dominate. Many spermatozoa within radial sperm sinuses. Central sperm sinuses large.
5. Spawning	Testis large, firm, creamy white. Milt released with slight pressure.	Central and radial sperm sinuses enlarged and filled with sperm. Spermatids and spermatozoa dominate.
6. Spent	Testis flaccid and reduced in size. Whitish grey and bloody.	Connective tissue dominates. Residual spermatozoa present. Sperm sinuses reduced in size.

* *P. auratus* undergo prematurational sex inversion (Francis and Pankhurst 1988)

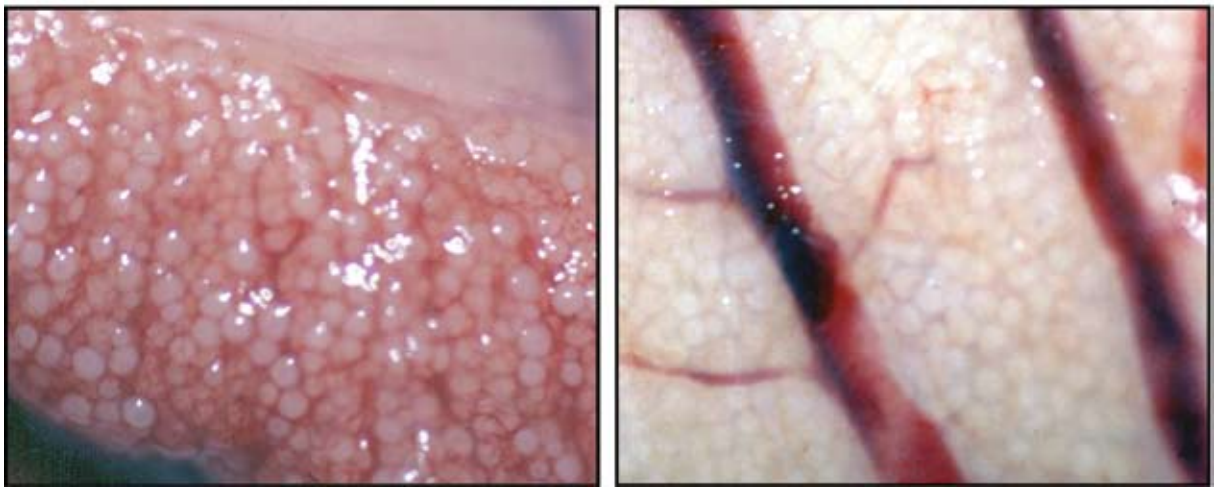
Appendix 3

Close-up macroscopic views of developing (F3) ovarian tissue (F2-3 using the simplified macroscopic staging system) of Spanish mackerel (*Scomberomorus commerson*). 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 (from Mackie and Lewis 2001).



Appendix 4

Close-up macroscopic views of developed (F4) ovarian tissue of Spanish mackerel (*Scomberomorus commerson*). The right photograph shows blood vessels and opaque yolk globule stage oocytes viewed through the ovarian wall. In the left photograph the ovary has been cut to expose the tightly packed yolk globule stage oocytes within the lamellae (from Mackie and Lewis 2001).



Appendix 5

Close-up macroscopic views of a pre-spawning (F5a) ovary of Spanish mackerel (*Scomberomorus commerson*). Blood vessels and translucent hydrated oocytes are clearly visible through the gonad wall in the left photograph. The ovarian tissue has been exposed in the right photograph to show the irregular texture of the tissue due to the mix of translucent hydrated and opaque yolk globule stage oocytes (from Mackie and Lewis 2001).