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Aspects of reproductive biology of five key fish species in the Murray Mouth and Coorong

K. J. M. Cheshire, Q. Ye, J. Fredberg, D. Short and J. Earl

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EXECUTIVE SUMMARY

This report examines the reproductive biology of five fish species (*Acanthopagrus butcheri, Aldrichetta forsteri, Rhombosolea tapirina, Afurcagobius tamarensis* and *Pseudaphritis urvillii*) in the Murray Mouth and Coorong of South Australia. Specifically, this study aims to outline a baseline of key aspects of reproductive biology (including sex ratios, spawning time and duration, size at first maturity and macroscopic and microscopic characteristics of ovaries) for the selected species during drought conditions. Fish were collected monthly between July 2007 and July 2008. All fish were sexed, weighed and measured. Their gonads were removed and weighed to determine gonadosomatic index (GSI) and classified macroscopically. Size at first maturity estimated for all mature females. A random subsample of ovaries was also selected for microscopic staging using histology. Sex ratios for each of the species varied throughout the sampling season, however, all species generally showed ratios heavily weighted towards female fish. This may be attributed to method of collection, or differences in behaviour, habitat preference and movement between the sexes. Results from size at maturity indicate that most species, with the exception of *A. forsteri*, exhibit plasticity in size at maturity either between regions or as a result of environmental change or external stressors. Seasonal spawning guilds were identified for all species, consisting of spring (black bream), spring/summer (Tamar goby), summer/autumn/winter (yellow eye mullet) and winter (greenback flounder and congolli) spawners. All species were identified as multiple batch spawners, i.e. they develop and release batches of oocytes on multiple occasions throughout the reproductive season. Microscopic analysis indicated that spawning for *P. urvillii* was unlikely to have occurred in the Coorong during this study.

This study suggests that the low flow conditions in the Coorong in the drought year (July 2007 to July 2008) may have had direct impacts on the reproductive biology of the key species present. Ensuring appropriate salinity ranges and connectivity between fresh, estuarine and marine waters is integral for the spawning and recruitment of many Coorong species. This is the first study undertaken in the Coorong to determine size at maturity for some of the targeted species, and to provide a detailed assessment of microscopic reproductive staging. The results of this study provide a baseline of key aspects of reproductive biology during drought conditions.

1. INTRODUCTION

In order to maximise reproductive success, teleost fish exhibit a wide range of reproductive diversity. The reproductive strategy of a fish is multifaceted, and requires an understanding of timing (including age at first maturity, seasonal timing and temporal patterns of reproduction), spawning habitat, behaviour, allocation of resources, fecundity, batch size and egg features (Wootton 1998). This diversity is frequently exhibited in differences in the oocyte development (cellular processes, size, organisation) and reproductive pattern (hermaphroditic or gonochoristic) (de Vlaming *et al.* 1982). Reproductive cycles in fish are generally characterised by changes in gonad size. The methodology for examining these changes range from a visual assessment of the external appearance of the gonad (macroscopic analysis), which is rapid but superficial, to histological staging (microscopic analysis), which is comprehensive but time-consuming (Somarakis *et al.* 2004). The application of understanding reproductive biology is vast. Previous studies include: control of pest species (Smith and Walker 2004), formation of marine protected areas (Soh *et al.* 2001; Karakulak *et al.* 2004), minimum catch size restrictions and the allocation of fishing seasons (Booth and Buxton 1997; Appleford *et al.* 1998; Hesp *et al.* 2004), protection of commercially important species (Fowler *et al.* 1999; Ye *et al.* 2002) and elucidated seasonal migrations among other reproductive patterns (Irwin and Bettoli 1995; McKinley *et al.* 1998; Kestemont *et al.* 1999). Understanding reproductive strategies is, therefore, vitally important for defining management and conservation strategies for species that may be at risk.

Estuaries are the interface between terrestrial, freshwater and marine environments. They are areas of extremely high productivity often recognised as biodiversity hotpots (Edgar *et al.* 2000; Edgar and Barrett 2002). Habitat loss and the declining health of coastal and estuarine habitats due to anthropogenic and climate change related disturbances are increasing in Australia and around the world. These impacts are likely to compromise estuarine ecosystems, potentially resulting in large-scale alterations of fish assemblages and subsequent decreases in biodiversity. Estuaries are considered among the most anthropogenically degraded habitats worldwide, despite their importance for coastal productivity and sustaining biodiversity (Edgar *et al.* 2000; Edgar and Barrett 2002).

The influence of freshwater inflows to estuaries on fish communities is not well understood (Gillanders and Kingsford 2002; Whitfield 2005). Freshwater inflows to estuaries result in abiotic

(changes to water quality, nutrients, and changed geomorphology) and biotic (changes to primary and secondary productivity, habitat availability, migrations, and biological responses) effects, and these changes can have a significant impact on fish communities (Hart and Finelli 1999; Alber 2002; Robins and Ye 2007). Estuaries have a vast sphere of influence by providing spawning and 'nursery' areas, and food and habitat for many species of invertebrates and fish (Blaber 1987; Potter *et al.* 1990; Beck *et al.* 2001). Whilst there are few fish species that complete their life cycles within estuaries, many fish will utilise estuaries for spawning and recruitment of juveniles (Loneragan and Potter 1990; Potter and Hyndes 1999).

The Murray Mouth and Coorong is not a typical estuary. It has been described as a reverse estuary, where salinity increases with distance from the mouth, generally ranging between saline in the Murray Mouth and hypersaline in the Northern and Southern Lagoons (Geddes and Bulter 1984). Since the construction of five barrages in the 1940s to prevent intrusion of saltwater into the Lower Lakes of the Murray River (which reduced the estuary to 11% of its original size), the region has suffered significantly from anthropogenic disturbances associated with a lack of consistent seasonal freshwater inflows to the estuary (Ferguson *et al.* 2008). In the past decade this was compounded by the over allocation of freshwater water resources and a severe drought from 2001-2010, which resulted in little/no freshwater flows over the barrages. Consequently, the lack of freshwater flow resulted in closures of the estuary's mouth due to sand and sediment build up. As a result, a continuous dredging operation was undertaken from 2002-2010 to keep the mouth open (Jennings *et al.* 2008). This changed the Murray Mouth and Coorong to effectively a tidal marine inlet with reverse estuary influence (Jennings *et al.* 2008). The ecological health of the Coorong with respect to its biodiversity and productivity was described as poor by Geddes (2005); given the continued degradation of the region due to lack of flows, the fish community was possibly at an historical low point during 2006-2008 (Noell *et al.* 2009). The Coorong is a Ramsar-listed Wetland of International Importance and is listed as one of the six Icon Sites under the Living Murray Initiative. The system also supports the multi-species Lakes and Coorong Fishery.

Five fish species were selected based on the results of previous community studies conducted in the Coorong. These species included: three commercially-important species - black bream (*Acanthopagrus butcheri*), yelloweye mullet (*Aldrichetta forsteri*) and greenback flounder (*Rhombosolea tapirina*); an important trophic level small-bodied estuarine species - Tamar goby (*Afurcagobius tamarensis*); and a species of conservation significance - congolli (*Pseudaphritis urvillii*). Reproductive information

available from previous studies for selected species is summarised in Table 1. Coorong specific information on reproductive characteristics (size at maturity, sex ratios, spawning season and mode) is available for the commercially important species (i.e. *A. butcheri*, *A. forsteri* and *R. tapirina*). However, there is a paucity of information for *A. tamarensis* and *P. urvillii*, with respect to many of these reproductive characteristics, and no available information on size/age at maturity and sex ratios, indicating clear knowledge gaps for these species. Furthermore, there is little data on the link between freshwater inflows and the spawning and recruitment of fish species in the Coorong (although see Molsher *et al.* 1994; Arthington and Marshall 1999; Pellizzari 2001; Jennings *et al.* 2008).

This report describes the reproductive biology of five key species of the Murray Mouth and Coorong of South Australia. The original aims of this study were to examine aspects of reproductive biology and influence of flow induced environmental conditions on spawning success of five key species in the region; however, with no freshwater inflows to the region since October 2006 and continuation of a drought that lasted more than five years, the aims were revised.

The primary aim of this study is to provide baseline information on the temporal variability of the reproductive biology and characteristics for the five key fish species during drought conditions, by investigating the several aspects of their biology including: (i) sex ratios; (ii) timing and duration of the spawning season; (iii) and size at maturity. This study will also provide the first detailed assessment of microscopic characteristics and staging of ovaries for all five key commercially targeted fish species within the Coorong.

Table 1. Life history styles of target species adapted from Noell *et al.* (2009). Each species was categorised as; C = catadromous, E = Estuarine, E and M = Estuarine and Marine, M = Marine and O = Marine opportunist (using the criteria of Potter and Hyndes 1994). References are numbered and listed at the end of this table.

		Reproduction				Early life stage		
Common name (Scientific name)	**Life history / adult habitat**	**Size/Age at maturity**	**Sex ratios (n_m:n_f)**	**Spawning: season and mode**	**Spawning habitat/ behaviour**	**Egg and larval attributes**	**Habitat preferences**	**Water quality preferences/ tolerances**
Black bream (*Acanthopagrus butcheri*)	**E** Completes entire life cycle within the estuary [1]. Pelagic. Structurally complex, low salinities and high dissolved oxygen [2].	Varies among estuaries in WA, 129-169 mm, and 1.9 to 4.3 years [3-4]. Coorong: Males 3 years old (140 to 180 mm FL) Females 4 years old (210 to 250 mm FL) [5].	Swan River Estuary 1.19:1.0 [4]. Coorong: Ranged from 1:00:0.58 to 1:8 [6].	August to December [7]. Multiple, repeat spawner that forms dense spawning aggregations [8].	Pelagic spawning [9-10].	Pelagic neutrally buoyant eggs. Larvae are planktonic [2].	Vegetation important for settlement. [9-10]. Juveniles preference shallow, littoral fringes of estuaries for woody debris and snags [2].	Estuarine but prefers lower salinities and high dissolved oxygen DO [9-10].
Yelloweye mullet (*Aldrichetta forsteri*)	**O** Estuaries preferred, but also brackish and inshore coastal waters and tidally inundated salt marshes. Pelagic. Found over sand and muddy substrates to depths of 10-20 m. [5, 11-15].	2 to 3 years [16-17]. Coorong: Males 220 mm FL Females 230 mm FL [17].	No data.	Two populations: Western winter spawning and eastern summer spawning. Coorong population possibly a mix of east and west [17]. Coorong: January to March. Single spawner [17].	Pelagic spawning in offshore habitats [18] In SA, estuaries [12, 17].	Pelagic eggs [17].	Late larvae/early juvenile stages migrate back into estuaries [18]. Juveniles remain in shallow banks of estuaries and beaches [18-19].	No data.

Common name (Scientific name)	Life history / adult habitat	Reproduction				Early life stage		
		Size/Age at maturity	Sex ratios (n_m:n_f)	Spawning: season and mode	Spawning habitat/ behaviour	Egg and larval attributes	Habitat preferences	Water quality preferences/ tolerances
Greenback flounder (*Rhombosolea tapirina*)	**E andM** Inhabits estuaries and coastal waters (up to 100 m deep). Benthic. Sand silt and muddy substrates in sheltered bays, estuaries and inshore coastal waters [11, 19-20].	In Tasmania 219 mm females, and 190 mm males [22]. Coorong: No data	No data.	June to October [21-22]. Serial prolonged spawner [21-22]. Coorong: No data	Deeper regions in tidal rivers, estuaries and offshore [21-22]. Coorong: No data	Pelagic buoyant eggs, 0.7 -1.0 mm in diameter. Undeveloped pelagic larvae 1.9 mm at hatch. Settlement and metamorphosis occurs 35 days post hatch [18, 21-24].	Shallow, un-vegetated sand and mudflat habitats [15, 25-26].	Wide range of salinity tolerances [19].
Tamar goby *(Afurcagobius tamarensis)*	**E** Quiet waters of estuaries, coastal lakes and lower river reaches. It prefers still or slow-flowing waters with a mud or silt substrate and cover provided by aquatic vegetation, rocks or fallen logs [27-28]	No data.	No data.	Spring [29-30] October to December [31]. Ubiquitous spawners, spawning over a protracted period of more than five months [32]. Coorong: No data	Male builds a burrow and performs display to entice the female [29]. Benthic/ burrowing. Prefers silt and mud bottoms with aquatic vegetation [14, 27, 33]. Coorong: No data	Not described.	No data.	Tolerates low DO conditions if able to perform ASR [34]. Recorded in salinities ranging from 10 to 30 ppt [35].

Common name (Scientific name)	Life history / adult habitat	Reproduction				Early life stage		
		Size/Age at maturity	Sex ratios (n_m:n_f)	Spawning: season and mode	Spawning habitat/ behaviour	Egg and larval attributes	Habitat preferences	Water quality preferences/ tolerances
Congolli (*Pseudaphritis urvillii*)	**C** Mainly inhabiting estuaries and riverine environments [14]. Benthic. Freshwater environments as an adult. When in freshwater prefers slow-flowing streams with leaf litter, rocks, sunken logs and overhanging banks [14].	No data.	No data.	Autumn- Spring. (April to September in SA) [28, 36-39, 44]	Spawning occurs in marine [44] and estuarine waters [40].	Eggs undescribed. Only postflexion larvae described. [41]	Prefers areas with aquatic vegetation when in estuaries [40]	Extremely hardy species being able to withstand direct transfer from freshwater to saltwater, and vice versa, without signs of distress [39, 42].

[1]: Chaplin *et al.* (1998), [2]: Norriss *et al.*(2002), [3]: Sarre and Potter (2000), [4]: Sarre (1999), [5]: Hall (1984), [6]: Ferguson and Ye (2008), [7]: Harbison (1974), [8]: Cashmore *et al.* (2000), [9]: Nicholson and Gunthorpe (2008), [10]: Nicholson and Gunthorpe (2006), [11]: Kailola *et al.* (1993), [12]: Higham *et al.* (2005), [13]: Thomson (1996), [14]: Gomon *et al.* (1994), [15]: Edgar and Shaw (1995), [16]: Chubb *et al.* (1981), [17]: Harris (1968), [18]: Jenkins (1986), [19]: Last *et al.* (1983), [20]: Ferguson (2007), [21]: Kurth (1957), [22]: Crawford (1984), [23]: Crawford (1986), [24]: May and Jenkins (1992), [25]: Connolly (1994), [26]: Jenkins *et al.* (1997), [27]: Larson *et al.* (1996), [28]: Cadwallader and Backhouse (1983), [29]: Lintermans (2007), [30]: Adams *et al.* (2004), [31]: Noell *et al.* (2009), [32]: Newton (1996), [33]: Higham *et al.* (2002), [34]: Gee and Gee (1991), [35]: Gee and Gee (1995), [36]: Hammer *et al.* (2009), [37]: Koehn and O'Connor (1990), [38]: SKM (2003), [39]: Andrews (1996), [40]: Hortle (1978), [41]: Neira *et al.* (1998), [42]: Merrick and Schmida (1984), [43]: Thomson (1957), [44] Crook (2010).

2. METHODS

Sampling regime

To examine the reproductive biology of the selected species, fish were collected from the Murray Mouth and Coorong (Figure 1) on 13 sampling occasions, at approximately one-month intervals between July 2007 and July 2008.

A. butcheri, A. forsteri and *R. tapirina* were collected by commercial fishers, using monofilament gill nets, with mesh sizes ranging from 2 to 6 inches. *Pseudaphritis urvillii* and *A. tamarensis* were sampled by SARDI researchers using single winged (6 mm mesh) fyke nets. Fyke nets were set overnight, and positioned with the wing facing the bank. Initially, the aim was to collect at least 30 fish per species per month from a range of sites, however, due to the unpredictability of sample collection, the number of individuals varied for each species and sometimes exceeded 30 (Table 2). In total 1,805 individuals were examined for analyses (Table 2).

Sample processing and macroscopic analysis

Monthly samples of adult fish for each species were processed in the laboratory (Table 2). All fish were processed within 24 hours of capture. Each fish was measured for total length (TL) to the nearest mm, weighed to the nearest gram, and dissected for the removal and analysis its gonads. Once removed, the gonads were examined to determine the sex of the fish and weighed to 0.1 g. Gonadosomatic indices (GSI) were calculated as:

GSI = [Wg/Wf]*100%

where, Wg = gonad weight, Wf = gonad-free fish weight.

Ovaries were classified macroscopically to one of five stages of development based on size, colour and visibility of oocytes (Fowler *et al.* 1999) (Table 3). Males were staged macroscopically and classified to one of three stages of development (Table 3).

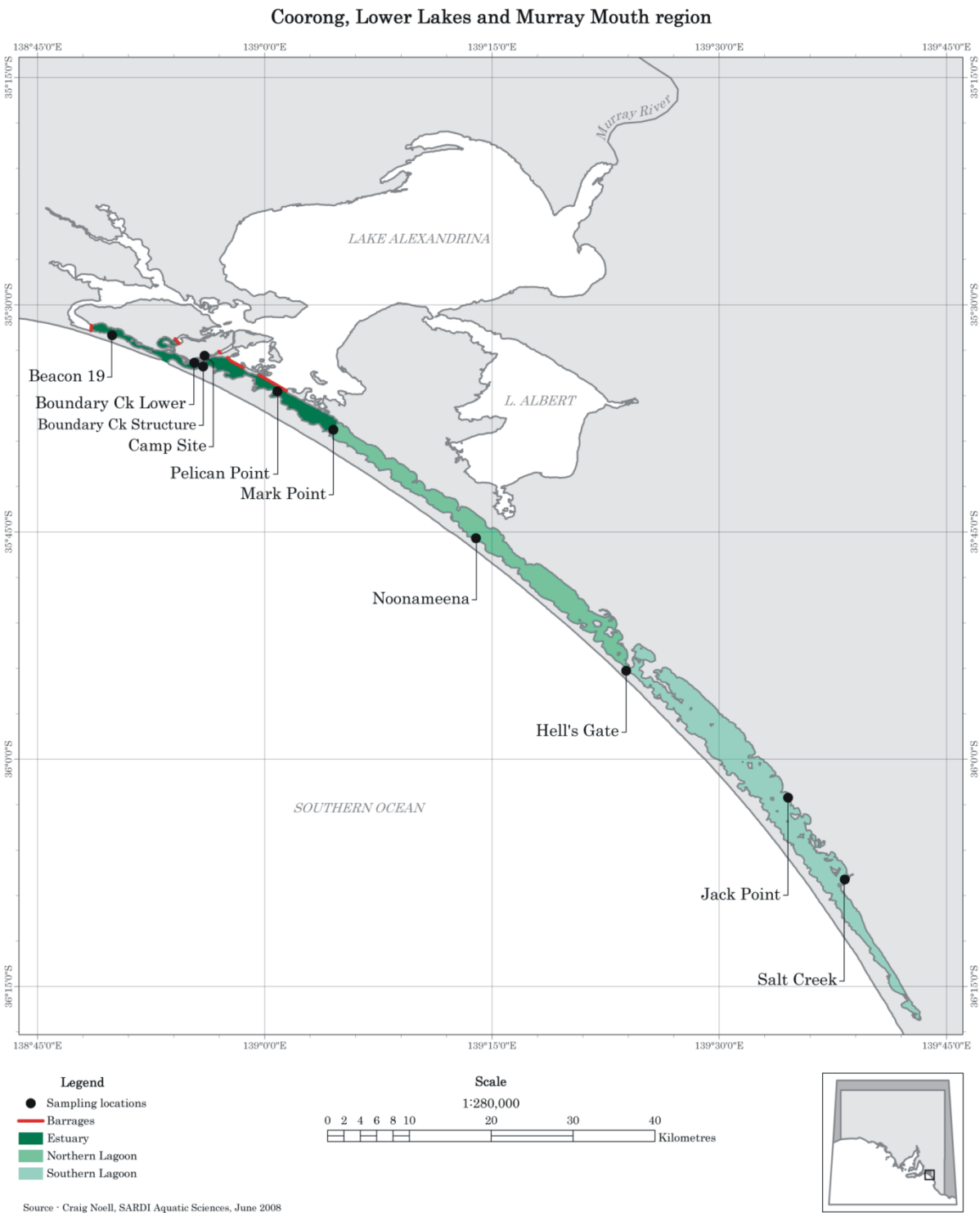


Figure 1. Map showing the Murray Mouth, Lower Lakes and Coorong. Dark green shading indicates the Murray Mouth, light green shading indicates the Northern Lagoon and light blue shading indicates the Southern Lagoon subregions.

Table 2. Summary of the total catch for the five selected species from the Murray Mouth and Coorong used for macroscopic staging (F = female; M = male).

Sample type	Month	*A. butcheri*		*A. forsteri*		*R. tapirina*		*A. tamarensis*		*P. urvillii*	
		F	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**
Fishery	Jul-07	18	13	21	9	27					
	Aug-07	5	2	45	15	34					
	Sep-07	30	17	35	9	10	1				
	Oct-07	4	1	60	13						
	Nov-07	9	3	1	4	9	1				
	Dec-07	1		25	4	6					
	Jan-08			74	16						
	Feb-08	11	2	15	13	3					
	Mar-08										
	Apr-08			84	24						
	May-08			55	5						
	Jun-08	4	1			7					
	Jul-08			52	11						
Fishery Total		**82**	**39**	**467**	**123**	**96**	**2**	**0**	**0**	**0**	**0**
Research	Jul-07					2		28		4	1
	Aug-07					2		29		20	5
	Sep-07	6		1				27	10	7	2
	Oct-07			1	1	1	1	75	29	11	
	Nov-07							81	13	30	
	Dec-07							35	28	44	
	Jan-08	2		1		3		59	16	22	1
	Feb-08							7		32	1
	Mar-08							12	1	64	6
	Apr-08			4	1			20	13	51	4
	May-08							6	7	37	25
	Jun-08										
	Jul-08	1		5		1		41	20	42	3
Research Total		**9**	**0**	**12**	**2**	**9**	**1**	**420**	**137**	**364**	**48**
GRAND TOTAL		**91**	**39**	**479**	**125**	**105**	**3**	**420**	**137**	**364**	**48**

Table 3. Classification of development of ovaries and testes for macroscopic staging (see Fowler *et al.* 1999).

Gonad Stage	Macroscopic characteristics
F1- Immature	Ovaries small and undeveloped, clear or translucent are showing little or no colouration.
F2- Developing	Ovaries small but larger than F1 and have become more orange/yellow/white (varies between species), no individual oocytes discernible.
F3- Developed	Ovaries larger and turgid, yellow/orange in colour, individual oocytes discernible.
F4- Hydrated	Large ovaries, taking up a large space in gut cavity, with hydrated oocytes easily discernible.
F5- Regressing /Spent	Ovaries are large, similar in size and colour to F2 stage, however more flaccid with a granular appearance.
M1- Immature	Undeveloped testes usually dark in colour.
M2- Developing	Developing testes whereby they are larger and become grey - white in colour but no milt present.
M3- Developed	Developed testes that are large and white in colour and milt is present.

Microscopic analysis

From the fish collected for macroscopic staging, a random subsample of ovaries was collected monthly for each species for more detailed analysis by histological preparation and microscopic examination (Table 4). For such ovaries, a segment was removed from the centre of each lobe and preserved in a fixative of 5% formalin.

Microscopic analysis was performed on histological sections of the formalin preserved tissue following methods outlined in Fowler *et al.* (1999). Tissue was sectioned at 6 μm and stained with haemotoxylin and eosin. Slides were examined at 100 x magnification and classified according to the most advanced stage of oocyte development, level of atresia and presence/absence of post ovulatory follicles (Farley and Davis 1998). Oocytes were classified as unyolked, partially yolked, advanced yolked, migratory nucleus or hydrated (Fowler *et al.* 1999) (Table 5). The estimated abundance of atretic oocytes relative to advanced yolk was classified as no atresia, <10%, 10-50%, >50% and 100%. The presence/absence of post-ovulatory follicles was also noted (Table 5). Microscopic characteristics were used to classify the ovaries into one of six stages of development (Table 6). The microscopic and macroscopic stages are aligned, however the microscopic staging allowed the spent/regressing stage to be separated based on presence/absence of post-ovulatory follicles and level of atresia.

Table 4. Summary of the numbers of female fish of each species used for histology from the Murray Mouth and Coorong.

Sample type	Month	*A. butcheri*	*A. forsteri*	*R. tapirina*	*A. tamarensis*	*P. urvillii*
Fishery	Jul-07	13				
	Aug-07	3	13	16		
	Sep-07	20	14	8		
	Oct-07	1	16			
	Nov-07	14	1	5		
	Dec-07	1	12			
	Jan-08		21			
	Feb-08	8	6			
	Mar-08					
	Apr-08		33			
	May-08		4			
	Jun-08	4		7		
	Jul-08		22			
Fishery Total		**64**	**142**	**36**	**0**	**0**
Research	Jul-07					
	Aug-07			2	15	9
	Sep-07	6		1	7	4
	Oct-07				3	
	Nov-07		3		10	
	Dec-07				9	6
	Jan-08	2		3	16	
	Feb-08				1	
	Mar-08					14
	Apr-08		1		2	13
	May-08				1	9
	Jun-08					
	Jul-08	1		1	10	17
Research Total		**9**	**4**	**7**	**74**	**72**
GRAND TOTAL		**73**	**146**	**43**	**74**	**72**

Table 5. Description of oocyte development stages, atretic oocytes and post ovulatory follicles observed in histological prepared sections of ovaries (after Fowler *et al.* 1999).

Oocyte stage	Microscopic characteristics
Unyolked	Oogonia small, cytoplasm blue. Nucleus large, central, several nucleoli occur at nucleus periphery.
Partially yolked	Oocyte developing, becoming larger, cytoplasm purple. White lipid granules throughout cytoplasm. Zona radiata present but thin.
Advanced yolked	Oocyte large, lipid granules and red yolk protein granules throughout cytoplasm. Nucleus still central. Zona radiata thick.
Migratory nucleus	Similar to advanced yolk except nucleus has moved to the peripheral cytoplasm. This represents the initiation of the hydration process.
Hydrated oocyte	Oocyte much larger with uptake of fluid, nucleus absent. Yolk plates occupy entire volume of cytoplasm or have fused to form a pink homogeneous mass. Often misshapen due to damage caused during preserving and preparation processes. Zona radiata and follicular layers greatly stretched.
Atretic oocyte	Zona radiata dissolved, oocyte misshapen and internal structure disintegrating.
Post-ovulatory follicle	Remaining follicular layers after ovulation. Initially it is large, highly convoluted with an obvious lumen. Convoluted nature becomes less apparent with age and lumen much reduced or closed.

Table 6. Classification of development of ovaries for microscopic staging (after Fowler *et al.* 1999).

Ovary stage	Microscopic characteristics
F1- Immature	Only unyolked oocytes present.
	No atresia
F2- Developing	Partially yolked and unyolked oocytes present.
	Low levels of atresia (<10%).
F3- Developed	Advanced yolked oocytes predominate.
	Partially yolked and unyolked oocytes also present.
	Low levels of atresia (<10%).
	Some post-ovulatory follicles may be present.
F4- Hydrated	All stages of oocyte development present, however advanced yolk and hydrated oocytes predominate.
	Hydrated oocytes predominate the mature (advanced and hydrated) oocytes.
	Low levels of atresia (<10%).
	Some post-ovulatory follicles may be present.
F5- Spent	Characterised by an absence of hydrated oocytes and a large number of post-ovulatory follicles. Density of post-ovulatory follicles > 1/mm2 on histology section.
	Low levels of atresia (<10%).
F6- Regressing	High levels of atresia (>50%).
	Absence of hydrated oocytes and post-ovulatory follicles.

Size at first maturity

The size at first maturity was measured for females of all key species. Those individuals with ovaries ≥ stage 3 during spawning season were defined as mature. Logistic curves were fitted to describe the percentage maturity at total length (TL cm) using the non-linear least squares (NLIN) procedure in SAS (Anon 1989) according to the equation:

$$P_m = \frac{100}{1+e^{-k(X-m)}}$$

where, P_m is % maturity, X is the TL (cm), k is a constant describing how rapidly fish mature, and m is the size at 50% maturity.

3. RESULTS

Black bream (*Acanthopagrus butcheri*)

Macroscopic analyses

Sex ratios, timing and extent of spawning season

The catch of *A. butcheri* in the Murray Mouth and Coorong was consistently dominated by female fish throughout the season (Figure 2). Males were present throughout most of the season with the exception of December, January and July 2008 (Figure 2).

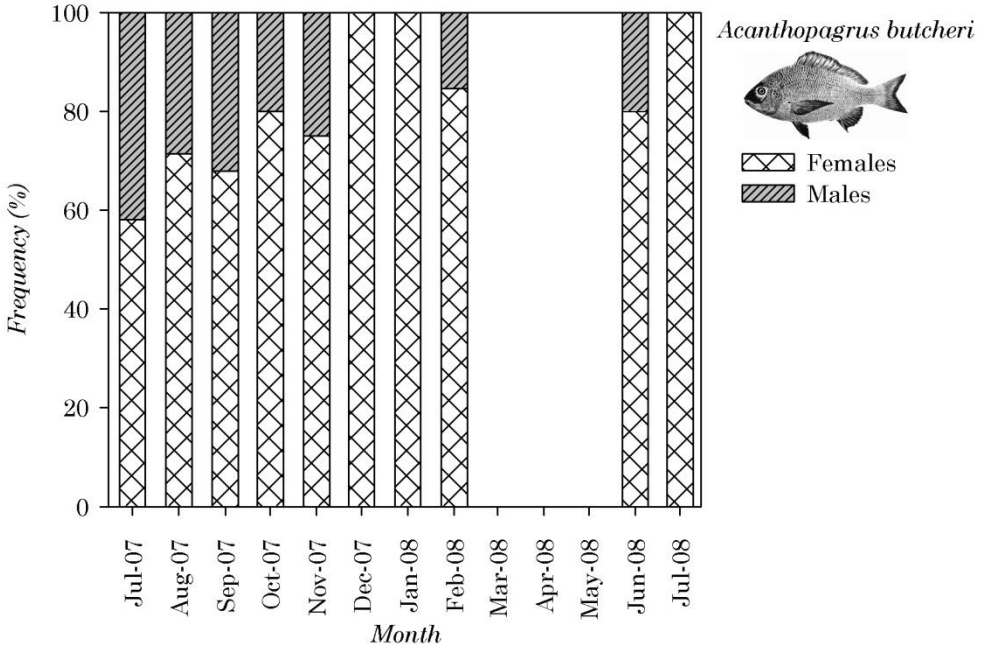


Figure 2. Temporal trends in sex ratios (frequency %) of *A. butcheri* in the Coorong from July 2007 to July 2008 (females n = 91, males n = 39).

GSIs for female *A. butcheri* increased from August to November in 2007, suggesting a spring spawning season in the Murray Mouth and Coorong (Figure 3a). Increasing female GSI followed the increase in water temperature early in the season, then dropped substantially in December 2007, following a dip in temperature and remained low for the rest of the sampling period (Figure 3a). GSI for males followed a similar pattern to females, increasing from July to November (Figure 3b). GSI was highest in males between July and February; although given the relatively small sample size and absence of male fish in December and January, this should be treated with caution.

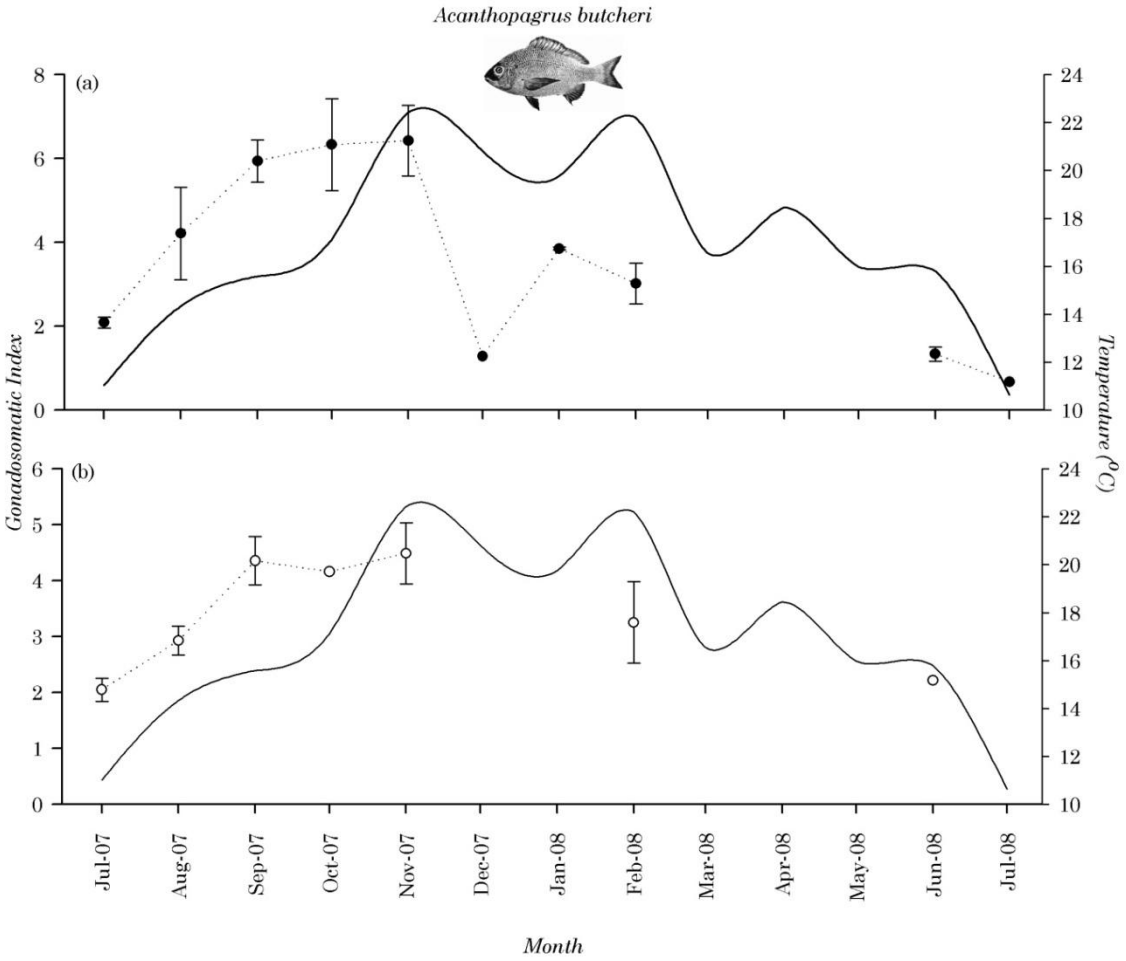


Figure 3. Temporal trends in gonadosomatic indices (± s.e.) (dotted line) for a) female (n = 91) and b) male (n = 39) *A. butcheri* in the Coorong from July 2007 to July 2008. Solid black line is mean monthly temperature.

Mature females (≥ stage 3) were collected throughout the sampling period, with the exception of March to May 2008, when no females were collected (Figure 4). In July 2007, the majority of females had ripe (stage 4) ovaries, whilst a small percentage (<20 %) had developed (stage 3) ovaries (Figure 4). From August to November 2007, the stages varied between developed and ripe (stages 3 and 4). In December only one female was collected, which was at the ripe stage (stage 4). During January and February only resting and developed stage (stage 2 and 3) ovaries were recorded. In June and July 2008, a small number of females were collected, with ovaries being at stages 2 or 3. No spent (stage 5) ovaries were recorded throughout the study period (Figure 4).

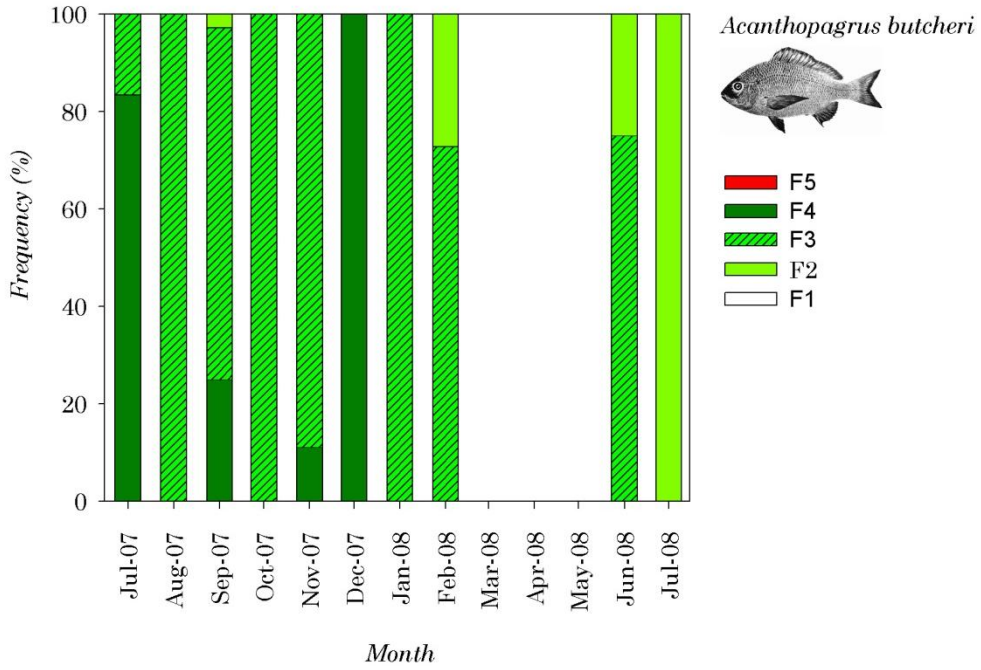


Figure 4. Temporal trends in macroscopic stages of gonad development (frequency %) for female *A. butcheri* in the Coorong from July 2007 to July 2008. (n = 91).

Size at first maturity

Half the population of female *A. butcheri* within the Coorong were sexually mature at ≥ 289 mm TL, whilst the smallest mature individual female was 270 mm TL (Table 7; Figure 5a). Half of the males were sexually mature at 340 mm TL, the smallest mature male was 251 mm TL (Table 7; Figure 5b). Parameters of the logistic maturity curves are provided in Table 7.

Table 7. Parameter estimates of the logistic curves of the size at first maturity of female and male *A. butcheri* from the Coorong. (TL=Total Length, SE=Standard Error, CL=Approximate 95% Confidence Limits, K = constant describing how rapidly fish mature, *p*= significance and N= # of individuals in sample).

Sex	TL (mm)	SE	95% CL	K	SE	95% CL	R-square	*p*	N
Female	289	2.4825	283.7-294.2	0.06	0.00785	0.0418-0.0751	0.0471	<0.0001	271
Male	340	11.0303	315.6-364.2	0.02	0.00382	0.0087-0.0255	0.1578	<0.0001	148

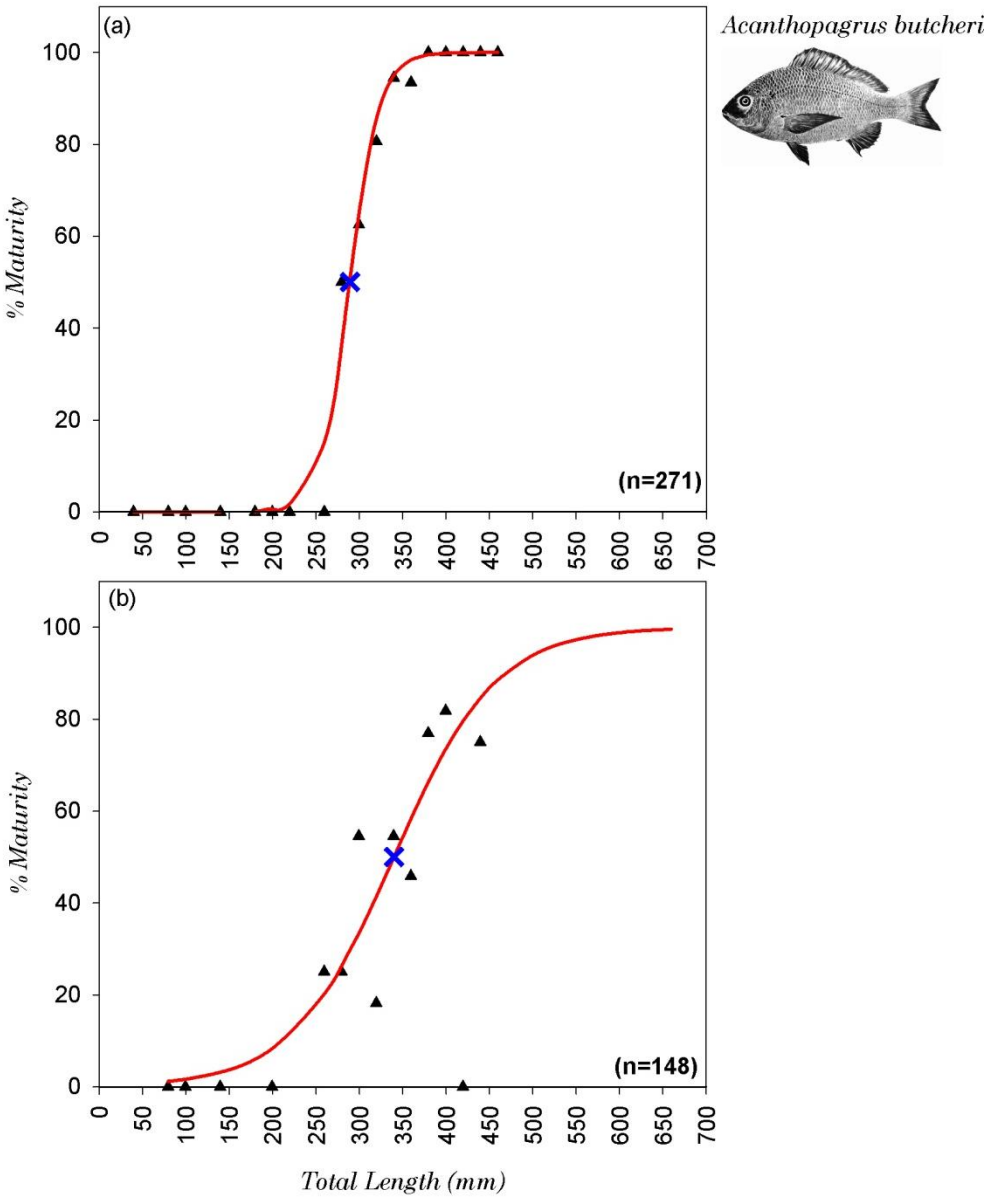


Figure 5. Size at reproductive maturity (% of population that is sexually mature) of a) female and b) male *A. butcheri* in the Coorong. Blue crosses indicate size when 50% of the population is mature (females *m*=289 mm and males *m*=340 mm).

Microscopic analyses

Microscopic characteristics of ovaries throughout development

Stage 1 (F1-immature) in *A. butcheri* ovaries was characterised as having only unyolked oocytes with no atresia present (Figure 6a).

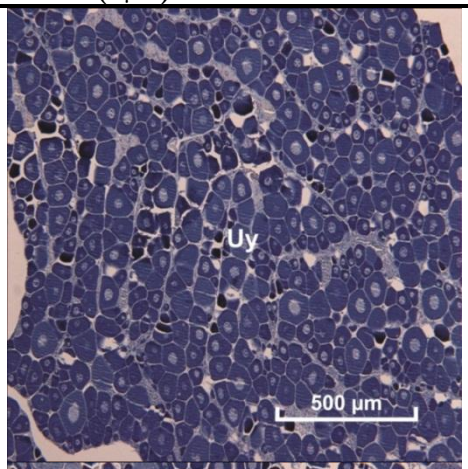
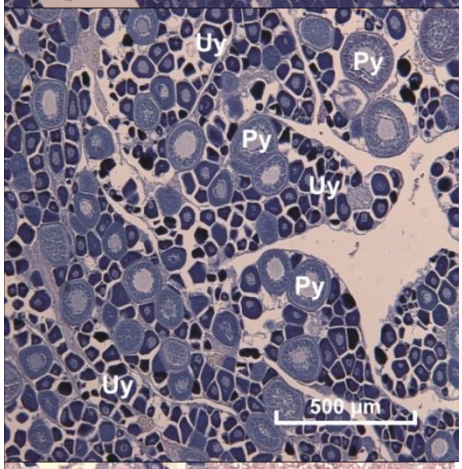
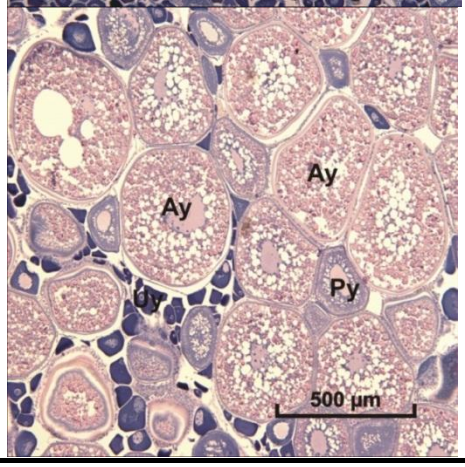
Stage 2 (F2-developing) showed signs of low levels of atresia, which were not present in the previous stage, along with partially yolked oocytes. Unyolked oocytes were also present in this stage, however, in lower proportions than that of stage 1 (Figure 6b).

Stage 3 (F3-Ripe), predominately consisted of advanced yolked oocytes, indicating that spawning was imminent for *A. butcheri* at time of capture. Partially yolked and unyolked oocytes were also present with low levels of atresia (Figure 6c).

Stage 4 (F4-Hydrated), in this stage for *A. butcheri,* all oocyte development were present, with predominately advanced yolk and hydrated oocytes, indicating optimum spawning condition. Low levels of atresia were also present with some post-ovulatory follicles (Figure 6d).

Stage 5 (F5-Spent) was characterised by an absence of hydrated oocytes and a large number of post-ovulatory follicles. However, also noted, was the persistence of low levels of atresia, which is consistent with the previous three stages (Figure 6e).

Stage 6 (F6-Regressing), unlike all the previous stages, had high levels of atresia present. Also observed was the absence of hydrated oocytes and post-ovulatory follicles, suggesting that spawning had occurred during the sample period (Figure 6f).

Microscopic staging and histological characteristic	Section (6 µm)
a. F1- Immature Only unyolked oocytes present. No atresia.	
b. F2- Developing Partially yolked and unyolked oocytes present. Low levels of atresia.	
c. F3- Ripe Advanced yolked oocytes predominate. Partially yolked and unyolked oocytes also present. Low levels of atresia.	

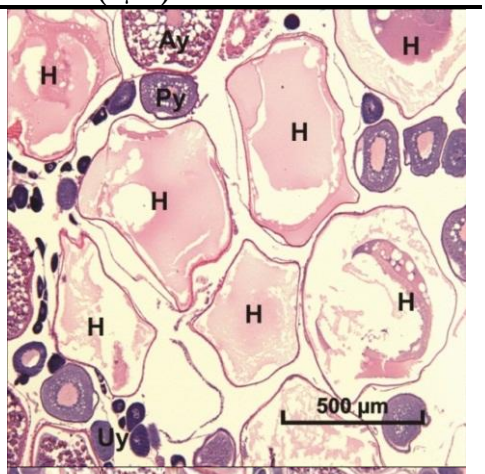
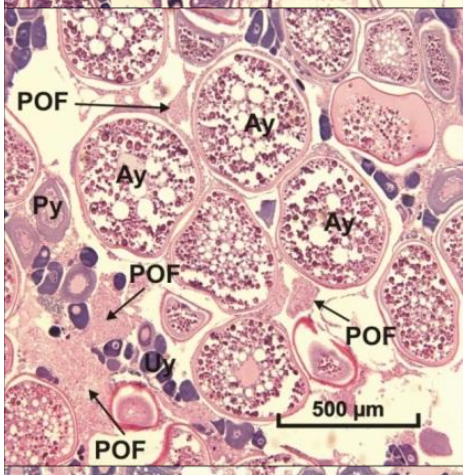
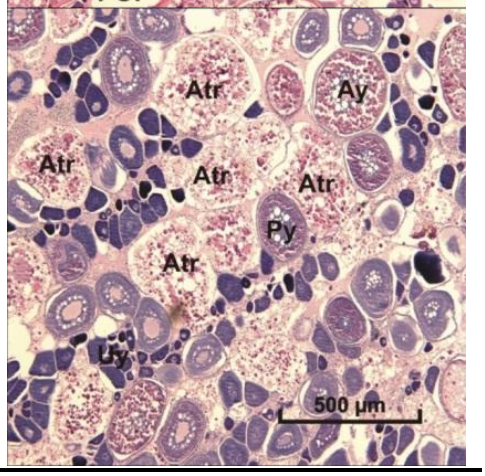
Microscopic staging and histological characteristic	Section (6 µm)
d. F4- Hydrated All stages of oocyte development present, however advanced yolk and hydrated oocytes predominate. Low levels of atresia. Some post-ovulatory follicles may be present.	
e. F5- Spent Characterised by an absence of hydrated oocytes and a large number of post-ovulatory follicles. Low levels of atresia.	
f. F6- Regressing High levels of atresia. Absence of hydrated oocytes and post-ovulatory follides.	

Figure 6. a.-f. Descriptions and digital images of microscopic staging and histological characteristics (section = 1µm) of different ovary stages in *A. butcheri*. Uy = unyolked oocyte, Py = partially yolked oocyte, Ay = advanced yolked oocyte, H = hydrated oocyte, POF = post-ovulatory follicle, Atr = atretic oocyte.

Yelloweye mullet (*Aldrichetta forsteri*)

Macroscopic analyses

Sex ratios, timing and extent of spawning season

The catch of *A. forsteri* in the Murray Mouth and Coorong during 2007 and 2008 was generally dominated by female fish, however, during November 2007 the sample was predominately male fish, and during February 2008 female:male ratios were almost 1:1 (Figure 7).

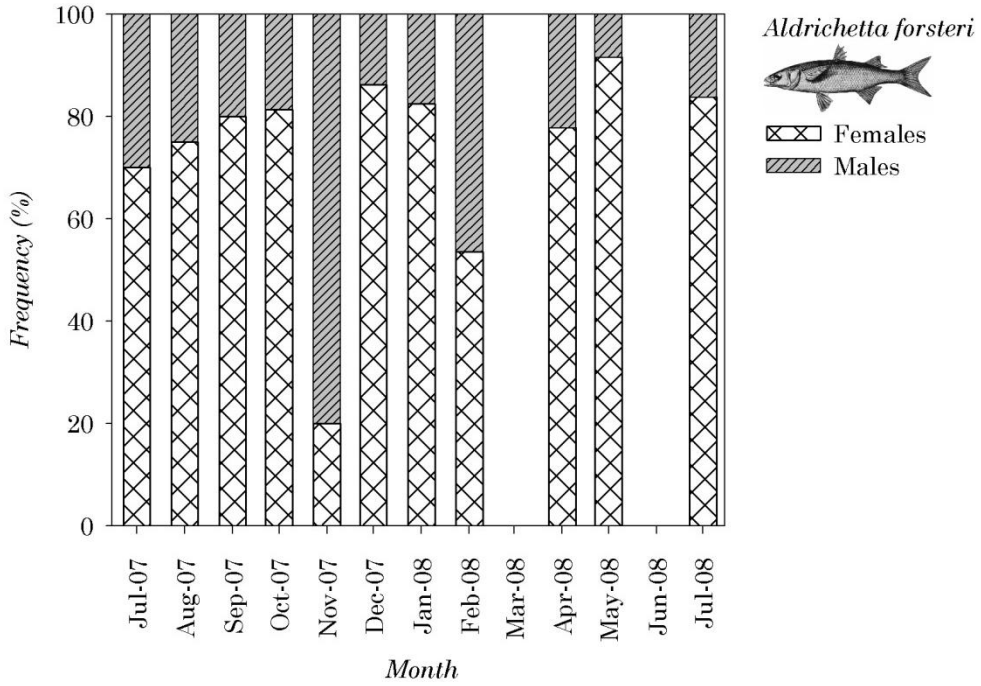


Figure 7. Temporal trends in sex ratios (frequency %) of *A. forsteri* in the Coorong from July 2007 to July 2008. (females n = 479, males n = 125).

GSI for female *A. forsteri* remained high throughout most months, except for the period of October-December. GSI for males followed an almost identical pattern to females (Figure 8). This suggests that *A. forsteri* has a protracted spawning season across summer/autumn/winter in the Murray Estuary and Coorong (Figure 8).

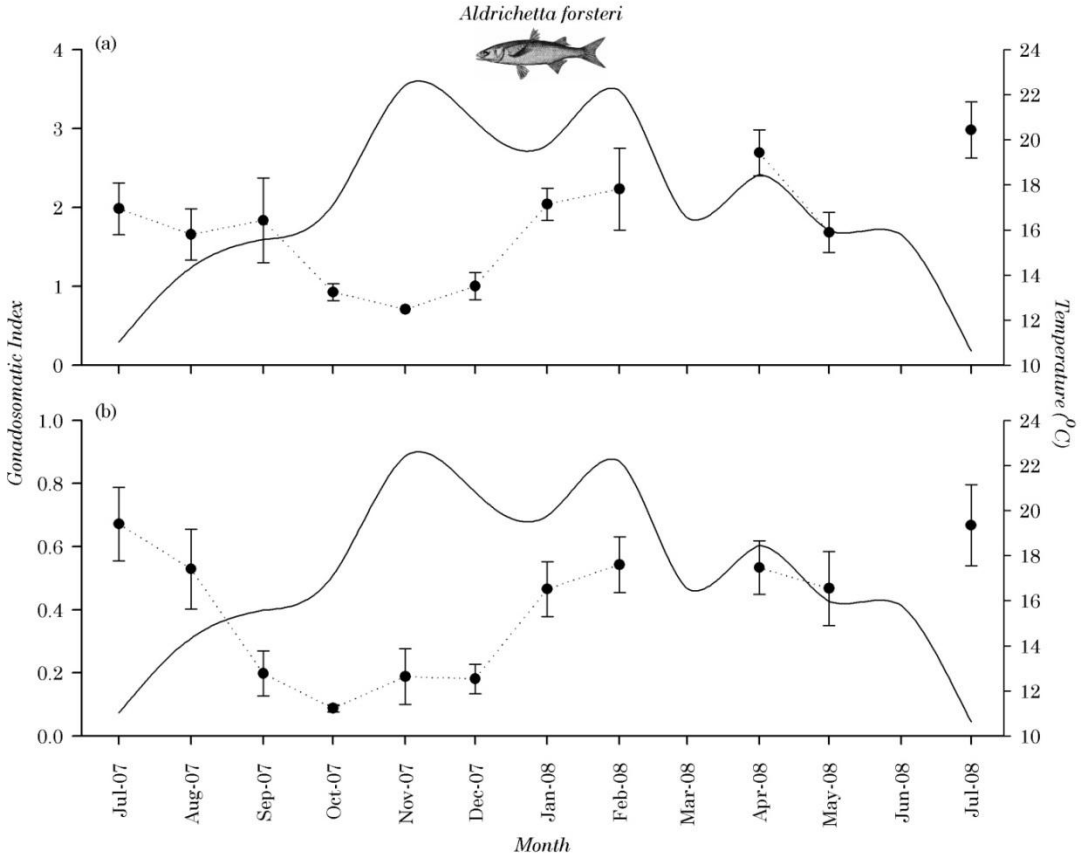


Figure 8. Temporal trends in gonadosomatic indices (± s.e.) (dotted line) for a) female (n = 479) and b) male (n = 125) *A. forsteri* in the Coorong from July 2007 to July 2008. Solid black line is mean monthly temperature.

Females with developed, hydrated, spent or regressing ovaries (≥ stage 3) were collected throughout the sampling period, although no samples were collected in March and June 2008, and only one female was collected in November 2007 (Figure 9). Patterns of ovarian development indicated an extended spawning period of *A. forsteri* with no distinct seasonality.

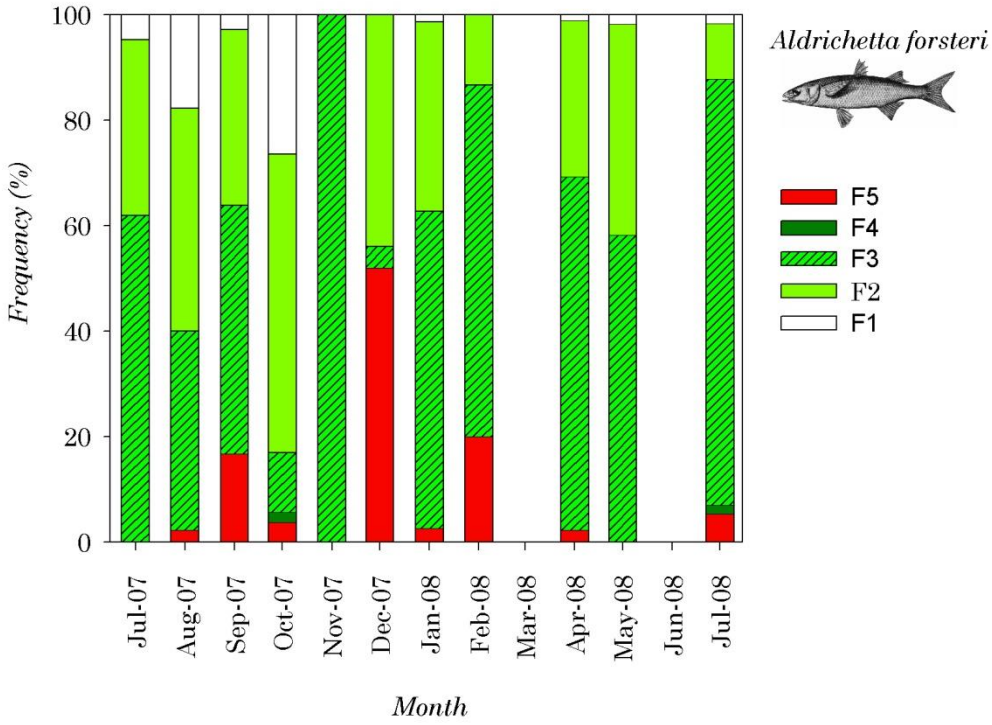


Figure 9. Temporal trends in macroscopic stages of gonad development (frequency %) for female *A. forsteri* in the Coorong from July 2007 to July 2008 (n = 479).

Size at first maturity

Half the population of female *A. forsteri* within the Coorong were sexually mature at the size ≥ 256 mm TL, while the smallest mature individual female was 226 mm TL (Table 8; Figure 10). Insufficient numbers of mature males were collected to develop a model to estimate size at 50% maturity. The smallest mature individual male (stage 3) was recorded at 220 mm TL. Parameters of the logistic maturity curves are provided in Table 8.

Table 8. Parameter estimates of the logistic curves of the size at first maturity of female *A. forsteri* from the Coorong. (TL=Total Length, SE=Standard Error, CL=Approximate 95% Confidence Limits, K = constant describing how rapidly fish mature, *p*= significance and N= #. of individuals in sample).

TL (mm)	SE	95% CL	K	SE	95% CL	R-square	*p*	N
256	2.8347	250.1-261.7	0.04	0.00457	0.0332-0.0519	0.1707	<0.0001	691

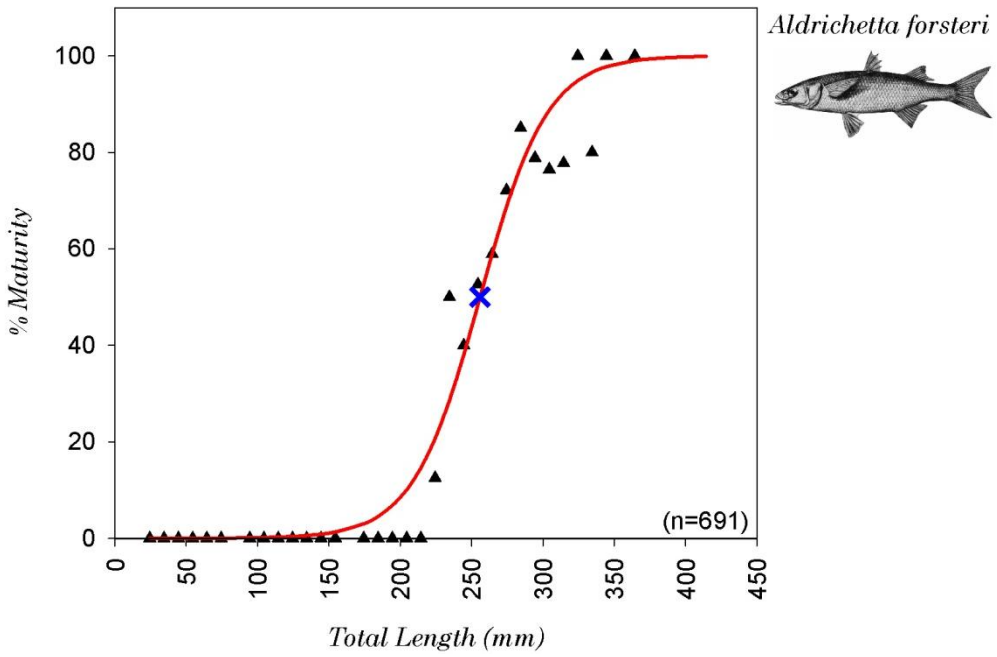


Figure 10. Size at reproductive maturity (% of population that is sexually mature) of female *A. forsteri* in the Coorong. Blue cross indicates size when 50% of the population is mature (females m=256 mm).

Microscopic analyses

Microscopic characteristics of ovaries throughout development

Stage 1 (F1-immature) in *A. forsteri*, was characterised as having only unyolked oocytes with no atresia present (Figure 11a).

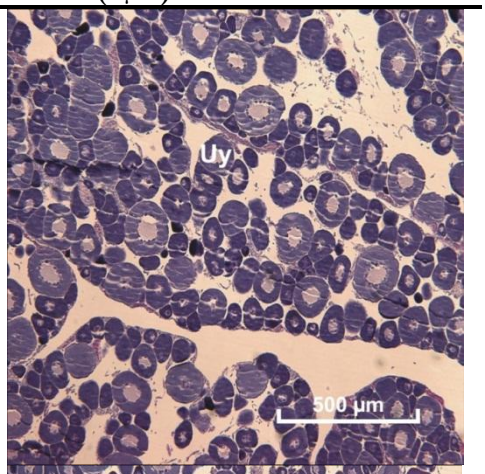
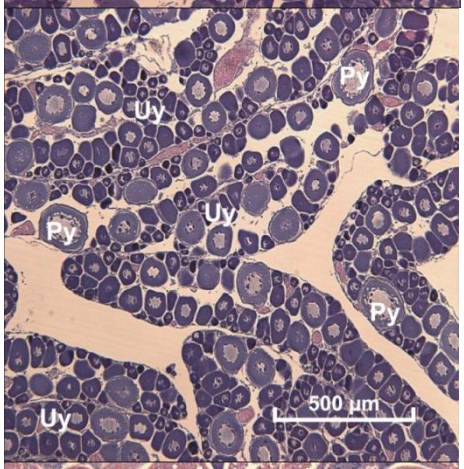
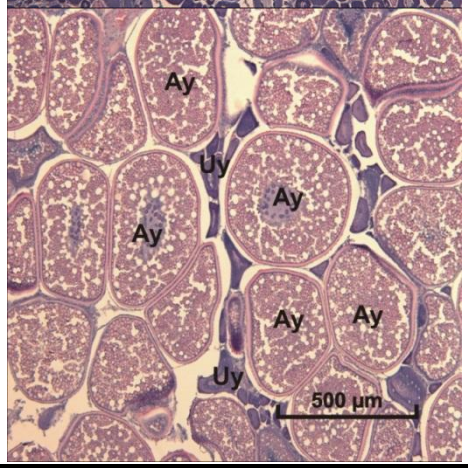
Stage 2 (F2-developing) showed signs of low levels of atresia, along with partially yolked oocytes. Unyolked oocytes were also present in this stage, however in lower proportions than that of stage 1 (Figure 11b).

Stage 3 (F3-Ripe), predominately consisted of advanced yolked oocytes. Partially yolked and unyolked oocytes were also present as with low levels of atresia (Figure 11c).

Stage 4 (F4-Hydrated), due to the lack of ripe females caught during sampling no image was available for *A. forsteri* for microscopic staging. However, by basing ovary development from Fowler *et al.* (1999), it could be implied that all oocyte development were present, with predominately advanced yolk and hydrated oocytes. Low levels of atresia were also present, as with some post-ovulatory follicles (Figure 11d).

Stage 5 (F5-Spent), was characterised by an absence of hydrated oocytes and a large number of post-ovulatory follicles. Also noted was the persistence of low levels of atresia (Figure 11e).

Stage 6 (F6-Regressing), unlike all the previous stages, this stage had high levels of atresia present. Also observed were the presence of a migratory nucleus and the absence of hydrated oocytes and post-ovulatory follicles (Figure 11f).

Microscopic staging and histological characteristic	Section (6 µm)
a. F1- Immature Only unyolked oocytes present. No atresia.	
b. F2- Developing Partially yolked and unyolked oocytes present. Low levels of atresia.	
c. F3- Ripe Advanced yolked oocytes predominate. Partially yolked and unyolked oocytes also present. Low levels of atresia.	
d. F4- Hydrated All stages of oocyte development present, however advanced yolk and hydrated oocytes predominate. Low levels of atresia. Some post-ovulatory follicles may be present. (Implied from Fowler *et al.* (1999))	no image available.

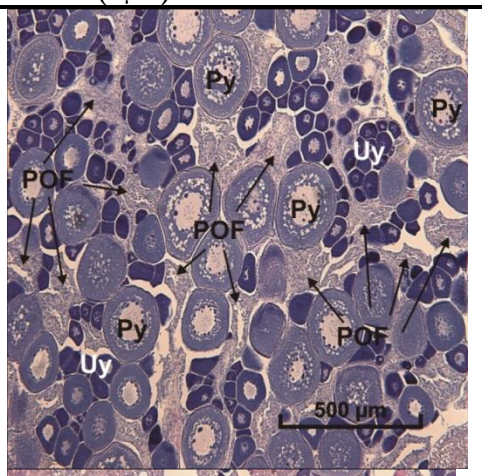
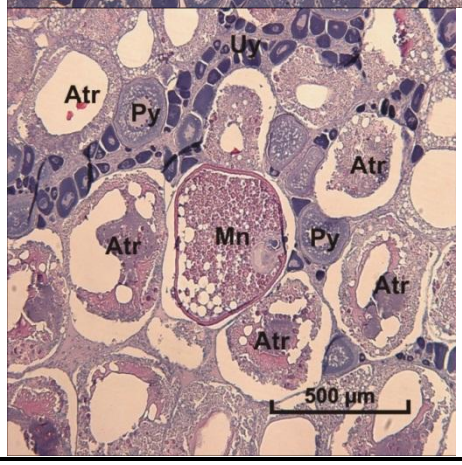
Microscopic staging and histological characteristic	Section (6 µm)
e. F5- Spent Characterised by an absence of hydrated oocytes and a large number of post-ovulatory follicles. Low levels of atresia.	
f. F6- Regressing High levels of atresia . Absence of hydrated oocytes and post-ovulatory follides Migratory nucleus present	

Figure 11. a.-f. Descriptions and digital images of microscopic and histological characteristics (section = 1µm) of different ovary stages in *A. forsteri*. Uy = unyolked oocyte, Py = partially yolked oocyte, Ay = advanced yolked oocyte, Mn = migratory nucleus, H = hydrated oocyte, POF = post-ovulatory follicle, Atr = atretic oocyte.

Greenback flounder (*Rhombosolea tapirina*)

Macroscopic analyses

Sex ratios, timing and extent of spawning season

The catch of *R. tapirina* from the Murray Mouth and Coorong during 2007 and 2008 was dominated by female fish. Only three males were collected throughout the sampling season (Figure 12).

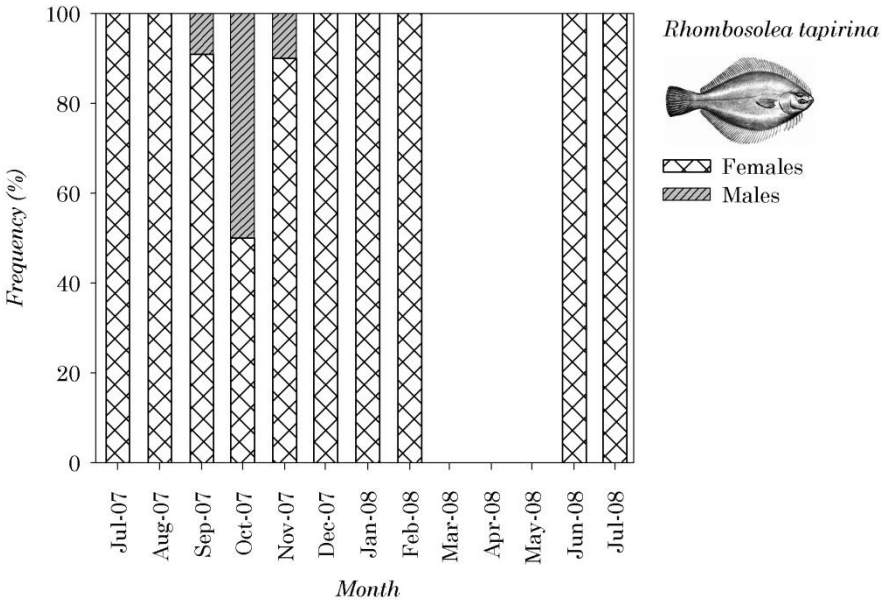


Figure 12. Temporal trends in sex ratios (frequency %) of *R .tapirina* in the Coorong from July 2007 to July 2008. (females n = 105, males n = 3).

GSI for female *R. tapirina* was highest in July 2007 with a subsequent decrease in GSI in the following months (Figure 13a). This is opposite to the peak in temperature suggesting that *R. tapirina* in the Murray Mouth and Coorong is a winter spawner (Figure 13a). Female GSI was lowest from November through to February (Figure 13a). GSI variation in males cannot be extrapolated to spawning season as only one individual was collected on three occasions (Figure 13b).

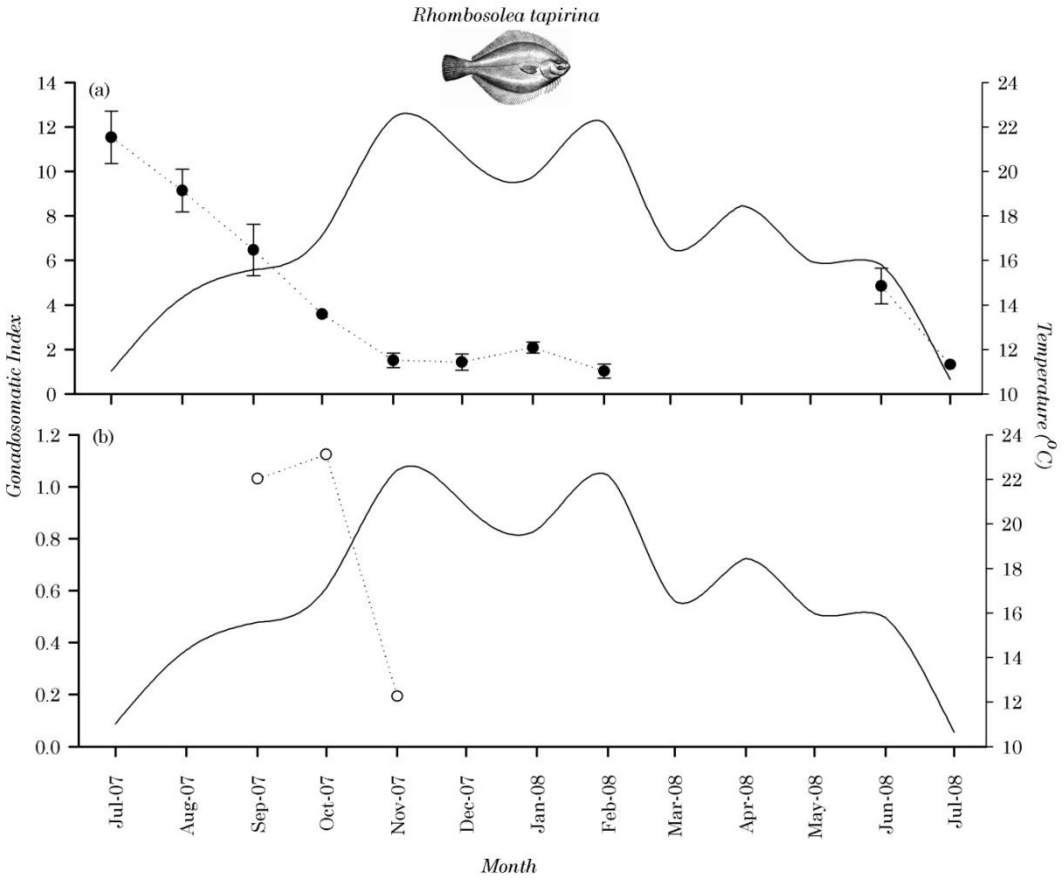


Figure 13. Temporal trends in gonadosomatic indices (± s.e.) (dotted line) for a) female (n= 105) and b) male (n = 3) *R. tapirina* in the Coorong from July 2007 to July 2008. Solid black line is mean monthly temperature.

Mature female (≥stage 3) *R. tapirina* were present throughout most of the season (Figure 14). Resting/developing (stage 2) and developed (stage 3) ovaries were recorded in July 2007. Temporal trends in macroscopic staging suggest that spawning condition was reached (stage 4) and that spawning occurred (stage 5) during August and September 2007 (Figure 14). Following this period, mature ovaries returned to a resting state (stage 3) and immature and developing (stage 1 and 2) ovaries were recorded from November 2007. Regressing (stage 5) ovaries were identified in January; unfortunately no samples were collected between March and May 2008 and fish appeared to be returning to spawning condition (stage 3 and 4) by June 2008 (Figure 14). These results are generally consistent with the temporal patterns of variation in female GSI (Figure 14).

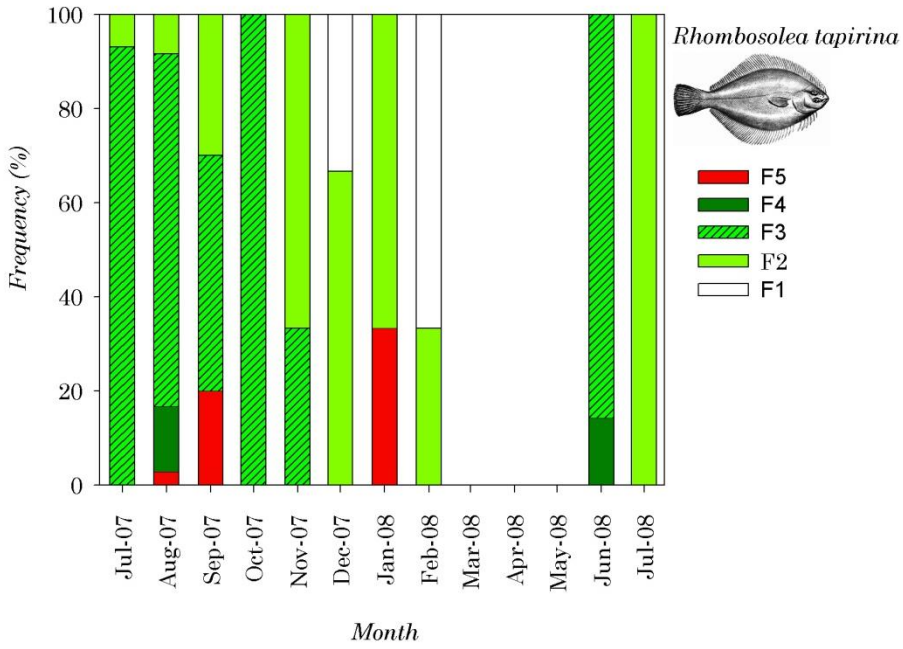


Figure 14. Temporal trends in macroscopic stages of gonad development (frequency %) for female *R. tapirina* in the Coorong from July 2007 to July 2008. (n = 105)

Size at first maturity

Half the female population of *R. tapirina* within the Coorong were sexually mature at ≥ 203 mm TL, whilst the smallest mature individual recorded was 211 mm TL (Table 9; Figure 15). Insufficient numbers of mature males were collected to develop a model to estimate size at 50% maturity. Parameters of the logistic maturity curves are provided in Table 9.

Table 9. Parameter estimates of the logistic curves of the size at first maturity of female *R. tapirina* from the Coorong. (TL=Total Length, SE=Standard Error, CL=Approximate 95% Confidence Limits, K = constant describing how rapidly fish mature, *p*= significance and N= # of individuals in sample).

TL (mm)	SE	95% CL	K	SE	95% CL	R-square	P	N
203	4.8318	191.5-213.8	0.1	0.0484	0.0183-0.2051	0.0545	<0.0001	219

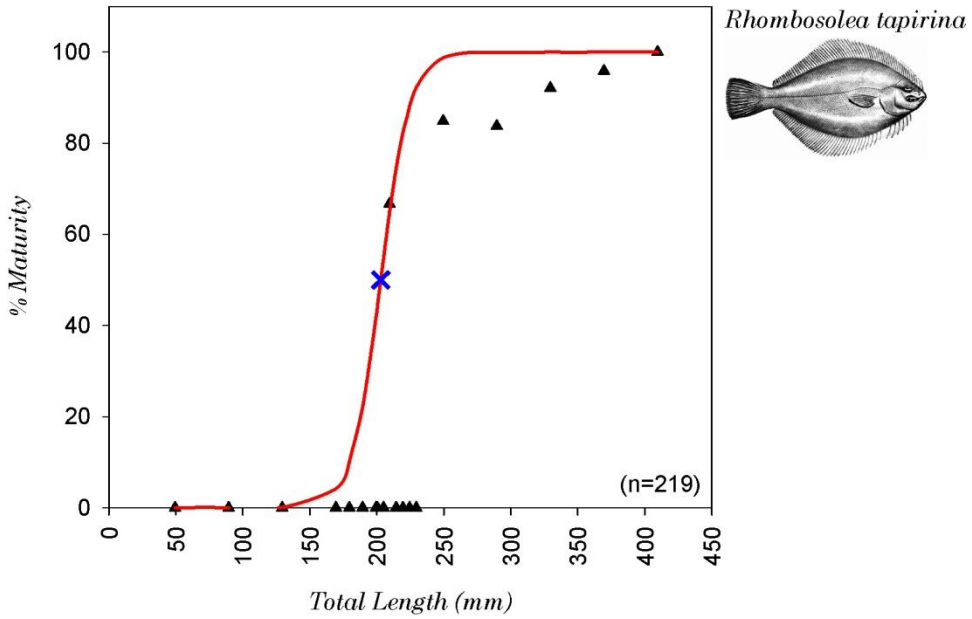


Figure 15. Size at reproductive maturity (% of population that is sexually mature) of female *R. tapirina* in the Coorong. Blue cross indicates size when 50% of the population is mature (females m=203 mm).

Microscopic analyses

Microscopic characteristics of ovaries throughout development

Stage 1 (F1-immature) in *R. tapirina*, was characterised as having only unyolked oocytes with no atresia present (Figure 16.).

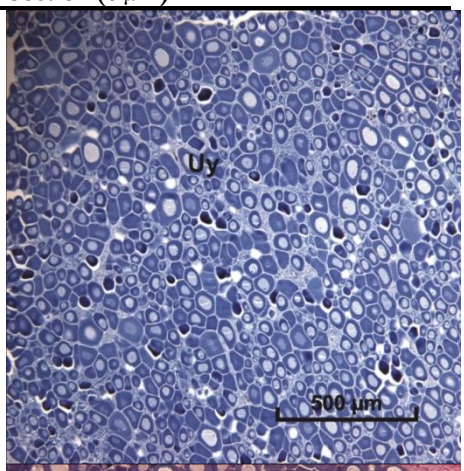
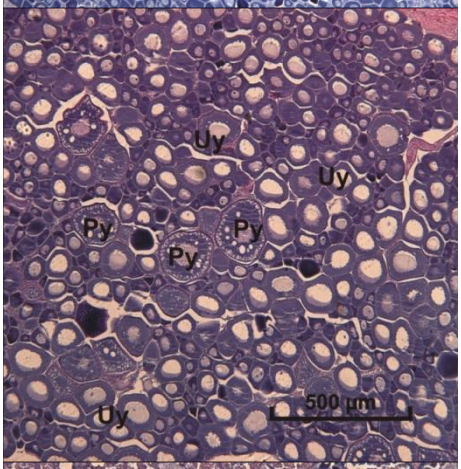
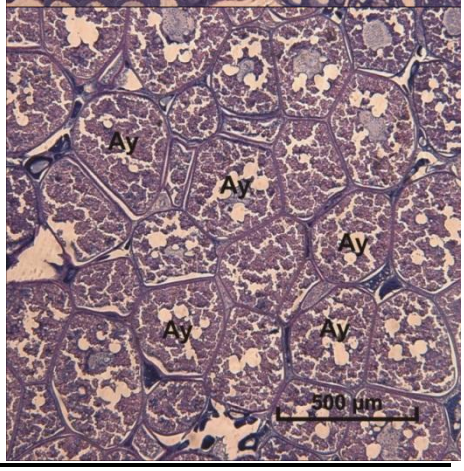
Stage 2 (F2-developing) showed signs of low levels of atresia, along with partially yolked oocytes. Unyolked oocytes were also present in this stage, however in lower proportions than that of stage 1 (Figure 16. b).

Stage 3 (F3-Ripe), predominately consisted of advanced yolked oocytes. Partially yolked and unyolked oocytes were also present as with low levels of atresia (Figure 16. c).

Stage 4 (F4-Hydrated), in this stage for *R. tapirina,* all oocyte development were present, with predominately advanced yolk and hydrated oocytes, indicating optimum spawning condition. Low levels of atresia were also present, as with some post-ovulatory follicles (Figure 16. d).

Stage 5 (F5-Spent), was characterised by an absence of hydrated oocytes and a large number of post-ovulatory follicles. Low levels of atresia were also present (Figure 16. e).

Stage 6 (F6-Regressing), although regressing ovaries were documented for *R. tapirina* in January 2008, no image was available for this particular study. However according to Fowler *et al.* (1999), it could be implied that this stage had high levels of atresia present, along with the absence of hydrated oocytes and post-ovulatory follicles (Figure 16. f).

Microscopic staging and histological characteristic	Section (6 µm)
a. F1- Immature Only unyolked oocytes present. No atresia.	
b. F2- Developing Partially yolked and unyolked oocytes present. Low levels of atresia.	
c. F3- Ripe Advanced yolked oocytes predominate. Partially yolked and unyolked oocytes also present. Low levels of atresia.	

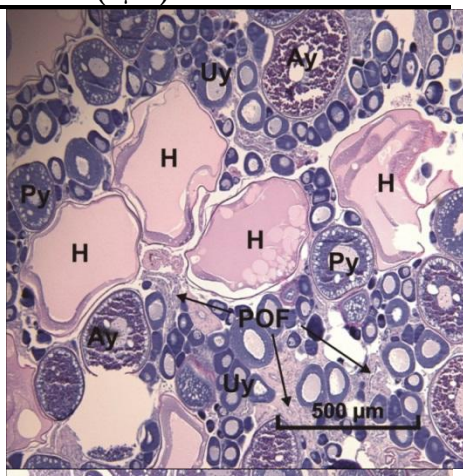
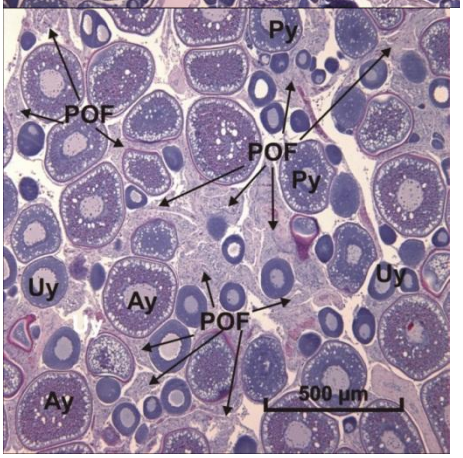
Microscopic staging and histological characteristic	Section (6 µm)
d. F4- Hydrated All stages of oocyte development present, however advanced yolk and hydrated oocytes predominate. Low levels of atresia. Some post-ovulatory follicles may be present.	
e. F5- Spent Characterised by an absence of hydrated oocytes and a large number of post-ovulatory follicles. Low levels of atresia.	
f. F6- Regressing High levels of atresia . Absence of hydrated oocytes and post-ovulatory follides (Implied from Fowler *et al.*(1999))	No image available.

Figure 16. a-f Descriptions and digital images of microscopic staging and histological characteristics (section = 1µm) of different ovary stages in *R. tapirina*. Uy = unyolked oocyte, Py = partially yolked oocyte, Ay = advanced yolked oocyte, H = hydrated oocyte, POF = post-ovulatory follicle.

Tamar goby (*Afurcagobius tamarensis*)

Macroscopic analyses

Sex ratios, timing and extent of spawning season

The catch of *A. tamarensis* from the Murray Mouth and Coorong was dominated by females, although sex ratios approximated 1:1 during December 2007 and April and May 2008 (Figure 17). Males were present throughout most of the season, although in low proportions (Figure 17).

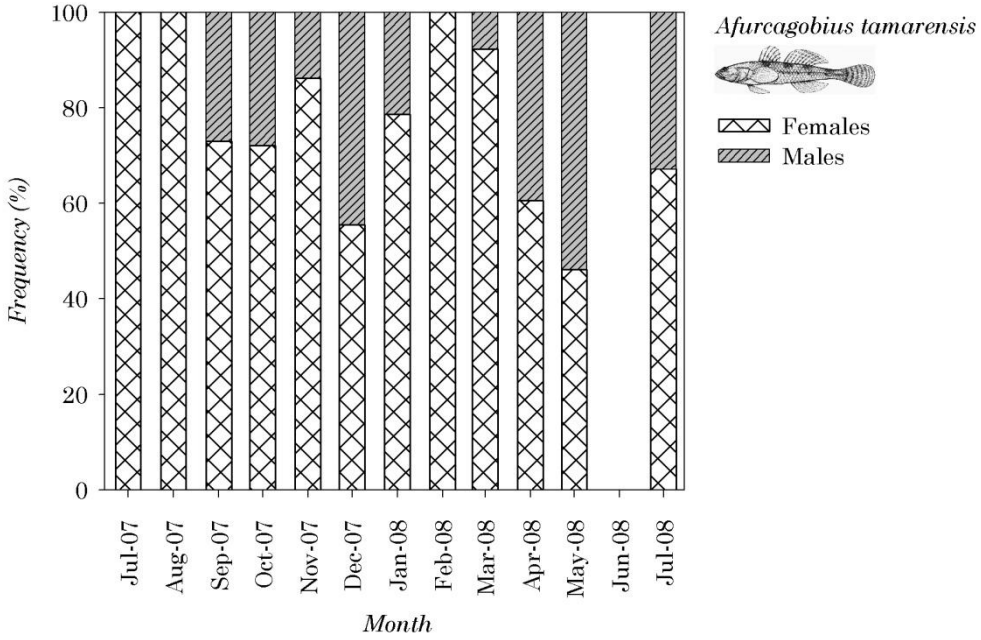


Figure 17. Temporal trends in sex ratios (frequency %) of *A. tamarensis* in the Coorong from July 2007 to July 2008. (females n = 420, males n = 137).

Female GSI for *A. tamarensis* appeared to be high between October and February, generally coinciding with the warmer temperatures (Figure 18a), suggesting a spring/summer spawning period. Female GSI was lowest during April and May 2008 (Figure 18a). Variation in male GSI followed a slightly different pattern to the females, peaking in November 2007 (Figure 18b).

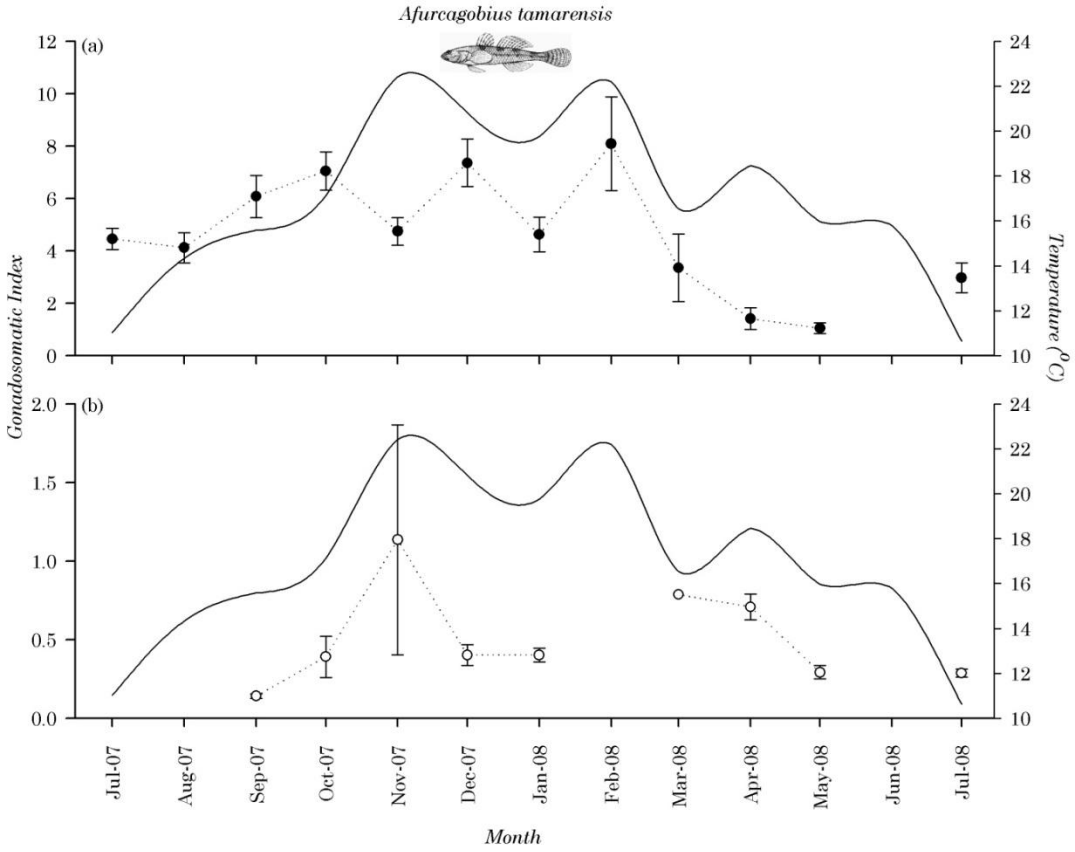


Figure 18. Temporal trends in gonadosomatic indices (± s.e.) (dotted line) for a) female (n = 420) and b) male (n = 137) *A. tamarensis* the Coorong from July 2007 to July 2008. Solid black line is mean monthly temperature.

Mature female (≥ stage 3) *A. tamarensis* were present throughout the season (Figure 19). Ripe females (stage 4) were recorded, although in low numbers from August 2007 to January 2008 suggesting a spring/summer spawning season (Figure 19). No regressing (stage 5) ovaries were recorded throughout the sampling period. Immature fish (stage 1) were recorded periodically between October 2007 and July 2008, and formed a large proportion of the females surveyed in April 2008 (Figure 19). The spawning period identified by ovarian development is generally consistent with that shown in female GSI patterns (Figure 19).

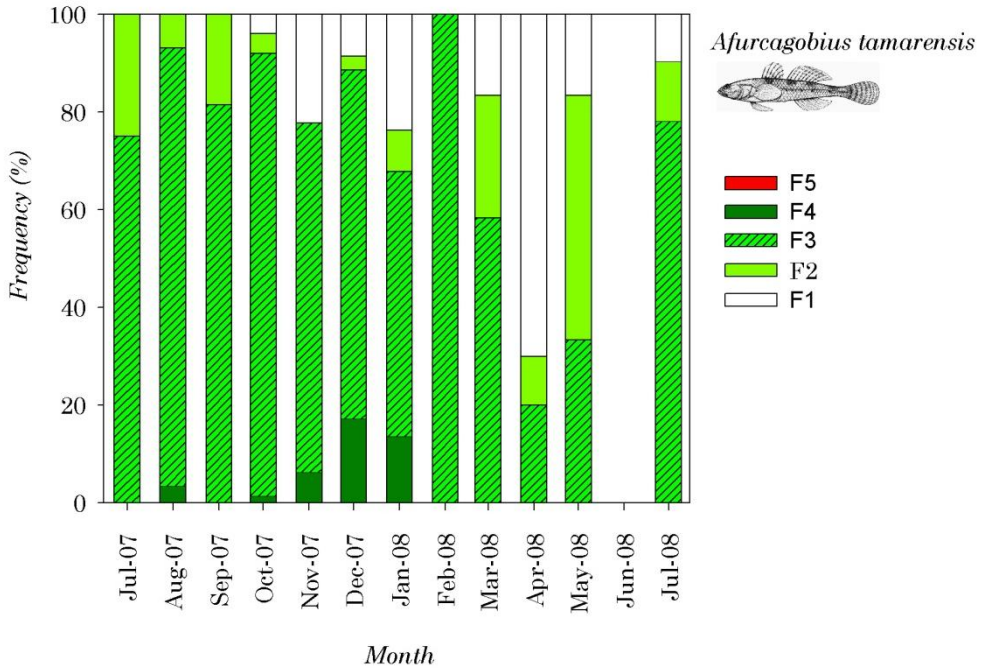


Figure 19. Temporal trends in macroscopic stages of gonad development (frequency %) for female *A. tamarensis* in the Coorong from July 2007 to July 2008. (n = 420).

Size at first maturity

Half the female population of *A. tamarensis* had reached sexual maturity within the Coorong at ≥ 53 mm TL, whilst the smallest mature female was 45 mm TL (Table 10; Figure 20). There were insufficient numbers of mature males collected to develop a model to estimate size at 50% maturity, however the smallest mature male collected (stage 3) was 65 mm TL. Parameters of the logistic maturity curves are provided in Table 10.

Table 10. Parameter estimates of the logistic curves of the size at first maturity of female *A. tamarensis* from the Coorong. (TL=Total Length, SE=Standard Error, CL=Approximate 95% Confidence Limits K = constant describing how rapidly fish mature, *p*= significance and N= # of individuals in sample).

TL (mm)	SE	95% CL	K	SE	95% CL	R-square	*p*	N
53	1.7365	49.1-56.6	0.15	0.0342	0.0739-0.2231	0.1787	<0.0001	348

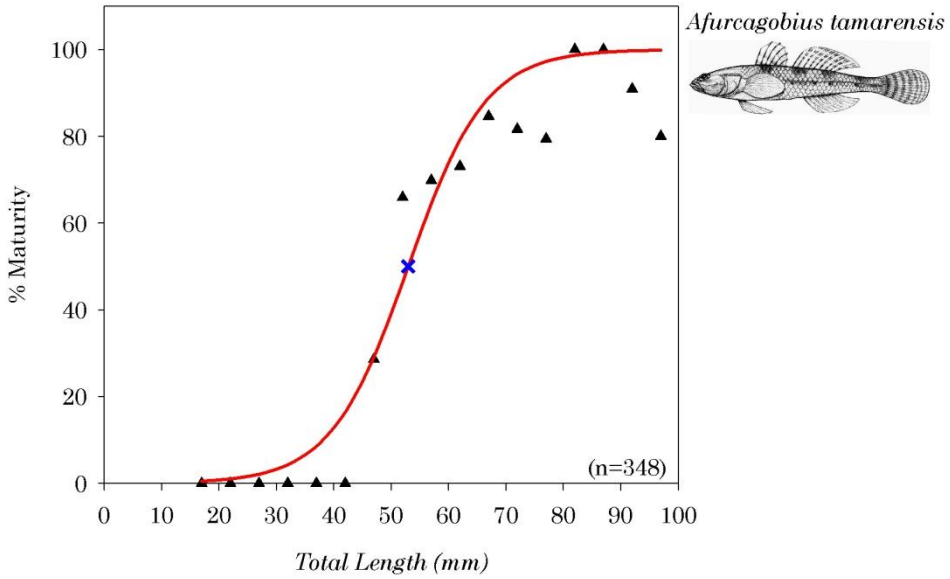


Figure 20. Size at reproductive maturity (% of population that is sexually mature) of female *A. tamarensis* in the Coorong. Blue cross indicates size when 50% of the population is mature (females m=53 mm).

Microscopic analyses

Microscopic characteristics of ovaries throughout development

Stage 1 (F1-immature) in *A. tamarensis*, was characterised as having only unyolked oocytes with no atresia present (Figure 21.).

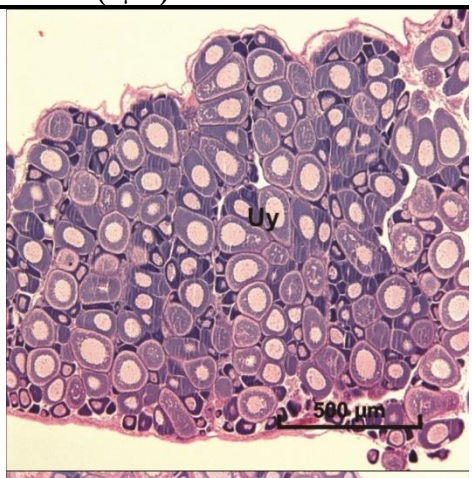
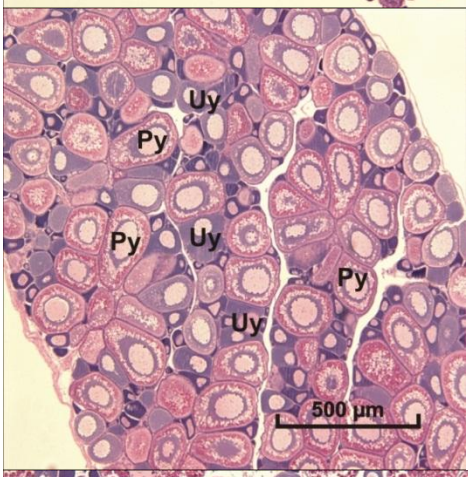
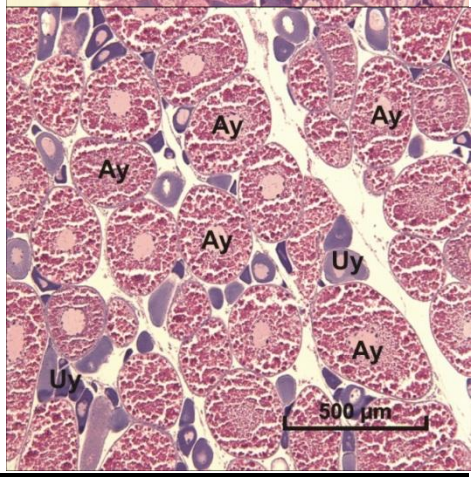
Stage 2 (F2-developing) showed signs of low levels of atresia, along with partially yolked oocytes. Unyolked oocytes were also present in this stage, however in lower proportions than that of stage 1 (Figure 21.b).

Stage 3 (F3-Ripe), predominately consisted of advanced yolked oocytes. Unyolked oocytes were also present as with low levels of atresia (Figure 21.c).

Stage 4 (F4-Hydrated), in this stage for *A. tamarensis,* all oocyte development was present; however, this predominately consisted of hydrated oocytes, indicating optimum spawning condition. Low levels of atresia were also present (Figure 21.d).

Stage 5 (F5-Spent), was characterised by an absence of hydrated oocytes and a large number of post-ovulatory follicles. Low levels of atresia were also present (Figure 21.e).

Stage 6 (F6-Regressing), regressing ovaries were not documented for *A. tamarensis* for the entire sampling period; therefore no image was available for this particular species. However according to Fowler *et al.* (1999), it could be implied that this stage had high levels of atresia present, along with the absence of hydrated oocytes and post-ovulatory follicles (Figure 21.f).

Microscopic staging and histological Characteristic	Section (6 µm)
a. F1- Immature Only unyolked oocytes present. No atresia.	
b. F2- Developing Partially yolked and unyolked oocytes present. Low levels of atresia.	
c. F3- Ripe Advanced yolked oocytes predominate. Partially yolked and unyolked oocytes also present. Low levels of atresia.	

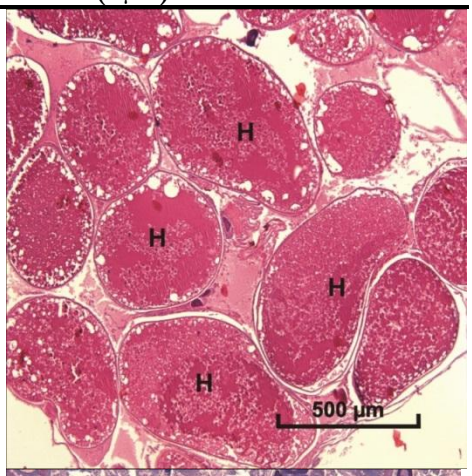
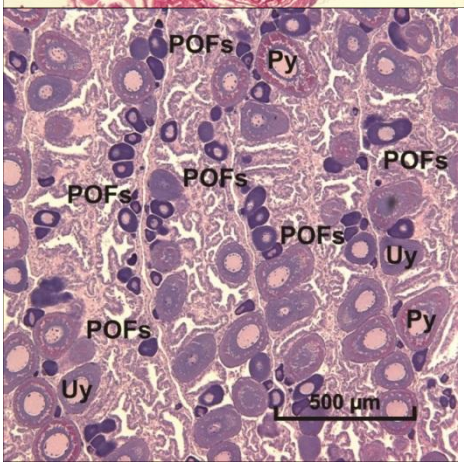
Microscopic staging and histological Characteristic	Section (6 µm)
d. F4- Hydrated All stages of oocyte development present, however advanced yolk and hydrated oocytes predominate. Low levels of atresia. Some post-ovulatory follicles may be present.	
e.F5- Spent Characterised by an absence of hydrated oocytes and a large number of post-ovulatory follicles. Low levels of atresia.	
f. F6- Regressing High levels of atresia . Absence of hydrated oocytes and post-ovulatory follides (Implied from Fowler *et al.*(1999))	No image available.

Figure 21. Descriptions and digital images of microscopic characteristics of different ovary stages in *A. tamarensis*. Uy = unyolked oocyte, Py = partially yolked oocyte, Ay = advanced yolked oocyte, H = hydrated oocyte, POF = post-ovulatory follicle.

Congolli (*Pseudaphritis urvillii*)

Macroscopic analyses

Sex ratios, timing and extent of spawning season

The catch of *P. urvillii* was dominated by females throughout the sampling season (Figure 22), with the highest percentage of males recorded in May 2008 (Figure 22).

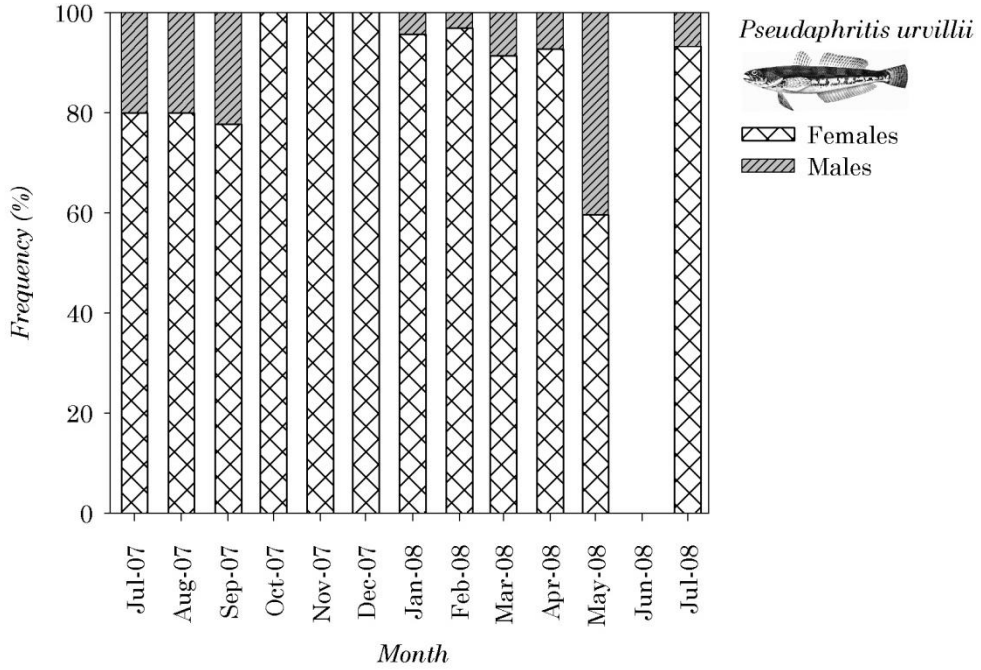


Figure 22. Temporal trends in sex ratios (frequency %) of *P. urvillii* in the Coorong from July 2007 to July 2008. (females n = 364, males n = 48).

The temporal variation in female GSI for *P. urvillii* indicated that GSI was highest from July to September 2007 (Figure 23a). Female GSI was low throughout the rest of the season under high temperatures during summer and appeared to rise again in May 2008 as water temperatures decreased (Figure 23a). Male GSI followed similar patterns to females, although due to smaller sample sizes and absence of males in some samples this should be interpreted cautiously (Figure 23b).

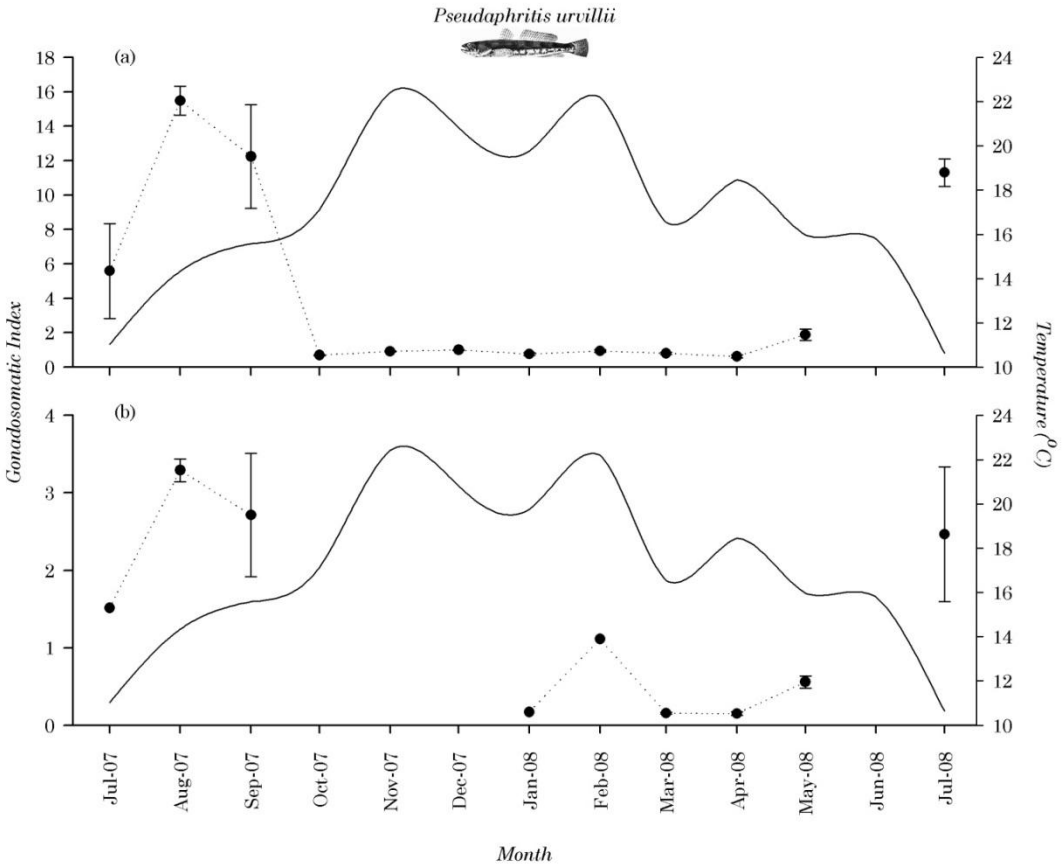


Figure 23. Temporal trends in gonadosomatic indices (± s.e.) (dotted line) for a) female (n = 364) and b) male (n = 48) *P. urvillii* in the Coorong from July 2007 to July 2008. Solid black line is mean monthly temperature.

Mature female (≥ stage 3) *P. urvillii* were recorded from July to September 2007, and April, June and July 2008 (Figure 24). From October 2007 to March 2008 only immature and developing ovaries were recorded. During July to September 2007 the majority of females collected had developed ovaries (Figure 24). However, no ripe (stage 4) or spent (stage 5) ovaries were documented using macroscopic staging (Figure 24). This pattern is consistent with the GSI for females and suggests an autumn/winter spawning period for *P. urvillii* in the Murray Mouth and Coorong.

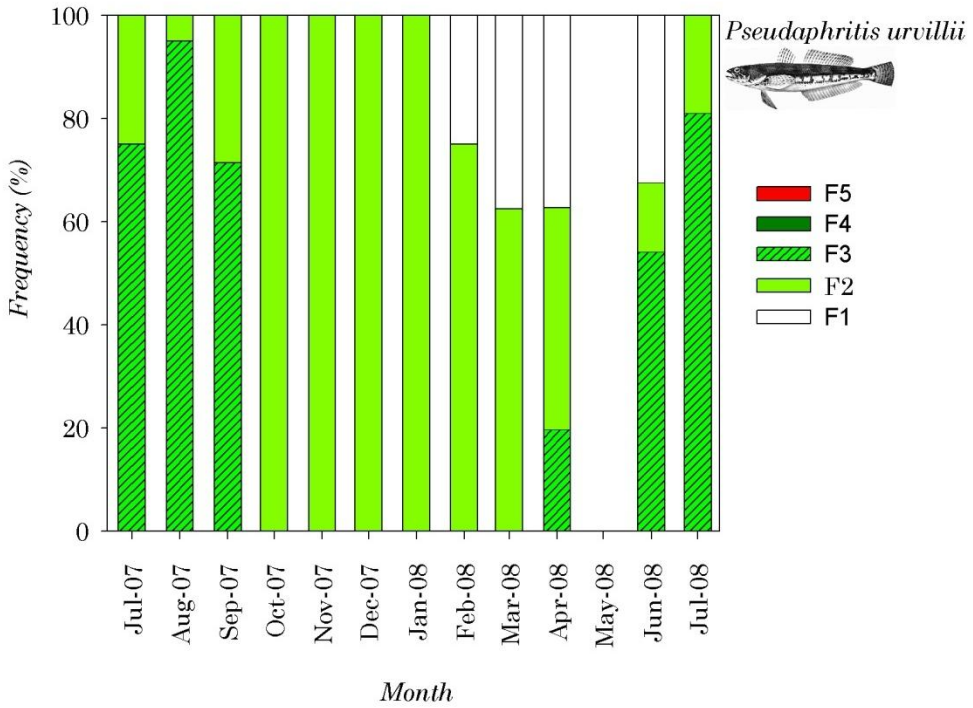


Figure 24. Temporal trends in macroscopic stages of gonad development (frequency %) for female *P. urvillii* in the Coorong from July 2007 to July 2008. (n = 364).

Size at first maturity

Within the Coorong half the sampled population of female *P. urvillii* were sexually mature at ≥ 165 mm TL, the same size of the smallest mature female collected (Table 11; Figure 25). There were insufficient numbers of mature males collected to develop a model to estimate size at 50% maturity, the smallest male collected was 103 mm. Parameters of the logistic maturity curves are provided in Table 11.

Table 11. Parameter estimates of the logistic curves of the size at first maturity of female *P. urvillii* from the Coorong. (TL=Total Length, SE=Standard Error, CL=Approximate 95% Confidence Limits, K = constant describing how rapidly fish mature, *p*= significance and N= # of individuals in sample).

TL (mm)	SE	95% CL	K	SE	95% CL	R-square	*p*	N
165	1.0866	162.2-166.9	0.13	0.0155	0.0929-0.1595	0.0233	<0.0001	86

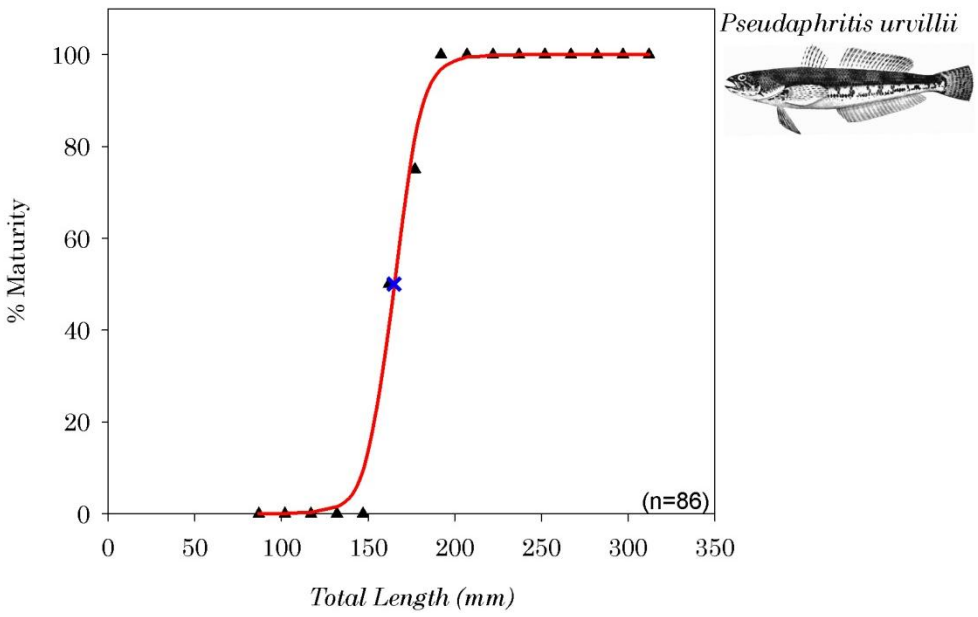


Figure 25. Size at reproductive maturity (% of population that is sexually mature) of female *P. urvillii* in the Coorong. Blue cross indicates size when 50% of the population is mature (females m=165 mm TL).

Microscopic analyses

Microscopic characteristics of ovaries throughout development

Stage 1 (F1-immature) in *P. urvillii* ovaries development, was characterised as having only unyolked oocytes with no atresia present (Figure 26.).

Stage 2 (F2-developing) showed signs of low levels of atresia, which were not present in the previous stage, along with partially yolked oocytes. Unyolked oocytes were also present (Figure 26.).

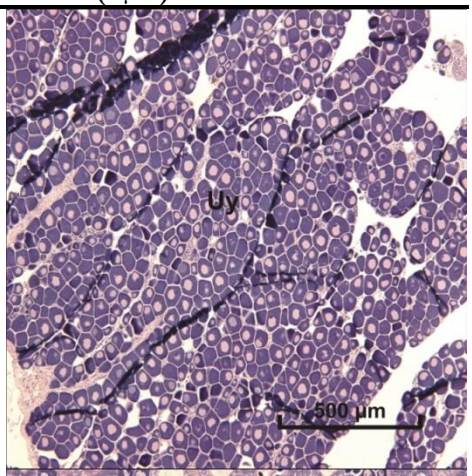
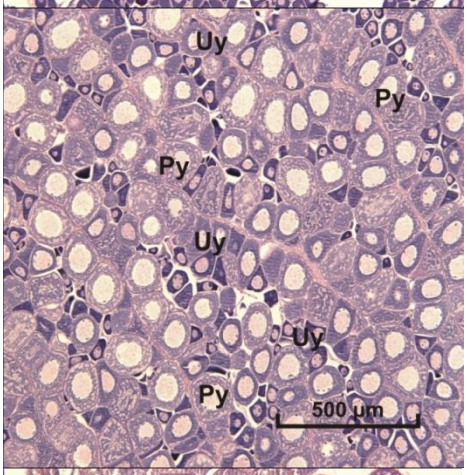
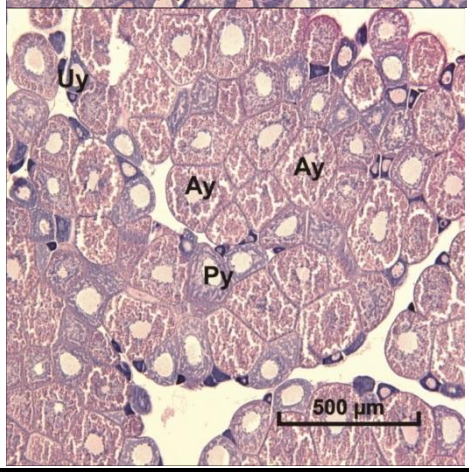
Stage 3 (F3-Ripe), predominately consisted of advanced yolked oocytes, indicating that spawning was imminent for *P. urvillii* at time of capture. Partially yolked and unyolked oocytes were also present as with low levels of atresia (Figure 26.).

Stage 4 (F4-Hydrated), in this stage, all oocyte development were present, with predominately advanced yolk and hydrated oocytes, indicating optimum spawning condition. Low levels of atresia were also present (Figure 26.).

Stage 5 (F5-Spent), no image was available for this stage, as no fish were recorded at this stage for the entire sample period, suggesting that an actual spawning event for this species may never of

occurred. However, according to Fowler *et al.* (1999), spent ovaries (stage 5) can be characterised by an absence of hydrated oocytes, large numbers of post-ovulatory follicles and low levels of atresia (Figure 26.).

Stage 6 (F6-Regressing), unlike all the previous stages, had high levels of atresia present. Also observed was the presence of advanced, partially and unyolked oocytes and the absence of hydrated oocytes and post-ovulatory follicles (Figure 26.).

Microscopic staging and histological characteristic	Section (6 µm)
a. F1- Immature Only unyolked oocytes present. No atresia.	
b. F2- Developing Partially yolked and unyolked oocytes present. Low levels of atresia.	
c. F3- Ripe Advanced yolked oocytes predominate. Partially yolked and unyolked oocytes also present. Low levels of atresia.	

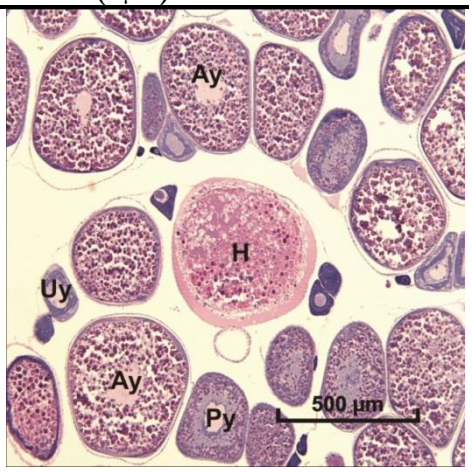
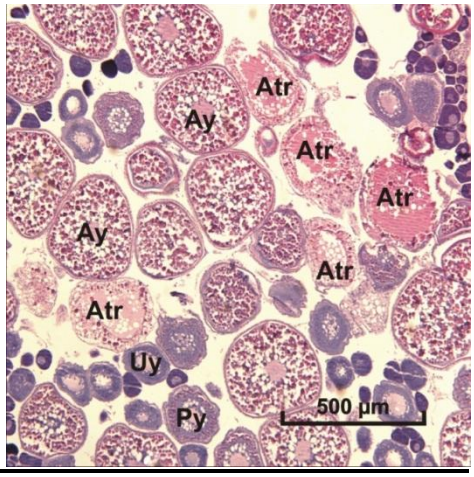
Microscopic staging and histological characteristic	Section (6 µm)
d. F4- Hydrated All stages of oocyte development present, however advanced yolk and hydrated oocytes predominate. Low levels of atresia. Some post-ovulatory follicles may be present.	
e. F5- Spent Characterised by an absence of hydrated oocytes and a large number of post-ovulatory follicles. Low levels of atresia. (Implied from Fowler *et al.* (1999))	No image available. None of the ovaries had POFs.
f. F6- Regressing High levels of atresia . Absence of hydrated oocytes and post-ovulatory follides	

Figure 26. Descriptions and digital images of microscopic characteristics of different ovary stages in *P. urvillii*. Uy = unyolked oocyte, Py = partially yolked oocyte, Ay = advanced yolked oocyte, H = hydrated oocyte, Atr = atretic oocyte.

4. DISCUSSION

Sex ratios

Differences in sex ratios are frequently a result of fishing pressure, growth rates, habitat preferences, environmental variability and/or movement. Sex ratios for each of the species varied throughout the sampling season; however, all of the species generally showed ratios heavily weighted towards female fish. Males dominated the catch in only two instances; *A. forsteri* during November 2007 and *A. tamarensis* in December 2007. In the current study the most likely factors explaining this result are sampling methodology and habitat segregation between the sexes, as there is no evidence that any of these species change sex.

Sex ratios in the commercially caught species, *A. butcheri*, *A. forsteri* and *R. tapirina* are likely to be influenced in part by the method of collection, whereby commercial methods may hold bias due to the sampling of spawning aggregations. The overall higher presence of females throughout the sampling period is likely biased towards the capture of the larger and more rapidly growing females (Harris 1968), given the mesh sizes of the gill nets and deeper habitats targeted for capture. Spawning aggregations of *A. butcheri* usually occur in the upper estuary near the interface between fresh and brackish waters (Kailola *et al.* 1993); both females and males congregate in single sexed schools for a continued spawning event (Hobday and Moran 1983). *R. tapirina* females are known to move into and congregate within deeper habitats prior to spawning, likely making them more susceptible to commercial netting practises (Kurth 1957; Crawford 1984; Ferguson 2007).

Another explanation for the sex ratio being dominated by females may be attributed to differences in behaviour, habitat preference and movement between the sexes. Male *A. forsteri* prefer shallow banks of estuaries and beaches, whilst females inhabit deeper habitats, such as channels and gutters (Higham *et al.* 2005). Movement into and out of spawning habitats may also account for shifts in sex ratios. Female *A. forsteri* could have moved out of the estuary after spawning, which according to the results of this assessment was between April and July 2007 and 2008 (winter). In February 2008, the male to female ratio was unitary. This suggests that males had moved into the estuary prior to spawning. There is limited information on the movement of *R. tapirina,* with conflicting theories. It has been suggested that *R. tapirina* are rarely found in marine waters adjacent to the Coorong, therefore they spend their entire life cycle within the Coorong lagoons (Hall 1984), yet Crawford (1984) suggests *R. tapirina* spawn in offshore habitats to allow for transportation of larvae to shallow, sandy, un-vegetated habitats. Male *A. tamarensis* build nests or burrows and perform a 'hopping'

courtship display to entice females (Lintermans 2007). This suggests that male *A. tamarensis* are less mobile than females and not as vulnerable to scientific sampling methods. Habitat segregation by sex may have played a factor in the one-sided sex ratio of female *P. urvillii* (Jennings *et al.* 2008). Female *P. urvillii* utilise fresh, estuarine and marine habitats within their life cycle, and therefore display a tendency for large scale movement (Hortle 1978; Crook *et al.* 2010). Males, however, are non-diadromous and more likely to be estuarine or marine residents which occasionally enter the lower freshwater reaches of rivers and streams (Hortle 1978; Crook *et al.* 2010). However, an increase in male presence was noted in May 2008 which could be attributed to the timing of spawning. Male *P. urvillii* may have begun to move and aggregate for spawning purposes, causing the sex ratio to equilibrate.

Size at first maturity

Size at first maturity represents a transitional point in the life of an individual where allocation of time and resources shifts from growth and survival to include reproduction (Wootton 1998). Size at first maturity varies between and within species, influenced by environmental change or external stressors (Stearns and Crandall 1984; Wootton 1998). Species that exhibit variations in size at maturity as a result of external pressures are said to have plasticity, this is most commonly exhibited by a shift to an earlier maturation in faster growing fish, though this is not always the case (Stearns and Crandall 1984). Three prominent patterns of decreased growth have been reported in the literature, both within and between populations, 1) delayed maturity at a smaller size, 2) delayed maturity at the same size, and 3) delayed maturity at a larger size (Stearns and Crandall 1984).

Female *A. butcheri* matured more rapidly when compared to males. When compared to different populations of *A. butcheri* across Australia, the population within the Coorong, during a period of severe drought, seemed to reach maturity at a size greater than those found in populations in southern Victorian and Western Australian estuaries. Both female and male bream in the Gippsland lake area reached sexual maturity at ≥ 216 mm (Norriss *et al.* 2002) and in the Swan River in W.A., sexual maturity was reached at ≥ 156 mm for females and 169 mm for males (Sarre 1999; Sarre and Potter 2000). This suggests that during periods of drought with no freshwater flushes or flows, sexual maturity in relation to size may slow, potentially due to factors such as the availability of food resources and salinity gradients optimal in the stages of reproductive development. In addition, potential differences may exist between populations from different geographical regions.

The results for *A. forsteri* in this study concur with results from Harris' (1968) Coorong study; female *A. forsteri* were found to be sexually mature at ≥ 230 mm TL, while males were found to sexually mature at ≥ 220 mm TL. This indicates that *A. forsteri's* reproductive development was not greatly affected by the drought conditions. This is most likely a result of being a marine species, more so than an estuarine species; that is, being less reliant on freshwater inflows or fluctuating salinities for optimum reproductive development.

This is the first study to determine size at maturity for the Coorong population of *R. tapirina*. Size at first maturity has been determined for *R. tapirina* populations in Tasmania where Crawford (1984) found that female *R. tapirina* reached sexual maturity at ≥ 219 mm TL whilst males were smaller at ≥190 mm TL. This indicates that *R. tapirina* in the Coorong (females only) reach maturity relatively faster than that of Tasmanian populations. This is most probably due to more optimum water temperatures involved in reproductive development.

Also, this is the first study to determine size at maturity for *A. tamarensis* and *P. urvillii,* within Australia. Thus the results indicating that female *A. tamarensis* reach sexual maturity at ≥ 53 mm TL and female *P. urvillii* reach sexual maturity at ≥ 165 mm TL can be used as a baseline during drought conditions for future reference. Results indicate that male *A. tamarensis* are likely to take a longer time to mature than their female counterparts. Contrastingly, the results for *P. urvillii* indicate that males would mature more rapidly than females. However, in both cases the lack of male fish available to the study limited the resolution and accuracy of the estimates of size at 50% maturity for males.

Timing and duration of the spawning season

The timing of the spawning period and the main spawning event is an important aspect of fish ecology, as it aims to place eggs, larvae and juveniles into the system under the optimal conditions for growth and survival (Bye 1984; Jobling 1995; Yaron and Sivan 2006). In temperate systems, spawning time is often correlated with day length, temperature and food availability. Seasonal timing of spawning within a year is likely to be driven by these factors, however, long term (preferably greater than 5 years) studies are required before environmental correlates for spawning activity can be accurately identified.

Using GSI, macroscopic staging and microscopic staging, this study has identified the spawning seasons for the five selected species in the Coorong under drought conditions. Spring and spring/summer spawners included *A. butcheri* and *A. tamarensis,* respectively. A previous investigation

into the spawning season of *A. butcheri* in the Coroong suggested a summer spawning season (Hall 1984). However, considerable variation in spawning times between different estuaries has been documented (Newton 1996; Haddy and Pankhurst 1998; Sarre and Potter 2000; Norriss *et al.* 2002). The spring period identified for *A. butcheri* spawning is not localised to South Australian estuaries (Harbison 1974) but has also been observed in Victorian estuaries such as the Glenelg River and Gippsland Lakes (Coutin *et al.* 1997; Norriss *et al.* 2002). *Acanthopagrus butcheri* has been documented to move downstream with freshwater flushes and then move upstream during summer months in order to find the appropriate salinity, dissolved oxygen content and habitat suitable for spawning (Norriss *et al.* 2002). For *A. tamarensis*, this study is the first to comprehensively assess the spawning season in the Coorong; however, the spawning season identified is consistent with results from Victorian estuaries where they have been seen to spawn from September to November (Cadwallader and Backhouse 1983). Spring and summer spawners are likely to utilise a time characterised by warmer temperatures, ensuring faster growth and more abundant source of food for developing larvae (Wootton 1998).

A. forsteri was identified as a summer/autumn/winter spawner, whereas *P. urvillii* and *R. tapirina* were found to be winter spawners. Timing and spawning seasons can vary greatly depending on location and stock structure. For example, there are two distinct populations of *A. forsteri,* i.e. eastern and western populations, whereby eastern populations spawn in late summer to autumn and western populations spawn in winter (Thomson 1957; Chubb *et al.* 1981; Lenanton 1982). A previous study suggested that the Coorong population was derived from both the eastern and western populations, with juveniles collected throughout the year in the Coorong (Pellizzari 2001). This supports the results of our study. Further, variation in spawning period could also be attributed to regional differences in estuarine habitat (Thomson 1957). For example, in Western Australia there is a marked tendency for sandbars to form at the entrances to estuaries during summer that effectively prevents *A. forsteri* in spawning condition from moving into coastal waters and estuaries for spawning (Chubbs *et al.* 1981). The spawning season of *R. tapirina* in Tasmania, based on gonadosomatic indices, occurred from June to October (Kurth 1957; Crawford 1984; Ferguson 2007). Due to the absence of ripe (stage 4) and spent (stage 5) ovaries in female *P. urvillii*, an actual spawning event may not have occurred within the sampling period inside the Coorong. However, the spawning season identified in this study is comparable to populations in eastern and western Victoria. Crook *et al.* (1999) identified that females started their spawning migration and were in optimum spawning condition in the cooler months between May and July. Female *P. urvillii* have

been documented to migrate downstream with the onset of freshwater flows, which historically would have occurred towards the end of winter. Both *A. forsteri* and *P. urvillii* are believed to spawn in the marine environment during late-autumn or winter, with developed larvae and juveniles then migrating back into the estuaries during spring (Jenkins 1986; Crook *et al.* 2010). This strategy places well developed larvae and juveniles into the estuaries during spring, allowing them to competitively take advantage of the warmer temperatures and abundant food resources.

Comparison of macroscopic and microscopic analyses

This report provides the first detailed assessment of microscopic staging for all five commercially targeted fish species within the Coorong. Results for all species demonstrated that the developmental stages observed in oocytes were very similar to those described for most other teleosts (Ye *et al.* 2002). Based on the classification and terminology used by Fowler *et al.* (1999), six stages and seven characteristics of oocyte development were identified through microscopic staging. When compared with macroscopic staging (5 stages), microscopic staging of female ovary development is more accurate and precise in determining reproductive development and type of spawning for each species, as all stages of oocyte development can be viewed instead of just advanced oocytes being viewed in macroscopic staging (Ye *et al.* 2002). In the current study, microscopic staging could differentiate between spent (stage 5) and regressing (stage 6) ovaries, through the presence or absence of atresia and post-ovulatory follicles (POFs), which cannot be observed macroscopically. However, although micro- and macroscopic stages were based on different characteristics and criteria, they were still roughly aligned for the purposes of this study.

Spawning modes

To maximise resources and suitable conditions, fish can also have discrete (short targeted period aimed at suitable conditions) or protracted (longer period, aimed at increasing the chance of encountering suitable conditions) spawning periods and single or multiple spawning events. Overall, the results indicated that all five species displayed asynchronous oocyte development which suggests that each species releases multiple batches of oocytes during their respective reproductive season. In each case, this was most evident during the latter stages of ovary development, whereby ovaries contained oocytes of all stages of development, including post-ovulatory follicles. Fish with asynchronous oocyte development are also referred to as 'serial' or 'multiple' batch spawners, as only a fraction of the complement of yolked oocytes are released (spawned) at any one time, while the

remaining oocytes continue to develop for spawning at a later stage (West 1990). Multiple batch spawning species typically have a protracted spawning season, in which batches of oocytes are released on multiple occasions throughout this period.

Previous studies on each of these species generally corroborate the findings of this study, with the exception of Harris (1968), which suggested that *A. forsteri* is a singular spawner within the Coorong lagoon region. However, this conclusion was drawn based on macroscopic analyses only, making it impossible to discern temporal variation in oocyte development (Harris 1968). Nevertheless, Newton (1996) also concluded that *A. tamarensis* is a serial spawner, as spawning was observed over a protracted period of more than five months. Similarly, *A. butcheri* are known to be multiple spawners in the Coorong (Cashmore *et al.* 2000) and *R. tapirina* are known to be batch spawners over a prolonged reproductive season (Kurth 1957; Crawford 1984), i.e. females having asynchronous oocyte development with the capacity for multiple ovulations within a reproductive season (Barnett and Pankhurst 1999). For *P. urvillii*, oocyte development was also classified as asynchronous. However, due to the lack of stage 5 (spent) fish and lack of POFs in histological sections in this study; it is possible that spawning for *P. urvillii* may not have occurred during this period of drought in the Coorong and Lower Lakes region. However, it has been suggested that female *P. urvillii* spawn in the marine environment, in which female ovaries containing POFs are unlikely to be present in fish collected in the study region (Hortle 1978; Crook *et al.* 2010).

Impacts of low flow conditions

Previous studies on Coorong spawning of *A. butcheri* suggested a summer spawning season (Hall 1984), rather than a spring spawning season as identified in this study. Summer spawning may have resulted following periods of high spring rainfalls whereby *A. butcheri* move downstream with freshwater flushes and then move upstream during summer months to find appropriate salinity, dissolved oxygen levels and habitat suitable for spawning (Norriss *et al.* 2002). However, in periods of drought fish may have been cued to spawn by increasing water temperatures and minor freshwater releases during the winter months, causing fish to spawn during spring.

Aldrichetta forsteri are known to move into freshwater on rising tides (Thomson 1996), however they are still predominately a marine estuarine-opportunistic species which can complete their entire life cycle in a marine environment (Potter and Hyndes 1994). This suggests that whilst freshwater flows would not hold much significance in terms of cueing a spawning event, they may be important in maintaining the quality and availability of spawning habitat. Access to suitable habitat will influence

the timing for spawning, for example in the western population of *A. forsteri,* during summer (period of little to no flow) there is a tendency for estuaries to close over by the formation of sandbars at the mouth which effectively prevents the movement of fish in spawning condition into the estuary (Chubb *et al.* 1981). Subsequently the opening of estuaries by heavy freshwater discharge during winter, permits spawning and supports the movement of juveniles into the protected estuaries for their first year of life. Although the Coorong and Lower Lakes area experienced severe drought during this study, and hence no heavy freshwater discharges, the Murray Mouth was constantly dredged to allow fish passage and tidal flushes to prevent the region becoming completely hypersaline.

Salinity levels also play a key role in the reproductive biology of *R. tapirina*, with optimum fertilisation rates occurring at salinities of 35-45 ppt (Hart and Purser 1995). Eggs of *R. tapirina* are said to be positively buoyant at salinities above 28 ppt and after fertilisation were tolerant of a wide range of salinities (15-45 ppt) (Hart and Purser 1995). Although adult *R. tapirina* are said to be tolerant to a wide range of salinities (Kailola *et al.* 1993), specific threshold values are still unknown. If low to no freshwater discharges continue for the Coorong and tidal flows decrease due to sand build up at the Murray Mouth, salinity levels may increase to a point where spawning and fertilisation of eggs for *R. tapirina* may be negatively affected. Further research will need to be undertaken to support this claim and to close the knowledge gap associated with the movement, reproduction and early life stages of *R. tapirina* in the Coorong.

Increased GSI and developed gonads were also associated with low water temperatures for some species, i.e. *P. urvillii* which is a winter spawner. However, there was no direct evidence of recent spawning activity for *P. urvillii*, suggesting that an actual spawning event may not have occurred within the sampling period and hence during drought conditions. As such, there is still some uncertainty in regards to the spawning mode of *P. urvillii* and whether it varies in response to environmental conditions. If spawning did not occur during the sample period, it could be directly attributed to the loss of fish passage from freshwater to estuarine habitats in the region, whereby females are unable to move into the Coorong lagoon area to disperse their eggs for fertilisation. Consequently, this may result in recruitment failure for this species (Zampatti *et al.* 2010) and if drought conditions persist it could potentially contribute to further declines in the abundance and distribution of this species (Zampatti *et al.* 2010).

In summary the low flow conditions in the Coorong may have direct impacts on the reproductive biology of the key species present. This may require interventions to ensure that the Murray Mouth

stays open, ensuring the salinity in the Coorong remains within a reasonable range for survival of eggs and larvae and that the passage between fresh and salt waters remains accessible.

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