Spawning and reproductive characteristics of Bight redfish and deepwater flathead in the Great Australian Bight Trawl Fishery

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NON-TECHNICAL SUMMARY

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Objectives:

1 Determine maturity ogives (proportion of population mature against length/age) and sex ratios of spawning Bight redfish and deepwater flathead.

2 Describe gonad maturation cycles and determine the relationship between number of eggs produced (fecundity) and length/age in female Bight redfish and deepwater flathead

3 Provide per recruit analysis to identify harvest strategies which allow optimum Bight redfish and deepwater flathead egg production

4 Provide Industry, GABMAC and stock assessment scientists with information on GABTF species biology.

Non Technical Summary:

Bight redfish (*Centroberyx gerrardi*) and deepwater flathead (*Neoplatycephalus conatus*) are the two principle commercial species captured along the continental shelf of the Commonwealth-managed Great Australian Bight Trawl Fishery (GABTF). The 2002 stock assessment indicated that in the case of Bight redfish, fishing mortality is continuing to rise, potentially resulting in a reduction of its biomass to a level below the trigger biological reference point. Given the recent doubling of fishing effort from five to ten Statutory Fishing Rights in the GABTF and the high uncertainty over the validity of existing Bight redfish and deepwater flathead reproductive indices, the present project was undertaken to produce more scientifically defensible maturity and fecundity relationships.

Gonad maturation in Bight redfish and deepwater flathead was investigated by applying macroscopic staging (gross anatomy) and histological staging (microscopic) techniques. Macroscopic staging was applied to 3000 preserved gonads collected during 2000–02, and a further 1650 freshly preserved gonads collected during the peak spawning period as part of the present study. Histological staging, a more accurate but time-consuming technique focusing on the microscopic development of oocytes, was applied to a subset of 868 preserved gonads. Histological examination indicated that gonad maturation was more advanced than that indicated by macroscopic staging. The histological examination reclassified a number of the smaller-sized individuals as mature instead of immature. The evidence from gonadosomatic index data, macroscopic staging and histological analyses suggests that in the Great Australian Bight both species are multiple spawners with asynchronous oocyte development. The spawning season of Bight redfish lasts 4 months (late January to early May), but is more protracted for deepwater flathead as it lasts for 8 months (October to May).

The sex ratio (females:males), or proportion of females in individual samples from the commercial catch, was ~1:1 (49% female) for Bight redfish and 1.4:1 (58% female) for deepwater flathead. During the deepwater flathead peak spawning period (December to March), the sex ratio was biased towards

females.

Size-at-maturity ogives, which indicate the proportion of fish in mature condition for a given length, were determined through both histological and macroscopic staging of gonads sampled during the 2000–02 and 2003/04 spawning periods. Samples from the spawning period, rather than the whole year, were used to reduce the incidence of misclassifying mature fish with redeveloping gonads as immature fish developing for the first time.

For Bight redfish, the size- and age-at-maturity ogives based on histological staging are deemed as knife-edged at 25 cm LCF and 9 yrs of age, respectively. The histological staging data indicate that all of the Bight redfish in the commercial catch of the GABTF are sexually mature. The smaller, immature component of the Bight redfish population may be found inshore (<100 m depth) outside the range of the current GABTF. Similar length-dependent spatial stock structuring occurs for the related species redfish (*Centroberyx affinis*) located on the south-east coast of Australia. The size and age at which 50% of the female Bight redfish population matures, based on macroscopic staging, was determined as 25 cm LCF and 9 yrs by logistic regression. These revised size estimates are lower than the earlier estimates of 32 cm LCF based on macroscopic staging of all gonads collected during 2000–02 and 28 cm LCF based on GSI data collected during 1988–1990.

For deepwater flathead, very few immature fish were represented in the sample to determine the size-at-maturity with logistic regression. The size- and age-at-maturity based on histological staging are thus best described as knife-edged at 40 cm TL and 3.8 yrs for this species. The size and age at which 50% of the female deepwater flathead population matures, based on macroscopic staging, was determined using logistic regression as 44 cm TL and 4 yrs. These size estimates are consistent with the earlier estimate of 45 cm TL based on the macroscopic staging of all gonads collected during 2000–02 and 45 cm TL based on GSI data collected during 1988-1990 for this species.

The continuous distribution of whole oocyte diameters suggests that the fecundity of both Bight redfish and deepwater flathead is indeterminate (ie. the number of oocytes to be spawned is not fixed prior to the onset of spawning). As an approximation to the estimate of annual fecundity, total fecundity (ie. number of oocytes of advanced development—yolked oocytes, nucleus migrated oocytes, and hydrated oocytes) was determined from eggs-per-recruit analysis. For Bight redfish the oocyte count, based on the standing stock of advanced oocytes, ranged from 0.62 to 1.54 million oocytes per fish. For deepwater flathead, the oocyte count ranged from 0.50 to 3.56 million oocytes per fish.

Bight redfish eggs-per-recruit analysis indicates that maximum egg production occurs at a size of ~25 cm LCF (7.5 yr) or slightly lower than the size of first capture. As such, setting a minimum size limit would have little effect on improving the Bight redfish reproductive capacity. The Bight redfish reproductive capacity of the stock is not compromised as previously suggested with the earlier estimate of size-at-maturity of 32 cm LCF.

Commercial catch sampling indicates that the deepwater flathead size and age of first capture in the GABTF is 30–35 cm TL (2 yr), well below the egg production peak of 42 cm TL (4 yr). It may be possible to improve the reproductive capacity of the deepwater flathead population by increasing the cod end mesh size to increase the size-at-first-capture.

Outcomes Achieved:

This project addressed some of the important information gaps identified during assessment workshops for Bight redfish and deepwater flathead. Improved stock assessments, which incorporate the latest biological parameters (size-at-maturity, fecundity and sex ratio), has lead to the setting of accurate Bight redfish and deepwater flathead TACs. Gear regulations have been proposed that include minimum mesh sizes and T90 extensions, which along with reducing discard species, allow small deepwater flathead to escape capture, and thereby increasing reproductive capacity of the flathead population.

This information has been provided to Great Australian Bight Fisheries Assessment Group (GABFAG) as part of formal and informal reporting arrangements. It has also been distributed to the public via media releases.

Keywords:

Bight redfish, *Centroberyx gerrardi*, deepwater flathead, *Neoplatycephalus conatus*, eggs per recruit, fecundity, reproduction, size- and age-at-maturity, spawning

Acknowledgments

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FINAL REPORT

2003/003 Spawning and reproductive characteristics of Bight redfish and deepwater flathead in the Great Australian Bight Trawl Fishery

Background

The Great Australian Bight Trawl Fishery (GABTF) is a multi-species fishery valued at approximately $7 million annually at the point of first sale. It provides fresh fish for markets in south-eastern Australia and has a growing export market to Asia. As a Commonwealth fishery, the GABTF is managed by the Australian Fisheries Management Authority (AFMA). The fishery covers an extensive area of southern Australian waters, from Cape Leeuwin in Western Australia to Cape Jervis in South Australia. Over most of this region, the fishery extends from the 200-m isobath to the outer limits of the Australian Fishing Zone (AFZ), but it also includes shallower waters between longitudes 125°E and 132°E. Only 10 vessels have Statutory Fishing Rights (SFR) to operate in the GABTF. There are two general components to the GABTF: a continental shelf–upper slope fishery (<200 m) for deepwater flathead (*Neoplatycephalus conatus*) and Bight redfish (*Centroberyx gerrardi*), and a seasonal deepwater slope fishery for orange roughy (*Hoplostethus atlanticus*). The combined annual catch of Bight redfish and deepwater flathead provides more than 50% of the total annual catch.

Bight redfish and deepwater flathead stock assessments in the form of age- and sex- structured population dynamics models have been developed by Bureau of Rural Sciences (BRS) (Wise and Tilzey 2000) as part of an AFMA funded project. The latest stock assessments, which include 2000 and 2001 catch data, were presented by Dr Brent Wise at the October 2002 GABFAG meeting. These assessments suggest that if the exploitation rate remains constant at the 2001 level, then the Bight redfish and deepwater flathead populations will be exploitable at a sustainable rate above a trigger reference point defined as 33% of the unexploited biomass. However, the Bight redfish stock assessment model suggests that fishing mortality has increased steadily since 1993, meaning that the Bight redfish biomass has the potential to drop below the reference point in the future.

The need for more detailed biological data, particularly reproduction indices, maturity ogives and fecundity relationships, was identified by GABFAG as a high priority requirement for stock assessments. During the recently completed FRDC Project 2000/169 'Assessment of Bycatch in the Great Australian Bight Trawl Fishery' (Knuckey and Brown 2002), some of this information was collected opportunistically. The length-weight relationships were consistent with those determined previously by Newton *et al.* (1994); however, the preliminary estimate of size-at-maturity (SOM) based on macroscopic staging of female Bight redfish was 4 cm greater than the previous estimate of 28 cm caudal fork length (LCF) (Newton *et al*. 1994). The differing estimates of size-at-maturity have major implications for the reproductive capacity of the stock and for sustainable fishing mortality and effort. For example, if the size-at-maturity (sexes combined) is 28 cm LCF (Newton *et al.* 1994), 80% of the 2000–02 Bight redfish onboard commercial catch would be mature compared with ~50% if the size-at-maturity (female) is 32cm LCF (Knuckey and Brown 2002). Similarly, a simplistic spawning biomass per-recruit model has shown that fishing mortality, and hence fishing effort, would need to be reduced by one third to maintain a target of 40% of the virgin biomass if size-at-maturity is 32 cm LCF (F = 1.45 and effort = 51 346 h for SOM of 28 cm LCF, F = 0.45 and effort = 15 963 h for SOM of 32 cm LCF).

The long-term sustainability of these two stocks may be further compromised by the fishing effort in the GABTF doubling since 2002 as the number of activated Statutory Fishing Right's increased from five to ten. For these reasons, additional data are required for improving the stock assessments for both species. The proposed study will provide the required maturity ogives and oocyte count–length relationships for both Bight redfish and deepwater flathead.

Need

Bight redfish (*Centroberyx gerrardi*) and deepwater flathead (*Neoplatycephalus conatus*) are the two principal commercial species captured along the continental shelf of the Commonwealth-managed GABTF and are worth an estimated $7 million per annum.

The 2002 stock assessment based on available data indicates that Bight redfish fishing mortality is rising and likely to reduce the biomass to below the trigger biological reference point. The outcomes of stock assessments are highly sensitive to the shape of the maturity ogive and oocyte count–length and oocyte count–age relationships. Given the current high uncertainty of the validity of existing Bight redfish and deepwater flathead maturity and fecundity relationships, GABFAG recommended that these be determined with high priority. More scientifically defensible maturity ogives and oocyte count–length and oocyte count–age relationships will markedly reduce uncertainty in the stock assessments.

Objectives

1 Determine maturity ogives (proportion mature against length/age) and sex ratios of spawning Bight redfish and deepwater flathead.

2 Describe gonad maturation cycles and determine the relationship between numbers of eggs produced (fecundity) and length or age in female Bight redfish and deepwater flathead.

3 Provide per-recruit analysis to identify harvest strategies which allow optimum Bight redfish and deepwater flathead egg production.

4 Provide industry, GABMAC and stock assessment scientists with information on GABTF species biology.

Methods

The present study aims to improve information on the reproduction through macroscopic and histological investigation of Bight redfish and deepwater flathead. The spawning cycles of these species are delineated based on the state of maturation of ovaries, requiring a detailed analysis of the reproductive season (using gonadosomatic indices), ovarian developmental stages, size-at-maturity and fecundity. Per-recruit analyses incorporates maturity and fecundity data, together with age and growth data, to investigate the effects of different levels of fishing effort and size/age at first capture on egg production of Bight redfish and deepwater flathead.

Sample collection and biological data

Samples of reproductive material from Bight redfish and deepwater flathead were sourced from the commercial catch of demersal fish trawlers working in the Great Australian Bight. Samples were collected either by trained field staff at fish processors based in South Australia or by the authors in the wet laboratory facilities at PIRVic, Queenscliff. Sampling was also undertaken in conjunction with the AFMA-funded GAB Integrated Scientific Monitoring Program by PIRVic onboard observers.

Length, measured to the nearest centimetre and mass weighed to the nearest gram were recorded from more than 3000 fish collected bi-monthly on an opportunistic basis during 2000–02, and a further 1600 fish collected monthly during the peak spawning periods of the present study. Length was measured as caudal fork length (LCF) for Bight redfish and as total length (TL) for deepwater flathead. The gonad of every fish was used to determine sex (male, female or indeterminate). Sagittal otoliths were removed for ageing purposes.

Gonad maturation

Macroscopic staging and GSI

Maturation of male and female gonads were assessed according to a set of macroscopic reproductive stage descriptions based on gross anatomical features (including immature, developing, ripe, running ripe, and spent). For Bight redfish, the seven macroscopic stage descriptions of the gonad maturation cycle of blue warehou (*Seriolella brama*), a deep sea teleost of southern Australia (Knuckey and Sivakumaran, 1999), were applied (Appendix 3). For deepwater flathead, the macroscopic stage descriptions of sand flathead (*Platycephalas bassensis*), an Australian temperate water flathead species (Jordan, 2001), were applied (Appendix 4). Staged gonads were removed, weighed to the nearest gram, and preserved in 10% neutral buffered formalin. During 2000–02, several gonads transferred from South Australia to Victoria had been frozen for 2–4 days prior to formalin preservation.

Gonadosomatic indices (GSI), which provide an indication of gonad maturation and spawning season in particular, were determined using the equation:

$$GSI = \frac{G}{W} \times 100\%,$$

where GSI is the gonadosomatic index, G is the gonad mass (g) and W is the whole fish mass (g).

Histological staging

Gonad maturation cycles are more accurately understood by histological examination (West 1990). Histological examination is essential for detecting changing conditions during the ovary maturation cycle, such as partial spawning, postovulatory follicles and atretic oocytes (Hunter & Macewicz 1985a, Hunter & Macewicz 1985b, Schaefer 1987, West 1990, Davis & West 1993, Marshall *et al*. 1993).

Histological examination required the dissected gonads to be cured in formalin for 4–10 weeks, by which time a transverse medial sub-sample of ~30 g of the preserved gonad was sectioned by a commercial pathology service and then returned for subsequent histological examination. The transverse medial material was blocked in paraffin wax and 6μm sections were cut, mounted, and stained in Harris' haematoxylin and eosin (Lunar 1968). Analysis of sexual staging relied on histological interpretations advocated by West (1990) as most appropriate for determining spawning cycles in the ovary. We followed the histological guidelines presented by Baedle (1996), Knuckey and Sivakumaran (1999), and Mackie and Lewis (2001) for Bight redfish and deepwater flathead.

Ovaries were staged on the basis of the most advanced type of oocytes present, regardless of their abundance (Wallace *et al*. 1987, West 1990, Baelde 1996). Oocyte development and maturation is a continuous process, which has been subdivided into various stages to simplify histological classification of ovaries. Descriptions and histological preparation examples of the microscopic stages of oocyte development for female Bight redfish and deepwater flathead are shown in Appendices 3 and 4, respectively.

The presence of postovulatory follicles in ovaries was used to identify females that had begun to spawn (Hunter & Macewicz 1985a, Hunter & Macewicz 1985b, Schaefer 1987). Individuals with postovulatory follicles, or with oocytes developed at least to the stage of exogenous vitellogenesis (appearance of yolk granules), were considered either to have spawned or to be capable of spawning (Bell *et al.* 1992).

The resorption of yolked oocytes and follicles, a procedure known as atresia, characterises the spawning period (Abaunza *et al.* 2003). The presence of atresia provides histological criteria to forecast the end of spawning and also to distinguish immature from mature individuals, which is essential for determining length- and age-at-maturity ogives (Hunter & Macewicz 1985a, Hunter & Macewicz 1985b, Schaefer 1987).

Oocyte measurement

The diameter of whole oocytes were measured and the distribution of these measurements plotted in order to classify fecundity as determinate or indeterminate (i.e. whether or not the number of oocytes to be spawned is fixed prior to the onset of spawning) (Hunter *et al.* 1985). Sub-samples of the preserved ovaries were mixed in small jars with water and shaken manually to dissociate the oocytes. Random

samples of one hundred oocytes from Bight redfish and deepwater flathead at various macroscopic stages of maturity (Appendix 3) were measured along the maximum diameter using image analysis software (Optimus).

At the same time, histological sections from the representative stage II to VI ovaries were selected and all individual oocytes, which had been sectioned through the nucleus, were staged and measured by taking the mean of the maximum and minimum diameter. The size frequency of the unyolked, yolked, and hydrated oocytes was then plotted for each gonad stage.

Size- and age-at-maturity

Size- and age-at-maturity ogives, which indicate the proportion of fish mature at any length or age, were determined for each species. A fish was classified as mature if the gonads were staged as developing, mature, running ripe, spent, resting or redeveloping. Maturity ogives were determined separately for macroscopically staged and histologically staged gonads. Data were pooled across the 2000–02 and 2003/04 collection periods for Bight redfish spawning during January–May, and for deepwater flathead spawning during October–May. Samples collected from the peak spawning periods instead of from the whole year were used to reduce the incidence of incorrectly classifying mature fish with redeveloping gonads as immature fish developing for first time (Abaunza *et al.* 2003). Age-at-maturity ogives for Bight redfish were determined where age estimates of otoliths removed during the present study were available (Stokie 2004).

A logistic curve was fitted to the data using a non-linear least-squares procedure weighted by the sample size of each length-class. The logistic regression equation takes the form of

$$\%mature = 100/(1+e^{a(b-c)})$$

where a is the parameter indicting the rate of increase in maturity, b is the parameter representing 50% maturity, and c is the 1-cm length-class or 1-yr age-class.

Oocyte count

Total fecundity is determined from the standing stock of advanced oocytes (yolked oocytes, nucleus migrated oocytes and hydrated oocytes) at the beginning of the spawning season (Hunter & Macewicz 1985a). Ovaries macroscopically staged as 'maturing', 'ripe' or 'running ripe' (stages IV–VI) were potentially suitable for the estimation of total fecundity. Upon more detailed histological examination, only those ovaries with advanced oocytes which showed no sign of previous spawning in that season (i.e. no postovulatory follicles and no sign of major atresia) were used to estimate the total fecundity.

For the total fecundity estimation, five to ten random samples of 0.1 to 0.2 g each were taken from the anterior, middle and posterior regions of each ovary of each specimen. These sub-samples were pooled to form a single composite sample of approximately 1 g. The mass of this sample was determined to the nearest 0.001 g. The number of advanced oocytes in each sample was counted under a binocular microscope and the total number of oocytes in each ovary was estimated using the gravimetric method of Hunter *et al.* (1985), Bagenal (1978), Cailliet (1986) and Knuckey and Sivakumaran (1999). Total fecundity is given by the equation

$$TF = \frac{c}{s} \times OW \ ,$$

where TF is the total fecundity, c is the number of advanced oocytes counted in a sample, s is the mass of a sample (g) and OW is the mass of the two ovaries combined (g).

The relationship between total fecundity and fish length, weight and age are obtained by plotting the data as a scatter-plot and fitting linear regressions up to the cubic polynomial form. The relative fecundity (Bagenal 1978), a term used to make observations on fish of different size more comparable, was defined as the number of maturing oocytes found in the ovary (total fecundity) per whole fish mass (g).

Per-recruit analyses

Maturity and fecundity data, together with age and growth data provided by the Central Ageing Facility based at the PIRVic Queenscliff Centre, were used in per-recruit analyses to investigate the effects of different levels of fishing effort and size/age at first capture on egg production of Bight redfish and deepwater flathead. A Microsoft Excel spreadsheet developed by Sanders (1995) and sourced from GABFAG scientific adviser, Dr Ian Knuckey (Fishwell Consulting Pty Ltd) was used to undertake the per-recruit analyses. For each species a separate per-recruit model was developed with species specific inputs including length weight parameters (Knuckey and Brown, 2002), von Bertalanffy growth parameters derived from 1990–2003 ages estimates and natural mortality estimates derived from life history parameters (Beverton and Holt, 1957; Gulland, 1983; Pauly, 1980). Size- and age-at-maturity and total fecundity relationships determined during the present study were also inputed into their species specific per-recruit model.

Results

Results are presented separately for Bight redfish and deepwater flathead.

Bight redfish

A total of 1,306 Bight redfish were collected during October 2000–July 2002 (usually bi-monthly), and a further 982 were collected during August 2003–May 2004 (usually monthly in the present study) from the Great Australian Bight for analysis of their reproductive condition (Table 1 and 2). The size ranges of male and female Bight redfish ranged 21–48 cm LCF and 23–52 cm LCF, respectively. Only 2 specimens were of indeterminate sex.

Table 1. Bight redfish samples taken during the 2000–02 collection period

Sex	2000					2001									2002										Total
	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	
F	nd	nd	86	nd	44	nd	54	nd	52	nd	45	nd	35	nd	nd	31	42	24	34	nd	84	51	nd	40	622
M	nd	nd	76	nd	53	nd	96	nd	43	nd	45	nd	50	nd	nd	47	46	38	46	nd	90	21	nd	31	682
U	nd	nd	1	nd	0	nd	0	nd	0	nd	0	nd	0	nd	nd	0	1	0	0	nd	0	0	nd	0	2
ALL	nd	nd	163	nd	97	nd	150	nd	95	nd	90	nd	85	nd	nd	78	89	62	80	nd	174	72	nd	71	1306

nd, no data

Table 2. Bight redfish samples taken during the 2003/04 collection period (present study)

Sex	2003					2004						Total
	A	S	O	N	D	J	F	M	A	M	J	
F	42*	nd	57	nd	26	25	84	160	67	34	nd	453
M	58*	nd	62	nd	14	39	69	181	42	22	nd	429
U	0	nd	0	nd	0	0	0	0	0	0	nd	0
ALL	100*	nd	119	nd	40	64	153	341	109	56	nd	882

* frames sampled not whole fish; nd, no data

Gonad maturation

GSI and macroscopic staging

Gonads were staged macroscopically and GSI values calculated for 1,075 female and 1,111 male Bight redfish. A further 42 female and 58 male Bight redfish frames collected during August 2003 were only staged macroscopically. A clear relationship was apparent between mean GSI and reproductive stage in females (Figure 1). GSI values were low for females with ovaries assigned to stages I–II, reflecting their immature status, and slowly increased in fish with ovaries allocated stages IIb–V, coinciding with the maturation of the ovary. GSI in stage VI ovaries was slightly less than stage V owing to some individuals

having already shed an unknown number of oocytes, resulting in loss of ovary mass (partially spent). Maximum GSI values for males (1.4–6.0%) were lower than those for females (0.2–8.6%) (Figure 2). Seasonal maturation of gonads begins in January, peaks in March and April, and finishes by May (Figure 3 and 4). The gonad development of males paralleled those of females.

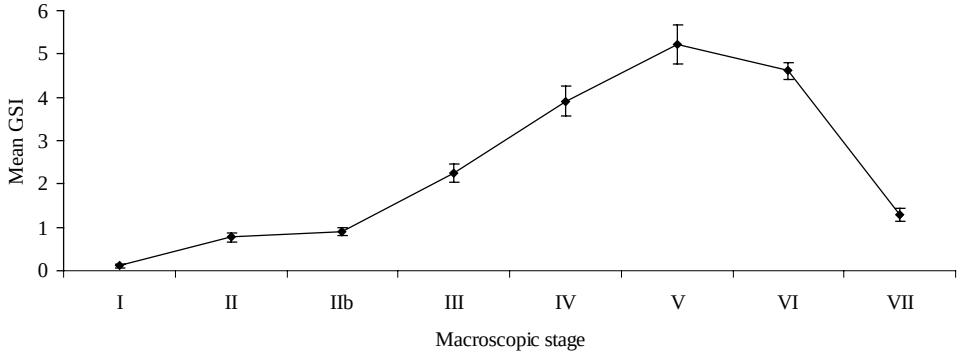


Figure 1. Mean GSI (± S.E.) of female Bight redfish plotted against macroscopic stage.

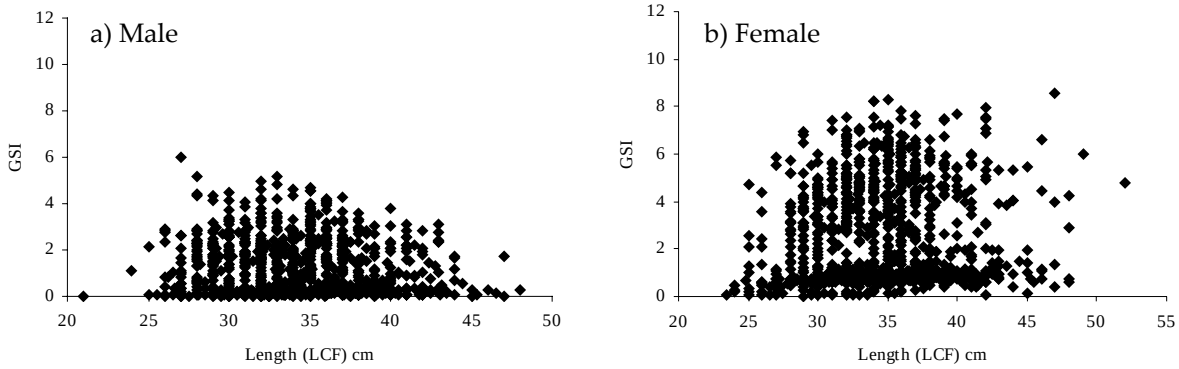


Figure 2. GSI of a) male and b) female Bight redfish plotted against length.

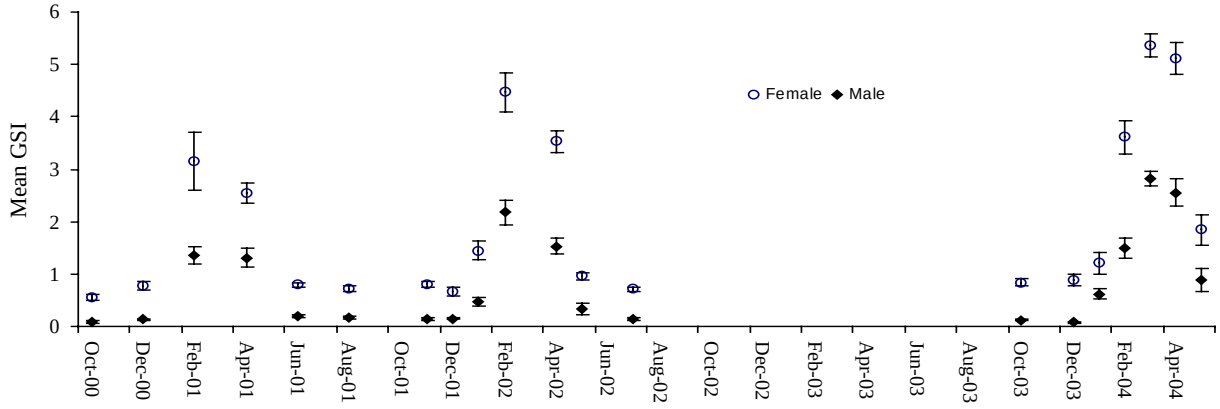
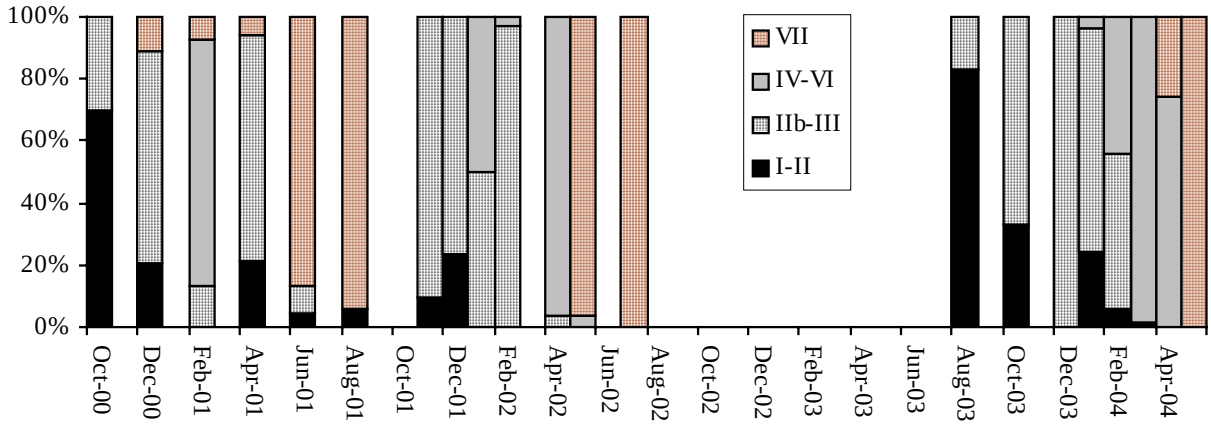


Figure 3. Mean monthly GSI (±S.E.) for male and female Bight redfish.

a) Male



b) Female

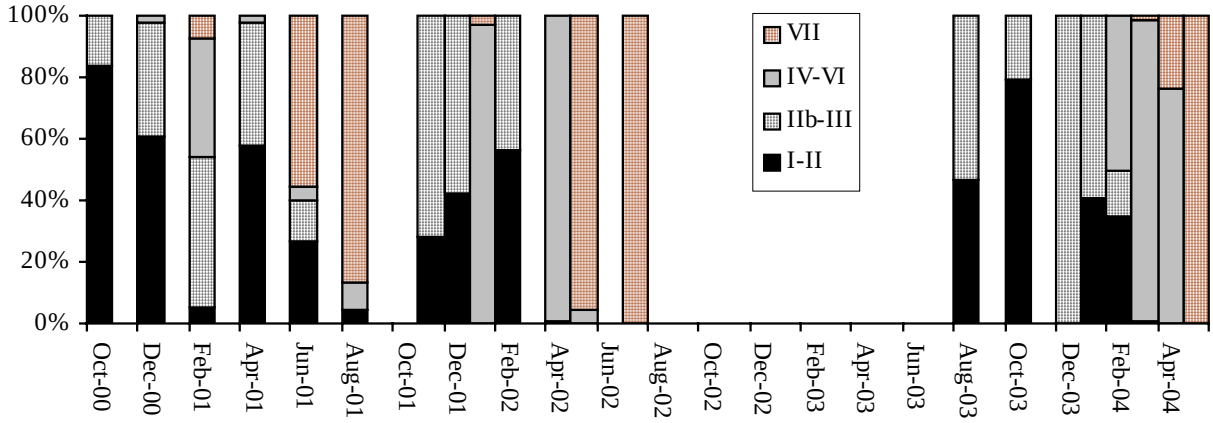


Figure 4. Monthly gonad development based on macroscopic staging for a) male and b) female Bight redfish.

immature =stage I–II; developing = stage IIb–III; spawning = stage IV–VI; spent = stage VII

Histology and microscopic staging

From the pool of preserved gonads collected during October 2000–May 2004, 407 ovaries were randomly selected for histological analysis to assess changes in the reproductive state of female Bight redfish. Of these, only 289 histological slides were suitable for the analysis. Histological slides discarded include those slides from frozen ovaries and older samples collected during 2000–02. Histological analysis was based on the following features: endogenous vitellogenic oocytes (i.e. accumulation of yolk globules in cytoplasm); exogenous vitellogenic oocytes (i.e. appearance of yolk granules); oocytes showing germinal vesicle migration (i.e. peripheral movement and breakdown of nucleus); mature oocytes (i.e. coalescence of yolk plates and hydration); postovulatory follicles (i.e. ruptured, empty follicles marking positions of ovulated mature oocytes with the degenerating follicle showing fewer loops and reduced in size); and atretic mature oocytes (i..e. resorption of unused mature oocytes). In mature ovaries, the cytoplasm of the largest oocytes is full of yolk granules and lipid droplets. Just prior to spawning, the hydration process

continues until ovulation, when the follicular epithelium surrounding the oocyte breaks and the egg is released. The follicular cells then form strings, which are folded in the space left by the egg. These postovulatory follicles undergo a rapid degeneration. Except for atretic mature oocytes, examples of the histological appearance of these features can be seen in Appendix 3.

In the sectioned material, oocytes less than 152μm in diameter constitute the reservoir of oocytes that are present year round in ovaries (Table 3). Of ovaries collected during the study period which were staged macroscopically as 'spent' (stage VII) based on histology, 43% had new and old postovulatory follicles. The mean diameter range of oocytes belonging to microscopic stages II to VI were 17–52 μm, 92–301 μm, 129–653 μm, 240–720 μm and 355–1272 μm, respectively (Table 3). The yolked oocytes (stage IV) were present during October to April. In the present study, oocytes stages from perinucleolar to hydrated (stages II to VI), were found in the macroscopically staged gonads.

Table 3. Description of microscopic stages in oocyte development of female Bight redfish

Stage	Stage Description	Histological description
I	Chromatin nucleolar	Very small oocytes, nucleus surrounded by a thin layer of dark-blue-stained cytoplasm (<17 μm)
II	Perinucleolar	Oocyte size increases slightly as dark-blue-stained cytoplasm thickens, nucleoli appear at at the periphery of nucleus (17–152 μm; n = 384, 51 ovaries)
III	Cortical alveoli	Appearance of cortical alveoli in pale-blue-stained cytoplasm, pink-stained zona radiata distinguishable, oil vesicles appearing, lampbrush chromosomes often visible in the nucleus (92–301 μm; n = 661, 96 ovaries)
IV	Yolk	Marked increase in oocyte size, cytoplasm filled with pink-stained yolk granules, cortical alveoli and oil vesicles increase in size and number (129–653 μm; n = 866, 45 ovaries)
V	Nuclear migration	Migration of nucleus to periphery of oocyte, fusion of yolk granules into yolk plates; fusion of oil vesicles into the oil droplet (240–720 μm; n = 344, 19 ovaries)
VI	Hydration	Further increase in size of oocytes, all yolk granules fused into a few plates (355–1272 μm; n = 307, 12 ovaries)
POF (new)	New postovulatory follicle	Remaining follicle soon after ovulation. It is large, highly convoluted with an obvious lumen, and may contain fine granular material. The layered nature of both cell types (thecal and granulosa) remains intact in lumen
POF (old)	Old postovulatory follicle	Convoluted nature much less apparent, lumen much reduced, even closed, and the thecal and granulosa cells no longer retain their orderly arrangement
α	α-atretic oocyte	Zona radiata dissolves, oocyte shape loses integrity yolk globules begin to disintegrate and are less regular in shape
β	β-atretic oocyte	Numerous disorganised granulosa cells surrounded by a thin thecal and blood vessel layer. Nucleus of some of the granulosa cells is pycnotic and many of the cells contain a large intracellular vacuole that may be empty or contain amorphous particles

The rates of atresia for both unyolked and yolked oocytes were generally low, when present, atretic oocytes represented between 10 and 20% of all oocytes (Table 4). A few ovaries, however, presented a high level of atresia (~70%) of their yolked oocytes. Histological examination revealed that 18% of the ovaries sampled were atretic and ~15% belonged to the first type of atresia (alpha stage atresia). Ovaries with atretic oocytes were observed during February to May in the present study, and during August and October during 2000–02. The occurrence of atretic oocytes was noted in all the ovarian maturity stages. Alpha atretic oocytes belong to histological oocyte stages III–VI (cortical alveoli, yolk, nuclear migration and hydration). Eight females presented the second type of atretic ovaries (beta stage atresia). Neither alpha nor beta atretic oocytes could be detected macroscopically, but were recognisable microscopically in the preserved samples. Atresia becomes increasingly noticeable as the spawning season draws to a close and the remaining advanced oocytes in the ovary are reabsorbed. In many individuals at the 'partially spent' and 'spent' stage (VI and VII), yolked oocytes in atresia were the most advanced type observed, indicating that spawning was over for these fish and reabsorption had started.

The presence of hydrated oocytes was detected by histological analysis from fifteen ovaries from the two collection periods. Only four macroscopically staged 'running ripe' ovaries (stage VI) from the present study period had hydrated oocytes.

Postovulatory follicles were found in 126 (43%) of all ovaries histologically examined. They were absent in ovaries at the early stage of yolk formation. New and old postovulatory follicles were present in ovaries belonging to macroscopic stages III, IV, V, and VI during February–May (Table 5). These females were partially spent, yet capable of spawning again that season.

Measurement of oocytes

A number of distinct groups of oocytes at different developmental stages were observed during oocyte measurement of Bight redfish ovaries. The size-frequency distribution of whole oocytes in ovaries shows the developmental sequence of maturation. Oocyte diameters clearly show a broad distribution, with peaks corresponding to size ranges shown in the macroscopic stages, with some overlap between stages (Figure 5, left). As maturation progressed, there was no clear gap between size-modes of unyolked and yolked oocytes, showing that the fecundity of Bight redfish is indeterminate. This continuous size distribution is confirmed in histological preparations of oocytes in Bight redfish ovaries (Figure 5, right)

Table 4. Distribution of the most advanced microscopic stages observed after macroscopic classification in Bight redfish ovaries

Microscopic stages	Mature virgin	Recovering	Developing	Late developing	Ripe	Running-ripe	Spent
Perinucleolar stage	3	5	2				6
Cortical alveoli stage	35	24	3				3
Yolk stage	1		18	2			1
Nuclear migration stage			2				
Hydration stage				4	2	1	
New POF stage			1	3	9	10	7
Old POF stage	1	1	7	16	8	58	5
Cortical alveoli oocytes in atresia	4	4	1				
Yolk oocytes in atresia	5	3	2	1	1	4	4
Nuclear migrated oocytes in atresia			5	6	1	1	
Hydrated oocytes in atresia			1		1	1	2
All oocytes in atresia				1	1	2	
Total	**49**	**37**	**42**	**33**	**23**	**77**	**28**

Table 5. Evidence of spawning of female Bight redfish. Presence of new (N) and old (O) postovulatory follicles is indicated

	2000		2001							2002				
	Oct	Dec	Feb	Apr	Jun	Aug	Sep	Nov	Dec	Jan	Feb	Apr	May	Jul
Presence of new (N) POF														
Presence of old (O) POF				O								O		

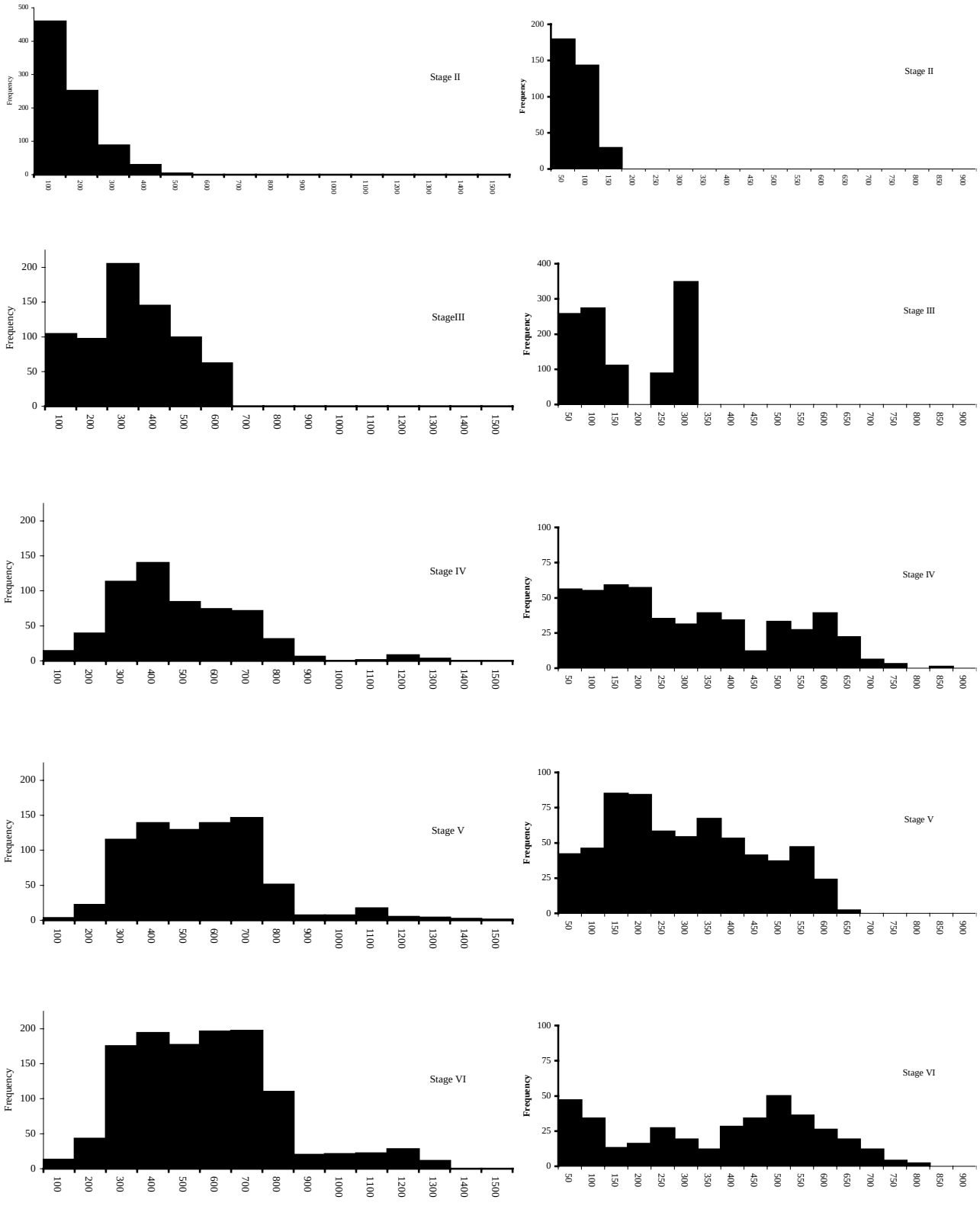


Figure 5. Frequency distribution of Bight redfish whole oocyte diameter (left) and oocyte diameter from histological sections (right) of different stages of gonad maturity.

Sex ratio

The proportion of female Bight redfish in individual samples taken from the commercial catch samples varied from 36 to 71%, with an average of 49% (sex ratio: ≅ 1:1; Female: Male) (Table 6).

Table 6. Summary of the proportion of female Bight redfish by month.

Year	Month	Female	Male	% Female
2000	10	86	76	53
2000	12	44	53	45
2001	2	54	96	36
2001	4	52	43	55
2001	6	45	45	50
2001	8	35	50	41
2001	11	31	47	40
2001	12	42	46	48
2002	1	24	38	39
2002	2	34	46	43
2002	4	84	90	48
2002	5	51	21	71
2002	7	40	31	56
2003	8	42	58	42
2003	10	57	62	48
2003	12	26	14	65
2004	1	25	39	39
2004	2	84	69	55
2004	3	160	181	47
2004	4	67	42	61
2004	5	34	22	61

Size- and age-of-maturity

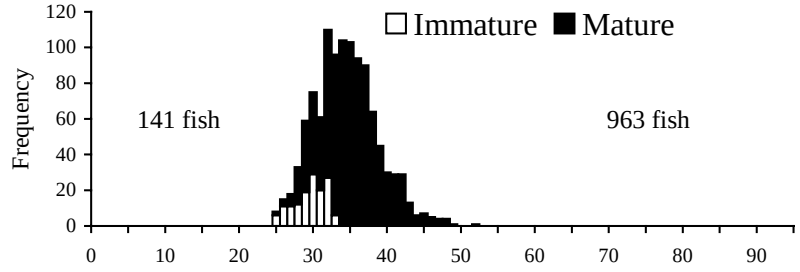
Separate size- and age-at-maturity ogives have been determined for female Bight redfish based on macroscopic and histological staging of gonads. Data were pooled over the 2000–02 and 2003/04 collection periods for the spawning period (January–May) and for the whole year (Figure 6). Ogives determined for the spawning period instead of the whole year reduce the incidence of incorrectly classifying mature individuals with redeveloping gonads as immature individuals with gonads developing for the first time.

The size at which 50% of female Bight redfish in the population is mature, based on macroscopic staging, was 28 cm LCF for all data pooled across two collection periods, and 25 cm LCF for data from the peak spawning period (Figure 7). The earlier estimate of 32 cm LCF, based on macroscopic staging of all data from the 2000–02 collection period, was an overestimation, with several mature individuals in the resting or redeveloping stage of the maturation cycle misclassified as immature.

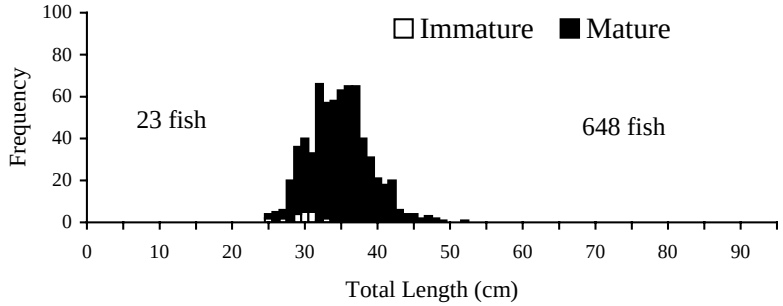
The size-at-maturity logistic regression for female Bight redfish, based on the more accurate histological staging technique was not determined, as all of the 289 histological samples examined during the spawning period were deemed mature. Potentially, only sexually mature individuals are recruited into the GABTF. As an approximation for input into the per-recruit analyses, the Bight redfish size-at-maturity based on histological staging is deemed as knife edged with all individuals greater than 25 cm LCF classed as mature (Figure 7).

The age-at-maturity logistic regressions for female Bight redfish, based on macroscopic and histological staging, were not determined. Over two–thirds of the 22 fish staged macroscopically as immature were estimated at 13–23 yrs (Figure 8). It is most likely that these fish were misclassified as immature. All of the 61 histological samples with age estimates, collected during the spawning period, were deemed mature. The age-at-maturity ogive for Bight redfish, based on histological staging, is deemed as knife edged, with all individuals 9+ yrs classed as mature.

a) Macroscopic stage (all)



b) Macroscopic Stage (spawning period Jan-May)



c) Histological Stage (spawning period Jan-May)

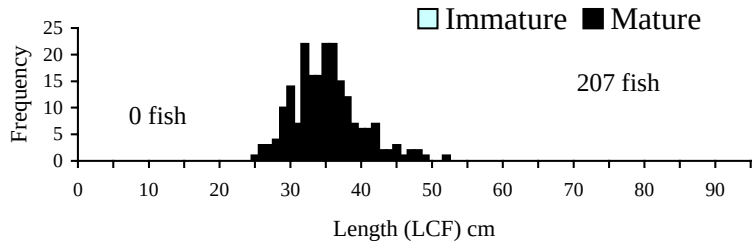


Figure 6. Size distributions of female Bight redfish staged as immature or mature based on a) macroscopic staging throughout the year and b) macroscopic and c) histological staging during the spawning period.

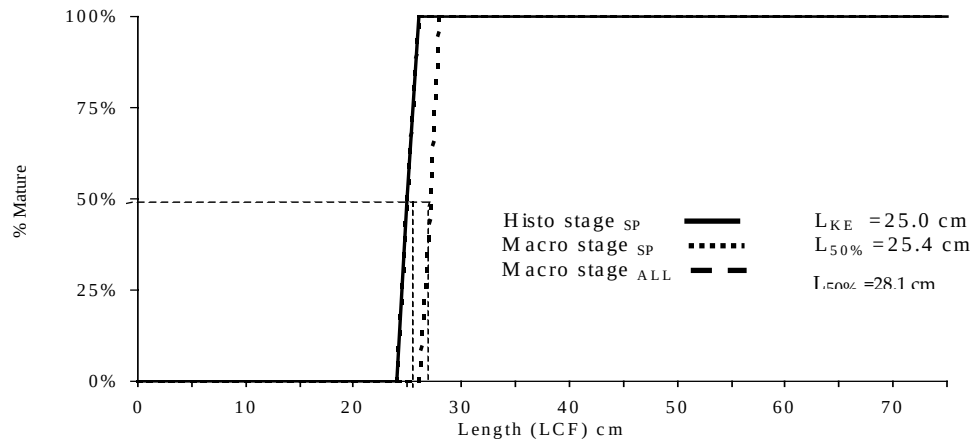
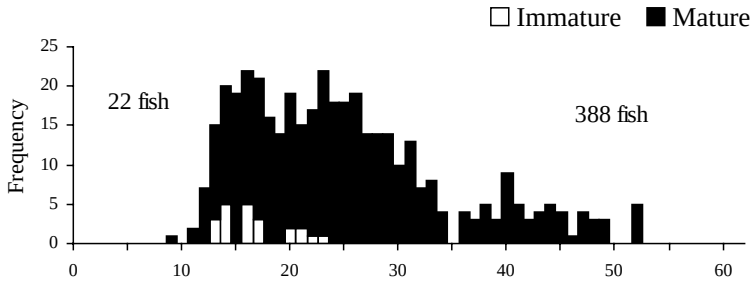


Figure 7. Maturity ogive for female Bight redfish plotted against length for ovaries staged macroscopically for all data and staged macroscopically and histologically for the spawning period (SP) only. Straight lines indicate length at 50% mature. (KE, Knife-edged maturity; $L_{50\%}$, Logistic curve maturity).

a) Macroscopic Stage (spawning period Jan-May)



b) Histological Stage (spawning period Jan-May)

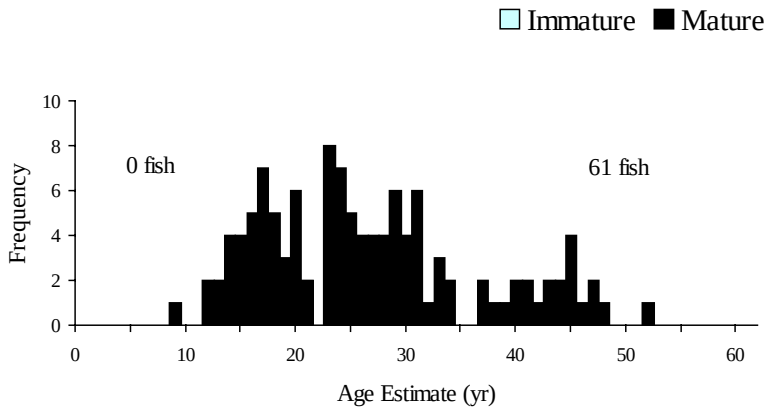


Figure 8. Age distributions of female Bight redfish staged as immature or mature based on a) macroscopic and b) histological staging during the spawning period.

Oocyte count

The total fecundity relationships with length, weight and age were based on oocyte counts from gonads staged histologically as 'running-ripe' (ie. no atresia and no postovulatory follicles present) (Figure 9). Of the 186 gonad samples examined histologically during the peak spawning period in 2003/04, only six samples were 'running ripe'. Postovulatory follicles were present in the majority of the gonad samples examined histologically, indicating that spawning had commenced (i.e. fish were 'partially spent'). The total fecundity, estimated from the standing stock of yolked oocytes, nuclear migrated oocytes and hydrated oocytes of 'running ripe' gonads, ranged between 0.62 and 1.54 million oocytes per fish. The total fecundity of Bight redfish increased exponentially with length and weight, but was not related to age. The total fecundity (TF) can be best described by fish length (LCF) with the following relationship:

$$TF = 386.54 \times LCF^{2.16;}\ n = 6;\ r^2 = 0.8612.$$

The relative fecundity of Bight redfish ranged between 0.55 and 0.84 million oocytes per kg. However, in contrast, no relationship between relative fecundity and fish length was observed.

Oocytes were counted in a further fifty gonads which were staged macroscopically as 'running ripe' but subsequently classified histologically as 'partially spent' (postovulatory follicles present). As expected, with spawning having already commenced, fewer yolked oocytes were observed in the 'partially spent' gonads with counts ranging between 0.33 and 1.47 million oocytes per fish (Figure 9).

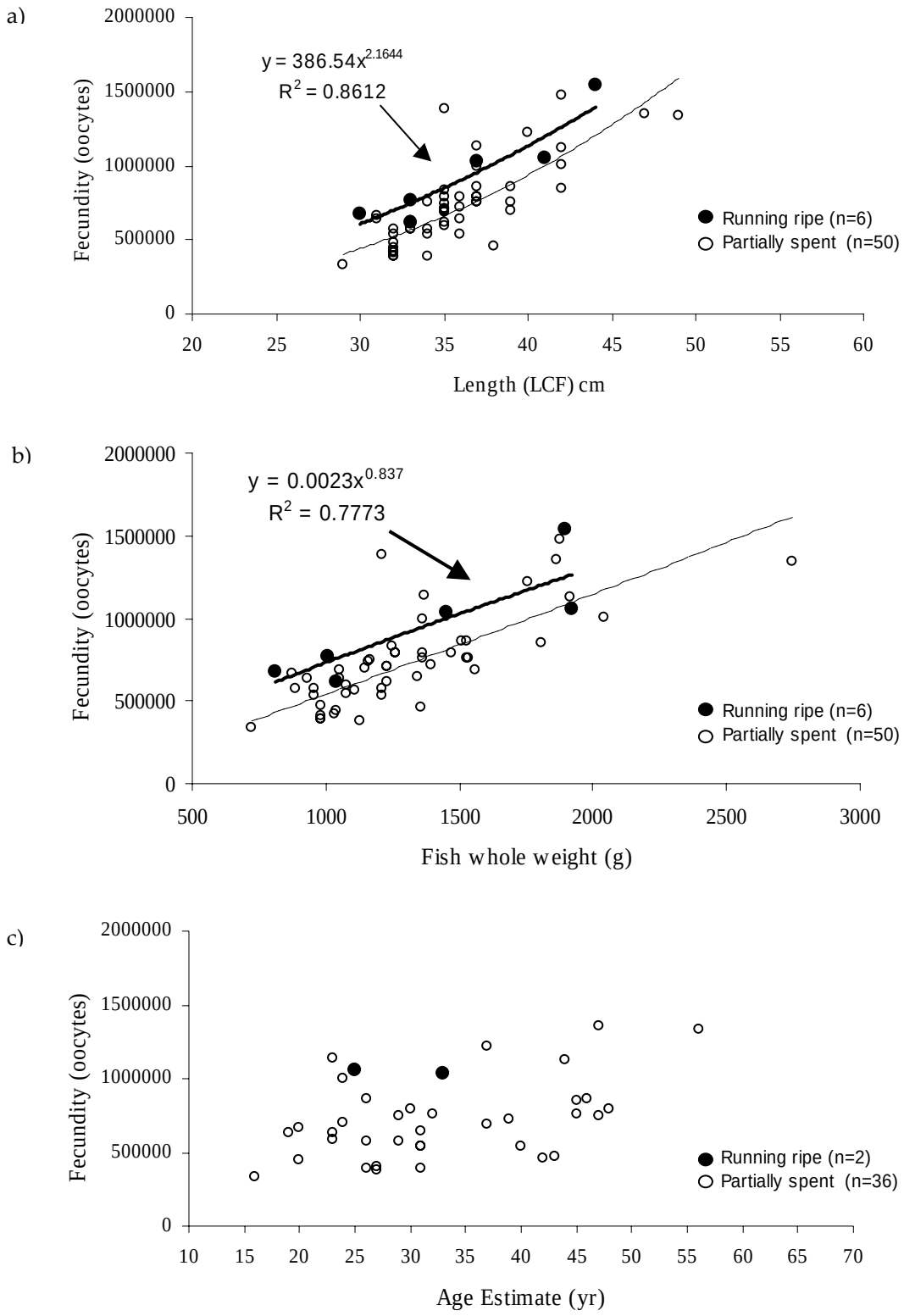


Figure 9. Fecundity relationship with a) length, b) weight and c) age for Bight redfish.

Separate oocyte counts of the 'running ripe' (no atresia, no postovulatory follicles) and 'partially spent' (postovulatory follicles present) gonads are presented.

Per-recruit Analyses

Biological parameters for egg per-recruit analyses of Bight redfish and deepwater flathead are presented in Table 7.

Egg production in Bight redfish peaks at ~25 cm LCF and age 7.5 yr for a range of natural mortality estimates (0.13–0.26), assuming no fishing mortality (Figure 10). The paucity of data of small Bight redfish (<25 cm LCF) resulted in a truncated bell shaped curve to describe egg production. The egg per-recruit analyses suggests that the actual peak in egg production occurs at a size and age smaller than the size and age of first capture (24 cm LCF; 7yr). A lower level of natural mortality (M=0.13) results in a greater proportion of larger and older fish contributing to total egg production. Egg production decreases exponentially with increased fishing mortality (Figure 11).

Table 7. Biological parameters for egg per recruit analyses of Bight redfish and deepwater flathead.

PARAMETERS		Bight redfish	Deepwater flathead
Weight (g):	a	1.28E-01	2.00E-03
W = a * L^b	b	2.56	3.33
Growth (year) :	Linf	38.19	69.82
Lt = Linf*(1-exp(-K(t-to)))	K	0.09	0.20
	to	-5.09	-0.64
Natural Mortality (year)	$M_{Beverton\&Holt}$	0.26	0.53
	$M_{Gulland}$	0.13	-
	M_{Pauly}	0.21	0.30
Maturity	a	22.18	1.62
	b	25.00	40.00
Fecundity	a	386.54	0.29
	b	2.16	3.76

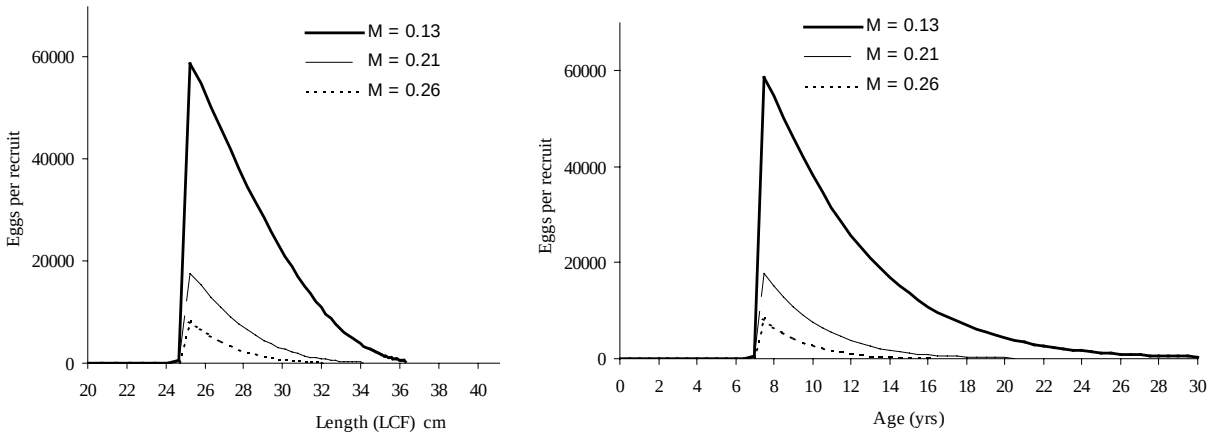


Figure 10. Eggs-per-recruit for female Bight redfish plotted against length and age for two different estimates of natural mortality (M). No fishing mortality has been incorporated into these analyses.

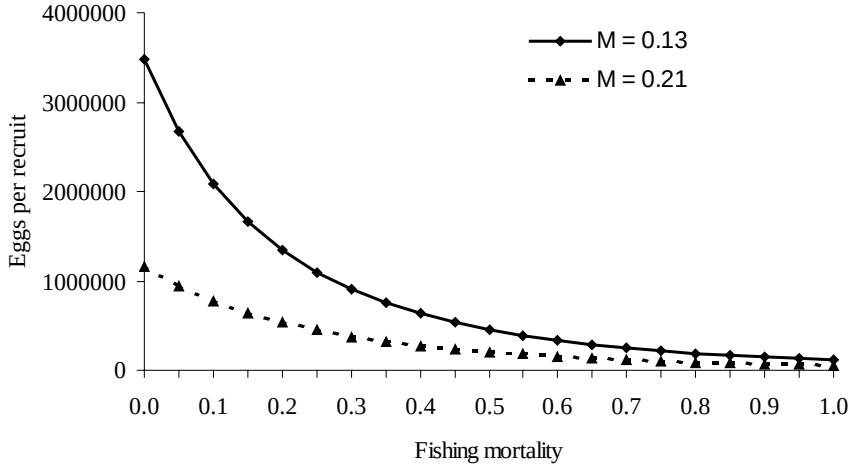


Figure 11. Eggs-per-recruit of Bight redfish for two different estimates of natural mortality (M) over a range of values of fishing mortality.

Deepwater flathead

Over 1,725 deepwater flathead were collected during October 2000–July 2002 (usually bi-monthly), and a further 667 were collected during August 2003–May 2004 (usually monthly) (Table 8 and 9). The size ranges of male and female deepwater flathead ranged 34–59 cm TL and 33–77 cm TL, respectively. Thirty-two specimens were of indeterminate sex.

Table 8. Deepwater flathead samples taken during the 2000–02 collection period

Sex	2000					2001											2002								Total
	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	
F	nd	nd	117	nd	35	nd	143	nd	40	nd	50	nd	34	49	nd	53	58	39	77	71	64	69	nd	33	932
M	nd	nd	67	nd	65	nd	62	nd	60	nd	50	nd	66	59	nd	52	28	59	1	2	135	6	nd	49	761
U	nd	nd	0	nd	0	nd	1	nd	0	nd	0	nd	0	2	nd	0	20	2	0	0	6	1	nd	0	32
ALL	nd	nd	184	nd	100	nd	206	nd	100	nd	100	nd	100	110	nd	105	106	100	78	73	205	76	nd	82	1725

nd, no data

Table 9.Deepwater flathead samples taken during the 2003/04 collection period (present study)

Sex	2003					2004						Total
	A	S	O	N	D	J	F	M	A	M	J	
F	25	nd	78	nd	73	43	84	47	38	53	nd	441
M	75	nd	87	nd	0	2	19	1	35	7	nd	226
U	0	nd	0	nd	0	0	0	0	0	0	nd	0
ALL	100	nd	165	nd	73	45	103	48	73	60	nd	667

nd, no data

Gonad maturation

GSI and macroscopic staging

Gonads were staged macroscopically and GSI values calculated for 1,373 female and 987 male deepwater flathead. A clear relationship was apparent between mean GSI and reproductive stage in females (Figure 12). GSI values were low for females with ovaries assigned to stages I–II, reflecting their immature status, slowly increasing in fish with ovaries allocated stages IIb–IV, coinciding with the maturation of the ovary. GSI in stage V–VI ovaries showed considerable variability owing to some individuals having already shed an unknown number of oocytes, resulting in loss of ovary mass (partially spent). Maximum GSI values for males (1–5%) were considerably lower than those for females (2–16%) (Figure 13). Seasonal maturation of gonads begins in August and continues through January to May (Figures 14 and 15).

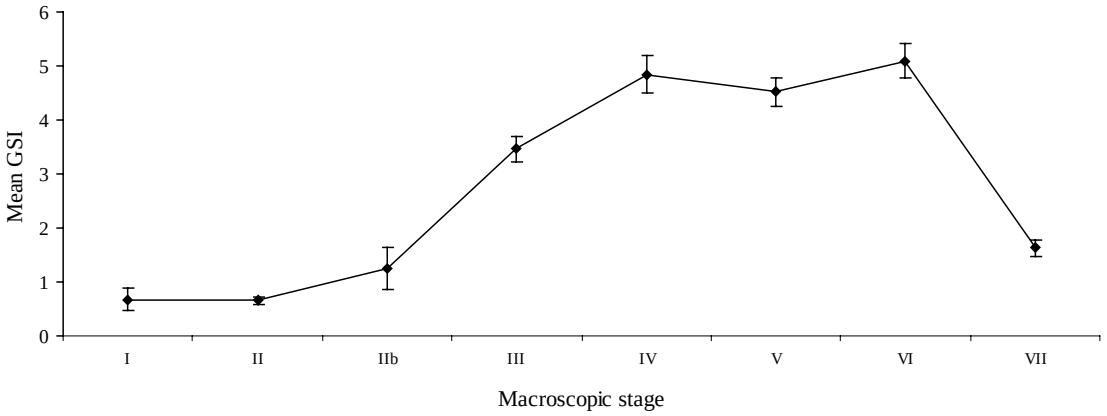


Figure 12. Mean GSI (±S.E.) of female deepwater flathead plotted against macroscopic stage.

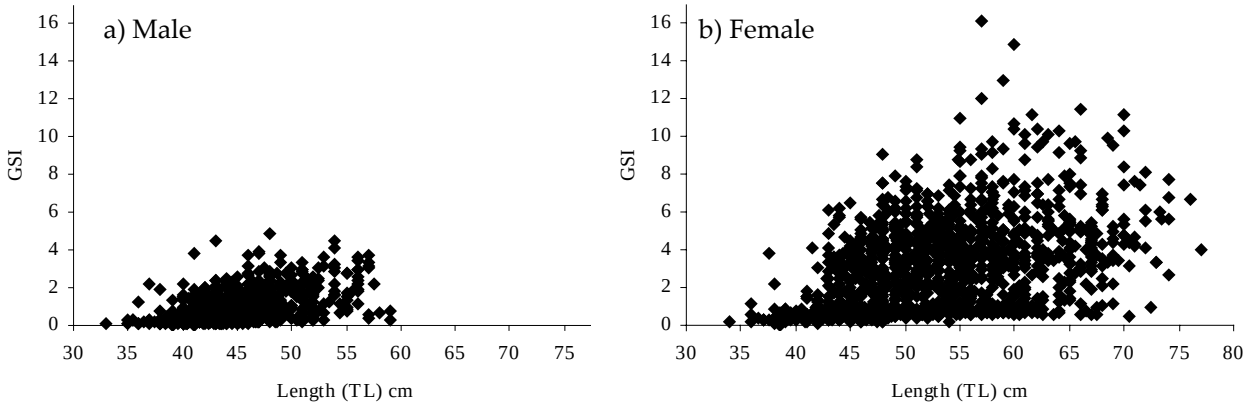


Figure 13. GSI of a) male and b) female deepwater flathead plotted against length.

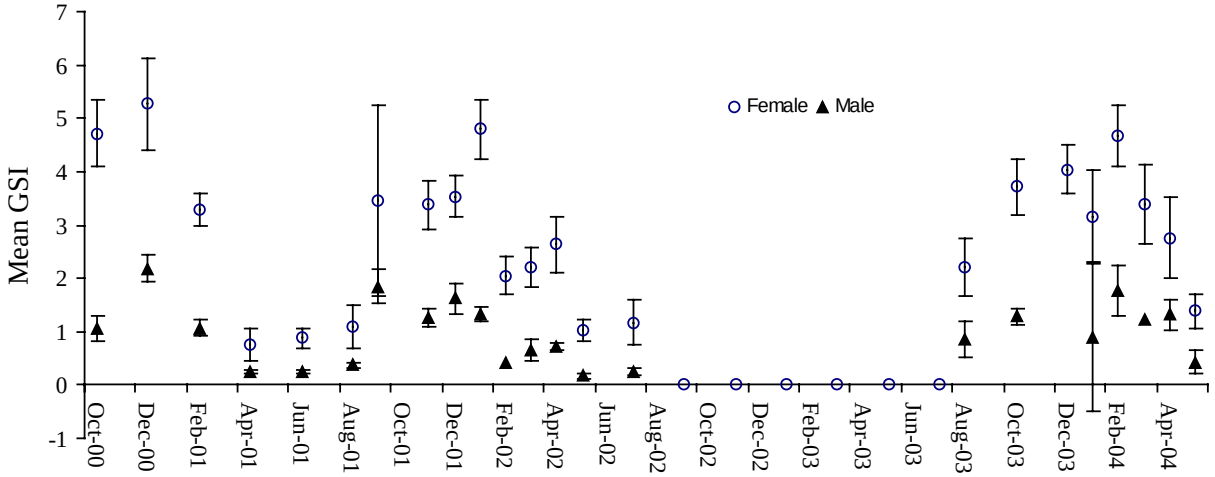


Figure 14. Mean monthly GSI (±S.E.) for male and female deepwater flathead.

a) Male

b) Female

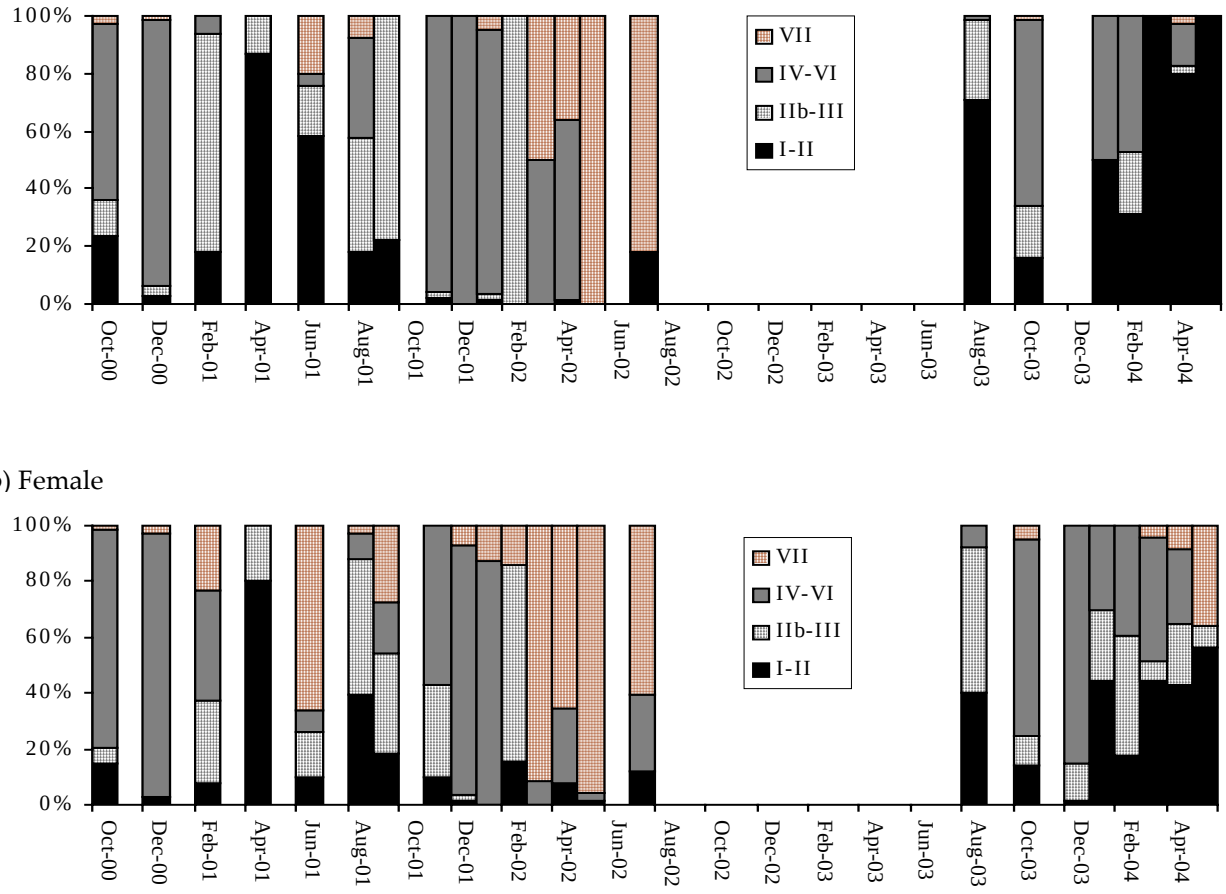


Figure 15. Monthly gonad development based on macroscopic staging for a) male and b) female deepwater flathead

immature =stage I–II; developing =stage IIb–III; spawning =stage IV–VI; spent =stage VII

Histology and microscopic staging
From the pool of preserved gonads collected during October 2000–May 2004, 552 ovaries were randomly selected for histological analysis to assess changes in the reproductive state of deepwater flathead. Of these only 430 histological slides were suitable for the analysis. Discarded slides include those slides from frozen ovaries and older samples collected during 2000–02

The mean oocyte diameter of less than 283 μm in sectioned material constitutes the reservoir of oocytes that is present year round in ovaries (Table 10). Out of 32 sexually indeterminate deepwater flathead, twenty fish were sub-sampled for histological examination. The results showed three were female whose oocytes were at perinucleolar and cortical alveoli stages. Of ovaries collected during the study period which were staged macroscopically as 'spent' (stage VII) based on histology, 30% had new and old postovulatory follicles. The mean diameter range of oocytes belonging to microscopic stages II to VI were 20–283 μm, 61–571 μm, 182–691 μm, 269–754 μm and 370–816 μm respectively (Table 10). In the present study oocytes stages from perinucleolar to hydrated (stages II to VI), were found in the macroscopically staged gonads

The rates of atresia for both unyolked and yolked oocytes were generally low and when present, atretic oocytes represented between 10 to 30% of all oocytes. However, few ovaries presented a high level of atresia (~80%) of their yolked oocytes. Histological examination revealed that 16% of the ovaries sampled were atretic and ~11% belong to first type of atresia (alpha stage atresia). Ovaries with atretic oocytes were observed throughout the year and occurrence noted in all the ovarian maturity stages (Table 10). Alpha atretic oocytes belong to histological oocyte stages III–VI (cortical alveoli, yolk, nuclear migration and hydration). Twenty-two females presented the second type of atretic ovaries (beta stage atresia). Neither alpha nor beta atretic oocytes could be detected macroscopically, but both were recognisable microscopically in the preserved samples. Atresia becomes noticeable as the spawning season draws to a close and the remaining advanced oocytes in the ovary are reabsorbed. In many individuals at the partially spent and spent stage (VI–VII) atretic yolked oocytes were the most advanced type observed, indicating that spawning was over for these fish and reabsorption had started.

The presence of hydrated oocytes was detected by histological analysis from 24 ovaries from the two collection periods. Only seven macroscopically staged 'running ripe' ovaries (stage VI) from the present study period contained hydrated oocytes.

Postovulatory follicles were found in 142 (33%) of all ovaries histologically examined. They were however, absent in ovaries at the early stage of yolk formation. New and old postovulatory follicles were present in ovaries belonging to macroscopic stages IIb, III, IV, V, and VI during October–June (Table 12). These females are partially spent yet capable of spawning again that season.

Some ovaries collected during the study period were staged macroscopically as 'spent' (stage VII), however on the subsequent histological analysis, no new or old postovulatory follicles were observed. These 'spent' ovaries showed cortical alveoli, yolked, nuclear migrated and hydrated oocytes in atresia, and few of them were in redeveloping stage, showing no sign of atresia.

Measurement of oocytes
In deepwater flathead with 'running-ripe' ovaries a number of distinct groups of oocytes at different developmental stages were observed during oocyte measurement. The size-frequency distribution of whole oocytes shows the developmental sequence of maturation. Oocytes diameter clearly shows a polymodal distribution, with peaks corresponding to size ranges shown in the macroscopic stages, with some overlap between stages (Figure 16, left). As maturation progressed, there was no clear gap between size-modes of unyolked and yolked oocytes, indicating that fecundity of deepwater flathead is indeterminate. This continuous, polymodal size distribution is confirmed in histological preparations of oocytes in 'ripe' (stage V) and 'running-ripe' (stage VI) ovaries (Figure 16, right).

Table 10. Description of microscopic stages in oocyte development of female deepwater flathead

Stage	Stage Description	Histological description
I	Chromatin nucleolar	Very small oocytes, nucleus surrounded by a thin layer of dark-blue-stained cytoplasm (<20 μm)
II	Perinucleolar	Oocyte size increases slightly as dark-blue-stained cytoplasm thickens, nucleoli appear at at the periphery of nucleus (20–283 μm; n = 335, 67 ovaries)
III	Cortical alveoli	Appearance of cortical alveoli in pale-blue-stained cytoplasm, pink-stained zona radiata distinguishable, oil vesicles appearing, lampbrush chromosomes often visible in the nucleus (61–571 μm; n = 541, 78 ovaries)
IV	Yolk	Marked increase in oocyte size, cytoplasm filled with pink-stained yolk granules, cortical alveoli and oil vesicles increase in size and number (182–691 μm; n = 1289, 71 ovaries)
V	Nuclear migration	Migration of nucleus to periphery of oocyte, fusion of yolk granules into yolk plates; fusion of oil vesicles into the oil droplet (269–754 μm; n = 591, 78 ovaries)
VI	Hydration	Further increase in size of oocytes, all yolk granules fused into a few plates (370–816 μm; n = 445, 32 ovaries)
POF (new)	New postovulatory follicle	Remaining follicle soon after ovulation. It is large, highly convoluted with an obvious lumen, and may contain fine granular material. The layered nature of both cell types (thecal and granulosa) remains intact in lumen
POF (old)	Old postovulatory follicle	Convoluted nature much less apparent, lumen much reduced, even closed, and the thecal and granulosa cells no longer retain their orderly arrangement
α	α-atretic oocyte	Zona radiata dissolves, oocyte shape loses integrity yolk globules begin to disintegrate and are less regular in shape
β	β-atretic oocyte	Numerous disorganised granulosa cells surrounded by a thin thecal and blood vessel layer. Nucleus of some of the granulosa cells is pycnotic and many of the cells contain a large intracellular vacuole that may be empty or contain amorphous particles

Table 11. Distribution of the most advanced microscopic stages observed after macroscopic classification in deepwater flathead ovaries

Microscopic stages	Immature	Mature virgin	Recovering	Developing	Late developing	Ripe	Running-ripe	Spent
Perinucleolar stage	3	5	1	1				7
Cortical alveoli stage		14	5	1	1		2	6
Yolk stage	1	2	1	6	21	15	14	11
Nuclear migration stage		1	1	10	17	16	26	7
Hydration stage		2	1	5	3	4	7	2
New POF stage		1		2	4	6		4
Old POF stage		1	2	19	34	37	16	16
Cortical alveoli oocytes in atresia	1	1						1
Yolk oocytes in atresia	3	7	2	9	4	6	5	22
Nuclear migrated oocytes in atresia		1					1	1
Hydrated oocytes in atresia		1						3
All oocytes in atresia						1		
Total	**8**	**36**	**13**	**53**	**84**	**85**	**71**	**80**

Table 12. Evidence of spawning of deepwater flathead. Presence of new (N) and old (O) postovulatory follicles is indicated

	2001						2002						2003		2004				
	Feb	Apr	Jun	Aug	Nov	Dec	Jan	Feb	Mar	Apr	May	Jul	Oct	Dec	Jan	Feb	Mar	Apr	May
Presence of new (N) POF					N					N			N	N	N		N	N	
Presence of old (O) POF	O		O		O	O	O	O	O	O			O	O	O	O	O	O	O

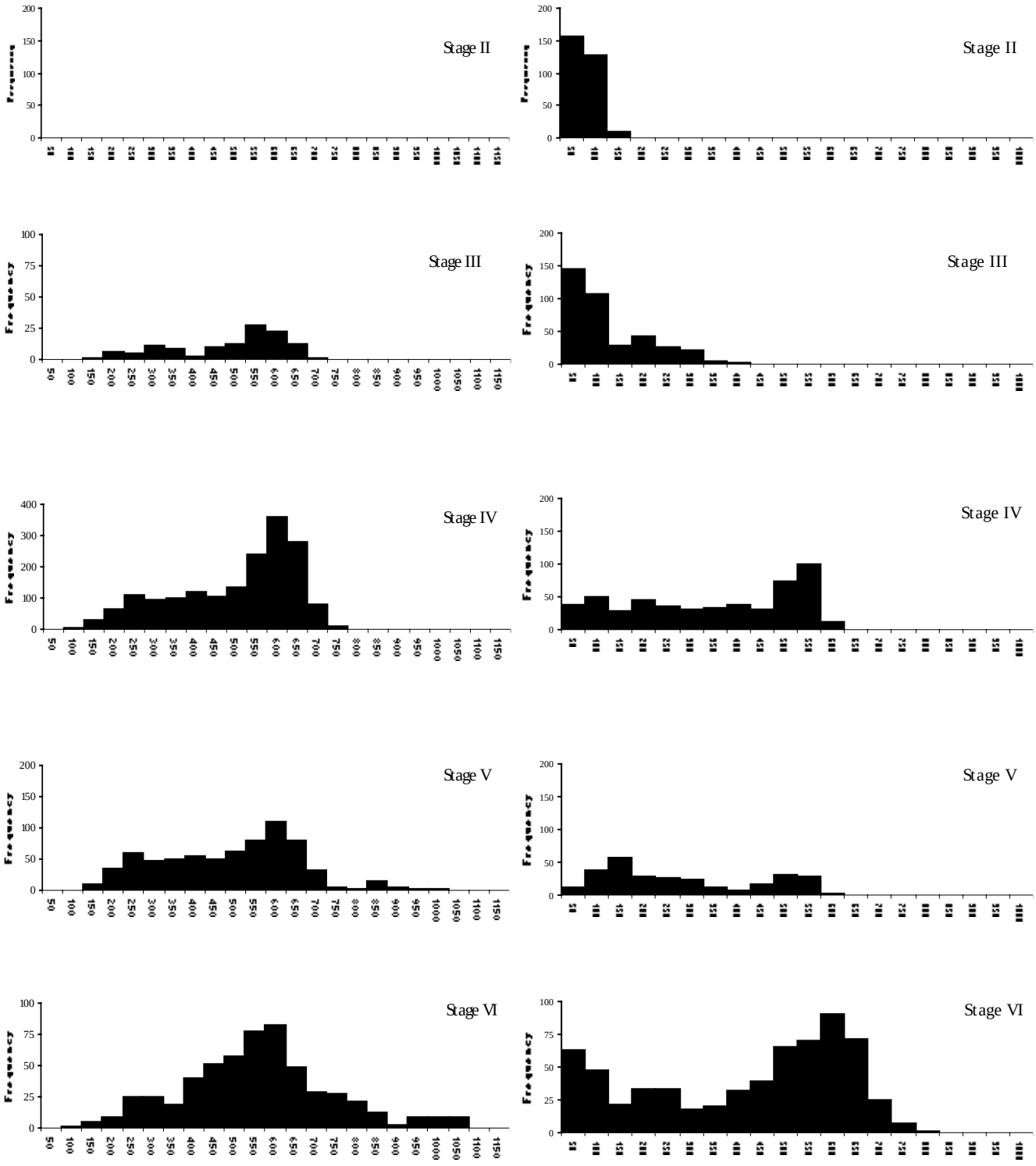


Figure 16. Frequency distribution of deepwater flathead whole oocyte diameter (left) and oocyte diameter from histological sections (right) of different stages of gonad maturity.

Sex ratio

The proportion of females taken from the commercial catch samples varied considerably from 25 to 100%, with an average of 58% (sex ratio: 1 male to 1.4 female) (Table 13). During the peak spawning period of deepwater flathead (December–March) the sex ratio was biased towards females. Whilst not investigated as part of this study, spatial variability in sex ratio and schooling behaviour by sex may occur in this species.

Table 13. Summary of the proportion of female deepwater flathead by month.

Year	Month	Female	Male	% Female
2000	10	117	67	64
2000	12	35	65	35
2001	2	143	62	70
2001	4	40	60	40
2001	6	50	50	50
2001	8	34	66	34
2001	9	49	59	45
2001	11	53	52	50
2001	12	58	28	67
2002	1	39	59	40
2002	2	77	1	99
2002	3	71	2	97
2002	4	64	135	32
2002	5	69	6	92
2002	7	33	49	40
2003	8	25	75	25
2003	10	78	87	47
2003	12	73	0	100
2004	1	43	2	96
2004	2	84	19	82
2004	3	47	1	98
2004	4	38	35	52
2004	5	53	7	88

Size-at-Maturity

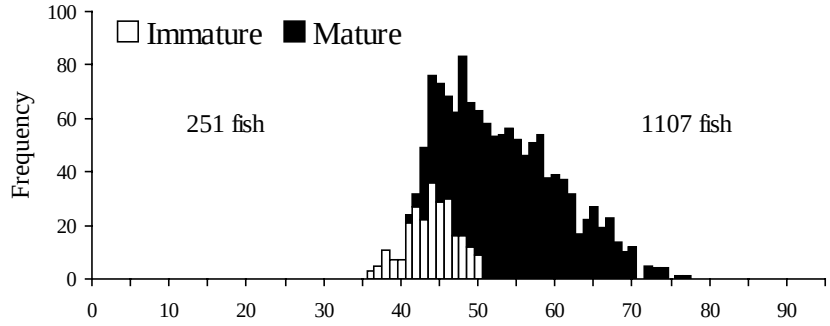
Separate size- and age-at-maturity ogives have been determined for female deepwater flathead based on macroscopic and histological staging of gonads. Data were pooled over the 2000–02 and 2003/04 collection periods for the spawning period (October–May) and for the whole year (Figure 17).

The size of deepwater flathead at which 50% of the female population is mature, based on macroscopic staging, was 44 cm TL for all data pooled across two collection periods (Figure 18), and 44 cm TL for data from the peak spawning periods (Figure 20). These estimates are consistent with an earlier estimate of 45 cm TL based on macroscopic staging of all data from the 2000–02 collection period.

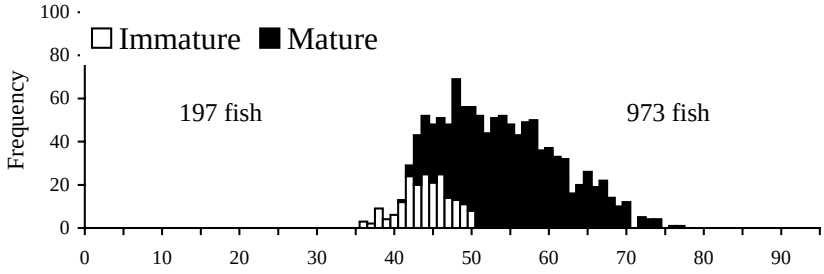
The deepwater flathead size at which 50% of the female population is mature, based on histological staging, was 40 cm TL for data from the peak spawning periods pooled across two collection periods (Figure 18). However, the logistic regression for these data was based on a small sample size (10 immature fish). A knife-edged size-at-maturity of 40 cm TL better describes the size-at-maturity than a logistic regression.

The age of deepwater flathead at which 50% of the female population is mature, based on the histological staging size of 40 cm TL and derived from von Bertalanffy growth parameters, is estimated at 3.8 yrs.

a) Macroscopic stage (all)



b) Macroscopic stage (spawning period Oct-May)



c) Histological stage (spawning period Oct-May**)**

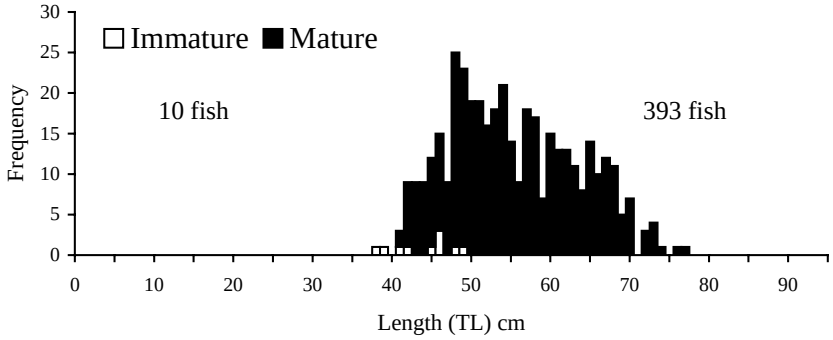


Figure 17. Size distributions of female deepwater flathead staged as immature or mature based on a) macroscopic staging throughout the year and b) macroscopic and c) histological staging during the spawning season.

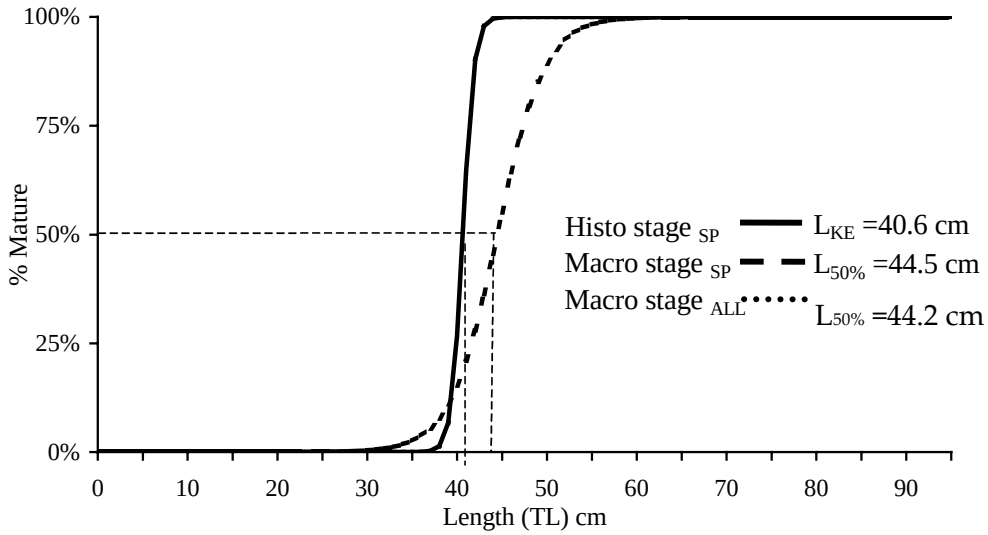


Figure 18. Maturity ogive for female deepwater flathead plotted against length for ovaries staged macroscopically for all data and staged macroscopically and histologically for the spawning period (SP) only. Straight lines indicate length at 50% mature. (KE, Knife-edged maturity; $L_{50\%}$, Logistic curve maturity)

Oocyte count

The total fecundity relationship with length and weight were based on oocyte counts from 25 gonads staged macroscopically as 'maturing', 'ripe' or 'running ripe' and histologically with no atresia and no postovulatory follicles present. The total fecundity estimated from the standing stock of yolked oocytes, nuclear migrated oocytes and hydrated oocytes gonads, ranged 0.50–3.56 million oocytes per fish. The total fecundity of deepwater flathead increased exponentially with length and weight (Figure 19). The total fecundity (TF) of deepwater flathead can be best described by fish length with the following relationship:

$$TF = 0.286 \times TL^{3.7639;}\ n = 25;\ r2 = 0.7705.$$

The relative fecundity of deepwater flathead ranged 0.31–1.18 million oocytes per kg. However no relationship between relative fecundity and fish length was observed.

Oocytes were counted from a further 5 gonads, staged macroscopically as 'running ripe' but subsequently classified histologically as 'partially spent' (postovulatory follicles present). As spawning had already commenced, as expected less yolked oocytes were observed in the 'partially spent' gonads, with counts ranging 0.73–1.98 million oocytes per fish (Figure 19).

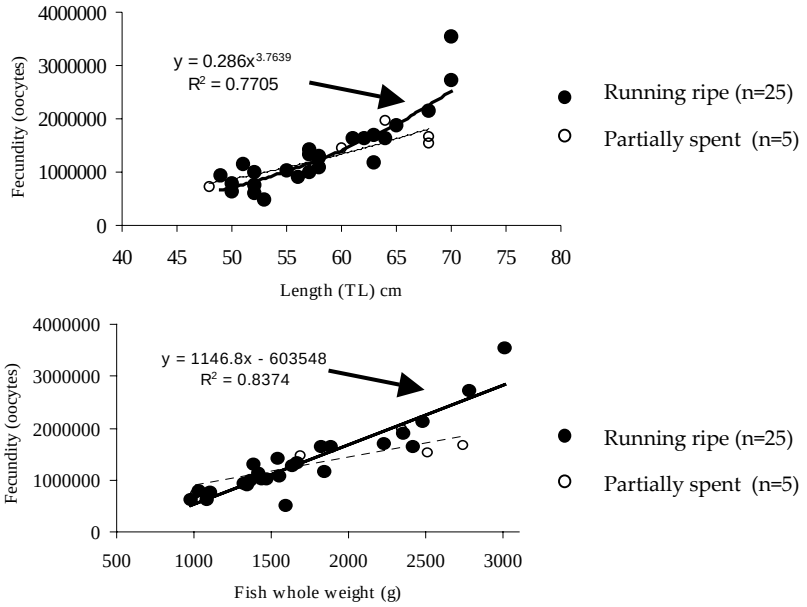


Figure 19. Fecundity relationship with length and weight for deepwater flathead. Separate oocyte counts of the 'running ripe' (no atresia, no postovulatory follicles) and 'partially spent' (postovulatory follicles present) gonads are presented.

Per-recruit Analyses

Biological parameters for egg per-recruit analyses of deepwater flathead are presented in Table 7. Deepwater flathead egg production peaks at 42 cm TL and age 4 yr for a range of natural mortality estimates (0.30–0.53), assuming no fishing mortality (Figure 20). Egg production decreases exponentially with increased fishing mortalities (Figure 21)

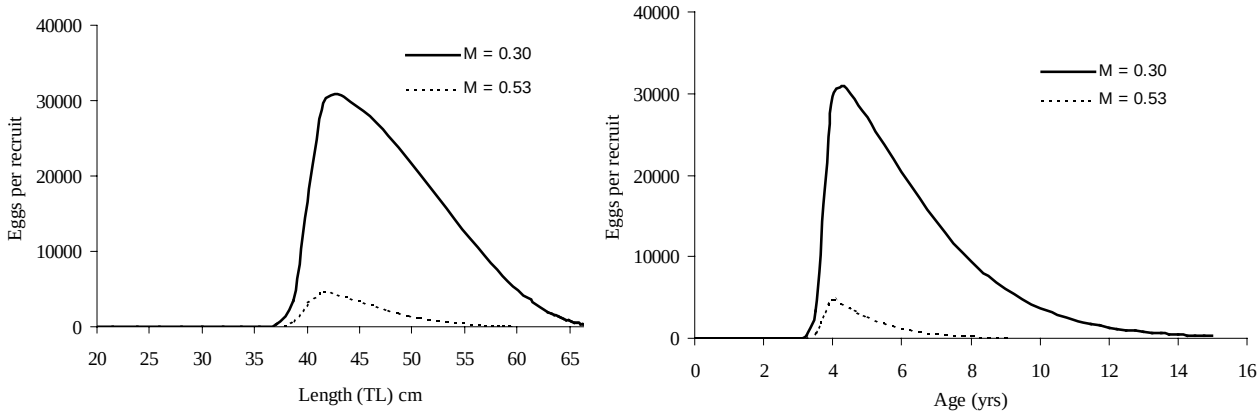


Figure 20. Eggs-per-recruit for female deepwater flathead plotted against length and age for two different estimates of natural mortality (M). No fishing mortality has been incorporated into these analyses.

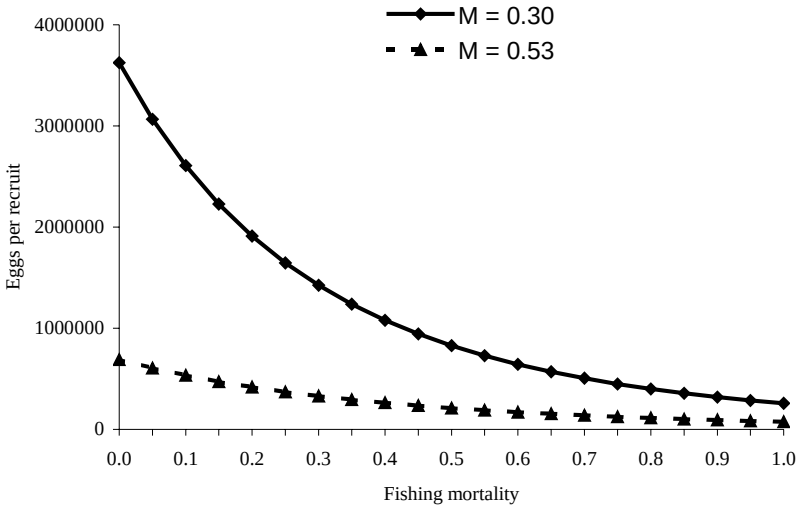


Figure 21. Eggs-per-recruit of deepwater flathead for two different estimates of natural mortality (M) over a range of values of fishing mortality.

Discussion

Bight redfish

Gonad maturation

The evidence from the GSI data and macroscopic staging suggests that the spawning season of Bight redfish in the Great Australian Bight can last for four months (late January to early May, peaking in March and April). The peak GSI value on its own does not indicate the peak spawning time, but rather a later developing stage. The high values of this index can be used to determine the spawning period of female Bight redfish when nuclear migrated and hydrated oocytes can be identified (Hunter & Macewicz 1985a). The subsequent histological analyses of ovaries, in particular the presence of nuclear migrated oocytes, hydrating oocytes and new and old postovulatory follicles, confirms the late summer to autumn spawning period for Bight redfish.

Size-at-maturity

Three basic assumptions underpin the size-at-maturity determination: that sampling is representative of the whole population, that the immature/mature status is not misclassified, and that sampling occurs from gonad development through to the end of spawning (Abaunza *et al.* 2003). For Bight redfish, whilst histological staging of ovaries was carried out to reduce the incidence of misclassifying maturity status, and sampling occurred over the spawning period, sampling was only undertaken within the GABTF. Small Bight redfish (<25 cm LCF), which reside outside the range of the fishery, were not represented in the sample. The histological analyses of this biased sample resulted in all Bight redfish samples being classed as mature. As such, a logistic regression was not undertaken to determine the size-at-maturity. The size-at-maturity of Bight redfish has been approximated to a knife-edged curve at 25 cm LCF and 9 years, which corresponds to the smallest fish histologically examined. To improve the size-at-maturity estimate, histological staging of gonads from the whole population needs to be undertaken, and include fish other than those in the commercial catch of the GABTF.

Size-at-maturity estimates undertaken previously, based on macroscopic staging, were almost certainly over estimated. Mature fish with redeveloping gonads appear to have been misclassified as immature with first time developing gonads. Size-at-maturity estimates for Bight redfish, based on macroscopic

staging of gonads collected throughout the year, were 32 cm LCF (Knuckey and Brown 2002), and 28 cm LCF (present study). GSI data collected during 1988–1990 suggested a size-at-maturity of 28 cm LCF for Bight redfish (Newton *et al.* 1994).

The absence of immature fish in the commercial catch gives rise to the question of what Bight redfish spatial stock structuring occurs in the Great Australian Bight. The related species Redfish, *Centroberyx affinis*, which occurs on the south-east coast of Australia, has shown a strong length-dependent offshore distribution, with small fish occurring more frequently in the shallow inshore waters (<60 m) and large fish in the deeper mid-shelf (90–125 m) and outer-shelf waters (125–165 m) (Chen *et al.* 1997). It is most likely that the small Bight redfish (<25 cm LCF) are inshore (<100 m depth), outside the current range of the GABTF. These inshore waters probably consist of small Bight redfish, predominantly immature, as well as larger mature fish.

Implications for the Bight redfish stock are that the reproductive capacity may not be compromised as previously suggested. The simple spawning biomass per-recruit model, sourced from the GABFAG modeller (Dr Brent Wise), indicted that fishing mortality, and hence fishing effort, would need to be reduced by one third to maintain a target of 40% of the virgin biomass. This was based on size-at-maturity (from 2000–02 data) of 32 cm LCF (F = 1.45 and effort = 51 346 h for SOM of 28 cm LCF, F = 0.45 and effort = 15 963 h for SOM of 32 cm LCF). From the present study, the size-at-maturity of 25 cm LCF suggests that the fishing effort could be increased 10 fold while maintaining a target of 40% of the virgin biomass (F = 4.00 and effort = 141 893 h). It should be noted however that the simple model is a tool to show how differing size-at-maturity estimates affect spawning biomass, but in itself does not provide an absolute estimate of spawning biomass.

Fecundity

The relative frequencies of oocytes at different development stages can be indicative of the reproductive mode for a species of fish (Hunter and Macewicz 1985; Hunter *et al.* 1992; Davis and West 1993, Macchi and Acha 2000). Such studies have shown that the characteristics that typically distinguish indeterminate fecundity include the co-occurrence of oocytes in all development stages; that oocytes range in size continuously from small to the advanced yolked oocyte stage; and there is no large hiatus between maturity classes of oocytes. The ovaries of the Bight redfish demonstrated all these characteristics, which suggest that oocytes continue to develop from the unyolked stage, through vitellogenesis to hydration throughout the spawning period of each fish. The data suggests therefore that the annual fecundity is indeterminate. The size distributions of oocytes at different stages of development also indicated that only a subset of, and not all, the advanced yolked oocytes became hydrated and were released on one occasion. This, in association with the simultaneous presence of different stages of development, indicates that oocyte development was asynchronous (Wallace and Selman 1981; West 1990).

Furthermore, the co-occurrence of hydrated oocytes and postovulatory follicles indicate that females spawn several times, at least within a few days of each other within a spawning season. These features indicate that Bight redfish is a multiple spawner with asynchronous oocyte development and indeterminate fecundity.

In the present study we have estimated the total fecundity, based on standing stock of yolked, nuclear migrated and hydrated oocytes, not the annual fecundity. For multiple spawning species with indeterminate fecundity such as Bight redfish, the total fecundity is an underestimation of the potential annual fecundity as it does not include *de novo* vitellogenises (formation of additional yolked oocytes during the spawning season) (Hunter *et al.* 1992, Abaunza *et al.* 2003). An accurate way to estimate annual fecundity is to take into account the spawning frequency, batch fecundity and rates of atresia. The appropriate sampling design should include the "postovulatory follicle" method as suggested by Hunter and Macewicz (1985) for estimating spawning fraction and frequency for multiple spawners. Also the design should include intensive time course sampling over a 24-h period to ensure capture of hydrated oocytes and understanding of postovulatory follicles formation during the peak spawning period (Isaac-Nahum *et al.* 1988). It should be noted that the parameters spawning frequency and batch fecundity are used for the estimation of spawning biomass using the daily egg production method.

Per-recruit analyses

This fishery population dynamics model describes how a fish stock biomass is increased through recruitment and growth of individuals and reduced by natural and fishing mortality. Recruitment occurs

through reproduction. One way to maintain the reproductive potential of a population is to minimise fishing impacts on immature fish. This may be achieved through applying gear restrictions, minimum capture lengths and/or area or seasonal closures, thereby allowing immature fish the opportunity to mature and contribute progeny to the population.

Per-recruit analysis indicates that maximum egg production occurs at a size ~25 cm LCF and 7.5 yrs of age, or slightly lower than the size of first capture, where egg per-recruit analyses has not peaked. As such, it is expected that a legal minimum length would have little effect on improving the Bight redfish reproductive capacity. Little or no fishing pressure is currently applied to the immature portion of the Bight redfish population. As discussed earlier, these immature fish almost certainly occur inside depths of 100 m within the Great Australian Bight, which is outside the current range of the trawl fishery. In essence these inshore grounds act as sanctuary for Bight redfish until mature individuals are recruited into the trawl fishery grounds in deeper waters (100-150 m). More importantly, the recent activation of all 10 Statutory Fishing Right's in the GABTF has increased fishing effort (F=0.44, Wise unpublished 2005). Until the end of 2002 only five Statutory Fishing Right's regularly worked (F=0.15, Wise unpublished 2005). This increase in fishing effort has resulted in a massive 66% reduction in the production of eggs per-recruit according to the model (M=0.13).

Whereas fecundity estimates based on oocyte counts have been incorporated into the simple per-recruit analyses, no consideration has been made of the adverse impact to the reproductive capacity of a population by the removal of older fish which may otherwise have produced further progeny. Recent work suggests that the relationship of egg survivorship with birth date (time in season), and age of parent stock needs to be investigated, as older fish may produce eggs of greater size, therefore leading to an increase in egg survivorship (Wright and Gibb 2005).

Deepwater flathead

Gonad maturation

The evidence from GSI data, macroscopic staging and histological analyses of the presence of nuclear migrated oocytes, hydrating oocytes and new and old postovulatory follicles, suggests that the spawning season of deepwater flathead in Great Australian Bight can last for eight months (October to May).

Studies on other Australian flathead species suggest spawning seasons of three to six months depending on species and location. In Tasmania sand flathead, *Platycephalus bassensis*, spawn up to six months between October and March (Jordan 2001) whilst in Victorian coastal embayments both sand flathead and rock flathead (*P laevigatus)* were found to spawn during August to November (Koopman *et al.* 2000; Brown 1977). In Western Australian estuaries the flathead species, *P. speculator* has been found to spawn during December to March (Hyndes *et al.* 1992).

In the present study, the GSI of deepwater flathead was quite variable, ranging from 2–16%. These values overlap, and slightly extend, the range previously observed in comparable Australian flathead studies. Jordan (2001) reported a GSI for sand flathead sampled from Tasmania from 1–18%. The mean GSI of deepwater flathead (0.6–5.1) was consistent with the mean GSI for sand flathead (1.8–6.5, Koopman *et al.* 2000) and the mean GSI for *P. speculator* (0.5–6.0, Hyndes *et al.* 1992).

The early stages of gonad development in female deepwater flathead, for example chromatin nucleolar oocytes (stage I), were difficult to determine microscopically. Four of the 29 ovaries staged macroscopically as stage I underwent histological analyses. These samples were found to be more advanced (perinucleolar (stage II), cortical alveoli (stage III) and yolked stages (stage IV)). This suggests that at the minimum size observed (34 cm TL) reproductive development had already started.

In the present study, histological examination showed that some ovaries contained oocytes in atresia during the protracted spawning season and that at any one time during the year some ovaries contained yolked oocytes. This implies that in the Great Australian Bight, any resting phase is very short and that there are always some female deepwater flathead with ovaries close to maturation. Note that although females with mature gonads appeared throughout the year, partially spent females (ie. individuals with the occurrence of both hydrated eggs and postovulatory follicles in the ovary) were only recorded during the spawning season.

Size-at-maturity

As discussed earlier for Bight redfish, the size-at-maturity estimation assumes that the sample is representative of the whole population, which includes immature fish. For deepwater flathead too few immature fish were represented in the sample for the determination of a logistic curve. As such the size-at-maturity based on histological staging was best described as knife-edged at 40 cm TL and 3–4 yrs of age.

From the present study, the size-at-maturity estimate based on histological staging (44 cm TL) is lower than that based on macroscopic staging, (45 cm TL, Knuckey and Brown 2002) derived from logistic curves, and is lower than an earlier estimate (45 cm TL) based on GSI data (Newton *et al.* 1994).

Fecundity

As was found for Bight redfish, histological examination of ovaries indicated that deepwater flathead is a multiple spawner with asynchronous oocyte development and indeterminate fecundity. The total fecundity, an underestimation of the annual fecundity when fecundity is indeterminate, ranged between 0.50 and 3.56 million oocytes per fish. The fecundity of deepwater flathead located on the continental shelf was twice that of rock flathead, a coastal embayment species. By comparison, the fecundity of rock flathead ranges between 0.05 and 0.50 million oocytes per fish (32 and 54 cm TL, Koopman *et al.* 2000) whilst fecundity of deepwater flathead (55 cm TL) averaged ~1 million oocytes (present study).

The estimation of annual fecundity of horse mackerel, *Trachurus trachurus* (Abaunza *et al.* 2003) may be useful in determining fecundity of deepwater flathead. Both species have indeterminate fecundity, are multiple spawners with asynchronous oocyte development, and have a protracted spawning season (up to 8 months). The annual fecundity estimate requires the measurement of spawning frequency, batch fecundity and rates of atresia throughout entire spawning season. The batch fecundity can be estimated quite accurately, however the fraction of females spawning, and hence the batch frequency, is more difficult to estimate. The sampling design for the annual fecundity estimate should include time course sampling (over 24 h-periods) to ensure capture of hydrated oocytes and understanding of postovulatory follicles formation in the peak spawning period (Isaac-Nahum *et al.* 1988). In the case of horse mackerel an individual female is believed to release 5 to 16 batches during the spawning period with a batch fecundity of ~200 oocytes per gram of fish (Abaunza *et al.* 2003).

Per-recruit analyses

Commercial catch sampling indicates that size and age of deepwater flathead at first capture in the GABTF are 30–35 cm TL and 2 yr respectively (Talman and Brown, 2004), considerably lower than the egg production peak of 42 cm TL and 4 yr (present study). Increasing the cod end mesh size to allow smaller deepwater flathead (<40 cm TL) to escape capture may increase the reproductive capacity of this species. As with Bight redfish, the recent activation of all 10 Statutory Fishing Right's has increased fishing effort in the GABTF (F=0.24, Wise unpublished 2005). Until the end of 2002 only 5 Statutory Fishing Rights were regularly worked (F=0.12, Wise unpublished 2005). The increased fishing effort has resulted in 33% reduction in the deepwater flathead production of eggs per-recruit, according to the model (M=0.30).

Benefits

This project has provided immediate benefit to the management of Bight redfish and deepwater flathead stocks in the Great Australian Bight. The project has improved the knowledge of the reproductive biology, and more importantly improved population parameters, for incorporation into stock assessment models for both Bight redfish and deepwater flathead. Improved parameter estimates reduce uncertainty of stock assessments and therefore increase the confidence of management decisions.

The results of the project will assist the Australian Fishing Management Authority in ensuring that the fisheries targeting Bight redfish and deepwater flathead are managed in a way consistent with ecologically sustainable development principles.

Further Development

Improved size-at-maturity estimates for Bight redfish should be possible if immature fish can be sourced from outside the GABTF commercial catch. These immature fish, potentially small sized Bight redfish (<25 cm LCF) are most likely within the 100 m isobath. A scientific survey directed at shallow waters during peak spawning period using a small trawl or bottom set mesh net (120 mm) may be required, as current inshore fishing operations never, or rarely encounter these small Bight redfish.

In addition to collecting small Bight redfish to further improve size-at-maturity estimates, the otoliths of these fish would be available to improve von Bertalanffy growth parameters. Presently there is a paucity of Bight redfish age data from fish less than 9 yrs of age. This lack of data presently distorts the shape of the growth curve dramatically.

Given that the present study suggests that both Bight redfish and deepwater flathead are multiple spawners with indeterminate fecundity, an adequate sampling design should be applied to accurately estimate the annual fecundity. The design needs to measure spawning frequency, batch fecundity, and rate of atresia throughout the entire spawning season.

Planned Outcomes

The study addresses some of the main information gaps on the biology Bight redfish and deepwater flathead identified at the 2000 Great Australian Bight fishery assessment group meeting. The project determined parameters for size-at-maturity and fecundity for Bight redfish and deepwater flathead. Improved stock assessments, which incorporate the latest age, growth and reproductive data along with standardised catch data, have been undertaken by the Bureau of Rural Sciences. Improved stock assessments are of particular importance considering the doubling of fishing effort in 2004 with the activation of all ten Statutory Fishing Rights. Improved stock assessments has lead to the setting of accurate Bight redfish and deepwater flathead TAC's. Gear regulations have been proposed that include minimum mesh sizes and T90 extensions, which along with reducing discard species, allow small deepwater flathead to escape capture thereby increasing reproductive capacity.

These results have been communicated to scientists, managers and industry members associated with the GABTF through informal discussions, through the extension material developed and distributed, and through presentations at the annual Great Australian Bight fishery assessment group meetings.

Conclusion

Bight redfish

The evidence from GSI data, macroscopic staging and histological analyses of the presence of nuclear migrated oocytes, hydrating oocytes and new and old postovulatory follicles, suggests that Bight redfish is a multiple spawner with asynchronous oocyte development and a short spawning season which runs from late January to early May.

The size of maturity of Bight redfish, based on histological staging of ovaries, can be considered knife-edged at 25 cm LCF and ~9 yrs of age. All samples from the trawl fishery commercial catch when histologically examined were classed as mature. The smaller, immature component of the population was not represented in the commercial catch. These small sized fish may be found inshore (<100 m), outside the current GABTF ground. An improved estimate of size-at-maturity will require the histological staging of gonads of a representative sample of these smaller fish, potentially sourced from inshore waters. The Bight redfish size-at-maturity estimate of 25 cm LCF based on histological staging is

less than the previous estimate of 28 cm LCF based on GSI data (Newton *et al.* 1994)

Evaluation of the Bight redfish ovaries suggests that the annual fecundity is indeterminate, with oocytes continuing to develop from the unyolked stage, through vitellogenesis to hydration throughout the spawning period of each fish. As such, the count of the standing stock of yolked, nuclear migrated and hydrated oocytes is the total fecundity, not the annual fecundity. The total fecundity, which ranged between 0.62 and 1.54 million oocytes per fish, is an underestimation of the annual fecundity as it does not include *de novo* vitellogenises (formation of additional yolked oocytes during the spawning season) (Hunter *et al.* 1992; Abaunza *et al.* 2003). To accurately estimate the annual fecundity, the spawning frequency, batch fecundity and rates of atresia are required.

Per-recruit analysis for Bight redfish, which incorporates the size-at-maturity and fecundity estimates determined during the present study, indicates that maximum egg production occurs at a size ~25 cm LCF and 7.5 yrs of age. This is slightly lower than the size of first capture (ie. egg per-recruit analyses has not peaked). As such, setting a minimum size limit would have little effect on improving the Bight redfish reproductive capacity. However, the doubling of fishing effort from four or five vessels in 2002 to all ten Statutory Fishing Rights being activated during 2004 has resulted in a three-fold reduction in the production of egg per-recruit, according to the per-recruit analysis.

Deepwater flathead

The evidence from GSI data, macroscopic staging and histological analyses of the presence of nuclear migrated oocytes, hydrating oocytes and new and old postovulatory follicles, suggests that deepwater flathead is a multiple spawner with asynchronous oocyte development and a protracted spawning season running from October to May.

Overall, the proportion of deepwater flathead that were female was 58%, with the sex ratio of samples biased towards females during the peak spawning period (December to March).

For deepwater flathead, too few immature fish were represented in the sample for determination of a logistic curve. As such the size-at-maturity, based on histological staging, is best described as knife-edged at 40 cm TL and 3–4 yrs of age. The size-at-maturity estimate for deepwater flathead of 40 cm TL based on histological staging, is lower than the previous estimate of 45 cm TL based on GSI data (Newton *et al.* 1994).

Histological examination of ovaries indicates that as maturation progresses, there was no clear gap between size-modes of unyolked and yolked oocytes, showing that the fecundity of deepwater flathead is indeterminate. The total fecundity based on the standing stock of yolked, nuclear migrated and hydrated oocytes, ranged between 0.50 and 3.56 million oocytes per fish. For species with indeterminate fecundity, such as deepwater flathead, the total fecundity is an underestimate of the annual fecundity, as the number of oocytes is not fixed prior to the onset of spawning since unyolked oocytes continue to mature.

Per-recruit analysis for deepwater flathead, which incorporates the size-at-maturity and fecundity estimates, determined during the present study, indicates that maximum egg production occurs at a size ~42 cm LCF and 4 yrs of age. This is higher than the size of first capture of ~30–35 cm TL; 2 yr. It may be possible to improve the reproductive capacity of the deepwater flathead population by increasing the cod end mesh size thereby increasing the size of first capture.

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Appendix 1- Intellectual Property

The intellectual property from this project will be shared between the Fisheries Research and Development Corporation and Primary Industries Research Victoria as outlined in the project contract. The Fisheries Research and Development Corporation will be acknowledged in all publications arising from the project.

Appendix 2 - Staff

Lauren Brown	Fisheries Scientist (PIRVic)
K. P. Sivakumaran	Fisheries Scientist (PIRVic)
Sonia Talman	Fisheries Scientist (PIRVic)

Appendix 3 – Bight Redfish

Developmental stages and macroscopic description of the gonads of male Bight redfish.

Stage		Macroscopic description
I	**Immature**	Testes very small, flat, and thread-like
II	**Early developing**	Testes flat/rounder in shape
		Testes occupy 20 to 70% of the length of body cavity
III	**Developing**	Testes lobed in formation
		Marked groove in the middle of each testis visible
		Testes occupy 40 to 70% of the length of body cavity
		Creamy or white milt sometimes present
IV	**Late Developing**	Testes very large
	/ Running-ripe	Testes occupy 40 to 70% of the length of body cavity
		Free-flowing milt
		Testes white or pinkish, sometimes bloodshot
V	**Spent & Resting**	Testes very bloodshot
		Testes occupy 20 to 50% of the length of body cavity
		Milt sometimes present
		Testes brownish and rubbery as they regress to resting stage

Developmental stages and macroscopic description of the gonads of female Bight redfish.

Stage		Macroscopic description
I	**Immature**	Small thread-like ovaries
		Ovaries pink and translucent
II	**Early developing**	Oocytes not visible
		Ovaries pink and translucent
IIa	**First-time Developing**	Ovary wall thin and transparent
IIb	**Redeveloping**	Ovaries flaccid, ovary wall thick
		Ovary colour pink/greyish to yellow-orange, and opaque
III	**Developing**	Small oocytes becoming visible, still translucent
		Ovaries sometimes change from pink to yellow-orange
		Ovaries occupy 20 to 70% of the length of body cavity
IV	**Late Developing**	Small opaque oocytes clearly visible
	(yolked)	Ovary wall thin and transparent
		Ovaries occupy 20 to100% of the length of body cavity
V	**Ripe**	Large transparent (hydrating) oocytes visible among
		Ovaries occupy 70 to100% of the length of body cavity
VI	**Running-ripe**	Hydrated oocytes larger, easily expressed from ovaries
		Ovaries occupy 70 to100% of the length of body cavity
VII	**Spent & Resting**	Some residual oocytes visible within translucent material
		Ovaries flaccid, greyish ovary wall thickened and wrinkled
		Ovaries occupy 20 to 70% of the length of body cavity

Whole oocyte description of Bight redfish and deepwater flathead.

Stage Description	Oocyte development
Unyolked oocytes	Very small oocytes, nucleus surrounded by a thin layer of dark-blue-stained cytoplasm. Cytoplasm homogeneous, brownish and transparent, comparatively large dark nucleus. Oocytes more or less spherical cytoplasm thickened, dark, granular, but still translucent, nucleus still visible
Yolked oocytes	Oocytes dark, completely opaque, size increasing with development, nucleus occluded
Nuclear migrated oocytes	Occurrence of partly translucent oocytes (hydrating), yolk plates visible
Hydrated oocytes	Occurrence of very large, almost totally translucent oocytes, oil droplet visible

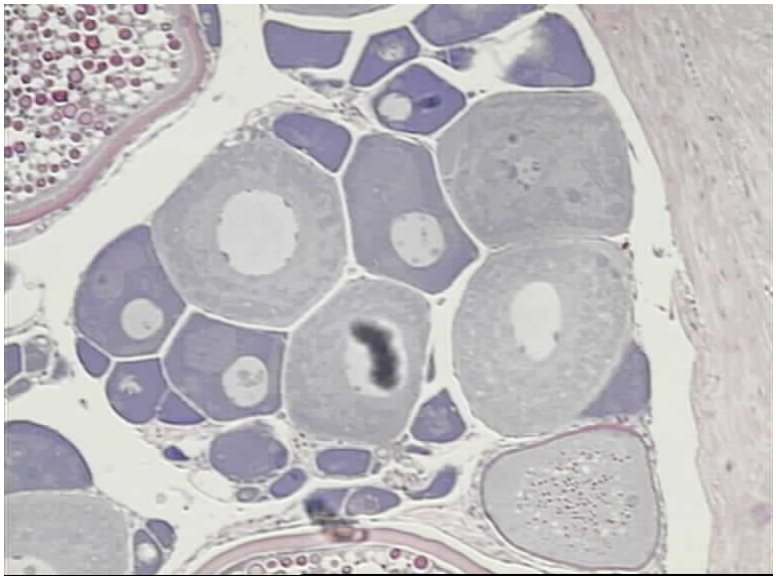

Fig 1. Perinucleolar stage: Oocyte size increases slightly as dark-blue-stained cytoplasm thickens, nucleoli appear at the periphery of nucleus [Bight redfish].

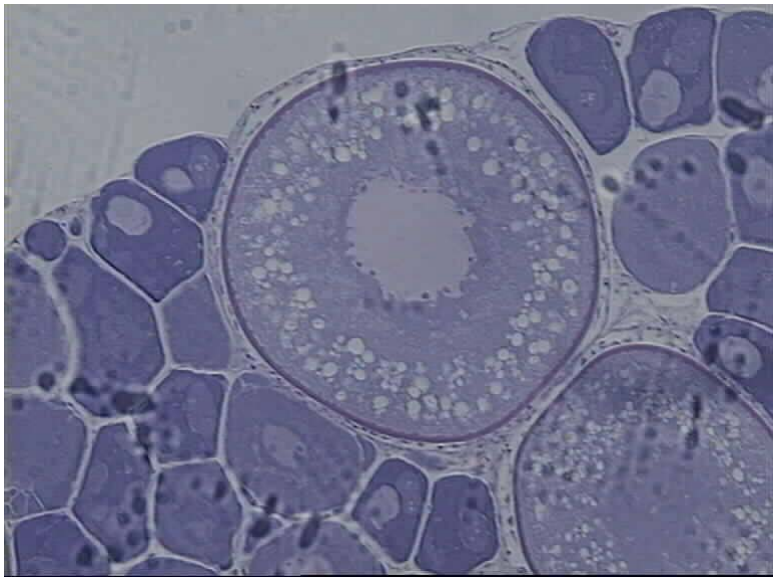

Fig 2. Cortical alveoli stage: Appearance of cortical alveoli in pale-blue-stained cytoplasm, pink-stained zona radiata distinguishable, oil vesicles appearing, lampbrush chromosomes often visible in the nucleus [Bight redfish].

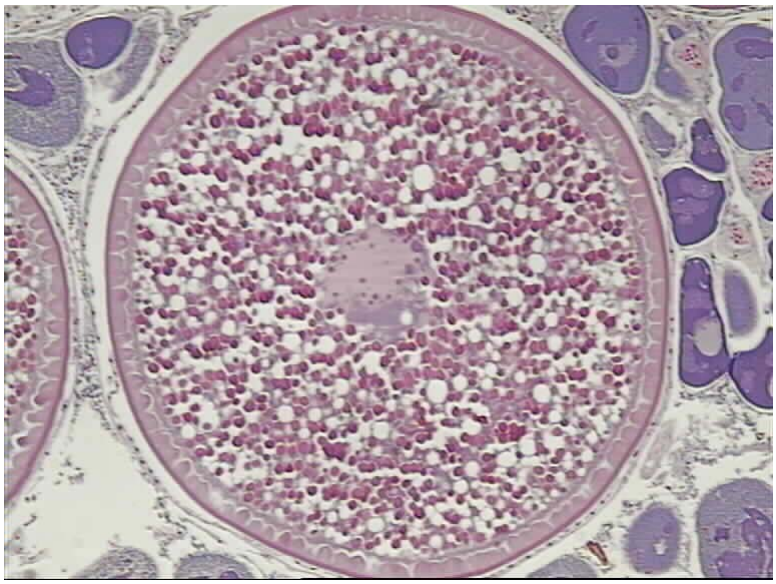

Fig 3. Yolk stage: Marked increase in oocyte size, cytoplasm filled with pink-stained yolk granules, cortical alveoli and oil vesicles increase in size and number [Bight redfish].

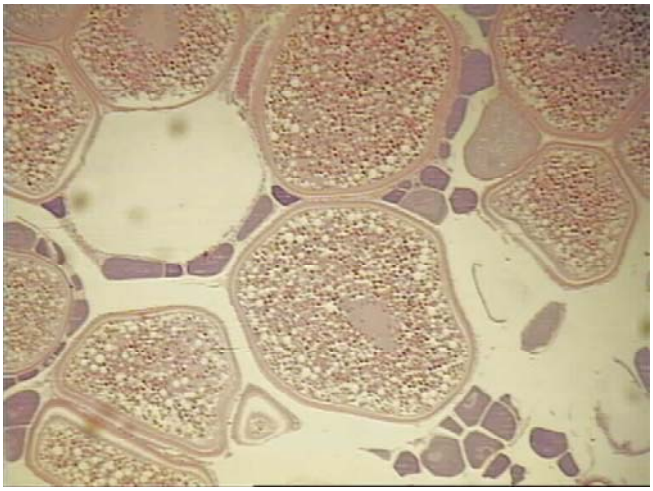

Fig 4. Early nuclear migration stage: Migration of nucleus from the centre of oocyte, fusion of yolk granules into yolk plates; fusion of oil vesicles into the oil droplet started [Bight redfish].

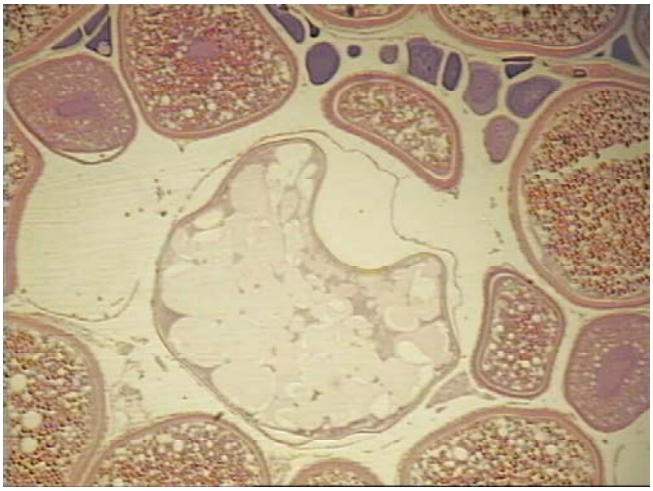

Fig 5. Hydration stage: Fusion of yolk granules into yolk plates and fusion of oil vesicles into the oil droplet completed [Bight redfish].

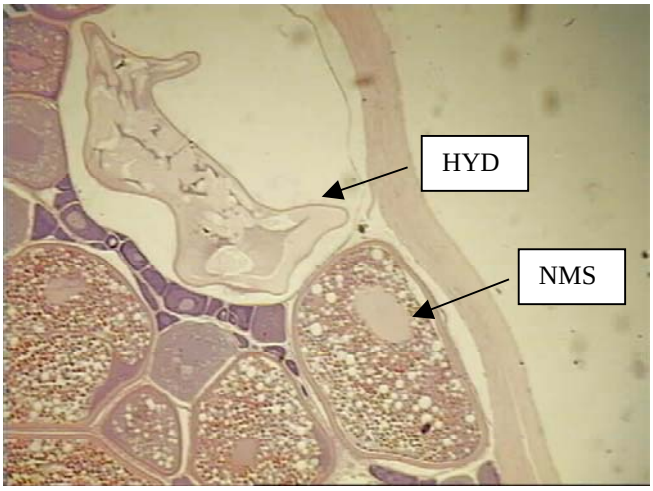

Fig 6. Nuclear migration (NMS) and Hydration (HYD) oocytes are present [Bight redfish].

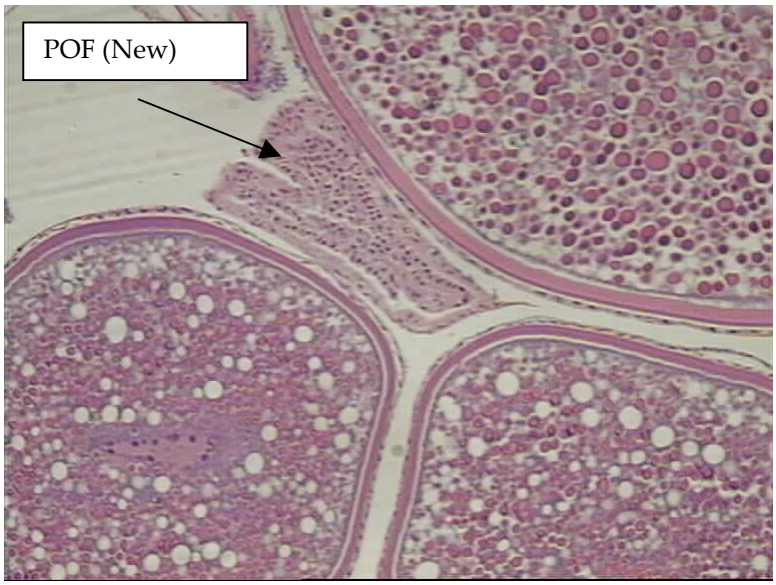

Fig 7. Postovulatory follicle POF (new): Remaining follicle soon after ovulation. It is large, highly convoluted with an obvious lumen, and may contain fine granular material. The layered nature of both cell types (thecal and granulosa) remains intact in lumen [Bight redfish].

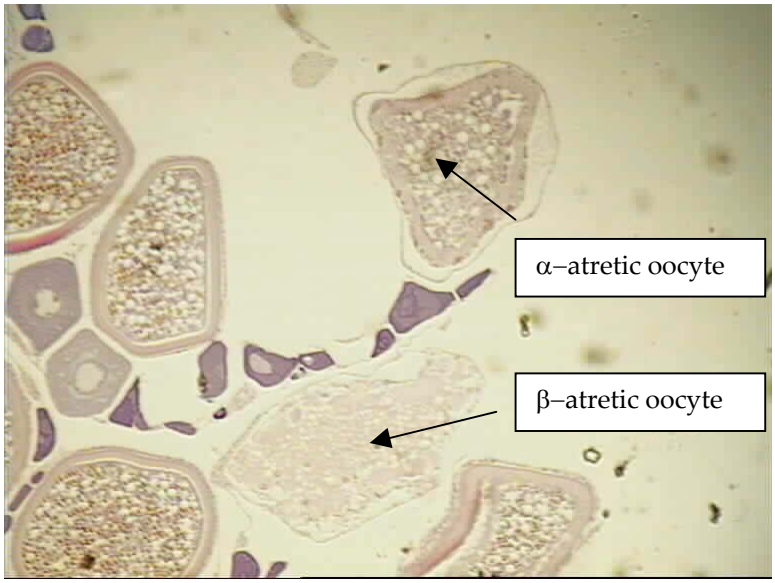

Fig 8. Yolked Oocytes in atresia: α–atretic and β–atretic oocytes are present [Bight redfish].

Appendix 4 - Deepwater flathead

Macroscopic description of gonad stages in reproductive development of deepwater flathead.

Stage	Stage description	Female	Male
I	Immature (virgin)	Small strap, less than ¾ of body cavity. Firm texture. (F1).	Small strap, less than ¾ of body cavity. Firm texture. (M1).
IIa	Mature Virgin	Virgin – Small strap with rounded edge at least ¾ of body length, pink and transparent. (F2a).	Virgin – Small strap with sharp edge at least ¾ of body length, pink and opaque . (M1b).
IIb	Recovering	As long as body cavity, bloodshot and flabby at posterior. (F2b).	As long as body cavity, bloodshot and flaccid at posterior. (M2b).
III	Developing	Almost length of body cavity, opaque and becoming yellow. Ova not discernible .(F3)	Almost length of body cavity, opaque and becoming larger. (M3).
IV	Late Developing	Full length of body cavity, opaque and yellowish pink. Ova discrete. (F4).	Full length of body cavity and larger. (M4).
V	Ripe	Full length of body cavity and swollen occupying all available space. Ovary and ova become translucent. (F5).	Full length of body cavity and swollen occupying all available space. No milt expressed with slight pressure (M5).
VI	Running ripe	Eggs expressed with slight pressure. Ovary oinkish, clear and granular. (F6).	Milt expressed with slight pressure. Testes granular. (M6).
VII	Spent	Slack and bloodshot. Few residual oocytes present. (F7).	Flaccid and bloodshot. (M7).

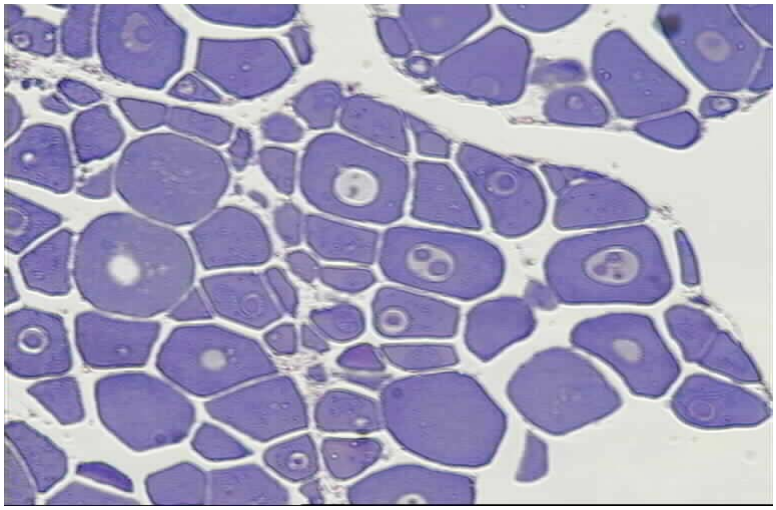

Fig 9. Perinucleolar stage: Oocyte size increases slightly as dark-blue-stained cytoplasm thickens, nucleoli appear at the periphery of nucleus [deepwater flathead].

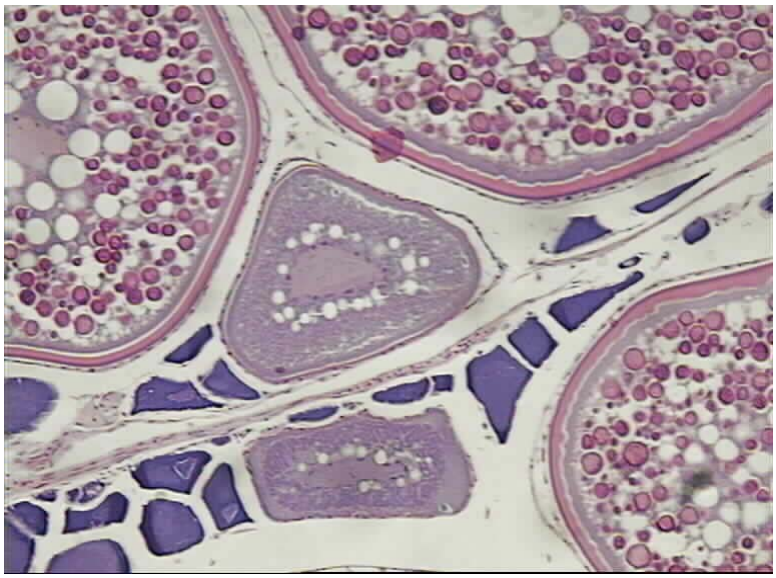

Fig 10. Cortical alveoli stage: Appearance of cortical alveoli in pale-blue-stained cytoplasm, pink-stained zona radiata distinguishable, oil vesicles appearing, lampbrush chromosomes often visible in the nucleus [deepwater flathead].

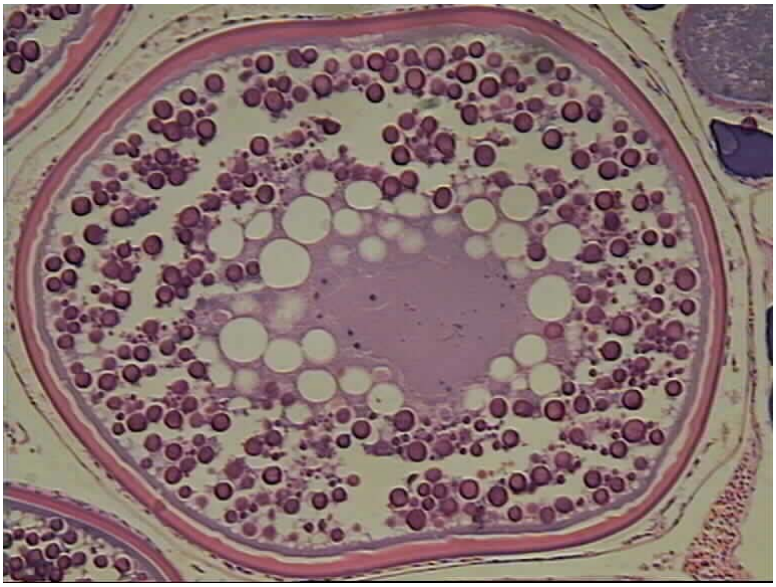

Fig 11. Yolk stage: Marked increase in oocyte size, cytoplasm filled with pink-stained yolk granules, cortical alveoli and oil vesicles increase in size and number [deepwater flathead].

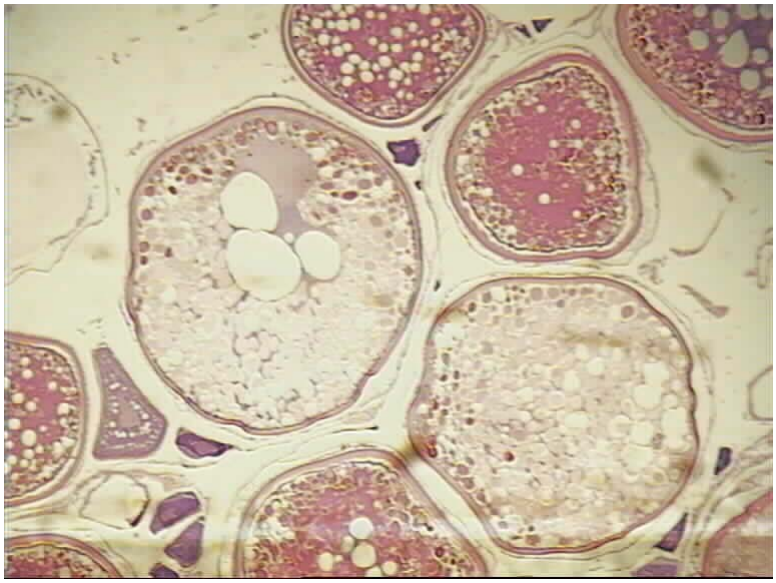

Fig 12. Nuclear migration stage: Migration of nucleus to periphery of oocyte, fusion of yolk granules into yolk plates; fusion of oil vesicles into the oil droplet [deepwater flathead].

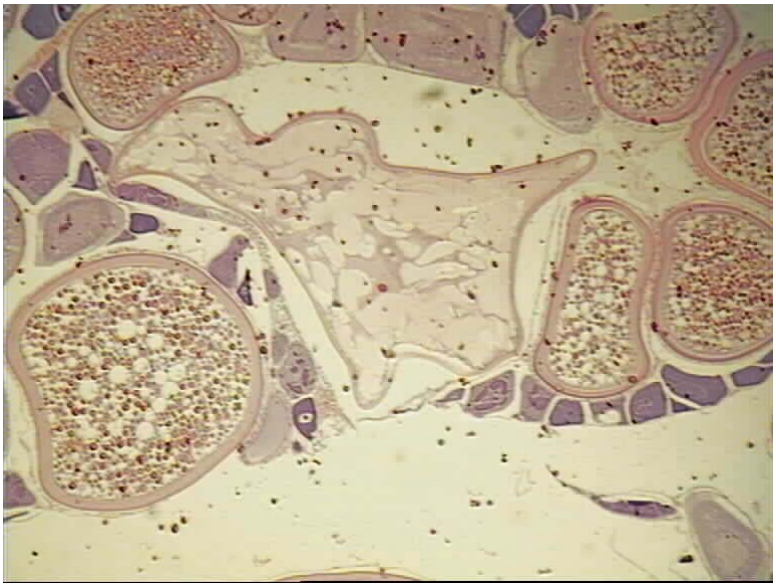

Fig 13. Hydration stage: Fusion of yolk granules into yolk plates and fusion of oil vesicles into the oil droplet completed [deepwater flathead].

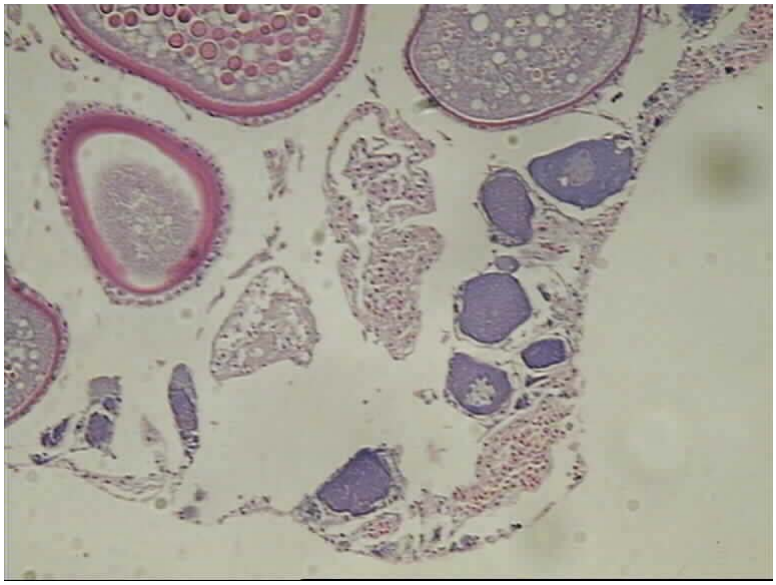

Fig 14. Postovulatory follicle (new): Remaining follicle soon after ovulation. It is large, highly convoluted with an obvious lumen, and may contain fine granular material. The layered nature of both cell types (thecal and granulosa) remains intact in lumen [deepwater flathead].

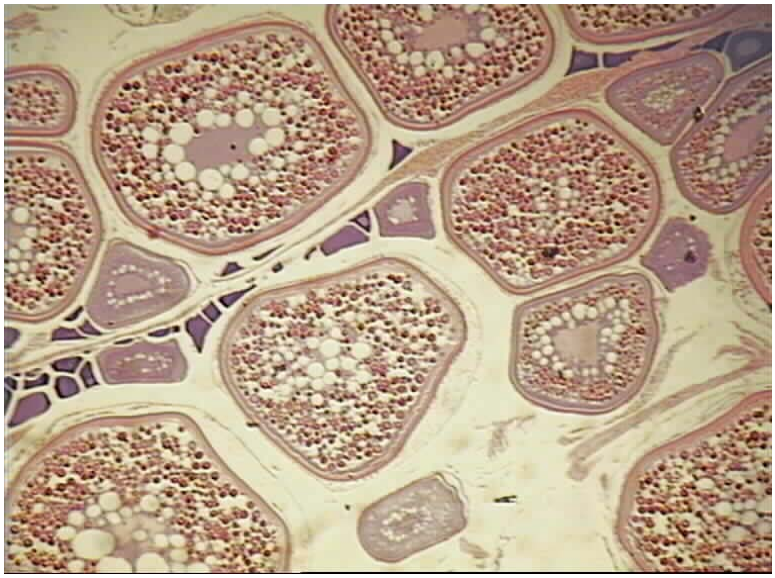

Fig 15. Yolked, Cortical alveoli and Perinucleolar oocytes are present [deepwater flathead].