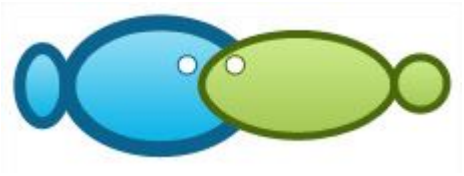

Comparing spawning, larval development, and recruitments of four mussel species (Bivalvia: Mytilidae) from South Australia

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Abstract. This study describes spawning, larval development, and new recruits of four mytilid species. Each of the mussel species was induced by standard thermal shock and natural seawater temperature changing. Fertilized eggs were incubated at a density of 30 mL^{-1}. When fertilized eggs reached D-stage, larvae were also stocked at the same density and fed a microalgae diet. New recruits were determined monthly after allowing them to settle on substratum provided and removed after one month in the field. The results show that *Mytilus galloprovincialis* spawned in June to October, *Brachidontes erosus* in November to January, *Brachidontes rostratus* in January to March, while *Trichomya hirsuta* spawned in October to December. *M. galloprovincialis* released four times as many eggs as the other mytilid species investigated. Eggs diameter of *B. erosus* ranged from 91 to 106 µm, which were larger than other species observed. *M. galloprovincialis* reached settlement stage in 16 days, *B. rostratus* and *T. hirsuta* in 15days, and *B. erosus* in 9 days. Larvae of *B. erosus* were larger in size compare to other species at the early D-stage, but this changed when metamorphosis occurred. A peak of new recruits following spawning time occurred in different time for each species.

Key Words: spawning, larval development, settlement, metamorphosis, new recruit, mytilid.

Introduction. The marine mussels *Mytilus galloprovincialis* (Lamarck, 1819), *Brachidontes rostratus* (Dunker, 1857), *Brachidontes erosus* (Lamarck, 1819), and *Trichomya hirsuta* (Lamarck, 1819) are commonly occurring in the South Australian marine waters where they usually are found in patches. *M. galloprovincialis* occurs throughout the southern temperate region from Western Australia to New South Wales and Tasmania (McDonald et al 1991). *B. rostratus* and *B. erosus* have limited distributions and are found in temperate Australian waters from Western to South Australia, and then to Tasmania (Edgar 2000). *T. hirsuta* is distributed from South Australia, Tasmania, and Queensland, then up to North of Australia (Edgar 2000). In an earlier study, the blue mussel in southern Australia was first described as and endemic subspecies of the northern European *Mytilus edulis* L. and subsequently named *Mytilus edulis planulatus* Lamarck, 1819, based on a perceived morphological observation of a more flattened shell than its European counterpart (Wisely 1964; Wilson & Hodgkin 1967). However, later genetic studies have found the species to be the cosmopolitan species *M. galloprovincialis* (McDonald et al 1991; Gosling 1992; Hilbish et al 2000; Svane 2011).

Although these species may share the same environment, they have different reproductive patterns such as time of gonad maturation, spawning, larval availability, settlement, and recruitment. Generally, mussels spawn when gonad matures. It seems that gonad maturation and subsequent spawning may occur in winter and spring at lower latitudes, and be extended into summer at higher latitudes (Dix & Ferguson 1984; Myrand et al 2000).

After releasing their eggs and sperm in the seawater column where they become fertilized, the fertilized eggs subsequently develop through D-stage, veliger, and pediveliger stages. Each developmental stage has different morphological characteristics, including shell shape, size, growth, behavior and physiology (Bayne 1965; Redfearn et al 1986). Duration of larval life in the water column may also vary between species. Differences in these characteristics reflect different larval ecology and may influence adult ecology (Mokady et al 1993).

Many studies have been conducted on maturation, spawning, and larval development of mytilid species such as *Mytilus planulatus* Lamarck, 1819 (*M. galloprovincialis*) (Wilson & Hodgkin 1967), *M. edulis* (Linnaeus, 1758) (Myrand et al 2000), *M. trossulus* (Gould, 1850) (Toro et al 2002), *Perna canaliculus* (Gmelin, 1791) (Buchanan 2001), *Xenostrobus pulex* (Lamarck, 1819) and *Brachidontes variabilis* (Krauss, 1848) (Morton 1988), and on larval development of *M. edulis* from the Northeast Atlantic (Bayne 1965), New Zealand (Redfearn et al 1986), from the Northwestern Atlantic (Fuller & Lutz 1989), and then *Brachidontes solisianus* (d'Orbigny, 1842) (Monteiro-Ribas et al 2006). Soares et al (2008) studied an extract compound from brown seaweed as one of settlement cues for the larvae of mytild species, *Perna perna* (Linnaeus, 1758). Furthermore, both Ompi (2010) and Carl et al (2012) have also described settlement behavior of *M. galloprovincialis* as a laboratory study. Plantigrade settlement of *Mytilus coruscus* (Gould, 1861) on biofilm formation has been shown by Wang et al (2012), while Morello & Yund (2016) have shown a behavior of competent blue mussel (*M. edulis*) larvae to positive and negative settlement cues. However, limited information on spawning, larval development, and recruitment has been available for mytilid species from the South Australia Sea and with exception for the larvae of *M. galloprovincialis*, no information for the larvae of *B. rostratus*, *B. erosus*, and *T. hirsuta* has been available.

This study was designed to compare spawning activity among mytilid species, and furthermore, to investigate whether any differences in number of eggs and sizes, larval sizes and development may be applied among mytilid species investigated, and finally, to investigate new recruits of four mytild species in Port Lincoln and surrounding waters of South Australia.

Material and Method. *M. galloprovincialis* were collected from mussel beds in Boston Bay, Port Lincoln (34°42'92"S, 135°52'79"E), *B. erosus* from Tumby Bay (34°23'57"S, 136°06'11"E, *B. rostratus* from Coffin Bay (34°36'20"S, 135°27'82''E) and *T. hirsuta* from Louth Bay (34°33'92"S, 135°56'83"E) South Australia, Australia. Mussels were removed from their habitat at monthly intervals from January 2001 to May 2002 by carefully cutting their byssus and immediately transferred to the hatchery of Lincoln Marine Sciences Centre, Port Lincoln.

In the hatchery of Lincoln Marine Sciences Centre, Pt Lincoln, South Australia, mussels were cleaned and placed in flow tanks supplied with natural running seawater. Each species collected was placed separately in one flow tank with running seawater, where spawning was observed. At the same time, thirty mussels of each species were induced to spawn by thermal stimulation (Strathmann 1992). Mussels were placed in a polyethylene tray (50 x 35 x 15 cm) provided with flow-through seawater and two aquarium heaters were placed on the bottom at both sides of the tray.

Rapidly increasing of the seawater temperature from 14 to 22°C using the heaters was carried out in the winter. Both in- and out-flow of running seawater were closed when heaters were on, and were opened to let seawater flew slowly when seawater temperature reached 22°C. In the summer, 0.5 to 1 kg of saltwater ice cubes was introduced into polyethylene tray to reduce the seawater temperature down to 14°C. When the seawater temperature reached 14°C, both in and out-flow of polyethylene tray were opened to let the running seawater flow into the tray until the seawater temperature reached 22°C. Seawater temperature of Southern Australia fluctuates from 12 to 17°C in the winter (June-August) and from 18 to 26°C in the summer (December-February) (Shepherd & Thomas 1989). An 8°C temperature increase was in the range used by Strathmann (1992).

When mussels started to release eggs and sperm, they were separated and kept individually in 500 mL plastic containers filled with 200 mL filtered seawater. Mussels were removed from spawning containers when spawning ended. Then the water containing eggs from each female of each species was diluted to 500 mL before a 0.5 mL suspension of eggs was taken and placed in a hemocytometer before being counted and photographed using an Olympus BX52 Nomarski DIC microscope equipped with a camera. Diameters of eleven eggs from each sample were measured using a calibrated ocular micrometer. At least three replicate samples from each species were taken and measured.

Eggs and sperm produced after inducing spawning were filtered separately through sieves with 100 µm mesh sizes to remove debris. For *B. erosus*, the debris was removed by using sieve with 150 µm mesh size. About 100,000 eggs were suspended into 200 mL of 0.45 µm filtered seawater (FSW) in a 1 L Pyrex® glass beaker. Several drops of sperm were added into each of beaker until the seawater looked slightly cloudy (Strathmann 1992). To ensure that most of the eggs were fertilized, eggs and sperm were left in the beaker for at least one hour.

Fertilized eggs were stocked at a density of 30 mL^{-1} (Strathmann 1992) in three replicate 1 L Pyrex® glass beakers containing 900 mL of FSW. All beakers were transferred to a modified larval culture system with automatic stirring and connected to controlled temperature conditions at 19±2°C (Strathmann 1992). When the larvae reached the D-stage, they were collected from each beaker on a 25-µm nylon sieve mesh and filled to 1 L larval rearing Pyrex® glass beaker containing FSW at a density of 30 larvae mL^{-1} in three replicates (Strathmann 1992). All beakers were also transferred to the modified larval culture system with automatic stirring at the same temperature until most of them were competent to settle, which was characterized by the appearance of eyes, foot, umbo and the large adductor muscle (Bayne 1965; Mokady et al 1993). Larvae were allowed to settle on the substrate of adult byssus threads. It was removed from adults by cutting, and was placed at the bottom of beaker, when a stage competent to settlement was reached.

D-stage larvae were fed with *Isochrysis* sp., and *Chaetocerus* sp. was added during the last larval stage at concentrations suggested by Strathmann (1992). The development was observed every three hours from fertilization of eggs to D-stage. Samples of 0.5 mL were removed from each beaker, placed into haemocytometer, and then each of development stage was observed, photographed, and the size was measured to the nearest µm by using microscope accommodated with a micrometer eye piece (Redfearn et al 1986) and a camera as mentioned above. Quick observation of larval development stage was performed every six hours. Time and sizes (shell length, height (width), hinge line, and length of umbo) were recorded when every new stage appeared. The larvae were also sampled every 48 h, at the same time when FSW was changed. The larvae were placed in the haemocytometer, and the size of larvae was measured as mentioned above.

Keough & Downes (1982) defined settlement as when attachment to a substratum occurs with the onset of metamorphosis, while recruitment is when an observer counts a settled organism. At this stage, recruits might grow and mortality might also occur. Many studies have used shell size to differentiate among settlement and new recruits. For example, Widdows (1991) used a range of shell length from 250 to 400 µm to identify new settlers. *M. edulis* larvae were competent to settle at size of 250 µm (Bayne 1965). In this study, new recruits are defined as larvae attached to the substratum with sizes larger than 400 µm and less than 1000 µm. Larvae were allowed to settle on nylon scourers, diameter 6 cm and area 28 cm^2, used as artificial collectors. The scourers were conditioned for two weeks in running seawater in a laboratory tank before deployment into the field. The scourer was mounted on top of bricks. Four bricks were placed at each location, where each brick with the scourer was placed haphazardly into each of mussel patch. The bricks with the scourers were replaced monthly from January 2001 through May 2002.

Living recruits were extracted in the laboratory by using a combination of washing through a sieve and an anesthetic technique. Each scourer was washed and the water

sieved through a 400 µm mesh to collect the new recruits. Then each scourer was transferred to a 250 mL plastic container with a solution of magnesium chloride isotonic with filtered seawater in order to relax the new recruits. After 15 minutes, the scourer was shaken several times to extract all recruits and then they were examined under a dissecting microscope for sorting and counting.

Non-parametric Kruskal-Wallis test (Fowler et al 1998) was applied to determine whether differences in number of eggs between mytilid populations were significant. The size of each development stage was also tested for differences between species investigated by this non-parametric test. Differences in the percentage of new recruits over 17 months for each species were tested using a One-Way ANOVA of the square root transformed data (Fowler et al 1998). Post hoc comparisons were performed using Turkey's test (Sokal & Rohlf 1981). This test was run separately for each species.

Results and Discussion. Spawning varied in time for each species and it did not occur throughout the year of observation in this study. When induced by thermal shock, spawning in *M. galloprovincialis* varied with time, and, furthermore, not all individuals released gametes. In June 73% of the specimens spawned, 53% in September, and 89% in October (Table 1). Spawning of all of these mussels (mass spawning) in flow tank supplied with natural running seawater occurred only in June.

Table 1

Spawning of mytilid species in the hatchery of Pt Lincoln Marine Sciences Centre, South Australia (√ = mass spawning)

Species	*Dates*	*Introduced mussels*	*Induced thermal shock (%)*	*Natural running seawater in tank*
M. galloprovincialis	7 June 2001	15	73	√
	6 September 2001	15	53	
	17 October 2001	18	89	
B. erosus	19 November 2001	20	10	
	20 December 2001	15	53	
	22 January 2002	20	100	√
B. rostratus	20 January 2002	30	17	
	24 February 2002	35	100	√
	21 March 2002	30	73	
T. hirsuta	20 October 2002	9	11	
	24 November 2002	12	17	
	3 December 2002	12	83	√

For *B. erosus*, spawning activity started in November, when 10% of induced mussels spawned. In January 2002, all of the mussels induced (100%) spawned. Spawning of *B. erosus* maintained in tank supplied with natural running seawater was also observed in January. For *B. rostratus*, spawning started in January 2002, when 17% of induced mussels spawned. Major spawning was observed in February 2002, when all induced mussels (100%) released their eggs and sperm. Spawning of *B. rostratus* kept in tank supplied with natural running seawater was also observed in February 2002. Induced spawning for *T. hirsuta*, which is also called hair mussel, started in October and the major spawning was observed in December (83%) (Table 1). *T. hirsuta* kept in the tank supplied with natural running seawater spawned in December.

Kruskal-Wallis test revealed that the number of released eggs differed significantly among species ($K = 13.94$, $df = 3$, $p < 0.001$). *M. galloprovincialis* released larger number of eggs than the other three species (Figure 1). The eggs of *M. galloprovincialis* were pink to orange in color and measured 61 to 70 µm in diameter. The eggs of *B. erosus* were dark brown in color and measured 91 to 106 µm in diameter. The eggs of *B. rostratus* were brown in color and measured 76 to 81 µm in diameter. For

T. hirsuta, eggs were orange to yellowish in color and measured 81 to 86 µm in diameter. All eggs were full of yolk enclosed by a vitelline membrane.

Statistical analysis showed that egg diameters were significantly different among species (K = 308.8, df = 3, p < 0.01). Eggs of *B. erosus* had the largest diameter, followed by *T. hirsuta* and *B. rostratus*, with *M. galloprovincialis* having the smallest egg diameter.

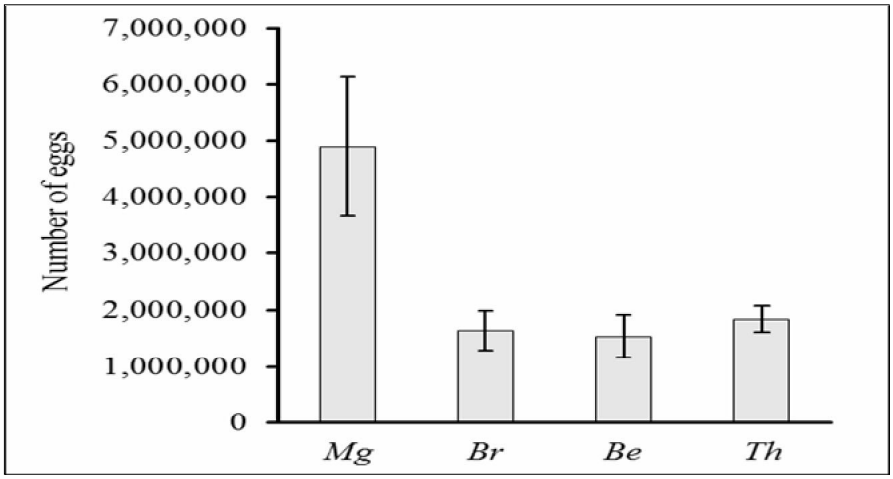

Figure 1. Mean number of eggs released by the four mytilid species investigated: *Mg = M. galloprovincialis*, *Br = B. rostratus*, *Be = B. erosus*, *Th = T. hirsuta* (Bars: 95% confidence interval).

The shape of the eggs through the development from fertilization to the trochophore stage changed for all the four species investigated. The eggs became asymmetrical as soon as the polar body appeared and the first division took place until the trochophore stage. Movements of the embryos were observed shortly after cilia were protruding from the vitelline membrane. After hatching, the trochophore of all species investigated swam close to the surface. A bundle of cilia known as an apical tuft was easily observed in all species investigated. Egg diameter was constant from fertilization until the trochophore stage for *M. galloprovincialis*, *B. rostratus*, and *T. hirsuta*, while differences in diameter from fertilization until trochophore stage for *B. erosus* occurred.

Development of fertilized eggs to early veliger stage of each species investigated varied in time. Early veliger stage characterized by straight hinges and is known as D-stage was recorded in 24 hours for *M. galloprovincialis*, for *B. rostratus* in 54 hours, *B. erosus* in 48 hours, and *T. hirsuta* in 18 hours (Table 2). Kruskal-Wallis test revealed a significant difference in length (K = 91.5, df = 3, p < 0.001), width (K = 93.78, df = 3, p < 0.001), and hinge line (K = 95.19, df = 3, p < 0.001) between species investigated at this early D-stage. Length, width, and hinge-line were larger in *B. erosus* than in the other species investigated (Table 2). The smallest size in length, width, and hinge-line appeared in *M. galloprovincialis* (Table 2). However, the size of larvae changed when larvae reached settlement and metamorphosis stage (Table 2). The larvae of *M. galloprovinclialis* were significantly larger in length (K = 34.08; df = 3, p < 0.001) and width (K = 38.04; df = 3, p < 0.001) than the other three species at settlement and metamorphosis stage (Table 2). Umbonal length in larvae of *B. erosus* was significantly larger compare to other three species (K = 14.32; df = 3; p < 0.001).

Each larval species showed an initial thin transparent shell, which is called the prodissoconch (PI). Another shell zone, known as prodissoconch II (PII), which was marked by growth lines, appeared. Each species of larvae had a ciliated velum that emerged from the valves during swimming. The velum remained inside the shell when at rest. Early umbo shape of all larval species was similar, which was broadly rounded or indistinct and broadly rounded. Earliest umbo larva was first seen on day 6 in *M. galloprovincialis* and *B. erosus*, on day 7 in *B. rostratus*, and on day 9 in *T. hirsuta* respectively. The umbo of *M. galloprovinciallis* was knobby when metamorphosis took place.

Table 2

Kruskal-Wallis test in sizes of length, width, and hinge line at different stage development of four mytilid species investigated from South Australia. Mg = *M. galloprovincialis,* Be = *B. erosus*, Br = *B. rostratus*, and Th = *Trichomya hirsuta*

Development stages	*Size (µm)*				*Kruskal-Wallis test (K)*		
	Mg	*Be*	*Br*	*Th*	*K*	*df*	*p*
D-veliger							
Length	99.6±3.5	123.6±5.6	104±4.2	109±7.1	91.47	3	*
Width	76.6±2.7	104±6.6	83.2±5.2	92.2±8.1	93.79	3	*
Hinge line	73.3±3.1	97.5±7	78.8±2	81.2±4.6	95.19	3	*
Early umbo							
Length	162±9.8	197.8±13.2	183±7.6	202.7±9.4	94.42	3	*
Width	138±6.5	158.2±16.4	132±7.6	161.4±11.8	76.38	3	*
Hinge line	-	-	-	-			
Umbo length	79.8±5	129.4±4	116.5±3	114.5±6.6	109.3	3	*
Early eye							
Length	214±10.2	214.2±7.3	232.4±14	269.4±15	48.1	3	*
Width	194±13.5	171.3±7.5	184.5±16	225.6±16	48.2	3	*
Hinge line	-	-	-	-			
Umbo length	91.7±7.3	134.6±6.2	113.5±2.5	112.5±6	66.5	3	*
Metamorphosis							
Length	384±15.9	310.8±22.8	257±34	318±23	34.04	3	*
Width	319.6±12	300±22	216.6±14.3	304±19	38.08	3	*
Hinge line	-	-	-	-			
Umbo length	127.6±16.7	158±9	110±4.8	137±22	14.32	3	*
Ages							
Early D-veliger	24 hours	48 hours	54 hours	18 hours			
Early umbo	6 days	6 days	7 days	7 days			
Early eye	16 days	9 days	15 days	15 days			

Stars indicate significant difference ($p < 0.05$).

Many larvae swam close to the bottom or attached to the bottom when the eyespot was formed. Attachment to substrata available at glass bottom occurred on day 16 for *M. galloprovincialis*, day 15 for *B. rostratus* and *T. hirsuta*. Each species used the foot when crawling at the bottom of the glass. The larvae swam or crawled close to the bottom before attachment. When no substratum, such as byssus threads and shells, was available in the beakers, the larvae subsequently swam back into water column or continuously crawled on the Pyrex glass bottom. *B. erosus* larvae aggregated and swam close to the bottom of glass on day 9, and subsequently a day after settled in patches at the bottom when substratum was introduced.

New recruits of *M. galloprovincialis* were found in the field throughout the year from January 2001 until April 2002 (Figure 2a). One-way ANOVA revealed that the mean percentage new recruits of *M. galloprovincialis* differed significantly between times ($F_{16,51}$ = 20.741, $p < 0.001$) (Table 3). A peak with large percentage of new recruits was observed in October and November 2001 (Figure 2a). Pairwise comparisons showed that the difference in mean percentage of new recruits between October and November 2001 was not significant (post-hoc Turkey's test, $p > 0.05$). A significantly larger percentage of new recruits was observed in November 2001 than in January, February, March, and until June 2001 (post-hoc Tukey's test, $p < 0.05$; Figure 2a), and also a significantly larger number of new recruits in November 2001 than in December 2001 until May 2002 was seen (post-hoc Tukey's test, $p < 0.05$; Figure 2a).

Table 3

One factorial ANOVA on percentage of recruitment for each of the four species with time as the main effects

Source	*df*	*SS*	*MS*	*F*	*P*
M. galloprovincialis					
Between groups	16	3.558.452	222.403	10.964	0.000***
Within groups	51	1.034.551	20.285		
B. erosus					
Between groups	16	52.228	3.264	12.827	0.000***
Within groups	51	12.979	0.254		
B. rostratus					
Between groups	16	491.670	30.729	3.107	0.001**
Within groups	51	504.407	9.890		
T. hirsuta					
Between groups	16	1.793	0.112	3.465	0.000***
Within groups	51	1.64	0.032		

**: $p < 0.01$; ***: $p < 0.001$.

Recruitment as a function of time from January 2001 to May 2002 for *B. erosus* differed significantly (One-way ANOVA, $F_{16,51}$ = 1.161, $p < 0.001$) (Table 3). A peak with significantly larger percentage was observed in February 2001 than in other months of 2001 and 2002 (post-hoc Turkey's test, $p < 0.05$) (Figure 2b). Low percentage of new recruits occurred in March until December 2001. Mean percentage of new recruits remained low in January 2002, before reaching a large peak in February 2002 and then decreased again in March, and remained about the same level from March until May 2002.

In *B. rostratus* recruitment occurred throughout the year with a large peak in March 2001 and another peak in April 2002. A significant effect of time on new recruits of *B. rostratus* was evident (One-way ANOVA, $F_{16,51}$ = 8.672, $p < 0.001$) (Table 3). Pair wise comparisons revealed that mean percentage of new recruits was significantly larger in March 2001 than in other months, as well as in April 2002 than in other months (post-hoc Turkey's test, $p < 0.05$; Figure 2c).

For *T. hirsuta*, new recruits appeared in January, February, March, April until June 2001 (Figure 2d). A large peak of new recruits was observed

in March until May 2001, and again in November, December 2001, and March 2002. A significant effect of time on new recruits of *T. hirsuta* occurred (One-way ANOVA, $F_{16,51}$ = 9.689, p < 0.001) (Table 3).

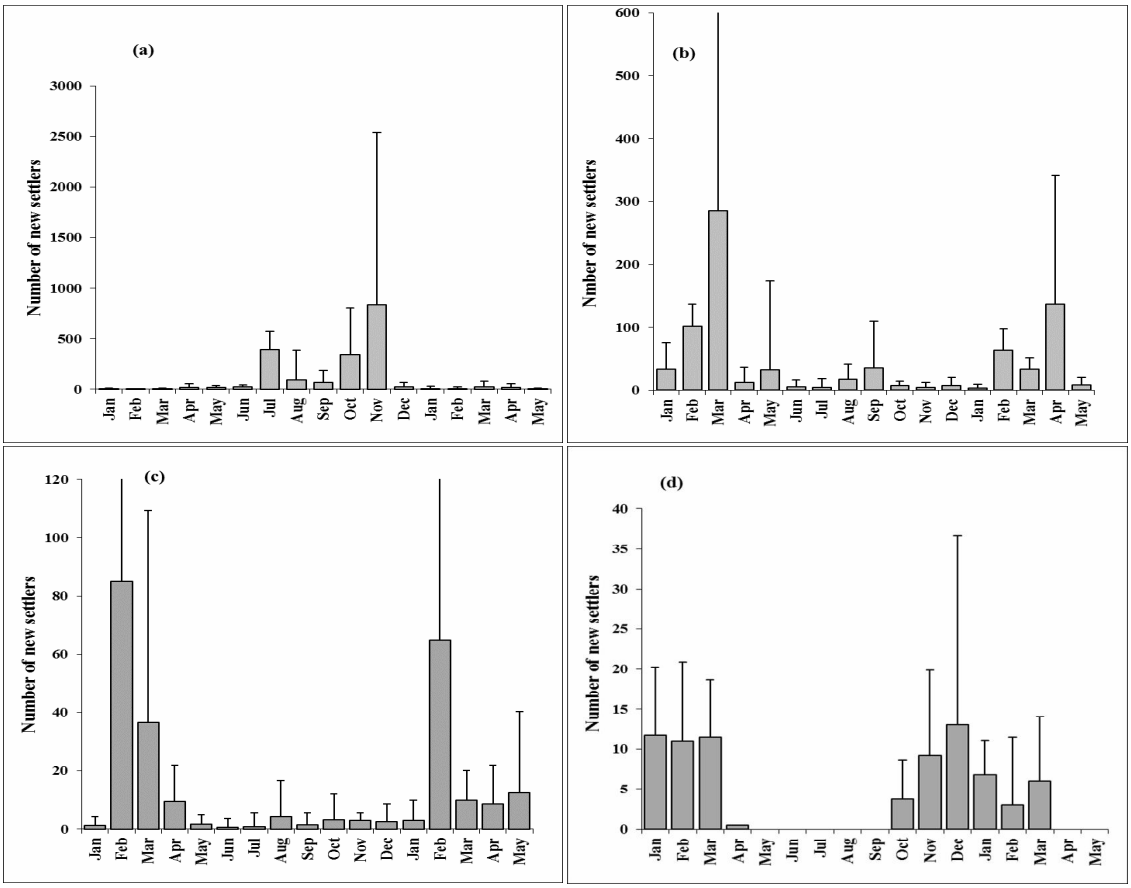

Figure 2. Mean percentage of new recruits of the four mytilid species: (a) *M. galloprovincialis*; (b) *B. erosus*; (c) *B. rostratus*; and (d) *T. hirsuta* investigated from January 2001 to May 2002 (Bars: 95% confidence interval).

The South Australian mytilids *M. galloprovincialis*, *B. rostratus*, *B. erosus*, and *T. hirsuta* populations found at Port Lincoln and the surrounding seawaters showed variation in spawning activity. In this study, *M. galloprovincialis* preferred spawning in cold seawater, which was most apparent from June to October, and up to warm seawater, when it appeared in September to October (spring). The spawning time of this species was similar to that of mussel in Fremantle waters, Western Australia (Wilson & Hodgkin 1967), and *Perna canaliculus* in New Zealand waters (Buchanan 2001). Shepherd & Thomas (1989) reported that seawater temperature in the Spencer Gulf of South Australia, including Port Lincoln, was about 13°C in winter and reached up to 26°C in summer. This seawater temperature in the winter of Port Lincoln and surrounding seawaters of South Australia was about the same as seawater temperature during the summer in northern hemisphere temperate regions such as in Sweden (Loo & Rosenberg 1983) and Finland (Antsulevich et al 1999), when mussel species, such as *M. edulis* spawns.

B. rostratus seems to prefer a high seawater temperature to spawn, which was in January to March, while *B. erosus* spawned when temperatures were warm to hot in November (spring) to January (early summer). Spawning of *B. rostratus* in this study was similar to *B. variabilis* in Western Australia (Wilson & Hodgkin 1967) and in Hongkong (Morton 1988), where spawning occurred when hot seawater occurred.

In a study of reproductive cycles of *T. hirsuta* in Queensland waters, Goggin (1994) showed that gonad development and spawning occurred throughout the year with three peaks. Environmental factors such as seawater temperature, salinity, and food

availability might affect mussel spawning (Sprung 1983; Hickman et al 1991; Seed & Suchanek 1992). In this study, spawning of the hair mussel appears to be more influenced by seawater temperature, since this mussel spawned in warm seawater in October to early hot seawater in December.

M. galloprovincialis produced about 7 million eggs in this study, which was slightly less than mussel in Sydney Harbor (Wisely 1964). However, *M. galloprovincialis* produced about four time larger number of eggs than the other three species investigated in this study. A large number of eggs is characteristic of marine invertebrates that produce planktonic feeding larvae (Young et al 2002), which stay in the water column longer than non-feeding lecithotrophic larvae.

This study suggests that larvae of both *B. rostratus* and *T. hirsuta* are grouped together with *M. galloprovincialis* as planktonic feeding larvae, because their egg diameter is less than 85 µm (Young et al 2002). *B. erosus* is likely to have a facultative lecithotrophic development with a short pelagic larval stage (Young et al 2002). It has a large egg diameter ranging from 91 to 106 µm. Then, the larvae of *B. erosus* might depend on planktonic food at the end of the larval stage.

Because spawning in the species investigated occurred at different times, the larvae of these species will also be available at different times in the seawater column of Port Lincoln and surrounding waters of South Australia. Mussel larvae may remain in the seawater column from two to four weeks before descending to the sea bottom to find suitable substrata, metamorphose, develop into juveniles, and finally to the adult stage (Widdows 1991; Young et al 2002).

Duration of larval stages in this study seem to be in agreement with the result of previous studies of mussel larvae, in which duration of free-swimming larval period depended on environmental factors such as temperature. Mussel larvae were sensitive to low temperature (Widdows 1991) and duration of larval stages were positively correlated with an increase temperature (Hrs-Brenko 1978; Satuito et al 1994). In the present study larvae developed over a range of temperatures. Temperate species such as *M. edulis* developed in normal condition and reached settlement stage in 22-32 and 18 days at 16 and 18^{o}C respectively (Sprung 1983; Pechenik et al 1990). Another temperate species, *M. galloprovincialis* was reported to have normal condition and settled in 12 days and 26-32 days at 22-26^{o}C and 18^{o}C respectively (Hrs-Brenko 1978; Satuito et al 1994). The larvae of New Zealand mytilids such as *M. edulis*, developed in normal condition and reached settlement stage in 14 days, when they were reared at 25^{o}C, and *Modiolarca impacta* and *Perna canaliculus* developed also in normal condition and reached settlement stage in 22 and 26 days respectively when reared at 22±2^{o}C (Redfearn et al 1986). In this study, the larvae of *M. galloprovincialis* reached settlement stage on day 16, *B. rostratus* and *T. hirsuta* on day 15, when they were reared at a temperature of 19±2^{o}C. However, the larvae of *B. erosus* reached a settlement stage on day 9, which is a shorter time than the other three mytilid larvae investigated. Overall, larval development of each species studied may not be controlled by seawater temperature alone, but also by specific life history traits as lecithotrophic or planktotrophic development as mentioned above. The present study is the first to describe duration time as free-swimming larvae for *B. rostratus, B. erosus* and *T. hirsuta*.

Development of South Australian mytilid species examined in this study followed the typical pattern for mytilids reported by other authors (Redfearn et al 1986; Buchanan & Babcock 1997). For three of the species reported here, *M. galloprovincialis*, *B. rostratus*, and *T. hirsuta* size remained the same from fertilized eggs through development to trochophore stage, but shape changed. For *B. erosus*, both size and shape changed when reaching trochophore stage. All mussel larvae had eyes and a large ciliated foot when reaching the stage competent of settlement. The larvae descended, swam and crawled at the bottom. Eyes, foot, swimming and crawling at the bottom are characteristics of late stage of mytilid larvae, which is called pediveliger (Young et al 2002). The larvae of *B. erosus* had eyes on day 9, which was different from the other three species in this study. In the laboratory, the larvae swam and crawled at the bottom, and metamorphosed a day after a suitable substrate was available.

Other differences between the species investigated were the size and shell shape along the development from D-stage until reaching metamorphosis. The size of early D-stage larvae of *B. erosus* was larger than *M. galloprovincialis*, *B. rostratus*, and *T. hirsuta*, but then *B. erosus* was smaller than *B. rostratus* and *T. hirsuta* at the end of larval stage. D-stage larvae of *M. galloprovincialis*, *B. rostratus, B. erosus*, and *T. hirsuta* in this study ranged in length from 93 to 101 µm, 100 to 104 µm, 124 to 130 µm, and 102 to 116 µm respectively, which is within the range of other mytilid species from New Zealand, such as *M. edulis*, *Perna canaliculus* and *Xenostrobus pulex* with lengths of D-stage larvae ranging from 86 to 136 µm, 75 to 135 µm, and 83 to 143 µm respectively (Redfearn et al 1986).

B. rostratus and *B. erosus* were larger at the earlier D-stage than other *Brachidontes* species, such as *B. solisianus* from Brazil marine water. Mean size of *B. solisianus* at D-stage veliger was 90 µm in length and 70 µm in height (Monteiro-Ribas et al 2006), while early D-stage larvae in this study was 104 µm in length and 83 µm in width (height) for *B. rostratus* and 124 µm in length and 104 µm in width (height) for *B. erosus*. With exception of *M. galloprovincialis* larvae, this is the first study on the larval development of three species, *B. rostratus*, *B. erosus*, and *T. hirsuta* from the family Mytilidae.

A peak number of new recruits varying in time among mytilid species might not only be linked to spawning time, but it might also be caused by differences in larval mortality, larval dispersal, settlement, metamorphosis and post-metamorphic mortality. This study revealed that the time of spawning differed among species. Once mussels spawned, larvae become available in the water column (Pineda 2000; Arribas et al 2016). However, larvae might need some time to grow and develop to the end of the larval stage when they descend, settle, metamorphose, and attach to substrata provided. The mytilid larvae settle and metamorphose on specific substrata (Ompi 2010; Carl et al 2012), where in this study the mussels attached on the mussel bed particularly on their adult shells and their adult byssus threads. Component materials from adults of marine bivalves become important settlement cues for bivalves larvae, for example, marine mussels (Ompi 2010, 2016; Carl et al 2012; Khalaman & Lezin 2015) as well as others bivalves such as oysters (Suquet et al 2014; Holm et al 2015; Lodeiros et al 2017). A growing in number of settling and attachment on favorable substrata perform aggregation or patch formation (Christensen et al 2015). Growing to certain sizes after attaching to attracting substrata as well as being preyed on by predators before being collecting could explain the differences in the number of new recruits observed during this study. The first settlers might grow to size of new recruits, while the latest settlers might not grow to the size of new recruits (Erlandsson et al 2008), which may have occurred in this study.

Conclusions. This study offers the first description of differences in south Australian Mytilid larvae. Variations in spawning time, sizes and shape during larval development were confirmed. *M. galloprovincialis* spawned in cold seawater, while *B. erosus* and *B. rostratus* released gametes in hot seawater, and then *T. hirsuta* spawned in warm seawaters. *M. galloprovincialis* produced larger number of eggs than other three species. *B. erosus* had larger eggs of diameter than other three Mytilids. The early D-veliger of *B. erosus* was larger in size length than other three larval species. However, *M. galloprovincialis* was larger in size length than the other three Mytilid species when it reached settlement stage. *B. erosus* reached settlement stage in short time comparing to other Mytilids. Different conditions including local condition, larval availability in water column, substrata availability, survival and growth of new settlement might determined variation in number of new recruits along the study time among four south Australian Mytilid species. Three Mytilid species, *B. erous*, *B. rostratus*, and *T. hirsuta* might compete both in space and food available in south Australian sea waters, since settlement of their competent larvae occurred most in the same time in warm to hot seawater.

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