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Original Paper

SETTLEMENT BEHAVIOUR AND SIZE OF MUSSEL LARVAE FROM THE FAMILY MYTILIDAE (*Brachidontes erosus* (Lamarck, 1819), *Brachidontes rostratus* (Dunker, 1857), *Trichomya hirsutus* (Lamarck, 1819), and *Mytilus galloprovincialis* Lamarck, 1819

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ABSTRACT

This study examines the settlement behaviour of the mytilids Mytilus galloprovincialis, Brachidontes erosus, Brachidontes rostratus, and Trichomya hirsutus larvae in response to different substrata: which were byssus threads of these four mussel species, coconut thread, and Polyvinyl chloride (PVC). The number of settlers on different substrata in the laboratory was analysed separately for each species using One-way ANOVA. A significant effect of substratum was found for all species tested. Larvae of T. hirsutus and B. erosus settled preferentially on conspecific byssus threads, while B. rostratus and M. galloprovincialis showed a similar trend. Settlement data from the field was analysed using two-way ANOVA with species and substrata as the main effect. Settlement was effected by species, but not by substrata. However, the overall settlement pattern indicated a conspecific preference with the lowest number of settlers on PVC substratum. Small size of settlement larvae of B erosus comparing to settlement larvae of T hirsutus, M galloprovinciallis, and B rostratus was observed.

Keywords: Mytilidae, mussels, larvae, settlement, substrates, Byssuss threads

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INTRODUCTION

Due to high mortality, settlement is a risky stage in the life history of many benthic marine invertebrates, including marine mussels (Thorson (1950). At this stage, many species of mussel larvae become photonegative, and tend to swim close to the bottom and search for suitable substrate for settlement (Bayne, 1965). Searching consists of repeatable swimming and crawling on substratum surfaces and settlement constitutes attachment by secretion of byssal threads (Lutz and Kennish, 1992). Many studies show that a suitable substrate is important at this stage. If a larva gets a stimuli or cue from a substrate, it will respond by attachment; if no stimuli, by rejection (Lutz and Kennish, 1992). If a larva does not settle, it swims back into the water column or continues searching. Mussel larvae can stay in the water column for several days up to months (Bayne, 1965). However, larvae may get swept away by currents from suitable substrate and remain in the water column where they are at high

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risk of being preyed on. Settling larvae metamorphose into spat or juveniles. Juvenile stages might have better adaptations to the benthic environmental conditions compared to larvae. As a result, there may be a reduction in risk might occur after settlement and metamorphosis.

Many studies show that at the end of larval stages, substrate cues may stimulate larvae to settle and metamorphose. Filamentous substrata were found to stimulate larvae of marine mussels to settle. Larvae of *Mytilus edulis* settled on filamentous substrata as reported by Bayne (1965); Suchanek (1978); Lane *et al.,* (1985). It is believed that mussel larvae settle first on filamentous algae, and then after reaching a certain size, migrate to mussel beds. Lane *et al.,* (1985) suggested that post larvae migrate by using the foot and long byssus threads to facilitate movement from filamentous algae to mussel beds. This pattern of settlement is known as primary and secondary settlement hypothesis. However, many studies have shown results in contrast to this primary and secondary settlement hypothesis. For example, mussel larvae of *Mytilus edulis* settle directly on adult mussels as shown by McGrath *et al.,* (1988); Svane and Ompi (1993). Direct settlement on adult mussels was also shown by mussel larvae of *Mytilus galloprovinciallis* (Cáceres-Martínez *et al.,*1994), *Geukensia demissa* (Nielsen & Franz 1995) and tropical box mussel, *Septiver billocularis* (Ompi 1998). Erlandsson & McQuaid (2004); McQuaid and Lindsay (2005) reported that larvae plantigrate of *Perna perna* attach on red algae, *Gelidium pristoides*, as well as on adult mussels. Settlement on different types of artificial substrate was also shown by larvae of *M galloprovinciallis* (Cáceres-Martínez, 1998; Ramírez & Cáceres-Martínez, 1999) and *M edulis* (Kamermans *et al*., 2002).

Mussel larvae can settle on different substrata, leaving the question of whether filamentous algae and other substrate stimulate larvae to settle. Whether larvae respond to various available substrate in early settlement might differ among larval species. Biological, physical, and chemical characteristics of a substratum appear to be important factors in determining settlement of most marine benthic invertebrates. One species may respond and settle on a particular substratum, while another species might not. In nature, marine mussels have patchy or aggregated distributions (e.g; Okamura 1986; Svane and Ompi 1993; Dolmer and Frandsen 2002; Rius *et al.,* 2006). Many factors might explain this pattern including settlement behaviour responding to substrate cues. However, in many cases, an individual can be observed isolated from an aggregation. In this case, many environmental factors such as topography, strong currents, and predators can modify the influence of substrate cues.

Results from many field and laboratory studies show that size at settlement varies. For example, Bayne (1965) reported that mussel larvae of *M edulis* at primary settlement in the laboratory were 260 µm in length. Variation in size from 248 µm to 321 µm of larvae settling on different filamentous substrata in the laboratory was also reported (Ester and Pechenik 1987). In a field study, Dare *et al.,* (1983) report the shell length of primary settlement larvae in Morecamble Bay (NW England) was 230 – 400 µm, and secondary settlers was 500 µm. Variation in length of Mytilid larvae settling in Vostok Bay and Possjet Bay of Peter the Great Bay in the Sea of Japan was also reported (Semenikhina *et. al.,* 2008). For example, larvae of *M. galloprovinciallis* settled at approximately 330 – 350 µm, larvae of *M trossulus* settled at approximately 300-330 µm, and larvae of *Septiver keenae* measured180 – 190 µm when settling.

This variation in shell length at settlement stage could be a species characteristic, though duration of observation time should be considered when differences

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in size length for the same species of new settlers are observed.

Most knowledge of the settlement patterns and size length of larvae when settling of *Mytilids* has come from studies of *Mytilus edulis* (see Bayne, 1964; Eyster & Pechenik, 1987), *Mytilus galloprovincialis* (see Lane *et al*., 1985; Cáceres-Martínez, 1998), *Mytilus californianus* (see Petersen, 1984), and *Perna perna* (Erlandsson *et al*., 2008). Little is known about the settlement behaviour and size of settling larvae of mussels from other genera of the family Mytilidae such as *Brachidontes* and *Trichomya* or of South Australian *Mytilid* species (See Wilson & Hodgkin 1967; Booth, 1977).

The question is thus whether pre-competent veligers exercise any substratum choice and if so, what are the preferences for settlement? Is there any size difference among species when settlement does take place? In this study I test experimentally whether four species of south Australian mytilids show settlement preference for six types of substrata, four of which are byssus threads from conspecifics. I also measure the size of four species of mytilids when settlement takes place. Knowledge of settlement patterns of these species may allow us to better understand adult distribution and abundance.

MATERIALS AND METHODS

Sampling

Four species of South Australian Mytilids (*Mytilus galloprovincialis*, *Brachidontes rostratus*, *Brachidontes erosus* and *Trichomya hirsutus*) were sampled during the spawning period. *Mytilus* were collected from mussel beds in Boston Bay, Port Lincoln, *B. erosus* from Trinity Heaven at Tumby Bay, *B. rostratus* from Coffin Bay, and *Trichomya hirsutus* from Louth Bay, South Australia, Australia.

Spawning and Development

The four species of mussels were taken to Lincoln Marine Science Centre, Port Lincoln, South Australia. *M. galloprovinciallis* was sampled and treated in the laboratory during June until July 2002, *T. hirsutus* in December 2002, and *B. erosus* and *B. rostratus* in January 2003. Mussels were cleaned and about 30 mussels were induced to spawn in the laboratory using temperature shock as suggested by Strathmann & Fernald (1992). After spawning, eggs and sperm were separately filtered using 100 μm mesh sieve to remove debris. About 27,000 eggs were suspended in 200 ml of filtered seawater (1 μm) in one litre Pyrex glass beakers (Mokady *et al*., 1993), and two to three drops of diluted sperm were mixed with the eggs. An hour after introducing sperm, 800 ml of seawater was added and the beaker transferred to a modified larval culture system (Strathmann and Fernald 1992). After about 24 hours, when the larvae reached the D-stage, the culture water was replaced with fresh filtered seawater. After that, the culture water was changed every 3 days. Larvae were held at a concentration of 20 larvae ml^{-1} at 20 – 22^{o}C. The larvae were maintained in this system until most of them were competent to settle, which is characterized by the appearance of eyes, foot, umbo, and the large adductor muscle (Mokady *et al.,* 1993)

Settlement Substrate

The settlement experiments were conducted as four separate experiments, one for each species. Six different substrate were chosen byssus threads of *Mytilus galloprovincialis, Trichomya hirsutus, Brachidontes erosus, Brachidontes rostratus*, coconuts fibers, and PVC. Each substratum, including coconut fibre and PVC, was replicated four times.

The byssus is secreted by the foot and can be divided into stem, threads, and plaque or pad (Lucas *et al*., 2002). Particularly at

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the stem of the byssus treads was cut as close as possible to the muscle and washed several times in filtered seawater before being mounted into holes drilled into the PVC plates. All types of substrata were arranged in a random pattern on PVC plates (24 cm x 24) cm. Each plate was divided into 24 squares of 1x1 cm. A 3 mm diameter hole was drilled in the centre of each of the 20 squares. The remaining 4 squares with no holes were considered as controls. Bundles of the four byssus and the coconut fibre substrata were fit into each hole by using small pointed tweezers until they were level with the plate.

Settlement Experiments

Each of the plates was placed on the bottom of a one litre glass beaker and filled with 900 ml of filtered sea waters. About 1000 competent larvae were pipetted into each beaker. The beakers were placed in the larval culture system at temperature of 20 – 22^{o}C. The experiments were run for 48 hours. After 48 hours each plate was removed carefully from the glass beaker and placed directly under a dissecting microscope to observe and record settlement.

Field Experiments

The same substrate and number of replicates per plate used in laboratory was also used in the field experiments. The plate size was also the same as was used in the laboratory experiment. However, four plates in each bed were applied. Each plate was attached to a brick using filamentous nylon string. In December 2002, the bricks were attached to ropes and suspended just below the low water mark from a jetty with dense patches of *M. Galloprovincialis*. The same number of bricks with experimental plates was also distributed in a *B. rostratus* bed in Coffin Bay, in a *B erosus* bed in Trinity Heaven at Tumby Bay, and in a *T hirsutus* bed in Loud Bay in December 2002. The plates were left in the field for one month. The plates were subsequently retrieved and the settled larvae recorded using a dissecting microscope.

Data Analysis

The settlement data of each larval species from the laboratory experiments was analysed using one-way ANOVA with species as the main effect, while data from the field experiments was analysed using two-way ANOVA with species and substrata as the main effects. The analysis was run using Super ANOVA (Abacus Concepts, 1991). To fulfil the requirement of ANOVA, the data were log (x + 1) transformed when required to obtain homogeneity of variances. Post hoc comparisons were performed using Student-Newman Keul's test (SNK-test) (Sokal and Rholf, 1981). Size length data obtained both from the laboratory and field were analysed using Kruskal-Wallis test (Fowler *et al.,* 1998).

RESULTS AND DISCUSSION

Laboratory experiment

A comparison of settlement of the four larval species on six different types of experimental substrate (byssus threads of four conspecific species, coconut threads, and PVC plates) showed a significant effect of substratum on number of settlers of each larval species (**Table 1**). All species showed a clear trend of preference for conspecific byssus threads, but with some significant variations.

An SNK post hoc test revealed that a significantly greater number of *M. galloprovincialis* larvae settled on conspecific byssus threads and byssus threads of *T. hirsutus* than on byssus threads of *B. rostratus*, *B. erosus*, coconut threads, and PVC (**Fig. 1**). *B. rostratus* larvae settled preferentially on byssus of its conspecific, on *B. erosus* and *T. hirsutus*. An SNK-test confirmed this with no significant differences

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in settlement on byssus of these three species but with a significant difference between byssus of *M. galloprovincialis*, PVC and coconut fibres (**Fig. 1**).

Table I. One factorial ANOVA on number of settlers for each of the four species with substratum as the main effects (**: P<0.01; ***: P<0.001). Data were log (x +1) transformed.

Source	df	SS	MS	F	P
M. galloprovincialis					
Between groups	5	1.591	0.318	4.44	0.002 **
Within groups	66	4.731	0.072		
Total	71	6.322			
B. rostratus					
Between groups	5	5.556	1.111	14.286	0.000 ***
Within groups	66	5.134	0.078		
Total	71	10.690			
B. erosus					
Between groups	5	3.165	0.633	10.671	0.000 ***
Within groups	66	3.914	0.059		
Total	71	7.079			
T. hirsutus					
Between groups	5	4.828	0.966	11.863	0.000 ***
Within groups	66	5.372	0.081		
Total	71	10.200			

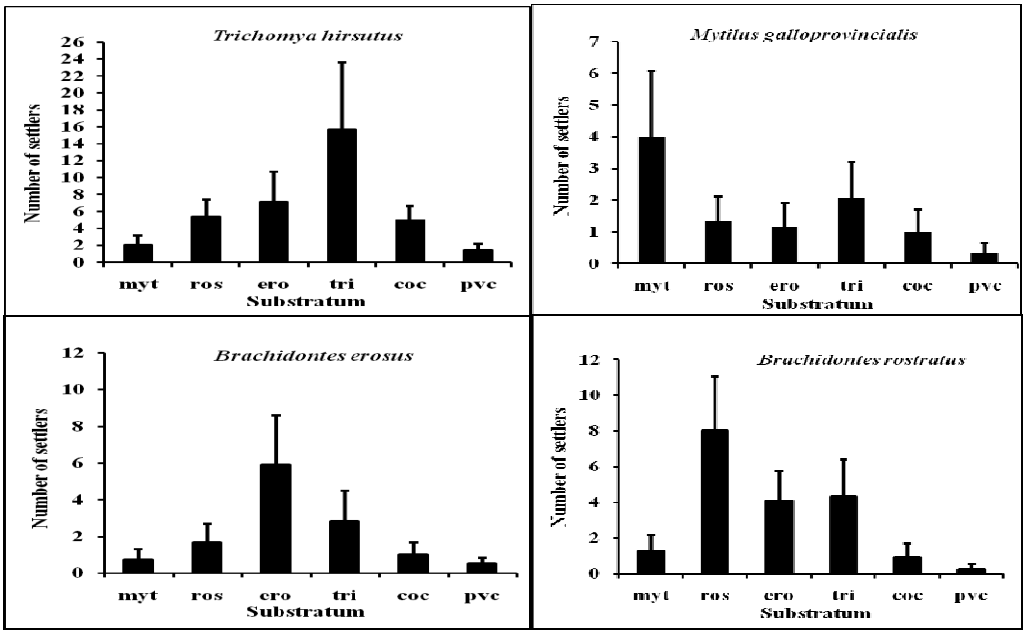

Fig1. Mean number of settlers of each species, *Trichomia hirsutus*, *M galloprovinciallis*, *Brachidontes rostratus*, and *Brachidontes erosus* on six types of substrata, myt=byssus threads of *M galloprovincallis*, ero=byssus threads of *B erosus*, ros=byssus threads of *B rostratus*, tri=byssus threads of *T hirsutus*, coc=coconut threads, and pvc=Polyvinyl chloride (PVC). (Error bars: 95 % confidence interval)

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For T. Hirsutus, a pattern of preferential settlement on conspecific byssus threads was found with a significant difference between this substrate and other substrate. No significant difference between B. rostratus, B. erosus and coconut fibre was evident. Neither was a significant difference in number of settlers on byssus threads of M. galloprovincialis, coconut fibre or PVC was evident (**Fig. 1**)

Field Experiment

The various types of settlement substrate that were placed in the field received few settlers. However, a pattern of settlement on conspecific and coconut threads appears for *T hirsutus*, *B rostratus*, and *B erosus,* but it is not clear for *M galloprovinciallis* larvae. PVC was not a favourable substratum for larval species of *B rostratus* and *B erosus*, since no settlement was found on this substratum. The result of settlement data of the four species observed in the field is presented in **Fig. 2**.

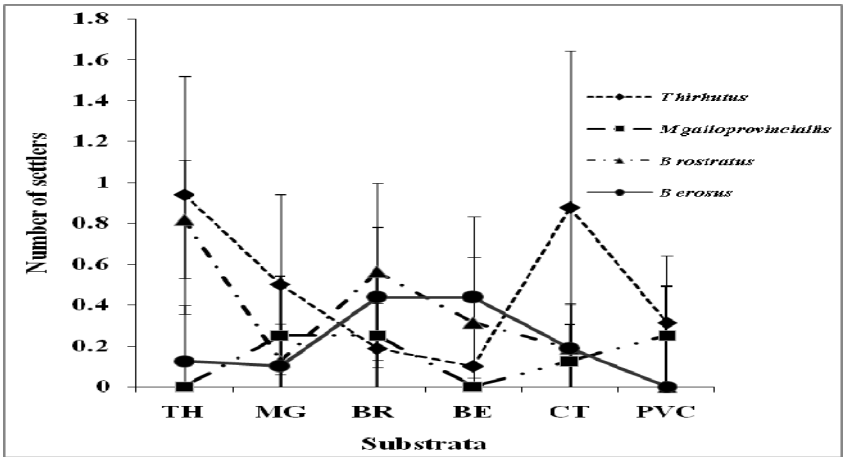

Fig 2. Interaction plots of a two-way ANOVA of number of settlers, with species and substrata as main effects. (Error bars: 95% confidence interval).

The settlement data were analysed using Two-Way ANOVA with larval species and substrate as the main factors (**Table 2**). The results show a significant effect of species ($P<0.05$), but no effect of substrate on settlement ($P>0.05$). A significant interaction was evidenced ($P<0.05$). The pattern of interactions is caused by settlement data on each substratum not consistent between species (**Fig. 2**).

Table 2. Two-way ANOVA. Number of settlement after transformation to log (X + 1), with Species and substrata as the main affect (**: P<0.01; n.s: not significant).

Source	df	SS	MS	F	P
Specie	3	0.299	0.100	4.811	0.003 **
Substrata	5	0.135	0.027	1.304	0.261 n.s
Secies*substrata	15	0.853	0.057	2.750	0.000 **
Error	360	7.449	0.021		

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The results showed that settlement was significantly different among species (P<0.05). A significantly higher number of settlers of *T hirsutus* than others species was evident (SNK-test: P<0.05). However, no difference in number of settlers among *M galloprovincialis* and *B rostratus* was found (SNK-test, P>0.05). The number of settlers was also not different among *M galloprovincialis* and *B erosus* (SNK-test, P>0.05), as well as among *B erosus* and *B rostratus* (SNK-test, P>0.05). The number of settlers for all of species is shown in **Fig. 3.**

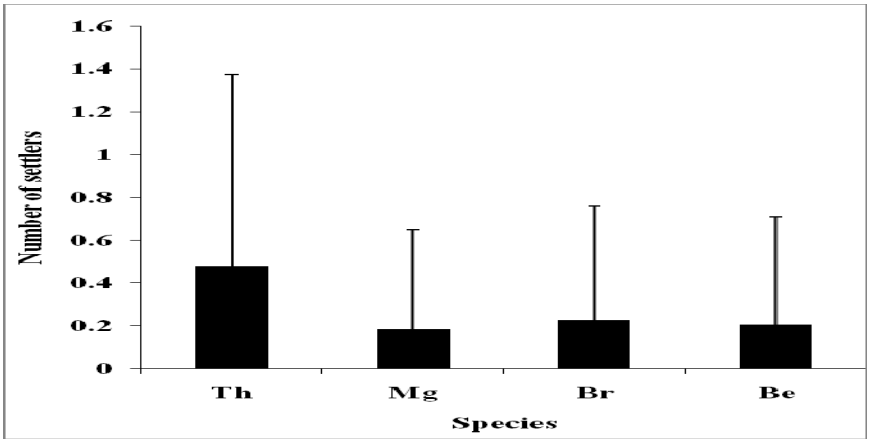

Fig 3. Mean number of settlers among four species, *Trichomia hirsutus*, *M galloprovinciallis*, *Brachidontes rostratus*, and *Brachidontes erosus*.(Error bars: 95 % confidence interval).

Therefore, the settlement data on each substratum for each species were pooled. An analysis settlement of each species was performed using one-way ANOVA, as shown in **Table 3.**

Table 3. One-way ANOVA. Number of settlement after transformation to log (X + 1), with species as the main effect (**: P<0.01)

Source	df	SS	MS	F	P
Species	3	0.299	0.100	4.483	0.004 **
Error	380	8.437	0.022		

Size

Settlement of larval mussels, *Brachidontes erosus*, *Brachidontes rostratus*, *Trichomya hirsutus*, and *Mytilus galloprovincialis* was observed in the laboratory. Larvae were characterized as having gills, reduced or absent velum, a foot and varied with sizes.

Size of *M. galloprovincialis* larvae varied from 228 to 278 µm (average 249 ± 13.25, 95 % confidence interval), larvae of *B. rostratus* ranged from 233 to 253 µm (ave. 245 ± 8.19,95 % confidence interval), *B. erosus* larvae were measured from 202 to 273 µm long (ave. 230.79 ± 10.23, 95 % confidence interval), and larvae of *T. hirsutus* larvae were 243 to 309 µm (ave. 278 ± 12.1, 95 % confidence interval).

Size length data of settlement larvae were statistically analysed by Kruskal-Wallis test. The settlement larvae were significantly different in size length (Kruskal-Wallis test, K=25.310, df 3, P<0.05). Settlement larvae of *T hirsutus* were larger in size length than

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other species of settlement larvae, where settlement larvae of *B erosus* was smaller in size length than other species. The size length of settlement larvae is shown at **Fig 4**.

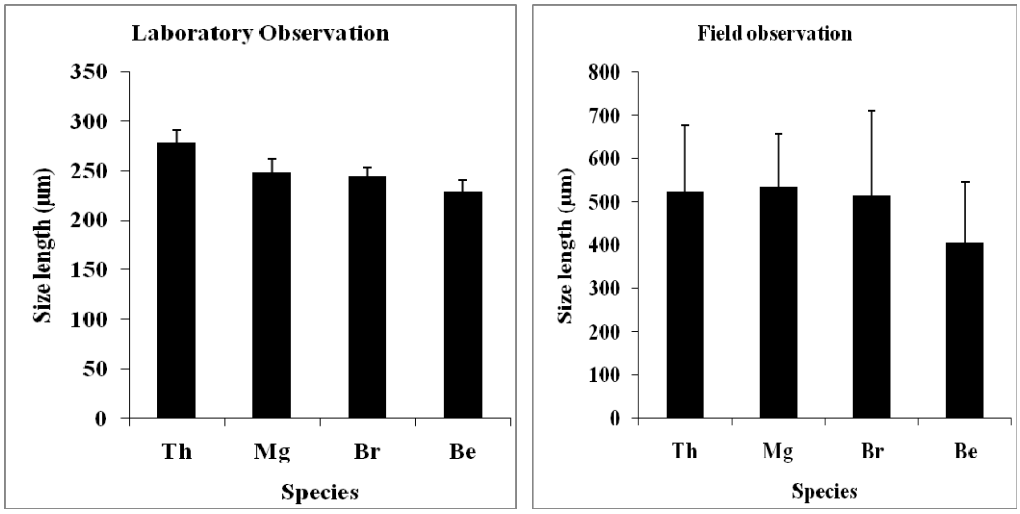

Fig 4. Mean shell length of each of settlement species, observed from Laboratory and field studies. (Error bars: 95 % confidence interval).

Settlement of each species larvae attaching on substrata in the field also varied in size length (**Fig. 4**). *M galloprovinciallis* was 296 until 953 µm (average 536.40 ± 161 µm 95 % confidence interval), larvae of *B rostratus* ranged from 273 until 892 µm (ave. 514 ± 198, 95 % confidence interval), larvae of *B erosus* ranged from 263 until 547 µm (ave. 407 ± 138, 95 % confidence interval), and settlers of *T hirsutus* was ranged from 324 until 928 µm (ave. 524 ± 138, 95 % confidence interval). The data was analysed using Kruskal-Wallist test. It shows that settlement larvae on six substrata for each species were not different among species (Kruskal Wallist test, P>0.05).

Settlement behaviour

Variation of settlement Mytilid larvae may be affected by larval response to different substratum cues. This study showed that in the laboratory, *T. hirsutus, B. erosus*, *B. rostratus*, and *M. galloprovincialis* pediveliger larvae preferentially settle on conspecific byssus threads but with different ability for selection. Larvae of *T. hirsutus* and *B. erosus* significantly settled on their own conspecific byssus threads, while larvae of *B. rostratus* and *M. galloprovincialis* settled in relatively high numbers on other species' byssus threads. However, the trend of highest mean settlement on conspecific byssus threads was clear for all four species. Coconut fibre was also a favourable substratum for *T hirhutus*. The number of larvae that settled on coconut fibre was low for the other three species and number settling on PVC was low for all four species. These results were confirmed in field experiments, particularly for *M. galloprovincialis*, *B. rostratus*, and *B erosus.*

Byssus threads are known to have the same physical characteristics but vary in diameter, length and colour. The byssus surface is covered with smooth hairs. All of these different physical structures might be recognized as settlement cues by each species of larvae. Many settlers produced byssus threads and attached on substrata,

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while others move and crawled on the substratum. The same behaviour was also reported for larvae of *Mytilus edulis*, particularly settling on the byssus of conspecifics such as reported by Lane *et al.,* (1985), Eyster & Pechenik (1987).

Competent larva of each species settled on conspecific byssus threads, and others substrata available might imply that all of competent larvae from these four species can settle on any substrata available. The pattern of direct settlement on substrata favourable is as settlement behaviour for the four of South Australian species, which are *M. galloprovinciallis*, *T. hirsutus*, *B. rostratus*, and *B. erosus*. The settlement behaviour is in accordance with the behaviour of settling directly on substrata related to any adult component. For example, mussel larvae of *Mytilus edulis* settle directly on adult mussels as shown by McGrath *et al.,* (1988); Svane and Ompi (1993). This settlement pattern was also reported for species of *M. galloprovinciallis* (Cáceres-Martínez *et al.,*1994), tropical box mussel, *Septiver billocularis* (Ompi, 1998), *Perna perna* and *Gelidium pristoides* (Erlandsson and McQuaid 2004; McQuaid and Lindsay 2005).

Many larval species have well-developed sensory organs at the end of their larval stage and may use these to identify settlement cues (Luz and Kennish 1992). Eyes are well developed at settlement in the four Mytilid species used in this study and could be clearly observed under a dissecting microscope. The foot is another organ that may help larvae to discriminate between different substrata. According to Richard *et al*. (1992), at the late larval stage of Mytilids, the foot has nine types of glands, each with a specific function during settlement.

The behaviour of detaching byssus threads as known for *M. galloprovincialis* larvae, when larvae tend to swim back into the water column in the early settlement (Bayne 1965), can also explain the variability of substrata preferences. *B, rostratus* may also have this behaviour. In contrast, larvae of *T. hirsutus* and *B. erosus* may stay firmly on their favourable substrata rather than swim back into the water column. Because many substrata were offered rather than just two, a large variance is to be expected as a consequence of the probability of finding any one type of substratum.

The larvae responded similarly to coconut fibers, which may be a consequence of its physical properties. Coconut fibre is oval in cross section, while byssus is round. The colour is also different. Recently, many kinds of non-marine material are introduced into the sea. Many of these may not only mimic the natural habitat for many marine invertebrates, but also attract larvae of many organisms including mussels (Svane and Petersen, 2001).

Rare settlers on PVC from all larval species were recorded. King *et al.,* (1992) observed that *Mytilus edulis* larvae settled on roughness or scarred surfaces rather than on smooth surfaces. Petersen (1984) observed that *Mytilid* larvae settled preferentially in holes and cracks in substratum surfaces. A PVC surface is smooth compared to other substrata and is likely to be rejected by the larvae, which consequently move to more preferable substrata, including different types of byssus threads as well as coconut fibres.

Settlement in the field may be influenced by many factors such as mortality, spawning season, and larval duration in the water column (Hunt and Scheibling, 1997). In this study, high number of *T. hirsutus* settlers compared to other settlers might have been caused by spawning season as well as mortality. December can be a peak spawning time for this species. As a result, larvae might be plentiful in the water column, as well as settling on suitable substrata. Consistently low numbers of settlers for *M. galloprovinciallis* might have been caused by a later spawning season, as described for South Australian Mussels (Ompi, 2003). Low spawning activity for *B. rostratus* and *B. erosus* might have resulted

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in low settlement. January and February can be a period of low spawning activity for these two species. Low numbers of settlers for these two species was evident.

Size at settlement

Development of pigmented eyes and a foot at the settlement stage was evident for all species. Size varied among species. Mussel larvae can range from 195 to 300 µm when they settle and metamorphose (Lutz & Kennish 1992). In the laboratory study, all of the species ranged within these sizes. Settlers of *B. erosus* ranged from 202 µm up to 273 µm, *T. hirsutus* ranged from 243 to 309 µm, and *B. rostratus* ranged from 233 to 253 µm. The range of size length of settlers was larger in the field. *B. erosus* ranged from 263 up to 547 µm long, *M galloprovinciallis* ranged from 296 to 953 µm, *T hirsutus* ranged from 324 to 928 µm, and *B rostratus* ranged from 273 to 892 µm.

Variance in size can be a species characteristic when settling. It can also be caused by length of time settlers have been on the substrate before sampling. Completion of metamorphosis and growth might occur on settlement larvae with size > 300 um, since changes in shell thickness and colour of many of settlers was observed. For example, brown and dark colour was observed for *B. rostrtus*, blue and dark color for *B. erosus*, and brown with hair for *T. hirsutus*. Particularly for *M. galloprovinciallis* had a thick transparent shell with gills appearing. Instead of crawling along the substrata, it is possible for this larva to swim back into the water column.

Among the four mussel species, small size can be a characteristic for *B. erosus* compared to other mussel species when settling. Many Mytilid species can have even smaller size lengths when settling. For example, *Septiver keenae* larvae were 180 to 190 µm, and *Crenella decussata* were 190 to 200 µm when settling in Possjet Bay of Peter the Great Bay in the Sea of Japan (Semenikhina *et al.,* 2008). Others Mytilid species observed from the same Bay have a large size, such as *Mytilus indeterminate*, ranging 280 to 300 µm, *Mytilus coruscus*, ranging from 330 to 350 µm, and *Mytilus trossulus*, ranging from 300 to 330 µm. The three South Australian species, *M. galloprovinciallis*, *T. hirsutus*, and *B. rostratus* can be grouped as having a large size when settling.

CONCLUSSION

Pediveliger larvae of *T. hirsutus, B. erosus*, *B. rostratus*, and *M. galloprovincialis* preferentially settle on conspecific byssus threads, with different ability for selection. Larvae of *T. Hirsutus, B. erosus,* and *B.rostratus* when reaching the competent stage for settlement, responded quickly to the substratum available by testing. *M. galloprovincialis* was more likely to test different substrata and tended to swim back into the water column. Pediveliger larvae were varying in size when settling indicating a species character. Larvae of *T. hirsutus*, *B.rostratus* and *M. galloprovinciallis* were large in size, while larvae of *B. erosus* were small in size when settling.

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Table I. One factorial ANOVA on number of settlers for each of the four species with substratum as the main effects (**: P<0.01; ***: P<0.001). Data were log (x +1) transformed.

Source	df	SS	MS	F	P
M. galloprovincialis					
Between groups	5	1.591	0.318	4.44	0.002 **
Within groups	66	4.731	0.072		
Total	71	6.322			
B. rostratus					
Between groups	5	5.556	1.111	14.286	0.000 ***
Within groups	66	5.134	0.078		
Total	71	10.690			
B. erosus					
Between groups	5	3.165	0.633	10.671	0.000 ***
Within groups	66	3.914	0.059		
Total	71	7.079			
T. hirsutus					
Between groups	5	4.828	0.966	11.863	0.000 ***
Within groups	66	5.372	0.081		
Total	71	10.200			

Table 2. Two-way ANOVA. Number of settlement after transformation to log (X + 1), with Species and substrata as the main affect (**: P<0.01; n.s: not significant).

Source	**df**	**SS**	**MS**	**F**	**P**
Specie	3	0.299	0.100	4.811	0.003 **
Substrata	5	0.135	0.027	1.304	0.261 n.s
Secies*substrata	15	0.853	0.057	2.750	0.000 **
Error	360	7.449	0.021		

Table 3. One-way ANOVA. Number of settlement after transformation to log (X + 1), with species as the main affect (**: P<0.01)

Source	**df**	**SS**	**MS**	**F**	**P**
Species	3	0.299	0.100	4.483	0.004 **
Error	380	8.437	0.022		

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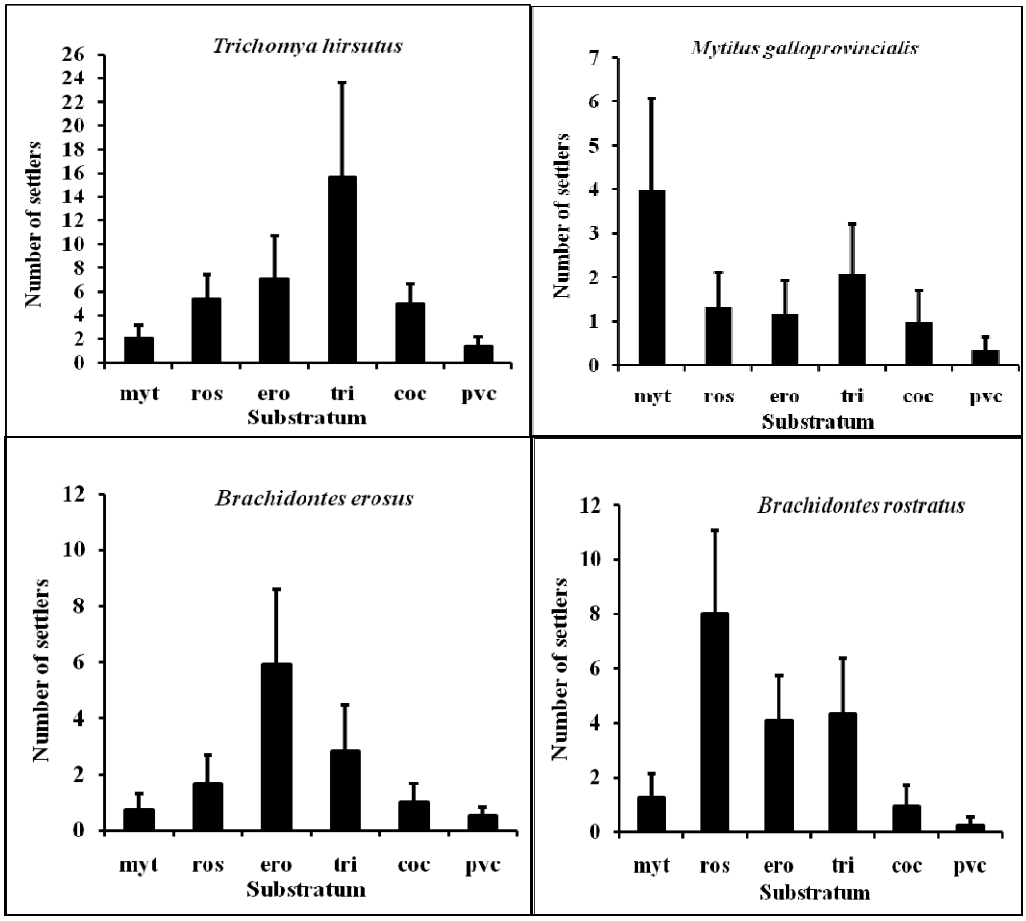

Figure 1. Mean number of settlers of each species, *Trichomia hirhutus*, *M galloprovinciallis*, *Brachidontes rostratus*, and *Brachidontes erosus* on six types of substrata, myt=byssus threads of *M galloprovincallis*, ero=byssus threads of *B erosus*, ros=byssus threads of *B rostratus*, tri=byssus threads of *T hirhutus*, coc=coconut threads, and pvc=Polyvinyl chloride (PVC). (Error bars: 95 % confidence interval)

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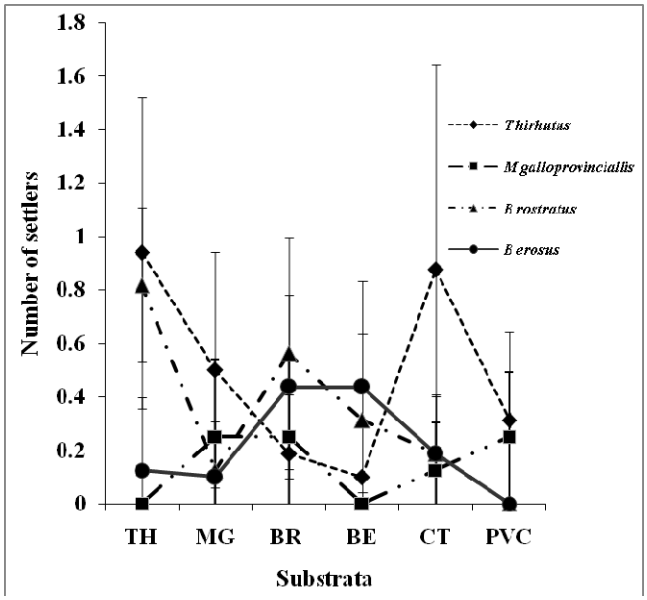

Figure 2. Interaction plots of a two-way ANOVA of number of settlers, with species and substrata as main effects. (Error bars: 95% confidence interval).

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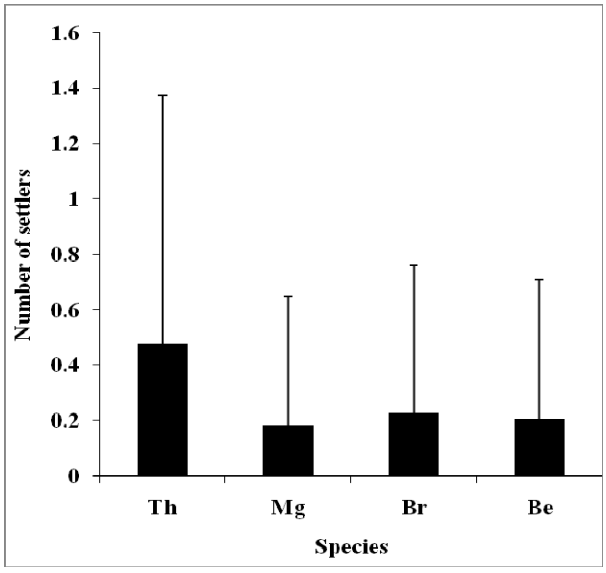

Figure 3. Mean number of settlers among four species, *Trichomia hirhutus*, *M galloprovinciallis*, *Brachidontes rostratus*, and *Brachidontes erosus*. (Error bars: 95 % confidence interval).

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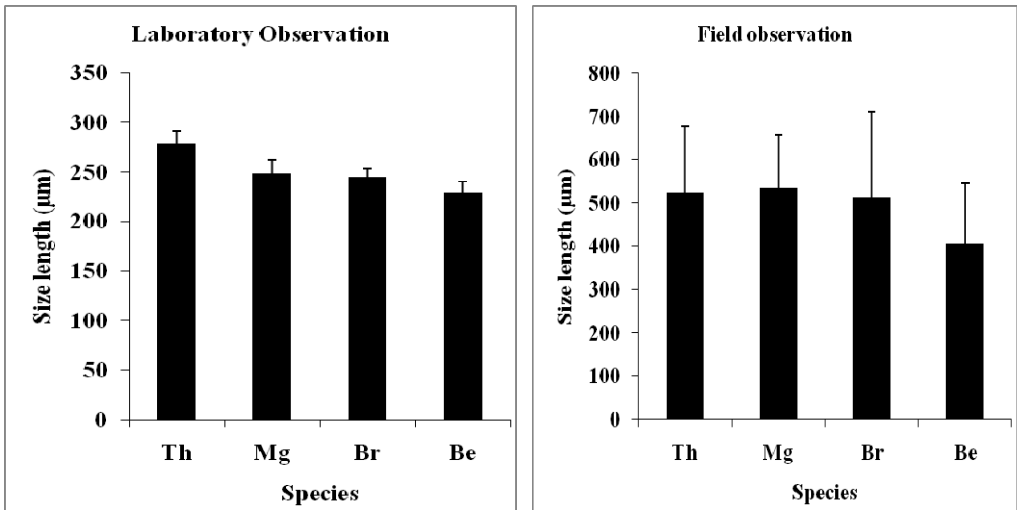

Figure 4. Mean shell length of each of settlement species, observed from Laboratory and field studies. (Error bars: 95 % confidence interval).