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Delayed timing of successful spawning of an estuarine dependent fish, black bream

Acanthopagrus butcheri

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ABSTRACT

In this paper, we investigate the period of successful spawning for black bream *Acanthopagrus butcheri*, an obligate estuarine species in southern Australia that typically spawn in spring and early summer. However, back-calculated spawning dates of juveniles sampled in Gippsland Lakes, Victoria from February to May 2016 indicated that spawning was concentrated over a short period in the Austral mid-summer (January), with a second spawning in late summer and early autumn (late February–early March). Ichthyoplankton sampling in the tributary estuaries from October to early December collected substantial numbers of fish larvae, dominated by gobiids, eleotrids and retropinnids of freshwater origin, but no *A. butcheri*. The lack of *A. butcheri* larvae was consistent with the delayed successful spawning indicated by juvenile otolith data. Freshwater flows declined from late winter to summer, with consistent salinity stratification of the water column. Dissolved oxygen (DO) concentrations were generally very low below the halocline. These conditions may have delayed the upstream spawning migration of adults or may have been unsuitable for survival of eggs and newly-hatched larvae. Longer-term predictions for climate change in southern Victoria, including the Gippsland Lakes region, are for lower winter–spring freshwater flows, potentially benefiting the reproductive success of *A. butcheri* through

high water-column stratification, but only if DO concentrations are not compromised by a lack of high winter flows needed to flush low DO water from the system.

KEYWORDS

climate change, Gippsland Lakes, larval fish, otolith daily increments, successful spawning, water quality

1 | INTRODUCTION

The addition of new recruits to a fish population depends on the suitability of environmental conditions for both adult spawning and the survival of eggs and larvae (Sissenwine, 1984; Secor & Houde, 1995; Houde, 2008). Estuaries are important areas for fish production (Blaber & Blaber, 1980; Beck *et al.*, 2001), but estuarine environments around the world are becoming increasingly degraded (Cottingham *et al.*, 2014). Environmental conditions in estuaries are highly dynamic due to the interaction of fresh and marine waters (Kurup *et al.*, 1998; Roy *et al.*, 2001). This interaction determines water quality (salinity, dissolved oxygen, turbidity *etc.*), as well as primary and secondary productivity, that may influence spawning behaviour (Hindell *et al.*, 2008) as well as survival of eggs and larvae (North & Houde, 2001; 2003; Williams *et al.*, 2013). Thus, variability in factors such as freshwater flows and marine incursions can strongly influence the period of successful spawning of fish in estuaries and is reflected by the distribution of birth dates of juveniles recruiting to the population (Sakabe *et al.*, 2011).

The black bream *Acanthopagrus butcheri* (Munro 1949) is an iconic estuarine-dependent fish species in southern Australia that has important commercial and recreational fishing value. *Acanthopagrus butcheri* has a protracted spawning period with asynchronous gonad development and spawns daily (Haddy & Pankhurst, 1998). The spawning period for *A. butcheri* in southern Australia generally occurs in spring and early summer (Butcher, 1945; Haddy & Pankhurst, 1998; Sarre & Potter, 1999). For example, eggs and larvae of *A. butcheri* from

tributary rivers entering Gippsland Lakes, eastern Victoria, sampled over 2 years were present from September to the beginning of December (Williams *et al.*, 2012).

There is evidence for variability in the timing of successful spawning of *A. butcheri* within a season. Based on adult reproductive indices, Walker and Neira (2001) found peak reproductive activity of *A. butcheri* in Gippsland Lakes in spring across multiple years, but in one season there was a secondary smaller reproductive peak in late summer. Nicholson *et al.* (2008) recorded *A. butcheri* eggs and yolk-sac larvae in the Glenelg and Hopkins Estuaries of western Victoria from September to December but mostly in October–November. However, Newton (1996) did not record *A. butcheri* eggs and larvae in the Hopkins Estuary until November, when concentrations peaked, but eggs and larvae continued to be recorded in lower concentrations until February. Nicholson *et al.* (2008) suggested that this difference in the timing of spawning may have been related to higher freshwater flows in the early spring in the Newton (1996) study, delaying the development of suitable salinity conditions for spawning. Similarly, using daily increments in otoliths to back-calculate spawning dates, Sakabe *et al.* (2011) found that spawning began in October in a year with a relatively dry spring, but was delayed until December in the following year after a wet spring.

The environmental characteristics of estuaries are undergoing long-term change. For example, climate change predictions for southern Australia are for reduced cool-season (winter and spring) rainfall (Chiew *et al.*, 2011; Hobday and Lough, 2011; CSIRO & Bureau of Meteorology, 2015). This change will affect freshwater flows and estuarine water quality,

including salinity structure, dissolved oxygen concentrations, turbidity and nutrient delivery (Alber, 2002), with potential consequences for estuarine fish (Gillanders *et al.*, 2011). Climate change effects may be exacerbated by human activities such as water abstraction, diversion and flow alteration (Kimmerer, 2002; Scavia *et al.*, 2002; Kingsford *et al.*, 2011), as well as artificial opening and closing of estuary entrances (Griffiths, 1999). These changes may lead to the long-term degradation of estuaries, for example, reduced flows together with increased nutrient concentrations can lead to increasing areas of anoxia (Valesini *et al.*, 2017; Cottingham *et al.*, 2018).

In this paper, we investigate the period of successful spawning for *A. butcheri* in Gippsland Lakes based on back-calculated spawning dates determined from daily otolith increments in juveniles. We hypothesize that the period of successful spawning is influenced by the environmental conditions in the estuarine system, particularly in relation to freshwater flows, salinity stratification and water quality. Plankton sampling data on fish larvae from the same spawning season is used to compare whether larval *A. butcheri* were present with the successful spawning period determined from juvenile otoliths.

2 | MATERIALS AND METHODS

2.1 | Study area

The present study was undertaken in the Gippsland Lakes, south-eastern Australia (Figure 1). The Gippsland Lakes are Australia's most extensive lagoon–estuarine system with an area of 600 km². The Gippsland Lakes system has a low tidal range (< 30 cm) and is connected to the open ocean by an artificial channel that was cut across the beach at Lakes Entrance in 1889 to stabilize the water level, create a harbour for fishing boats and open the lakes to shipping (Figure 1). The main stem of the Gippsland Lakes extends approximately 70 km, forming the largest navigable network of inland waterways in Australia.

This study focussed on the central basin of Lake King which is the entry point for three major tributaries, the Rivers Mitchell, Nicholson and Tambo (Figure 1). Lake Victoria and Lake King tend to be stratified with increasing salinity towards Lakes Entrance (Poore, 1982; Jenkins *et al.*, 2010) (Figure 1). Water temperatures in the Lakes reach a maximum of ~ 22°C in January and decline to a minimum of ~ 10°C in July (Webster *et al.*, 2001).

2.2 | Sampling methods

2.2.1 | Larval sampling

Plankton sampling for larval fish was conducted *c.* fortnightly from early October to early December 2015 at six sites in both the Mitchell and Tambo Rivers and at eight sites in the Nicholson River (Figure 1). The sampling method is described by Williams *et al.* (2012) but

briefly, at each site a 75 cm opening, conical plankton net with 333 μm mesh was towed obliquely between the surface and 1 m from the bottom at a speed of *c.* 1.5 km h^{-1} for 10 min. A General Oceanics 2030 flow meter (www.generaloceanics.com) was fitted to the entrance of the net to calculate volume of water sampled. Average volume of water sampled per tow was *c.* 150 m^3 . Samples were washed through a 250 μm mesh sieve and preserved in absolute ethanol.

A Hydrolab DS5X water quality multi-meter (OTT-Hydromet; www.ott.com) was used to measure depth (m), salinity, temperature ($^{\circ}\text{C}$) and dissolved oxygen (mg l^{-1}) profiles at each site.

2.2.2 | Juvenile sampling

Juveniles (young-of-the-year) were sampled at four sites (Figure 1) using a small seine in ~each month from February to May. The seine net was 15 m long and 2 m deep with a 2 mm knotless mesh. The seine was walked out in a broad arc and hauled into a bin by two operators using a pursing technique (Jenkins & Sutherland, 1997). Sampling depth ranged from 0.3–1.5 m. Seining was conducted during daylight hours and net hauls were distributed to sample vegetated habitat and bare sediment. Fish were identified to species, counted and measured (total length; L_T). Fish were placed on ice and then transported to the laboratory where they were frozen (-20°C) for later analysis.

2.3 | Laboratory methods

Fish larvae were sorted from plankton samples under a dissecting microscope. Three large volume samples were split to $\frac{1}{2}$, $\frac{1}{8}$ and $\frac{1}{16}$, respectively using a Folsom plankton splitter. Pelagic fish eggs were present in some samples, however, identification of *A. butcheri* eggs was not possible due to the effects of ethanol preservation on egg shape and opacity.

Juvenile *A. butcheri* were measured (L_T , mm). Sagittal otoliths were then removed using electrolytically-sharpened tungsten needles under a dissecting microscope with a polarising light source. They were embedded in crystal bond on glass microscope slides and measured using a compound microscope under a magnification of x50 with attached video camera. Leica Application Suite image software (www.leica-microsystems.com) was used to measure the length and width of the otolith.

Transverse cross-sections of otoliths were prepared. Otoliths were positioned with the proximal surface facing up, in crystal bond, on the edge of a microscope slide. This was done under x100 magnification, ensuring the dorsal and ventral sides of the otolith were parallel to the long edge of the slide and perpendicular to the short edges. The post-rostrum (posterior) end of the otolith was polished close to the core using 30 μm lapping film. The otolith was then positioned in the middle of a slide in crystal bond, with the polished side face downwards on the microscope slide and the rostrum (anterior end) facing up. The otolith was polished with 30 μm and 5 μm lapping film until increments from the primordium could be clearly seen.

The daily periodicity of increment formation in juvenile *A. butcheri* has been confirmed experimentally by Sakabe *et al.* (2011). Otolith increment counts were made using a compound

microscope under a magnification of x200. Triplicate counts were made on each otolith and the average (CV) for samples from individual months and sites ranged from 1.4 to 3.5%, well within the acceptable range of precision of < 5% (Campana, 2001). The average of the triplicate counts was used for back-calculation and under the assumption that the first increment was formed at hatching, an additional 2 days was added to the number of increments to give the spawning date (Sakabe *et al.*, 2011).

2.4 | Flow data

Flow (discharge, MI (10^6 l) d^{-1}) is recorded for each river system entering Gippsland Lakes and stored in the Victorian Water Resources Data Warehouse (www.data.water.vic.gov.au/monitoring.htm). Daily flow from 1 July 2015 to 30 June 2016 were extracted for the Mitchell (Site 224217), Nicholson (Site 223210) and Tambo (Site 223209) Rivers.

3 | RESULTS

The flow in the Mitchell River was 4000–7000 MI d^{-1} at the end of winter with a further pulse at the beginning of September (5000 MI d^{-1}), declining to 1000 to 3000 MI d^{-1} in October–November and further declining to < 500 MI d^{-1} from December to May (apart from small pulses

of flow in early January and early February; Figure 2a). Flows were much lower in the Nicholson with a baseline of under 100 MI d^{-1} and pulses of flow up to 600 MI d^{-1} that declined from late winter to summer (Figure 2b). Flows in the Tambo peaked near 5000 MI d^{-1} in late August followed by lesser peaks of 3000 MI d^{-1} at the beginning of September and 2000 MI d^{-1} in mid-November, after which the flow was $< 1000 \text{ MI d}^{-1}$ until late June (Figure 2c).

Water temperatures ranged from *c.* 15 to 19°C at the start of larval sampling in early October and subsequently from 18 to 20°C , increasing to *c.* 20 to 22°C at the end of sampling in early December. Surface salinities at upper to middle tributary sites were generally close to freshwater while bottom salinities were intermediate, ranging from about 15 – 20 (Table 1). Most of these sites were characterized by a relatively shallow halocline between the fresh and intermediate salinities. For sites further down the Nicholson and Tambo tributaries towards Lake King (Figure 1), surface salinities increased to 10 – 20 and bottom salinities increased to 15 – 25 (Table 1).

Dissolved oxygen (DO) was typically ~ 6 – 8 mg l^{-1} at the surface with very low levels of 0 – 1 mg l^{-1} near the bottom (Table 2). Levels of DO tended to drop sharply at the halocline. Levels of DO at the bottom were slightly higher for downstream sites near Lake King and towards the end of the sampling period (Table 2).

Larval fish were abundant in the sampling programme (Table 3), but none were positively identified as *A. butcheri*. At the upper, more freshwater influenced sites, the samples were dominated by larvae of the flathead gudgeon *Philypnodon grandiceps* (Kreff 1864) and the

Australian smelt *Retropinna semoni* (Weber 1895). Sites in the mid to lower estuaries were dominated by larvae of gobiids: largemouth goby *Redigobius macrostoma* (Günther 1861); Tamar goby *Afurcagobius tamarensis* (Johnston 1883); blue-spot goby *Pseudogobius* sp.; and, bridled goby *Arenigobius bifrenatus* (Kner 1865).

In contrast to larvae, juvenile *A. butcheri* were abundant in the seine net sampling programme. At site 390 in February, juvenile *A. butcheri* ranged from 24 to 36 mm L_T (Figure 3a). At the same site in March the L_T range was 35 to 47 mm (Figure 3b) and by April L_T was 51 to 80 mm (Figure 3c). At site 374 in February the L_T range was 27 to 46 mm (Figure 3d). Juvenile *A. butcheri* were much smaller at sites 386 (Figure 3e) and 410 (Figure 3f) in April, ranging from 11 to 22 mm..

Back-calculated spawning dates for juvenile *A. butcheri* collected from site 390 over 3 months indicated that the fish were from the same spawning cohort (Figure 4a–c). Juveniles collected in February were spawned from mid to late December (Figure 4a), while in March the spawning dates were from mid-December to early January (Figure 4b) and in April spawning was from late December to early January (Figure 4c). Back-calculated spawning dates for site 374 in February were similar to site 390, ranging from mid-December to very early January (Figure 4d). In contrast, hatching dates were 2 months later for site 386 in April, ranging from mid-February to early March (Figure 4e) and for site 410 in April, clustered at the end of February and the beginning of March (Figure 4f).

4 | DISCUSSION

The spawning period of *A. butcheri* in southern Australia typically occurs in spring and summer (Butcher, 1945; Haddy & Pankhurst, 1998; Sarre & Potter, 1999; Williams *et al.*, 2012).

However, in this study, successful spawning did not occur until nearly mid-summer and there was evidence that spawning also occurred at the end of summer–early autumn. The observed period of successful spawning was likely to be related to environmental conditions, with freshwater flow and water quality expected to be key variables. Plankton sampling over spring–early summer, using the same methods as Williams *et al.* (2012), failed to record any larvae of *A. butcheri*, suggesting that either spawning by adults did not occur in the tributary rivers in spring or that there was high mortality of eggs and newly hatched larvae.

Acanthopagrus butcheri eggs and larvae were recorded in a similar temperature range in earlier estuarine studies in Victoria. In the Mitchell Estuary, Williams *et al.* (2013) found *A. butcheri* eggs and larvae in water temperatures of 14 to 22°C over spring and early summer. A similar temperature range was recorded for presence of eggs and larvae in earlier estuarine studies in Victoria (Newton, 1996; Nicholson *et al.*, 2008). Therefore, delayed successful spawning of *A. butcheri* in this study did not appear to be related to anomalous water temperatures.

Williams *et al.* (2013) found that a well-developed halocline in the tributary rivers of Gippsland Lakes was associated with successful *A. butcheri* spawning. Reduced ovulation rate,

egg fertilisation and hatching success for *A. butcheri* has been observed in low salinity (< 10) water (Haddy & Pankhurst, 2000; Hassell *et al.*, 2008b). Previous field studies have found *A. butcheri* eggs and larvae to be most common below the halocline in salinities of 15 to 30 (Newton, 1996; Nicholson *et al.*, 2008; Williams *et al.*, 2013). Sakabe (2011) found that the successful spawning period for *A. butcheri* in unstratified conditions was associated with salinities of above *c.* 15 in the upper estuary. In the current study, therefore, salinity conditions below the halocline over spring should have been suitable for survival of eggs and larvae, although maximum salinities were lower than in previous studies where eggs and larvae of *A. butcheri* were abundant in salinities of 20–30 (Newton, 1996; Nicholson *et al.*, 2008; Williams *et al.*, 2013).

The levels of DO in the tributary estuaries were consistently very low below the halocline. DO is known to affect development and hatching rates of *A. butcheri* eggs, with no hatching at all at a DO saturation level of 30% (*c.* 3 mg l⁻¹) and larvae did not survive past Day 2 in water less than 55% saturation (Hassell *et al.*, 2008a,b), well above the levels seen below the halocline in the majority of samples in this study. Low DO (hypoxia) can develop in stratified conditions due to limited gas exchange across the halocline (Breitburg *et al.*, 2003). The development of anoxia in southern Australian estuaries is influenced by seasonal flow variation, where a lack of high winter flows can mean that low DO water formed under low-flow conditions in summer and autumn is not flushed from the system (Newton, 1996; Kurup & Hamilton, 2002). For Gippsland Lakes tributaries, Williams *et al.* (2012) compared a year with high winter flows (> 20 000 Ml d⁻¹)

¹ in the Mitchell Estuary) and a year with low winter flows and found substantial anoxia in the year of low flows and correspondingly lower concentrations of *A. butcheri* larvae. DO levels in the current study were even lower than those recorded by Williams *et al.* (2012; 2013) and therefore may have resulted in high egg and larval mortality.

High concentrations of larvae of other, primarily freshwater fish species suggests that water quality above the halocline in near freshwater salinities was sufficient for survival of these species. In contrast, the lack of larvae of marine species was consistent with the lower than marine salinities and very low DO levels below the halocline in the tributary river estuaries over the course of this study. The low to anoxic DO conditions below the halocline observed in this study are likely to have resulted from a lack of high winter flows to flush the system together with consistently strong stratification over spring preventing mixing of the oxygenated surface layer with the deoxygenated bottom layer (Newton, 1996; Kurup & Hamilton, 2002). A trend for DO levels below the halocline to increase towards the end of the sampling period for the tributaries may have continued to the point where egg and larval survival was possible from mid-summer to early autumn.

The delayed period of successful spawning observed in this study may have alternatively related to the movement of adults in relation to freshwater flows and water quality. In drought conditions in the Gippsland Lakes, adult *A. butcheri* were found to move into the tributaries (presumably for spawning) in winter and spring when salinities reached 15 to 20 around the halocline of these rivers (Hindell *et al.*, 2008). Using finer-scale sampling in the tributaries,

Williams *et al* (2017) found a similar seasonal pattern under drought conditions but also found that pulses of high freshwater flow resulted in the rapid movement of *A. butcheri* downstream to the Gippsland Lakes system. These movements in relation to freshwater flow in Gippsland Lakes are consistent with results from Tasmania (Sakabe & Lyle, 2010), where high-flow events resulted in the downstream movement of *A. butcheri* in the estuary. In this study, however, there were no substantial freshwater pulses (floods) through the system and salinities below the halocline should have been suitable for adult *A. butcheri*. However, there is also evidence that adult *A. butcheri* will avoid areas of low DO in estuaries (Cottingham *et al.*, 2014; Valesini *et al.*, 2017). It is therefore possible that the low DO conditions below the halocline in the current study may have deterred the adult *A. butcheri* from migrating to the tributary rivers for spawning during spring.

If *A. butcheri* in spawning condition were prevented from moving into the tributaries over spring it is possible that they spawned in higher salinity water in the lakes system, but this did not result in any recruitment of juveniles. This would be consistent with the results of Williams *et al.* (2012) who found *A. butcheri* eggs in both the lakes and the tributary rivers, but larvae were essentially only found in the tributaries, indicating that only the rivers provided a larval nursery habitat. Although suitable conditions of salinity and oxygen may have occurred in the lakes system, other features of the tributaries must make them more suitable for spawning and egg and larval survival. The high plankton productivity associated with the upstream reaches of the salt wedge, providing suitable food for larvae, is likely to be a key factor (North & Houde,

2001, 2003; Williams *et al.*, 2013). However, the full suite of conditions required for reproductive success by *A. butcheri* is still not fully understood and therefore requires further investigation.

The usual hydrological cycle for microtidal estuaries in Victoria is related to typical rainfall patterns. Highest rainfall and in turn highest flows, occur in winter and early spring (Newton, 1996). At this time, high-flow events tend to flush saline water from the estuary. Lower flows from late-spring to autumn allow the salt wedge to reform through the incursion of well-oxygenated marine water (Newton, 1996). Low flows and high stratification can then lead to de-oxygenation in summer and autumn (Newton, 1996). The formation of the salt wedge in spring typically coincides with the spawning of *A. butcheri*. However, environmental conditions may cause delayed spawning through two main mechanisms. One is where high flows extend longer than normal into spring, resulting in low salinity conditions unsuitable for spawning or larval survival, lasting until late spring or summer (Sakabe *et al.* 2010, 2011). An alternative mechanism, most likely the case in this study, is where winter flows are insufficient to flush the stratified, de-oxygenated bottom water from the system, so that stale water under the halocline persists through the spring and is again unsuitable for *A. butcheri* spawning or egg and larval survival. Lower flows over summer may allow for the incursion of well-oxygenated marine water, making conditions more suitable for spawning.

Climate change predictions for the Gippsland Lakes area are for reduced rainfall, primarily the result of rainfall decline in winter and spring (CSIRO & Bureau of Meteorology, 2015). This

trend may favour recruitment of *A. butcheri* in the Gippsland Lakes system where higher recruitment has been associated with lower freshwater flows and higher stratification in the tributary estuaries in the spring (Jenkins *et al.*, 2015), although if flows become too low then the tributary estuaries will be marine dominated and stratification will be reduced, to the detriment of *A. butcheri* spawning. Evidence from other southern Australian estuaries indicates that lower winter–early spring flows under climate change is leading to an increasing prevalence of hypoxia with adverse consequences for *A. butcheri* populations (Cottingham *et al.*, 2014, 2018; Valesini *et al.*, 2017). If lower winter flows and a lack of flushing leads to increased hypoxia in Gippsland Lakes tributaries over spring, this could also be detrimental to *A. butcheri* spawning. Furthermore, other species requiring high spring flows for recruitment, such as estuary perch *Percalates colonorum* (Günther 1863), are likely to be negatively affected by reduced flows (Jenkins *et al.*, 2015).

In summary, successful spawning of *A. butcheri*, relative to the expected spring–early summer period, was delayed until nearly mid-summer with a further spawning episode at the end of summer–early autumn. We conclude that this delay in spawning was most likely the result of low DO below the halocline throughout the spring period. This may have prevented the migration of spawning adults into the tributaries or resulted in high mortality of eggs and newly-hatched larvae. Climate change predictions for southern Victoria, including Gippsland Lakes, indicate a trend for lower winter-spring freshwater flows, which may have a positive influence on reproductive success of *A. butcheri* through high water-column stratification, but only if DO

concentrations are not compromised by a lack of substantial winter flows. Thus, high flows in winter and early spring may be necessary to maintain water quality, particularly adequate DO concentrations, for successful spawning of *A. butcheri*.

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Contributions

G.P.J. Study design, field sampling, laboratory analysis, data analysis, manuscript preparation.

J.A.K. Laboratory analysis, data analysis, manuscript preparation.

R.J.W. Study design, field sampling, manuscript preparation.

F.W. Study design, field sampling, manuscript preparation.

S.E.S. Study design, manuscript preparation.

P.L.M.C. Study design, manuscript preparation, funding.

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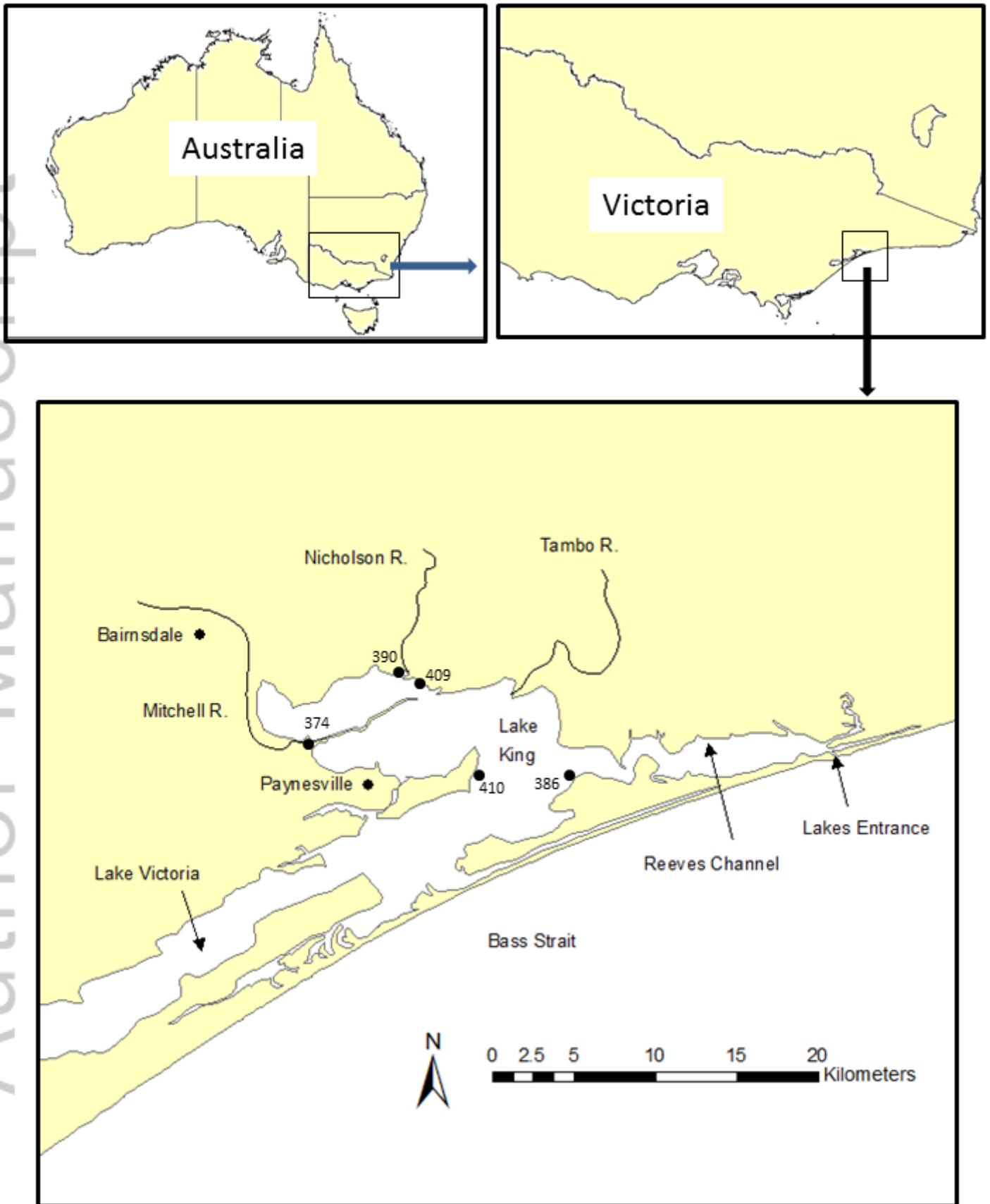
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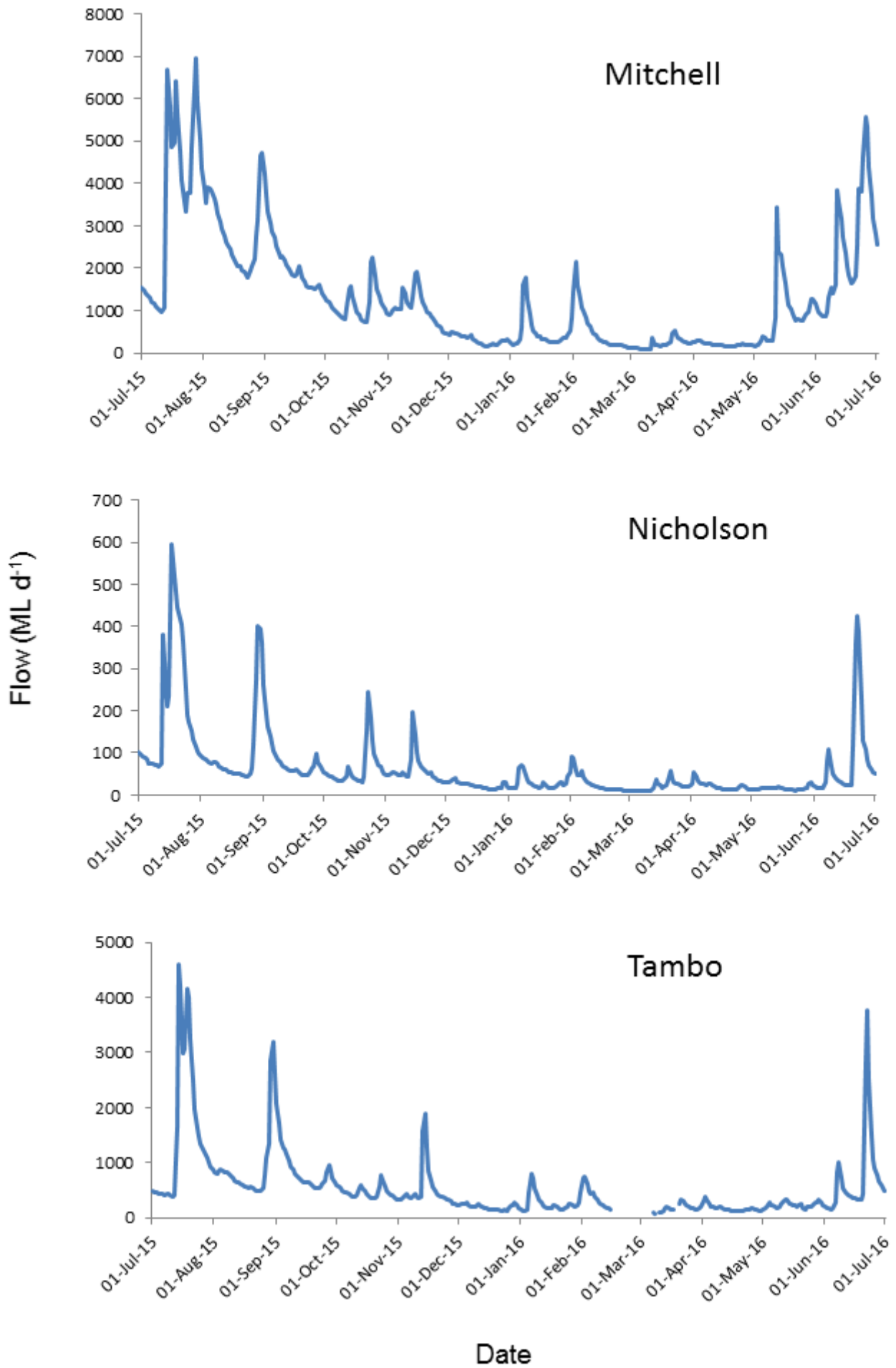
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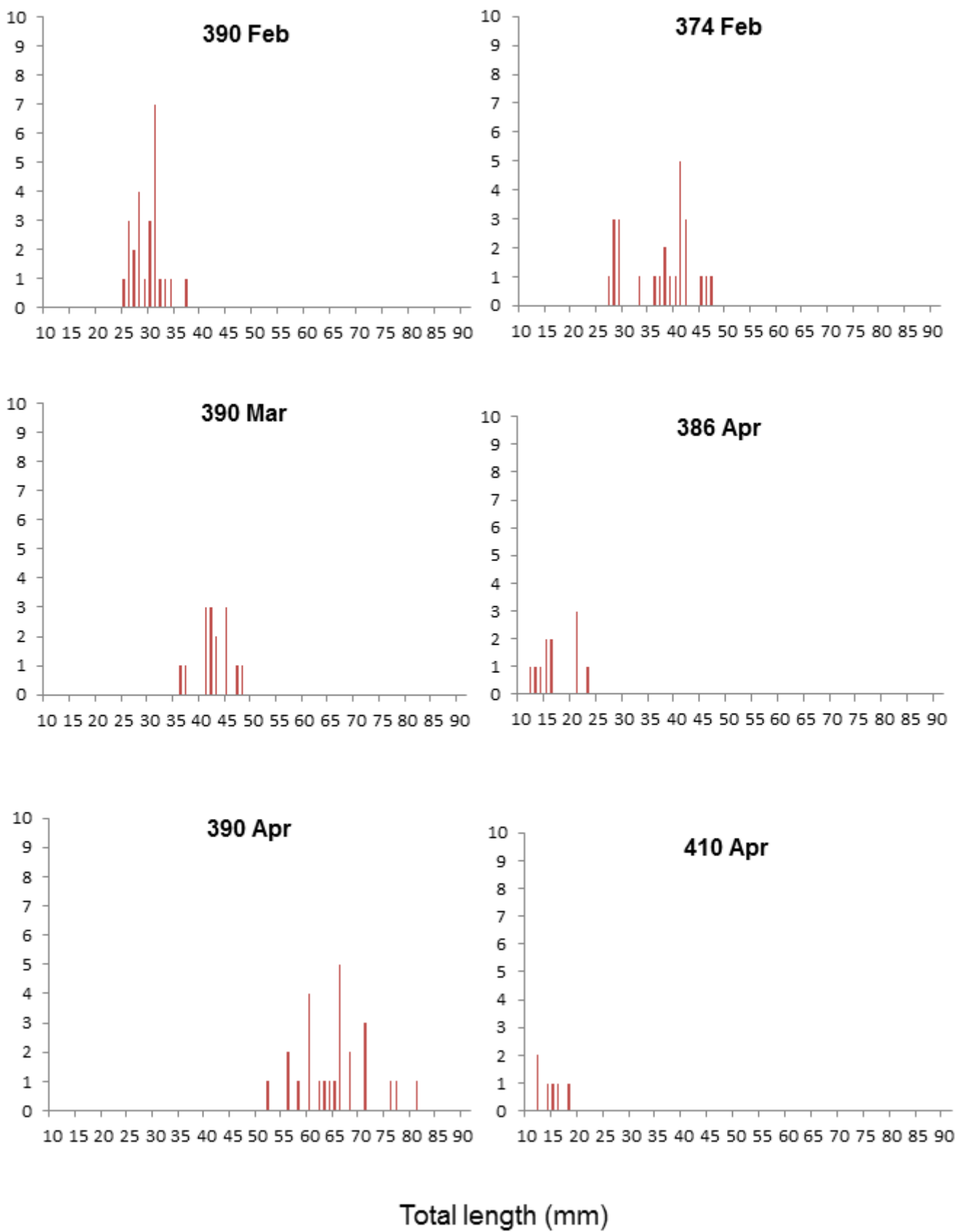
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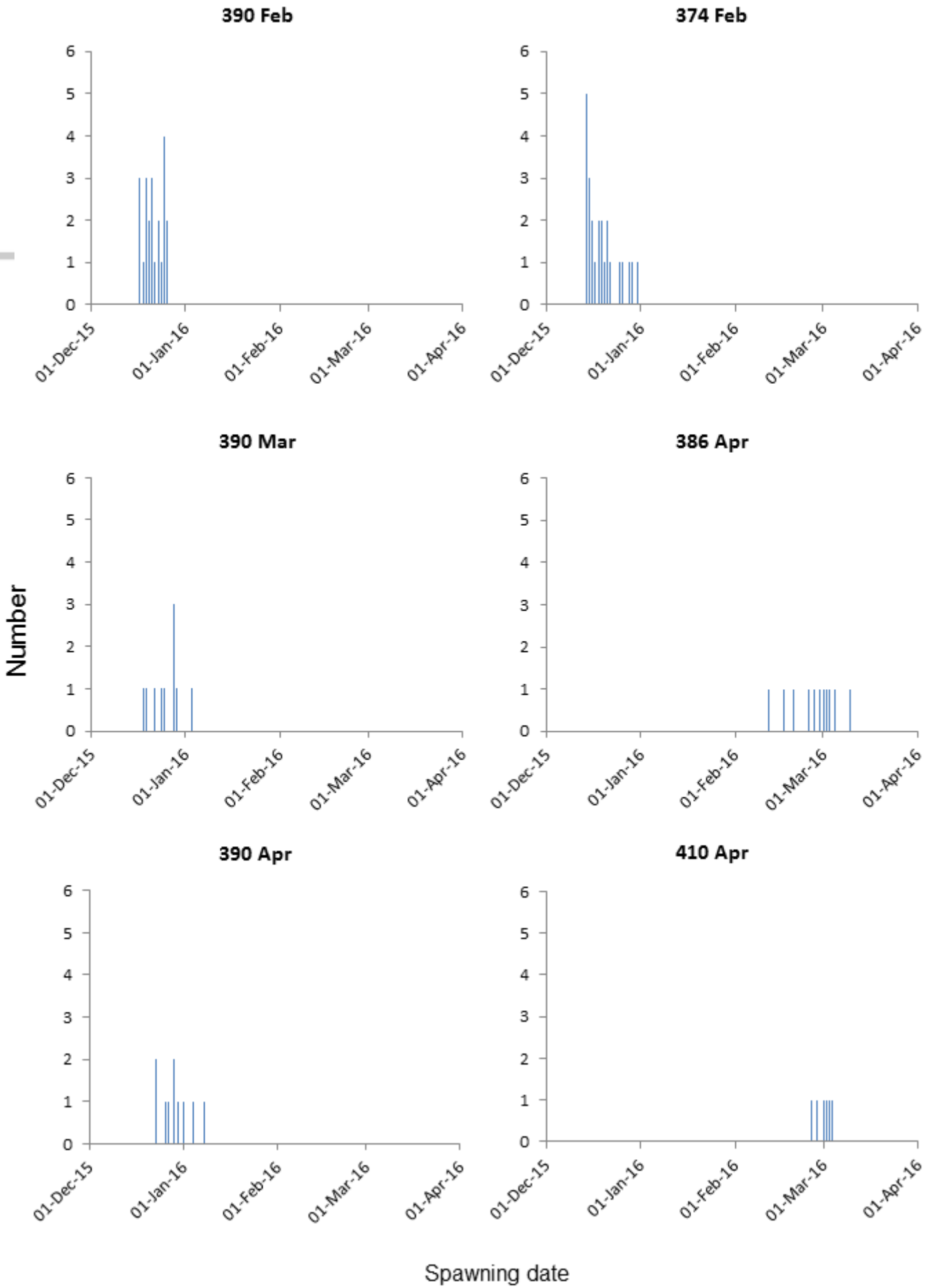


FIGURE CAPTIONS

FIGURE 1 Location of the State of Victoria within Australia, the Gippsland Lakes on the coast of Victorian and the Gippsland Lakes study sites: I, sites that were sampled for fish larvae).

Typesetter

- 1 Change the compass arrow to a simple latin cross.
- 2 Change Kilometers to km.

FIGURE 2. Daily freshwater flows in (a) Mitchell River, (b) Nicholson River and (c) Tambo River from July 2015 to June 2016.

Typesetter

- 1 Change Mitchell to (a), Nicholson to (b) and Tambo to (c).
- 2 Change (ML d⁻¹) to (ML day⁻¹).

- 3 Delete  from (a) and (b)

FIGURE 3. Total length (L_T)-frequency distributions of juvenile *Acanthopagrus butcheri* collected from four sites in Lake King (Figure 1) between February and April 2016.

Typesetter


- 1 Change Total length to L_T .
- 2 Change Number to Frequency (n).
- 3 Delete  from the top four panels

FIGURE 4. Frequency distributions of the back-calculated date of spawning of juvenile *Acanthopagrus butcheri* collected from four sites in Lake King (Figure 1) between February and April 2016.

Typesetter

1 Change Number to Frequency (n).

2 Delete  from the top four panels

TABLE 1 Surface and bottom salinities from sampling sites within three estuaries entering the Gippsland Lakes, October to December 2016. Sampling sites were numbered consecutively from upstream to downstream.

Date	Estuary	Depth	Sampling site									
			1	2	3	4	5	6	7	8		
6 October	Mitchell	Surface	0.1	0.4	0.8							
		Bottom	14.3	15.5	16.2							
7 October	Nicholson	Surface	1.6	3.7	6.0							
		Bottom	14.7	16.7	17.3							
19 October	Mitchell	Surface	*	*	*							
		Bottom										
20 October	Nicholson	Surface		*	*	*	*	*	*	*	*	*
		Bottom										
21 October	Tambo	Surface	0.5	0.9	1.0	2.8	1.9	18.8				
		Bottom	17.1	19.0	19.3	19.4	19.3	19.7				
22 October	Mitchell	Surface				1.2	1.9	2.5				
		Bottom				16.5	17.2	18.0				
10 November	Mitchell	Surface	0.4	0.4	0.8	1.1	1.2	1.5				
		Bottom	0.4	17.3	18.3	18.4	19.4	19.9				
10 November	Nicholson	Surface		0.5	4.8	5.5	4.7	7.3	11	14		
		Bottom		16.6	17.5	17.8	18.2	19.2	19.4	22.6		
11 November	Tambo	Surface	0.2	0.4	0.7	1.5	3.0	3.5				
		Bottom	19.8	20.8	21.9	23.2	23.6	23.8				
24 November	Mitchell	Surface	0.5	1.1	1.5							
		Bottom	19.3	19.7	19.9							
25 November	Nicholson	Surface	0.2	0.7	2.1							
		Bottom	17.7	17.5	18.3							
26 November	Tambo	Surface	3.5	1.9	3.8	9.1						
		Bottom	21.8	22.1	22.7	22.8						
8 December	Mitchell	Surface	0.6	1.0	1.2	1.7	*	*				
		Bottom	21.0	21.5	21.9	21.0						
8 December	Nicholson	Surface		2.1	3.8	3.7	5.0	9.8	11.3	14.2		
		Bottom		18.7	19.1	19.6	20.5	21.4	21.0	23.1		
9 December	Tambo	Surface	1.6	5.3	4.1	10.4	11.2	17.6				
		Bottom	23.4	23.8	24.2	24.5	24.6	25.5				

* Equipment malfunction

TABLE 2 Surface and bottom dissolved oxygen (mg l^{-1}) from sampling sites within three estuaries entering the Gippsland Lakes, October to December 2016. Sampling sites were numbered consecutively from upstream to downstream.

Date	Estuary	Depth	Sampling site									
			1	2	3	4	5	6	7	8		
6 October	Mitchell	Surface	9.2	8.8	8.7							
		Bottom	7.3	7.1	2.6							
7 October	Nicholson	Surface	5.8	4.3	3.4							
		Bottom	0.0	0.0	0.0							
19 October	Mitchell	Surface	*	*	*							
		Bottom										
20 October	Nicholson	Surface		*	*	*	*	*	*	*	*	*
		Bottom										
21 October	Tambo	Surface	0.0	0.0	0.0	8.8	0.0	9.6				
		Bottom	0.0	0.0	1.7	4.0	1.9	3.5				
22 October	Mitchell	Surface				7.7	7.4	7.5				
		Bottom				0.0	0.0	0.0				
10 November	Mitchell	Surface	8.8	8.4	8.0	8.1	8.4	8.6				
		Bottom	8.8	0.0	0.9	4.1	5.1	5.0				
10 November	Nicholson	Surface		6.8	5.2	7.5	9.1	9.2	9.4	8.8		
		Bottom		0.0	0.0	0.0	0.0	0.0	0.0	4.9		
11 November	Tambo	Surface	8.1	8.0	8.6	8.2	9.1	9.0				
		Bottom	0.9	2.0	2.6	4.1	6.6	5.8				
24 November	Mitchell	Surface	7.6	7.2	7.4							
		Bottom	0.5	0.8	0.6							
25 November	Nicholson	Surface	7.1	6.2	6.7							
		Bottom	0.0	0.0	0.0							
26 November	Tambo	Surface	6.3	8.2	8.2	8.4						
		Bottom	2.5	3.3	0.6	3.0						
8 December	Mitchell	Surface	6.5	6.8	6.9	8.4	*	*				
		Bottom	0.0	1.9	1.5	4.8						
8 December	Nicholson	Surface		4.5	3.3	5.4	7.4	6.9	7.1	7.9		
		Bottom		0.0	0.0	1.4	0.7	1.3	5.0	4.7		
9 December	Tambo	Surface	6.7	6.8	8.0	7.5	7.9	8.1				
		Bottom	2.1	3.3	5.2	6.7	7.5	6.5				

* Equipment malfunction

TABLE 3 Concentrations of fish larvae ($n\ 100\ m^{-3}$) collected from sampling sites within three estuaries entering the Gippsland Lakes, October to December 2016. Sampling sites

were numbered consecutively from upstream to downstream.

Date	Estuary	Site							
		1	2	3	4	5	6	7	8
6 October	Mitchell	86	79	38					
7 October	Nicholson	409	60	0					
19 October	Mitchell	553	8	9					
20 October	Nicholson		3	3	11	12	9	0	14
21 October	Tambo	8	7	0	123	166	18		
22 October	Mitchell				319	36	37		
10 November	Mitchell	232	107	53	47	19	103		
10 November	Nicholson		61	37	237	46	19	25	66
11 November	Tambo	7	12	31	21	0	22		
24 November	Mitchell	15	4	5					
25 November	Nicholson		31	56					
26 November	Tambo	6	27	233	43				
8 December	Mitchell	164	14	61	44	50	106		
8 December	Nicholson		59	9	101	64	43	29	69
9 December	Tambo	6	45	328	770	94	75		



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