**ESTUARINE CARIDEAN SHRIMP (*PALAEMON DEBILIS* DANA, 1852)
(DECAPODA: CARIDEA) CAN DIFFERENTIATE OLFACTORY CUES FROM
DIFFERENT MANGROVE SPECIES FOR MICROHABITAT SELECTION**

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ABSTRACT

Environmental cues in mangroves such as chemicals released from senescent leaf litter could help guide juvenile nekton to their nursery habitats. A laboratory olfactory choice experiment was conducted using a three-channel choice flume to assess the ability of juveniles of the caridean shrimp *Palaemon debilis* Dana (1852) to distinguish between water containing leachates from the leaf litter of the mangroves *Avicennia marina* (Forsk.) Vierh., *Rhizophora stylosa* Griff. and a mixture of the two species. Senescent leaves of these species of mangroves were soaked for 14 days in artificial seawater before being exposed to juvenile *P.*

debilis in the flume. *P. debilis* spent significantly more time in *R. stylosa* water, followed by mixed *R. stylosa* and *A. marina* water, and least time in *A. marina* water. The consistent preference for *R. stylosa* water suggests that the chemical cues from senescent leaves of this mangrove species might influence choice selection by the shrimp. The olfactory choice experiment was further investigated to assess if the preference for senescent *R. stylosa* leachate (water) was due to the water-borne chemicals (either from the senescent *R. stylosa* leaf litter water or aged *R. stylosa* detritus) serving as an attractant to the shrimps, by comparing it to aged *R. stylosa* detritus and artificial seawater. Juvenile shrimp preferred senescent *R. stylosa* water, therefore suggesting a role of chemical attractants in the shrimp's choice of habitat.

KEY WORDS: chemical cues; choice selection; juvenile nekton; mangrove litter; olfactory choice test

1.0 INTRODUCTION

One of the paradigms in mangrove ecology is the habitat's role as a nursery for juvenile nekton, where survival and thus contribution to the future adult population is enhanced by the benefits of shelter, food, and protection from predators (Nagelkerken 2009; Nagelkerken et al. 2008; Sheridan and Hays 2003). Most species of juvenile nekton found in mangroves, however, spawn offshore as adults so juveniles must possess effective means of locating their preferred nursery habitats (Beck et al. 2001; Dahlgren et al. 2006). The juveniles most likely utilise mangroves as a refuge area and to search for food during the high tide using environmental cues such as olfactory chemicals to home in on the preferred habitat (e.g. (Huijbers et al. 2008)). While coastal hydrodynamics play a strong role in transporting and trapping larvae in particular water bodies, environmental cues such as sound, magnetic field,

electric field, currents, light and water chemistry may play an important role in determining the final habitat chosen for settlement by juvenile nekton as reviewed by Kingsford et al. (2002).

Sensory cues are important in helping juvenile nekton (of both pelagic, benthic and plankton species) differentiate and select habitat for settlement. Olfactory cues play a role in helping juvenile fishes to detect suitable settlement habitat including chemicals from reefs (Atema et al. 2002; Gerlach et al. 2007), seagrasses (Arvedlund and Takemura 2006), mangroves (Huijbers et al. 2008), and coral reefs (Lecchini et al. 2013). Apart from single sensory cues, multiple sensory cues (olfaction, vision, and hearing) also influence settlement of coral reef fishes (Huijbers et al. 2012), with fishes using multiple sensory cues in sequence in their orientation towards their settlement habitat.

Apart from coral reef fishes, olfactory cues have been shown to play an important role in the recognition of habitats by crustaceans (Diaz et al. 2003; Lecchini et al. 2010; Welch et al. 1997), predator's avoidance (Chiussi 2003; Diaz et al. 2001; Forward Jr. et al. 2003), and the presence or absence of conspecifics (Diaz et al. 2003; Lecchini et al. 2010) and heterospecifics (Lecchini et al. 2010). Chemical cues are also used to detect the presence or absence of heterospecifics and identification of potential hosts by a parasitic barnacle (Boone et al. 2003) and symbiotic brachyuran crabs (Castro 2015). Cues from conspecific adults (Gebauer et al. 2002) and substrate (Diele and Simith 2007) can also delay or expedite metamorphosis. In general, crustaceans such as amphipods, lobsters, crayfishes, and crabs are known to use chemical cues to find mates, find favoured food and habitats, signal dominance, recognize known conspecifics, and assess the presence of predators (Hay 2011).

The primary sense used in detecting chemical cues over long distances in fishes is olfaction (Leis et al. 2011), as it is probably most effective in distant habitat detection. The olfactory system in crustaceans is through the antennular chemical sensing (Derby 2000). The

chemical senses in decapod crustaceans can be discovered by either their aesthetasc or non-aesthetasc pathways (Derby 2000). A study conducted by Horner et al. (2004) showed that both the aesthetasc and non-aesthetasc pathways are involved and showed functional overlap in the food localization behaviour by the Caribbean spiny lobsters.

While most cues are waterborne, some lipid-soluble compounds are still used as cues (Hay 2011). Chemical cues affect not only individual and population-level processes, but also community organization and ecosystem function. The chemical cues from phytoplankton-generated dimethyl sulphide (dispersed in either water or air) had a cascading effect on ciliates, zooplankton, fishes, sea birds, and potentially, desert plants, desert animals, and global weather patterns (Hay and Kubanek 2002).

Little is known about chemical cues guiding juvenile nekton to nursery sites in mangroves. While it has been shown that mangrove waters attracted the post-larvae of a reef fish (Huijbers et al. 2008), no studies have yet tested if chemical cues from the leaf litter of different mangrove species could determine the choice preference of juvenile nekton utilising mangrove areas. This ability is potentially important as different species of mangrove offer different structural complexities as well as biogeochemical environments relevant to survival of juvenile nekton.

Postlarvae of palaemonid shrimps (Natantia) made active habitat choices with a preference for macroalgae over live coral, dead coral, and sand in a laboratory based experiment (Lecchini et al. 2010). Laboratory studies conducted on reared juvenile coral reef fishes by Dixon et al. (2008) also showed that the olfactory preference was innate rather than learned, where the reared juvenile coral reef fish had the ability to exhibit the same response to the water treated with anemone scent and rainforest vegetation, similar with the field juveniles. Based on this ability, it is possible that juvenile nekton will be able to distinguish chemical cues from different species of mangroves. Understanding how and why juvenile

nektons are associated with mangroves may contribute in assessing the importance of mangrove diversity in habitat function and thus the delivery of beneficial ecosystem services. It is anticipated that chemical cues from leaf litter of mangroves are one of the most likely agents influencing the preferred habitat of juvenile nekton.

Different mangrove species may release characteristic chemicals at different rates during the decomposition process. Initial leaching of soluble chemicals happens most rapidly during the first 10-14 days of decomposition when up to 50 % of the dry mass could be lost (Lee 1989; Robertson et al. 1992; Steinke et al. 1993). Mangrove leachates comprise a diverse mixture of mainly soluble organic substances (Popp 1984a; Popp 1984b; Popp et al. 1984). These sugars, tannins, and other phenolic compounds may have distinctly different appeal to juvenile nekton as cues for determining habitat choice.

This is a laboratory study aimed to investigate whether juveniles of the caridean shrimp *Palaemon debilis* (Palaemonidae), a common species in mangrove-lined estuaries in the Indo West-Pacific, are able to differentiate chemical cues from different mangrove leaf litter leachates in their location of preferred choice and also to determine whether the functional chemicals might be serving as an attractant or deterrent.

2.0 MATERIAL AND METHODS

2.1 Study Site and Collections

Palaemon debilis larvae undergo seven zoeal and one megalopal stages before they reach the juvenile stage, with the megalopae transforming into the juvenile stage of around 7 mm (Shokita 1977). Based on field observation, the size structure of *P. debilis* present in the estuary of Tallebudgera Creek had a range of total length (rostrum to telson) of between 5 mm and 25 mm. The individuals that we used in the experiments were therefore first juveniles that would be actively seeking their eventual habitat. The behaviour of being

influenced by chemical cues from mangroves would also facilitate the choice of foraging habitats during the tidal migration of juveniles and adults.

Juvenile individuals of the common estuarine caridean shrimp (*P. debilis*) were captured by using a hand-net at intertidal pools adjacent to a mangrove forest consisting of two species of mangroves (*Avicennia marina* (Forsk.) Vierh. and *Rhizophora stylosa* Griff.) at Tallebudgera Creek (28°6'25"S, 153°26'55"E), southeast Queensland, Australia, during the low tide in autumn, April 2013. The mangrove forest along Tallebudgera Creek is approximately 44 hectares dominated by a mixture of *R. stylosa* and *A. marina*. Juvenile *P. debilis* have commonly been observed moving into the mangroves during high tide at this site although there is no formal report of their abundance. Juvenile *P. debilis* move into the mangroves during the high tide, perhaps driven by the mangrove cues to seek shelter and food. *P. debilis* was the most abundant invertebrate sampled during a separate field study conducted in the same area and there is a resident population in the estuary. There is little information available on the biology of *P. debilis*.

Individuals used in the experiment comprised only juveniles, which had a range of total length of between 7 and 10 mm measured from the tip of the rostrum to the posterior end of the telson (body length is between 5 and 7 mm). This size class was most frequent among shrimps trapped in the intertidal pools adjacent to the mangrove forest during the low tide. The adult size of this shrimp is approximately 35 mm (Poupin 2007).

The shrimps were maintained in a plastic pail in aerated seawater upon collection. All shrimps were transferred to a glass aquarium tank and kept in aerated seawater (salinity 28) at 20°C in the laboratory for 24 hours and shrimps were not fed during the acclimation period prior to the start of the experiments. This is to starve the shrimps so that it will initiate food searching response prior to the experiments. The salinity of 28 was chosen to match the salinity where the shrimps were captured.

2.2 Experimental Design

The experiments were carried out in a constant temperature room (20°C) with the use of a rectangular flow tank, which contained a three-channel choice flume, allowing three water sources to be used simultaneously during the choice test. This design avoided having too many between-test comparisons before a preference pattern could be established. This is a modified design of a two-channel choice flume used in previous studies (Atema et al. 2002; Huijbers et al. 2008). The design allows three water sources to flow through the channel flumes and mix at the downstream end of the flume, i.e. E - the starting position of the juvenile caridean shrimp / zone with mixed water from all three flumes (Fig. 1).

The test area (12 cm x 7.5 cm) of the three-channel choice flume had a water depth of 2.5 cm (Fig. 1). The time spent by the shrimp in each flume carrying a different water type was monitored in the observation area (Fig. 1). Three different types of water (see below) were gravity fed from three different respective storage tanks and flowed into the three individual flumes that were partitioned using 6 cm flume barriers. The storage tanks were connected to the rectangular flow-tank's water inlet according to the three individual flumes (left, middle and right) by a series of connectors, delivering the desired water type to specific flumes.

Artificial seawater (a mixture of 1.8 kg of artificial salt and 50 l filtered tap water, salinity 28) was used for soaking mangrove leaves as the experiment water and as a neutral control during the acclimation stage. The rectangular flow tank was placed in a large darkened tank to ensure the shrimp would not be unduly influenced by external disturbances, including the observer.

The flow speed was measured and maintained at 0.5 ml per second by filling a measuring cylinder with water exiting the flow-tank (Fig. 1) over a fixed amount of time (190

ml in 60 s) and a flume cross-sectional area of 6.25 cm². The water was released from the water storage tank to the tubes that were attached to the flow-tank inlets. The flow speed was selected based on preliminary tests, as it was strong enough to stimulate the shrimp to swim against the flow while not too strong to tire the animal.

At the beginning of the experiment, water was released from the storage tank until it filled up the flow tank. The control valve at the water outflow end was then opened and adjusted to release the water. This regulated the desired water flow and water level. This step was important in order to control the water level to prevent overflowing and to maintain the desired flow speed in the tank. The water flow for each flume was adjusted accordingly to achieve laminar flow. The flow speed was maintained the same for all experiments. A downstream mesh net was placed near the end of the flow tank to avoid the shrimp from getting washed out during the experiments.

Tests using non-toxic coloured food dyes (Queen brand) were conducted before the start of each experiment to ensure maximal flow separation following the flume barrier with minor turbulence (Fig. 2). The three flow streams separated up to the downstream mesh net, where the shrimp could swim freely between the water masses. This also ensured the flow speed was constant before the actual test. Juvenile shrimp were released at the downstream end of the flume and were free to move towards the preferred water source.

2.3 Olfactory Choice Experiments

2.3.1 Experiment 1

This experiment was run to see if there was any flume bias in factors other than odour influencing shrimp choice. It is anticipated that there will be slightly flume bias due to the flow rate and local conditions such as turbulence. Each juvenile shrimp was exposed to the same water type for all the flumes. The water sources used for water type A were a

combination of *Rhizophora stylosa* and *Avicennia marina* (RS + AM) water, water type B - *R. stylosa* (RS) water and water type C - *A. marina* (AM) water. At the start of each experiment, a shrimp was acclimated in the flow tank for 3 min in flowing artificial seawater to ensure removal of any potential residual chemical cues from the previous before exposing it to any possible chemical cues from the different water types (Experiment 1) or three water types for experiments 2 and 3. 3 min was chosen for acclimation as any potential residual chemical cues prior to the start of experiments would have been completely removed. Each shrimp was assigned to a sequence of 3 same water type-flume combinations (e.g. AAA, BBB and CCC). After each test (3-min) (e.g. AAA), the test was repeated with a different water type, but with all flume positions discharging the same water (e.g. BBB or CCC). The time spent in each of the three flumes was recorded during the 3-min testing period for each of the three tests. 3 min was chosen for the experimental runs as the shrimps would show its preferences in the first 3 min of exposure to the chemical cues (Huijbers et al. 2008). The experimental protocol was performed on 32 individuals of juvenile *P. debilis*. Each shrimp was used only once for each experiment. A complete experiment comprising 3 tests for each shrimp producing 9 data points. The total number of data points was therefore 288. There was a 1-min period for switching of water types to the rectangular flow-tank inlets between successive combinations. During the 1-min switching period, the shrimps were repositioned to E (Fig. 1) between the tests by exposing a hand shadow above the shrimp and this had influenced the shrimps to return to E (Fig. 1). A complete experiment for each individual lasted for 14 min (3 min acclimatisation, plus three tests for a total time of 9 min choice test, plus 2 min switching period). The unit for the time spent by the shrimps in each flume is in seconds. The control test was only conducted for Experiment 2.

2.3.2 Experiment 2

This experiment tested whether juvenile *P. debilis* was able to distinguish chemical signals from mangrove leaf litter leachates and their combinations. It is anticipated that *P. debilis* would be able to distinguish chemical signals from mangrove leaf litter leachates and choose the preferable cue. The water sources used were 1) a combination of *Rhizophora stylosa* and *Avicennia marina* (RS + AM) water, 2) *R. stylosa* (RS) water, and (3) *A. marina* (AM) water. Air-dried senescent mangrove leaves (100 g/type) were soaked and aerated in three separate storage tanks in 50 L of artificial seawater (salinity of 28 ppt) for the duration of two weeks. For the RS + AM water, 50 g of air-dried senescent leaves of each species were soaked in one tank. The soaking process reduced the concentration of some of the chemicals such as tannins (similarly to leaching process e.g. in Hernes et al. (2001)) and other phenolic compounds known to be strong deterrents to meiofauna (Alongi 1987a; Alongi 1987b) and standardised as well as conditioned the leaves to resemble the field condition, i.e. partially decomposed litter, in terms of their leaching state.

For all experiments 1, 2 and 3, water was drained out after the two-week soaking period and replaced with a new volume of artificial seawater, aerated and left for 24 hours before commencing the experiment. A 24 hour period was chosen to allow the leaching of significant soluble chemicals from the mangrove leaves before the test. Water from the same batch was used only for up to three days to ensure that the concentration of the chemical cues from the mangrove leaves were roughly equal throughout the replicate tests.

In general, the experimental protocol followed that used in Experiment 1. Each shrimp was assigned to a random sequence of six consecutive water type-flume combinations (e.g. ABC, BCA, CAB, ACB, BAC and CBA) in experiments 2 and 3. After each combination (3 min) (e.g. ABC), the test was repeated with a different combination of water type-flume position (e.g. BCA), to exclude a possible effect of flume position and also the possibility that a shrimp was remaining in the same water type without sensing the new

water types in the other flume positions. There was a 1-min period for switching of water types to the tank inlets between successive tests. The time spent in each of the three flumes was recorded during the 3 min testing period per water type-flume combination. For Experiment 2, the experimental protocol was performed on 32 individuals. Each juvenile shrimp was used only once for each experiment. A complete experiment comprising 6 tests for each shrimp producing 18 data points. The total number of data points was therefore 576.

A complete experiment for each individual lasted for 23 minutes (3 min acclimation, plus 6 tests where each test lasting for 3 min for a total time of 18 min choice test, plus 2 min switching period). Each shrimp had two values of time spent per water type-flume combination (e.g. in ABC and ACB, there are two values of time spent in water A of the same flume). These two values were calculated and the said value was the time spent in water A for the respective shrimp. The mean time spent in each of the three flumes per water type-flume combination for 32 individuals of juvenile shrimp was calculated from this.

2.3.3 Experiment 3

This experiment was conducted to confirm if the preference for *R. stylosa* water observed in Experiment 2 (see results of Experiment 2) was due to the waterborne chemicals (either from the senescent *R. stylosa* leaf litter water or aged *R. stylosa* detritus) serving as an attractant to the shrimps. The water sources that were used for this experiment were 1) *R. stylosa* water, 2) aged *R. stylosa* detritus water, and 3) artificial seawater only. The preparation of the senescent leaves of *R. stylosa* was similar to that in the first experiment. Two separate tanks were prepared for the other water sources. Air-dried, aged *R. stylosa* detritus was added into the storage tank while only artificial seawater was used in the third tank. All three water sources were aerated for 24 hours before commencing the experiment. Aged *R. stylosa* detritus was collected from the top 10 cm of the mangrove sediment and adjacent mudflat. The detritus was cleaned to remove excess sediment and air dried to remove surface water.

Upon drying, only *R. stylosa* fragments were selected and used for the experiment. The experimental protocol was similar to that of Experiment 2. Aged *R. stylosa* detritus was used in this experiment as it is anticipated that the shrimps will prefer decomposing than senescent *R. stylosa* leaf litter due to microbial enrichment in the former.

In all three experiments, artificial seawater was used during the 3 min acclimation stage as it contained no biogenic chemical cues. If the olfactory preference for any of the treatments reflected attraction due to the presence of leaf chemicals, the shrimp would be positively attracted to the leachate compared to the neutral control.

2.4 Data Analysis

For experiments 2 and 3, a two-way mixed model was used to test if water type in which the juvenile shrimp was offered the six different water-flume combinations affected their choice response. The mixed model allowed the analysis of whether respective water types in six different water-flume combinations had an effect on the shrimp (repeated measures), in terms of the time (dependent variable) spent at each respective water type-flume combinations. Time and the respective water type in six different water-flume combinations (treatment group) are fixed factors. The Shrimp (ID of each test subject) is a random variable. For the control test (Experiment 1), a one-way ANOVA was performed to test if the same water type-flume combinations affected the shrimp's choice response. For Experiment 1, a mixed model or repeated measures was not used as the levels of the repeated effect are not different for each observation within a repeated subject, i.e. all flumes carried the same water type.

Percentages of time spent in the six different water-flume combinations were arcsine square root transformed based on the proportion of time (for experiments 2 and 3 data sets) to meet the assumption of normality. For control test (Experiment 1) data set, Levene's test showed that homogeneity of variance had not been violated, hence the samples have equal

variances. For all experiments a normal Q-Q plot of residuals showed that data were normally distributed. All statistical analyses were conducted using SPSS version 21. Post-hoc Bonferroni test (for experiments 2 and 3 data sets) and Tukey HSD (for Experiment 1) were performed when there was a significant main effect.

3.0 RESULTS

Most shrimp showed a strong preference for one of the three types of water offered, but still occasionally switched to either one of the two other flumes during the experiments. Generally, within the 3 min test, the shrimps entered the flume that contained senescent *R. stylosa* leaf litter water more frequently compared to the other two water types. The 32 shrimps tested in each experiment showed a clear choice in 47 % (Experiment 1), 51 % (Experiment 2), and 53 % (Experiment 3) of the overall test time by remaining within a particular flume (section D, Fig. 1), rather than in the mixed water section (section E, Fig. 1). All animals tested (96 shrimps) showed clear choice during the experiments, by remaining within a particular flume.

3.1 Experiment 1 (Control Test)

The control test confirmed there was a significant effect of the time spent by the shrimps in the same water-flume combinations according to flume position. For water type A (RS+AM), the time spent in right flume was higher than the time spent in middle flume ($p = 0.00$; Fig. 3, Appendix 1). For water type B (RS), the time spent in both left and right flume were higher than the time spent in middle flume ($p < 0.001$, Fig. 3, Appendix 1). For water type C (AM), the time spent in the right flume was higher than the time spent in the left ($p = 0.014$) and middle ($p < 0.001$) flumes (Fig. 3, Appendix 1). All other interactions were not significant (Appendix 1). Therefore, the actual choice tests in Experiments 2 and 3, were conducted

using random sequences of six water type-flume combinations to eliminate flume bias by using the mean percentage of time spent by the shrimps for each water types in each respective flume.

3.2 Experiment 2 (Mixed Versus Individual Mangrove Odours)

The tests of fixed effects showed that shrimps spent significantly different amounts of time among the three water types ($F_{2, 195.7} = 4.15$, $p = 0.017$; Table 1). Post-hoc analyses with Bonferroni ($\alpha = 0.0167$) showed that the time spent in RS water was significantly higher than that in AM water ($p = 0.014$, Fig. 4; Appendix 2). There was no significant difference between the times spent in RS + AM water and either RS or AM water (Fig. 4; Appendix 2).

Table 1. Experiment 2. Results of a two-way mixed model ANOVA on the effects of water type (WT) and water type in which the juvenile shrimp was offered six different water-flume combinations (WF). Significant test results are highlighted in bold.

Source	Numerator df	Denominator df	F	p
WT	2	195.726	4.154	0.017
WF x WT	10	338.233	17.108	<0.001

The tests of fixed effects also showed a significant interaction of water types in six different water-flume combinations ($F_{10, 338.2} = 17.11$, $p < 0.001$; Table 1) based on time spent in the water types according to the six different water-flume combinations. Post-hoc analyses with Bonferroni ($\alpha = 0.0167$) showed the time spent in the water types varies according to the six different water-flume combinations (Fig. 4).

In the first water-flume combination (ABC), the time spent in AM water was significantly higher than the time spent in RS water ($p < 0.001$; Fig. 4, Appendix 3). There was no significant difference between the times spent in the AM water and RS + AM water

and also between the times spent in the RS + AM water and RS water (Fig. 4, Appendix 3).

In the second water-flume combination (BCA), the time spent in AM water was significantly lower than the time spent in either the RS + AM water ($p < 0.001$) and RS water ($p = 0.005$; Fig. 4, Appendix 3). There was no significant difference between the times spent in the RS + AM water and RS water (Fig. 4, Appendix 3). In the third water-flume combination (CAB), the time spent in RS water was significantly higher than the time spent in either the AM water or RS + AM water ($p < 0.001$; Fig. 4, Appendix 3). There was no significant difference between the times spent in the AM water and RS + AM water (Fig. 4, Appendix 3). In the fourth water-flume combination (ACB), the time spent in AM water was significantly lower than the time spent in either the RS water and RS + AM water ($p < 0.001$; Fig. 4, Appendix 3). There was no significant difference between the times spent in the RS water and RS + AM water (Fig. 4, Appendix 3). In the fifth water-flume combination (CBA), the time spent in RS water was significantly lower than the time spent in either the RS + AM water ($p < 0.001$) and AM water ($p = 0.005$; Fig. 4, Appendix 3). There was also significant difference between the times spent in the RS + AM water and AM water ($p = 0.016$; Fig. 4, Appendix 3). In the six water-flume combination (BAC), the time spent in RS + AM water was significantly lower than the time spent in either the RS water ($p < 0.001$) and AM water ($p = 0.002$; Fig. 4, Appendix 3). There was no significant difference between the times spent in the RS water and AM water (Fig. 4, Appendix 3). Generally throughout the total of six water-flume combination experiments, the result showed that the juvenile shrimp spent most of the times in RS water.

Following these results, we concluded that the water type and the interaction of water types in six different water-flume combination influenced the shrimp's preference towards the water types offered.

3.3 Experiment 3 (*Rhizophora stylosa* Odour Versus Artificial Seawater)

There was a significant effect of water type ($F_{2, 230.8} = 11.00$, $p < 0.001$; Table 2), with the shrimp spending more time in RS water and the least time in aged RS detritus water. The time spent in RS water was significantly higher (Bonferroni test; $\alpha = 0.0167$) than the time spent in aged RS detritus water ($p < 0.001$; Fig. 5, Appendix 4). There was no significant difference between the times spent in RS water and artificial seawater and also between the times spent in aged RS detritus water and artificial seawater (Fig. 5, Appendix 4).

Table 2. Experiment 3. Results of two-way mixed model for the effects of water type (WT) and water type in which the juvenile shrimp was offered six different water-flume combinations (WF). Significant test results are highlighted in bold.

Source	Numerator df	Denominator df	F	p
WT	2	230.888	11.004	<0.001
WF x WT	10	369.068	28.224	<0.001

As in Experiment 2, there was also a significant interaction of water types in six different water-flume combination ($F_{10, 369} = 28.22$, $p < 0.001$; Table 2), based on time spent among the water types according to the six different water-flume combination. Post-hoc analyses (Bonferroni test; $\alpha = 0.0167$) showed the time spent among the water types varies according to the six different water-flume combination (Fig. 5).

In the first water-flume combination (ABC), the time spent in RS detritus water was significantly higher than the time spent in either the artificial seawater and RS water ($p < 0.001$; Fig. 5, Appendix 5). There was no significant difference between the times spent in the RS water and artificial seawater (Fig. 5, Appendix 5). In the second water-flume combination (BCA), the time spent in artificial seawater was significantly lower than the time spent in either the RS water and the RS detritus water ($p < 0.001$; Fig. 5, Appendix 5). There

was also significant difference between the times spent in the RS water and RS detritus water ($p = 0.003$; Fig. 5, Appendix 5). In the third water-flume combination (CAB), the time spent in artificial seawater was significantly higher than the time spent in the RS water ($p < 0.001$; Fig. 5, Appendix 5). There was no significant difference between the times spent in the RS detritus water and either the RS water and artificial seawater (Fig. 5, Appendix 5). In the fourth water-flume combination (ACB), the time spent in the artificial seawater was significantly lower than the time spent in either the RS water and RS detritus water ($p < 0.001$; Fig. 5, Appendix 5). There was no significant difference between the times spent in the RS water and RS detritus water (Fig. 5, Appendix 5). In the fifth water-flume combination (CBA), the time spent in RS detritus water was significantly lower than the time spent in either the RS water or artificial seawater ($p < 0.001$; Fig. 5, Appendix 5). There was no significant difference between the times spent in the RS water and artificial seawater (Fig. 5, Appendix 5). In the six water-flume combination (BAC), the time spent in RS water was significantly lower than the time spent in either the artificial seawater and RS detritus water ($p < 0.001$; Fig. 5, Appendix 5). There was no significant difference between the times spent in the artificial seawater and RS detritus water (Fig. 5, Appendix 5). As in Experiment 2, generally throughout the total of six water-flume combination experiments, the result showed that the shrimp spent most of the times in RS water.

4.0 DISCUSSION

Our results of Experiment 1 showed that flume position affect the time spent by the shrimps in the flumes. The difference in affinity for the three flume positions may have something to do with the flow rate and local conditions such as turbulence. Our results of Experiment 2 and 3 indicate that juvenile *P. debilis* not only respond to water-borne chemical cues common in their habitat but are also able to differentiate chemical cues from different mangrove

species. Results of Experiment 2 and 3 also suggest that the leachate from *R. stylosa* contain chemical components that probably serve as attractants, contributing to the choice selection by the juveniles. The megalopae of the blue crab *Callinectes sapidus* (Rathbun 1896) have been reported to be able to discriminate between microhabitats using odours from aquatic vegetation, a behaviour that is also modified by factors such as additional odour from predators, ontogenetic stage, and tidal flow (Diaz et al. 2001; Forward Jr. et al. 2003). While these additional factors may play a part in determining habitat preference in *P. debilis*, our data clearly indicate a significant role of chemicals from mangroves in potential habitat location and choice selection by the shrimp.

The chemical components of mangrove litter leachates are diverse, comprising many chemicals that may serve as potential cues. There are also significant differences in chemical composition of leachates from members of Rhizophoraceae and Avicenniaceae (Popp 1984a; Popp 1984b; Popp et al. 1984) (Appendix 6). It is, however, unclear which group(s) of chemicals is the most effective chemical cue used by *P. debilis* and other juvenile nekton. Although there is no direct evidence that low molecular weight carbohydrates (LMWC) cues might be responsible in attracting the juvenile shrimp, several studies have suggested the importance of carbohydrates as settlement cues to non-crustacean larvae, e.g. corals (Morse et al. 1996; Morse et al. 1994), sacoglossans (Krug and Manzi 1999), and sea urchins (Williamson et al. 2000). It is known that amino acids and other small molecules (nucleotides and simple sugars or organic acids) are also attractants available in animals (and sometimes in plant) tissue but it is unclear whether the molecules cues are released sufficient enough in order to be detected (Weissburg et al. 2002). Hay (2011) argued that primary metabolites such as proteins, sugars, and amino acids are prime candidates for chemical cues leading to food, with amino acids apparently particularly effective in crustaceans. Identification of the exact active compounds is, however, challenging because of low environmental

concentrations, the use of ‘blends’ and sequences of multiple rather than single compounds as cues, and expected fast degradation of cue chemicals (Hay 2011). The specific composition of the three different types of mangrove leaf water has not been analysed, as the identification of specific chemical cues is complex (Lecchini et al. 2010).

The shrimp’s preference for the water types could also have resulted from the difference in the initial decomposition rates between the two mangrove species, influencing the leaching rate and thus the concentrations of the chemical cues. This result could be demonstrated by the juvenile shrimps in the actual field as well, hence guiding the shrimps to their preferred mangrove choice. In an experiment conducted at the same location where the leaves were collected, *R. stylosa* leaves lost more biomass followed by those in a mixture of *R. stylosa* and *A. marina* leaves or *A. marina* leaves, during the first 14 days of decomposition in the wet season (P. Natin, unpublished). Werry and Lee (2005) also observed that *R. stylosa* leaves lost more biomass during the first 14 days of decomposition compared to *A. marina* litter, probably resulting from a faster leaching rate. Following this result, the leaching rate and thus concentrations of chemical cues released from *R. stylosa* after the 14-day soaking period prompted the strongest response from the shrimp. In the first 10-14 days of decomposition, mangrove leaf litter loses 20-50 % of organic carbon by leaching (Lee 1989; Robertson et al. 1992; Steinke et al. 1993), with up to 30 % tannins leached during a single day of soaking (Hernes et al. 2001). This rapid release of soluble chemicals may provide important cues guiding juvenile nekton recruited from offshore waters to their nursery habitats in estuaries.

Results of Experiment 3 confirmed that the preference for *R. stylosa* water in Experiment 2 was due to soluble chemical cues that served as attractants to the shrimp. The attraction of juvenile shrimp to senescent mangrove leaf litter but not to detritus suggests that the attractant chemical cues of senescent *R. stylosa* litter probably originated from leaves

rather than microbes. This is due to the abundance of decomposers colonising the leaf litter would not have reached high enough levels in the first 14 days to produce a strong chemical signal as the initial leaching of soluble chemicals happens most rapidly during the first 10-14 days of decomposition. The low preference for detritus is intriguing because there is often an abundance of decomposing litter in the sediment of mangrove forests, where juvenile nekton seek refuge and shelter. It is possible that there is a stage-structured response by juvenile nekton to chemical cues from mangrove litter of different ages, i.e. juveniles of different ages may consider the same cues differently in accordance to their specific habitat requirements. Another potential reason may be the sulphide often associated with hypoxic mangrove mud from which the aged detritus was extracted. Sediment in *Rhizophora* stands are often oxygen poor and with high concentrations of sulphide. While not statistically significant when compared to the artificial seawater, there is a trend that aged detritus of *R. stylosa* was even less preferred by *P. debilis* (Fig. 4).

In mangroves, an acute ability to detect and move into the preferred habitat is crucial to the survival and growth of juvenile shrimp. While it is unclear what benefits the juvenile shrimp are seeking from *Rhizophora stylosa* forests, these are likely to be associated with either food availability or refuge from physical stress and predators. The benefits sought may be directly related to the effective chemicals in our experiment, i.e. using senescent mangrove leaf litter as food, or the chemicals may just serve as a proxy for non-chemical benefits, protection derived from the structural complexity of the root system of *R. stylosa*. Different mangrove species provide different environments for particular inhabitants in terms of the presence of complex vegetation structure, other organisms, and the size of sediment particles (Pinto et al. 2012). The microhabitats offered by mangrove forests for macrobenthos include the hard substrates (trunk, aerial roots, and tree foliage), soft sediment, and burrows for small benthos (Lee 2008).

Movement of soluble organic compounds from mangrove leaf litter could extend to some distances from the estuary, at least 1 km (Moran et al. (1991); Dittmar et al. (2001), and the chemical stimuli could serve as an effective guide to the juvenile shrimp in habitat selection at a spatial scale of kilometres based on the presence of local mangrove species. Even though the chemical cues at a distance would be a mixture of all available chemicals, the shrimp would still be able to differentiate individual cues when it is getting closer to the actual habitats. The effective concentration of chemical cues in mediating choice is unknown but it is likely that the signals can be detected at distances relevant to the need for juvenile shrimp to seek food and refuge between their low tide and high tide habitats. Juvenile penaeid shrimps are known to switch between refuge in mud banks during low tide and make excursions into the mangrove during high tide. It is unknown, however, whether such behaviour is just simple retreat-expansion into alternate habitats driven by tides, or if the shrimp actively seek out their preferred mangrove patches during the high tide using chemical cues. Other cues, such as changes in hydrostatic pressure, may also assist juvenile nekton locate their habitat over long-distances. *P. debilis* at the east coast of Sinai, Egypt, utilise mangroves as a refuge area (juveniles) and search for food on the sand bottom (adult) during the high tide. With the receding tide, they migrate to more sheltered areas near mangrove (*Avicennia marina*) aerial roots or pneumatophores (Fonds et al. 1981).

In conclusion, it has been shown that the juvenile *P. debilis* is able to detect and distinguish water-borne chemical cues from three different types of mangrove-leaf water. Chemicals released by *R. stylosa* leaf litter probably act as positive cues that guide juvenile shrimp to their preferred mangrove choice. There is still research to be undertaken, including identifying the chemical compounds involved, and at what spatial scale they are detected. For the experimental design, flow meters can be installed in the water inlet, upstream of each flume channel.

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Figures

Fig. 1. Rectangular flow-tank with 3-channel flume: Three different water sources (A, B and C) from 3 different tanks respectively are introduced into the flume. D - observation area. E - starting position of the juvenile caridean shrimp / zone with mixed water from all three flumes. F - water outlet from the flume. G - down current mesh net.

Fig. 2. Dye test prior to the experiment.

Fig. 3. Mean (± 1 SE) percentage of time juvenile *P. debilis* spent according to same water types in each flume.

Fig. 4. Mean (± 1 SE) percentage of time juvenile *P. debilis* spent according to water type-flume combination during the choice test (experiment 2). A = RS+AM (a combination of *R. stylosa* and *A. marina*) water type, B = RS (*R. stylosa*) water type and C = AM (*A. marina*) water type.

Fig. 5. Mean (± 1 SE) percentage of time juvenile *P. debilis* spent according to water type-flume combination during the choice test (experiment 3). A = RS (*R. stylosa*) water type, B = RS_Det (aged *R. stylosa* detritus) water type and C = ASW (artificial seawater) water type.

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Appendix

Appendix 1: Experiment 1. Results of post-hoc tests with Tukey HSD for time spent in same water type in each of the flume position. Significant test results are highlighted in bold.

Water type	Flume Position	Comparison	p
A (RS+AM)	Left	Middle	0.420
	Left	Right	0.052
	Middle	Right	0.001
B (RS)	Left	Middle	<0.001
	Left	Right	0.994
	Middle	Right	<0.001
C (AM)	Left	Middle	0.089
	Left	Right	0.014
	Middle	Right	<0.001

RS+AM = a combination of *Rhizophora stylosa* and *Avicennia marina* water, AM = *A. marina* water and RS = *R. stylosa* water.

Appendix 2: Experiment 2. Results of post-hoc tests with Bonferroni ($\alpha = 0.0167$) correction for time spent in water type. Significant test results are highlighted in bold.

Water type	Comparison	p
RS+AM	RS	0.835
RS	AM	0.014
AM	RS+AM	0.237

RS+AM = a combination of *Rhizophora stylosa* and *Avicennia marina* water, AM = *A. marina* water and RS = *R. stylosa* water.

Appendix 3: Experiment 2. Results of post-hoc analyses with Bonferroni ($\alpha = 0.0167$) for times spent in the interaction of water types x six different water-flume combinations.

Significant test results are highlighted in bold.

Water-flume Combination	Water type	Comparison	p
ABC	RS+AM	RS	0.095
	RS	AM	<0.001
	AM	RS+AM	0.028
BCA	RS+AM	RS	1.000
	RS	AM	0.005
	AM	RS+AM	<0.001
CAB	RS+AM	RS	<0.001
	RS	AM	<0.001
	AM	RS+AM	0.556
ACB	RS+AM	RS	0.258
	RS	AM	<0.001
	AM	RS+AM	<0.001
CBA	RS+AM	RS	<0.001
	RS	AM	0.005
	AM	RS+AM	0.016
BAC	RS+AM	RS	<0.001
	RS	AM	1.000
	AM	RS+AM	0.002

RS+AM = a combination of *Rhizophora stylosa* and *Avicennia marina* water, AM = *A. marina* water
and RS = *R. stylosa* water.

Appendix 4: Experiment 3. Results of post-hoc tests with Bonferroni ($\alpha = 0.0167$) corrections
for times spent in water type. Significant test results are highlighted in bold.

Water type	Comparison	P
RS	RS_Det	<0.001
RS_Det	ASW	0.165
ASW	RS	0.021

RS = *R. stylosa* water, RS_Det = *R. stylosa* detritus and ASW = artificial seawater

Appendix 5: Experiment 3. Results of post-hoc analyses with Bonferroni ($\alpha = 0.0167$) for
times spent in the interaction of water types x six different water-flume combinations.
Significant test results are highlighted in bold.

Flume Combination	Water type	Comparison	p
ABC	RS	RS_Det	<0.001
	RS_Det	ASW	<0.001
	ASW	RS	0.967
BCA	RS	RS_Det	0.003
	RS_Det	ASW	<0.001
	ASW	RS	<0.001
CAB	RS	RS_Det	0.147
	RS_Det	ASW	0.129
	ASW	RS	<0.001
ACB	RS	RS_Det	0.208
	RS_Det	ASW	<0.001
	ASW	RS	<0.001
CBA	RS	RS_Det	<0.001
	RS_Det	ASW	<0.001
	ASW	RS	1.000
BAC	RS	RS_Det	<0.001
	RS_Det	ASW	0.033
	ASW	RS	<0.001

855 RS = *R. stylosa* water, RS_Det = *R. stylosa* detritus and ASW = artificial seawater

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857 Appendix 6: Reported composition of leachates from leaves of mangrove species from

858 Rhizophoraceae and Avicenniaceae. The symbols ‘+’ and ‘-’ respectively indicate presence

859 and absence of the respective chemicals in the two families.

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Groups of chemicals	Rhizophoraceae	Avicenniaceae	References
(1) inorganic ions and organic acids			(Popp 1984a)
<i>Inorganic ions</i>			
SO ₄ ²⁻	+	+	
Mg ²⁺	+	+	
Na ⁺	+	+	
Cl ⁻	+	+	
<i>Organic acids</i>			
oxalate	-	+	
malate	+	+	
citrate	+	+	
quinic	+	+	
shikimate	+	+	
(2) low molecular weight carbohydrates (LMWC)			(Popp 1984b)

cyclitol	+	-	
pinitol	+	-	
sucrose	+	+	
glucose	+	+	
fructose	+	+	
(3) free amino acids, total methylated onium compounds (TMOC) and total nitrogen			(Popp et al. 1984)
Free amino acids	+	+	
TMOC	+	+	
total Nitrogen	+	+	
glycinebetaine	-	+	
(4) condensed tannins	+	+	(Hernes et al. 2001)
(5) lignin-derived phenols			(Marchand et al. 2005)
cinnamic phenols	+	+	
vanillic phenols	+	+	
syringic phenols	+	+	
(6)triterpenoids			(Koch et al. 2003)
taraxerol	+	-	
germanicol	+	-	
lupeol	+	-	
betulin	-	+	
lupeol	-	+	
β -sitosterol	-	+	