IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

LARVAL SETTLEMENT PREFERENCE OF FALSE LIMPET SIPHONARIA GUAMENSIS Q&G 1833.

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Abstract

All marine invertebrate larvae exhibit habitat preference for settlement which is mediated by positive or negative cues. Recruitment of the false limpet *Siphonaria guamensis (S.g)* occurred only on the rocks where the invasive crustose red alga (CRA) *Hildenbrandia prototypus* (*H.p*) is distributed, but there was no study to date on the larval settlement preference of *S. guamensis* This study was aimed to prove that the larvae of *S.guamensis* settles on the rocks covered by *H. prototypus*. The larval habitat preference of *S. guamensis* was investigated conducting experiments in the laboratory aquaria, exposing the larvae to native and invasive substrata that occur in the rocky coast of Visakhapatnam, east coast of India. Three different types of experiments were designed to study the settlement preference as 1. Each type of rock in separate aquarium (one set with rocks covered by *H. p* and another set with bare rocks, each set with 10 replicates), 2. All types of rocks covered by *H. p* placed in one aquarium and all types of bare rock substrata in another aquarium (named as 7 way experiment), 3. Pair-wise experiments, rocks covered by *H. p* coupled with bare rocks. Larvae rejected the bare rocks as substratum. Only very few larvae settled on those bare rocks consists of spat, live adults, dead shells of *S. guamensis* and thalloid algae (1:1). In the pair-wise experiments undertaken in the individual aquaria, settlement is significantly high on the rocks covered by *H. prototypus*.

Key words: *Siphonaria guamensis*, *Hildenbrandia prototypus*, habitat choice, larval settlement

1. Introduction

Many investigators have studied the development of eggs and settlement of larvae and survival of juveniles after spawning, in various limpets (Branch, 1975; Stimson and Black, 1975; Lewis and Bowmann, 1975). *Benhamina obliquata* was associated with crustose algae (Borland, 1950). *Kerguelenella stewartiana* associated with *Bostrychia arbuscula* (Knox, 1955).The veliger of *Kerguelenella stewartiana* from Stewart Island was passed within the egg and larva hatches out in the crawling stage which is known as a post-veliger. *Siphonaria pectinata* associated with *Bostrychia* species and also *Ulva* and *Enteromorpha* beds (Voss, 1959). *Siphonaria lessoni* settles in the areas where the rock substratum was covered by algae as they form the main food source (Bastida et al.1971).The earlier investigators reported that species of *Siphonaria* prefer clean rock surfaces without over growth by thalloid algae and surfaces which are not scoured by wave action. Each species of *Siphonaria* associated with specific thalloid /crustose algae (Borland, 1950; Knox, 1955; Voss, 1959; Garrity and

Levings, 1983).The larvae of *S. diemenensis* could settle in a wide variety of habitats and be flexible in their response to changing environmental conditions in their settlements pattern also (Quinn, 1988a & b). Larger eggs deposited by bigger limpets of *S. pectinata* at Bizerte channel, northern Tunisia and *S. lessoni* was due to the presence of more nutrients in the perivitelline fluid of the egg capsules in female phase (Pal and Hodgson 2003; Zabala et al. 2018). In the laboratory conditions, at temperature 13°-15° C, larval settlement occurred within 36-38 days after hatching of veliger larva in *S pectinata* at Bizerte channel (Slama et al. 2018).

Many investigators have studied the settlement preference of larvae in various limpets in response to positive or negative cues. Marine invertebrate larvae settle not only in response to cues but also to a range of chemical and physical factors. Natural cues from biofilms, macroalgae, crustose algae on the rock substrata and conspecific shells can induce settlement. In *Mytilus galloprovincialis* pediveligers responded to microbial filmed surface rather to an unfilmed surface. An extracellular product with MW < 5000 dalton, derived from culture medium of a bacterial strain C1.1 (*Pseudomonas - Alteromonas* group) induced the settlement of *M. galloprovicialis* larvae (Satuito, Shimizu & Fusetani, 1997). Exposure to neuropeptides initiated metamorphosis and settlement in *the sea urchin Strongylocentrotus intermedius* (Takahashi et al. 2002).

Bacterial populations on crustose coralline algae must be distinct from those growing on other marine surfaces in order to produce inducers that trigger settlement of some marine invertebrate larvae. On the west coast of cape peninsula, Oudekroal, South Africa, bacterial populations from coralline crusts are characteristic and distinct from those isolated from different neighbouring microhabitats (Johnson, Muir & Reysenbach, 1991). A number of crustose coralline algal (CCA) species can induce settlement in various species of corals (Heyward & Negri 1999; Raimondi & Morse 2000; Ritson - Williams et al. 2010). Ethanol or Methanol extracts of CCA have induced settlement in some coral species (Heyward & Negri 1999). The processes that contribute to larval settlement are complex with apparent physical substrate specificities inducing settlement in some species (Harrington et al. 2004). Provision of refuge from predation and added habitat complexity are important mechanisms that drive the larval forms to recognise their habitats (Crooks 1998, 2009; Gribben and White 2006; Gribben et al. 2009a, b). The identification of settlement cues associated with habitats suggests that marine invertebrate larvae have the ability to recognise specific compounds that either identify favourable habitats or initiate metamorphosis (Hadfield, 2011). Recruitment to structured surfaces perse may be considered adaptive because they offer a refuge from predation, larvae may settle in structured habitats for this reason (Gribben et al. 2009a).

The aim of the present study is to accept the hypothesis that the larvae of *S. guamensis* prefer to settle on the rock substrata covered by the Crustose red alga *Hildenbrandia prototypus.*

2. Material and methods

2.1. Limpet collection sites and Laboratory maintenance:

At the time of collection of *Siphonaria guamensis* for ecological studies, it was observed that the recruits of *S. guamensis* were present only on the rock surfaces covered by Crustose Red Alga *Hildenbrandia prototypus*. Hence the larval settlement preference of *S. guamensis* was investigated under laboratory conditions in the Wet Laboratory, Department of Marine Living Resources, Andhra University, Visakhapatnam, India.

Adult *S. guamensis* of size ranging from 8 to 10 mm shell length were collected at the low tide time from station 3 (Waltair Point 2) Visakhapatnam, one day after full moon and new moon from September to December 2012, and 2013 without breaking the edges of shell, immediately placing them in a clean 10 mm. diameter, 2 cm. deep petriplate containing a film of sea water of the study site, transported to laboratory within one hour after collection and transferred to 5L round flat bottom glass troughs filled with filtered sea water (FSW) of salinity 30g L^{-1} and temperature $28^0 \pm 2^0$ C for acclimation.

2.2. Experimental design

S. guamensis spawns naturally in the laboratory, on the 2nd day to the 3rd day after full moon or new moon as it was observed in the field. Since most spawns released in the night time, next day after spawning, the egg ribbons were removed from the trough where they were deposited, transferred into 250 ml finger bowls and thoroughly

cleaned with FSW for 3 to 4 times and finally maintained in 1L finger bowls @ 4 egg ribbons of 2 cm. length / 1 finger bowl. The total number of larvae transferred to each experimental aquarium from each finger bowl is equal.

Small rocks of varying shape and size 7 cm. length and breadth, 3 to 5 cm. height and covered by *H.prototypus* and having the required features such as 1. Rocks fully covered (RFC) by CRA *H. prototypus (H.p);* 2. RFC by *H.p* and on which recruits of *S. guamensis* are present; 3. RFC by *H.p* and on which adult *S. guamensis* are invaded; 4. RFC by *H.p* and on which dead shells of *S. guamensis* were kept; 5. RFC by *H.p* and on which *Cellana* sp. is present; 6. Rocks 50% surface covered by *H.p* and remaining 50% covered by thalloid algae (TA); 7. Bare rocks (10 rocks for each variety) were brought to the laboratory washed thoroughly with FSW and placed in the polythene tubs of 50 L. capacity each, filled with FSW of 30g L^{-1} salinity for 48 hours and finally transferred in to experimental aquaria filled with FSW. Every rock is again washed with fresh filtered sea water before placing the rocks in the experimental aquaria. Since the aquaria are shallow, it has been arranged in such a way that the height of water column is only 10 cm above the upper level of the rocks placed in the aquarium. One experimental aquarium is maintained only with filtered sea water, without offering substrata to find out whether larvae settles on the walls of aquarium or on the bottom.

The maximum and minimum room temperatures recorded in the experimental period was 34° and 28° C respectively and the water temperature was 32° and 25° C respectively. Sea water (30g L^{-1} salinity) used in all the experiments was filtered though 1cm cartridge filters. Substratum in all experimental aquaria has a standard surface area of 70 cm^2 and the size of experimental aquaria is 60 cm. X 30 cm. X 20 cm. and the same is maintained in all experiments.

From the studies on reproduction and spawning of *S. guamensis*, it was known that an adult limpet of size ranging from 8 mm to 10 mm shell length, released an egg ribbon of 2 cm. length, which contains 95 (n =150) egg capsules in a 2 mm. x 2 mm. x 2 mm. piece of egg ribbon. The mean hatching rate is 85%. The first free swimming veliger released 72 hours after the egg ribbon laid. Following the above observations FSW in the finger bowls was changed twice in a day until 60 hours after the egg ribbon was laid. Afterwards, the water in the finger bowls along with hatched larvae was released into the experimental aquaria. The number of larvae hatched and the number of empty egg capsules were counted with the help of an inverted Microscope (Carl Zeiss, model - primovert), in order to report the percentage hatching of larval forms.

From the studies on the larval development and settlement of *S. guamensis*, it was recorded that the first limpet settled 8 days after release of 1st larval form from the eggs ribbon. Following the above observations the experimental aquaria were made ready by the time that the first larval form released from the eggs ribbon. Three days old free swimming veligers (1800 ± 100) were released into experimental aquaria. Until then, the veligers were reared in 1L finger bowls @ 4 egg ribbons (2 cm. long) per one finger bowl and fed with mixture of microalgae *Nannochloropsis occulata*, *Isochrysis galbana* and *Chaetoceros* sp. @10,000 cells/ $larvae^{-1}$ d^{-1} in 1:1:1 ratio (O' Connor and Heisman 1997).

At the end of each trial, all the post-settlement juveniles (recruits) were removed from the rock substrata of experimental aquaria, swabbing with a sponge padand transferred to individual specimen tubes (Tarsons make) of size 5 cm. and fixed in 4% formalin. Following this, any unsettled larvae were sieved and fixed. Finally, the surfaces of the aquaria were carefully swabbed to remove any recruits that had settled directly on the walls/ bottom of aquaria. These larvae were also fixed for later counting. Rose Bengal is added to stain the recruits, prior to fixing to assist in later sorting and counting of recruits. The number of recruits on the surfaces of the aquaria and the number of unsettled larvae were counted under a dissecting microscope (10 x magnifications).

2.2.1. General Settlement Preference / 7- Way Experiments:

General settlement preference of larvae of S. *guamensis* was explored by offering larvae a choice of 7 different kinds of substrata viz., 1. Rocks fully covered (RFC) by CRA *H. prototypus (H.p);* 2. RFC by *H.p* and on which recruits of *S. guamensis* are present; 3. RFC by *H.p* and on which adult *S. guamensis* are invaded; 4. RFC by *H.p* and on which dead shells of *S. guamensis*; 5. RFC by *H.p* and on which *Cellana* sp. is present; 6. Rocks 50% covered by *H.p* and 50% covered by TA; 7. Bare rocks (two rocks for each of the 7 substrata of size 7x7x5 cm. each) were randomly placed to the replicate aquaria (n =10) before releasing veligers. One control was maintained

with rocks covered by CRA *H. prototypus*, since the hypothesis is "larvae of *S. guamensis* settles only on rocks covered by *H. prototypus*. Another 7 varieties of substrata were prepared with same combination of organisms but with bare rocks and kept in another aquarium, in order to study whether recruits, live adults of *S. guamensis*, dead shells *S. guamensis*, live *Cellana* or thalloid algae are responsible for settlement of larvae in the absence of rocks covered by *H. prototypus*. The results of the two 7- way experimental study was depicted in the table 1 a & b; figure 1 a & b.

Further investigations were carried out to confirm whether the habitat choice found in the 7 way experimental aquarium was maintained. To study whether larvae are having preference to any other substrata in absence of rocks covered by *H. prototypus*, experiments were conducted offering fewer substrata to larvae - specific substrata in each aquarium and paired substrata.

2.2.2. Study whether consistency in the larval settlement preference

2.2.2.1. Experiments with specific substrata in individual aquarium:

Larvae in the 1st experiment exhibited a strong preference for rock substrata covered by CRA *H. prototypus*. To test whether larvae show preference to any of the other substrata in the absence of rocks covered by *H.prototypus*, experiments were conducted with bare rocks on which recruits of *S. guamensis* (<3 mm.), bare rocks with live S. *guamensis* (>6 mm.), bare rocks with dead shells of S. *guamensis* (>6 mm.), bare rocks with live *Cellana* (>8 mm.) and bare rocks covered with thalloid algae (1:1). Each variety of rock substrata placed in individual aquarium to test whether settlement is induced by organisms placed on rocks in the absence of *H. prototypus*, when only single variety of substrata is available for settlement. The results of the experimental study was depicted in the table figure 2 a & b.

Larvae were offered a specific type of substratum consisting of 70 cm^2 area in each aquarium and 10 aquaria were maintained in total. All experiments were conducted simultaneously from single batch of larvae. Only actively swimming larvae released into the experimental aquaria.

2.2.2.2. Pair-wise Experiments:

To test whether the general pattern of habitat choice found in the 7 way experiment was maintained when larvae were offered fewer substrata, experiments were conducted with pair wise substrata i.e.,1. rocks with *H.p* and bare rocks; 2. rocks with *H.p* and bare rocks on which *S. guamensis* recruits are present; 3. rocks with *H.p* and bare rocks on which *S. guamensis* live adults are present; 4. rocks with *H.p* and bare rocks on which *S. guamensis* dead shells were kept ; 5. rocks with *H.p* and bare rocks on which *Cellana* live adults are present; 6. rocks with *H.p* and bare rocks partially covered by thalloid algae (1: 1), placing each pair in a single aquarium. Paired experiments were also conducted with bare rocks paired with bare rocks on which recruits of *S. g*; live adults of *S.g*; dead shells of *S.g*; live *Cellana* and thalloid algae were placed, placing each pair in a single aquarium, in order to test whether larvae show a preference for any other substrata other than *H.p* when only restricted choice is available. The results of the study were depicted in the table 3; figure 3 a - f.

Settlement involves the transition from a planktonic to benthic mode of life accompanied by processes facilitating attachment before metamorphosis; metamorphosis describes developmental changes where distinct and permanent morphological changes are undertaken to form a juvenile (Hadfield, 2011). In this study the use of terms settlement and metamorphosis are followed Hadfield's definition. Since attachment of larvae takes place as culmination of metamorphosis in the veliger larvae of *S. guamensis*, the term settlement was used to indicate both the processes.

2.3. Statistical analyses

The differences in the larval settlement in the 7 way settlement trails were analysed using Chi- square test on considering the mean number of larvae on each settlement substrata pooled across 10 experimental aquaria. The significance of difference in the variance obtained was tested by Tucky's test. All pair- wise experimental trails were analysed using paired t-tests. One way ANOVA was used to determine the significance of difference in variance of percent settlement of larvae between two individual substrata. For all experiments, percent settlement

was estimated as the total number of larvae settled on each treatment divided by total number of larvae released. Mean percent settlement (±SE) was taken into consideration to the graphical expression of data for ease of comparison among the experiments.

3. Results and Discussion

3.1. General Larval Settlement preference

Total number of larvae settled at the end of experiment varied among aquaria within each experiment and also between different experiments (Tables 1, 2 & 3). Larvae settle on the substrata covered by H.p or else died and reached the bottom of aquaria. The dead shells (Protochonchs) recovered for each aquarium in all experimental trails and preserved in 2% formaldehyde.

3.2. Seven- way Experiments

In the two sets of seven way experiments (one set of 10 replicates with rocks covered by *H. prototypus (H. p)* and another set of 10 replicates with bare rocks), the difference in the settlement of larvae (between the rock substrata of the two sets) is significant (χ^2 =26.858; DF =4; p<0.001). Settlement of larvae on the bare rock (although the spat, live adults and dead shells of *S. guamensis* (*S.g*) were placed on those rocks) was lowest (SE ±1.1).

3.3. Experiments with individual substrata each in separate aquarium

For the experiments conducted with individual substrata (with rocks covered by *H. p* and with bare rocks, each type in separate aquaria), the difference of variance in the percent settlement was significant between the trails with rocks covered by *H. p* ($F_{5, 54}$ = 64335.52; p<0.001). In general, settlement was highest on rock surfaces covered by *H. p* compared to all other surfaces although differences between rocks with *H. p* alone and rocks with *H. p* consist of spat of *S. guamensis* (*S. g)*; rocks with *H. p* and live adults of *S. g*; rocks with *H. p* and dead shells of *S. g* and rocks with *H. p* consist of thalloid algae (1:1) were not significant (all p >1.0).

The difference of variance in the settlement of larvae between the rocks covered by *H. p* and all types of bare rocks is significant. For experiments conducted with bare rocks consist of spat, live adults & dead shells of *S. g*, live *Cellana* and thalloid algae (1:1), each substrata keeping in individual aquaria, the settlement is very less. The difference of variance in the total settlement of larvae among trails is significant. (F=187.95; p<0.001). However, the difference of variance in the percent settlements are not significant between the bare rock substrata with spat of *S. g* and bare rock substrata with live adults of *S. g* & bare rock substrata with dead shells of *S. g* (Tuckey's Test *p* > 0.413). But the difference of variance in the percent settlements between the bare rocks with spat and bare rock with *S. g* live adults & between bare rocks with thalloid algae and dead shells of *S. g* is significant (Tuckey's Test p<1.0).

3.4. Pair-wise Experiments

For the pair wise experiments conducted with rocks covered by *H. prototypus* and bare rocks on which organisms placed, to eliminate the possibility of settlement inducement by recruits/live adult/dead shells of *S. guamensis*; live adult *Cellana* and thalloid algae, the difference of variance between the two experimental groups (each pair tested in a single aquarium) is significant. The rocks covered by *H. prototypus* and bare rocks on which 1. Spat of *S. guamensis* (t=98.42; df =9; P< 0.001), 2. Live adults of *S. guamensis* (t=813.03; DF=9; p < 0.001) 3. Dead shells of *S. guamensis* on bare rocks (t= 89.61; df=9; p<0.001) 4. Thalloid algae (t=94.273; df=9; p<0.001). 5. No settlement of larvae on the bare rocks consists of live *Cellana.*

Veligers of *S.pectinata* failed to settle in the laboratory (Voss, 1959). Attempts to induce settlement of the larva of *S. pectinata* in the laboratory were unsuccessful even though the larvae had been fed on *Chlorella* and offered a substratum occupied by limpets of the same species. But the veliger of *S. alternata* was (a feeble, intermittent swimmer) settled on the surface of the glass containers and lost its velum within three days after hatching (Zischke, 1979). So far, investigations on the larval settlement preference of siphonariid limpets are hardly undertaken.

The larval settlement preference of *S. guamensis* in the laboratory experiments coincided with the observed high abundances of *S. guamensis* recruits on the rock surfaces covered by crustose red alga *H. prototypus* in the field. Larvae exhibit a consistent positive response to the rock surfaces covered by *H. prototypus* and spat of *S. guamensis.* Larvae exhibited strong preference to settle on those rock surfaces covered by *H. prototypus* and also to settle on the rocks covered by *H. prototypus* on which spat, dead shells and live adults of *S. guamensis* and thalloid algae were placed in the individual laboratory experimental aquaria, 7 way experimental aquaria and also in the pair wise experimental aquaria.

Settlement is poor on rocks covered by *H. prototypus* invaded by *Cellana* sp. Larvae rejected the bare rock surfaces as substratum although exhibited little preference to settle on bare rocks invaded by thalloid algae (1:1), spat, live adults and dead shells of *S. guamensis.* No settlement on bare rocks invaded by *Cellana* sp.

Settlement response of *S. guamensis* larvae to *H. prototypus* in the field and in the laboratory experiments may be explained on the basis of availability of food. The larval preference to settle is, in the decreasing order in numbers, on the rock surfaces covered by *H. prototypus* invaded by recruits, dead shells & live adults of *S. guamensis* and thalloid algae also supports the same. Less numbers of larvae settled on those rocks where live adults present may be to avoid competition for food. In the pair-wise experiments, consistent higher settlement of larvae on the rocks covered by *H. prototypus* confirmed that there are no chemical cues operating from spat and live adults and dead shells of *S. guamensis* and thalloid algae. The response of *S. guamensis* larvae to the structure (shell topography) of adults is eliminated by their low settlement on rocks covered by *H.p* and on which live adults are present.

In the experiments conducted with specific type of substrata in individual aquaria, the percentage settlement is higher in the aquarium, where rock substrata covered by *H. prototypus* and no other organisms are present on that rock. The reason for this behaviour, the recruits may be responding to chemical cues from the CRA *H. prototypus*. Larvae have shown next preference to substrata covered by *H. prototypus* where spat, dead shells and live adults and thalloid algae in the order (Table 2; figure 2). Poor larval settlement on the substrata where *Cellana* sp. are present although rock substrata covered by *H. prototypus* suggest that preference of larvae to settle on rocks covered by *H. prototypus* is for post settlement survival. The low recruitment on the rocks where live adults of *S. guamensis* may also supports this opinion. The recruits may be facing competition for food as well as problem of bulldozing by the adult limpets. The larvae reject the bare rocks as substratum for settlement. The very poor settlement on the bare rocks on which the recruits, dead shells & live adults of *S. guamensis* present and no settlement on the bare rocks invaded by *Cellana* indicates the failure of post settlement survival of larvae.

Little increment in percentage settlement of larvae on the bare rock substrata covered by thalloid algae (although poor) when compared to the other experimental substrata offered, the thalloid algae may be providing refuge to the recruits.

The consistent positive response of *S. guamensis* larvae to the rock surface covered H. *prototypus* may be explained on the basis of its food habits since *S. guamensis* mainly feed on this crustose red algae *H. prototypus*.This crustose red alga may provide camouflage to the new recruits also.

High abundances of *Anadara trapezia* recruits on *Caulerpa taxifolia* compared to other native habitats in the field (Gribben et al. 2009). Larvae in the controlled laboratory experiments neither show strong preference for *Caulerpa taxifolia* nor rejected it as settlement surface, but exhibited a consistent positive response to adult *A. trapezia*. *C. taxifolia* is the most abundant macrophyte in Lake Conjola; recruits may not be able to find adults and simply settle on any substrata. *C. taxifolia* may provide a refuge from predation (Gribben & Wright, 2006; Gribben et al. 2009).

The present experimental studies revealed that the larval preference for *H. prototypus* suggest that the larvae responded to cues from the surface of the thallus of these crustose red algae or the chemical cues. Settlement cues for marine invertebrates are more likely to be waterborne. Invertebrates show a strong settlement response to specific water borne cues (Williamson et al., 2000; Swanson et al., 2004).

Since this study is designed to determine the larval preference to *H. prototypus* covered rock substrata, further studies may be required to establish whether the cues are associated with surface or chemical cues from the thallus. Components of biofilms such as bacteria and microalgae (eg. diatoms) found on the surface of host organisms have demonstrated positive effects on the recruitment of marine invertebrates (Keough & Raimondi, 1996; Huggett et al., 2006).

Habitat related cues associated with biofilms are important for settlement of several species of reef sponges (Whalan et al., 2008; Wahab, Nys & Whalan, 2011). In Great Barrier Reef sponge *Rhopaloeides odorabile*, coral rubble in addition to Crustose Coralline Algae enhanced the transition for initial settlement to metamorphosis, which was an important substratum for post - settlement survival (Whalan, Webster & Negri, 2012). The consistent larval response of GBR sponges *Conscinoderma mathewsi* and *R. odorabile* to CCA *Porolithon onkodes* indicates that CCA acting as signal that the larvae have entered a coral reef habitat where post settlement survival was assured (Whalan, Webster & Negri, 2012). The consistent response of larvae of *S. guamensis* to *H. prototypus* may also for post settlement survival where *H. prototypus* offers the settlers food and camouflage against predation.

The enhanced settlement in response to increased concentration of CCA extracts observed in laboratory studies on the GBR sponges *R. odorabile* and *C. mathewsi* indicates the sponge larvae possess chemosensory capabilities that play a role in the selection of microhabitat for recruitment (Whalan, Webster & Negri, 2012). The chemosensory ability has been demonstrated for several groups of marine invertebrates particularly for branches where sensory antennules are used to select optimal settlement sites (Maruzzo et al., 2011). The present study was designed to establish the fact that the settlement of *S. guamensis* larvae is influenced by a crustose red algae *H. prototypus*. The response of *S. guamensis* larvae to the structure (shell topography) of adults is eliminated by their low settlement on rocks covered by *H. prototypus* and on which live adults are present. Further work is required to explore the chemosensory ability of the larvae in the selection of microhabitat containing *H. prototypus* for recruitment. Components of bio films such as bacteria and microalgae (eg. diatoms) found on the surface of *H. prototypus* may also be taken into consideration for further investigations.

4. Conclusion

The experimental results demonstrated in this study accept the hypothesis that "the larvae of *Siphonaria guamensis* settle on the rocks essentially covered by *Hildenbrandia prototypus"*. This was proved by conducting three types of experiments in the laboratory aquaria. In general settlement preference of larvae was confirmed by 7 way experiment, where in the larvae were offered a choice of 7 different kinds of substrata. The larvae settled on the substrata covered by *H.p* or else died. In the experiments with individual substrata, each in separate aquarium, settlement was highest on the rock surfaces covered by *H.p* compared to all other surfaces. The differences in the percent settlement between rocks were not significant. To eliminate the possibility of settlement induced by conspecific shells, *Cellana* sp. and thalloid algae, the number of algae settled on the rocks covered by *H.p* among each pair of rocks was more than the bare rocks. The experimental results conclude the absence of removal of crustose red alga *Hildenbrandia prototypus* from the rocks will reduce the population of *Siphonaria guamensis* on the intertidal rocks of Visakhapatnam coast.

5. Acknowledgement

The first author thanks the Govt. of Andhra Pradesh State-India, Department of Social Welfare for funding. The first author thanks the Head, Department of Marine Living Resources for permitting to utilize the facilities of the wet laboratory for conducting experiments. The first author also thanks the second and third authors for their valuable suggestions reviewing and editing the manuscript.

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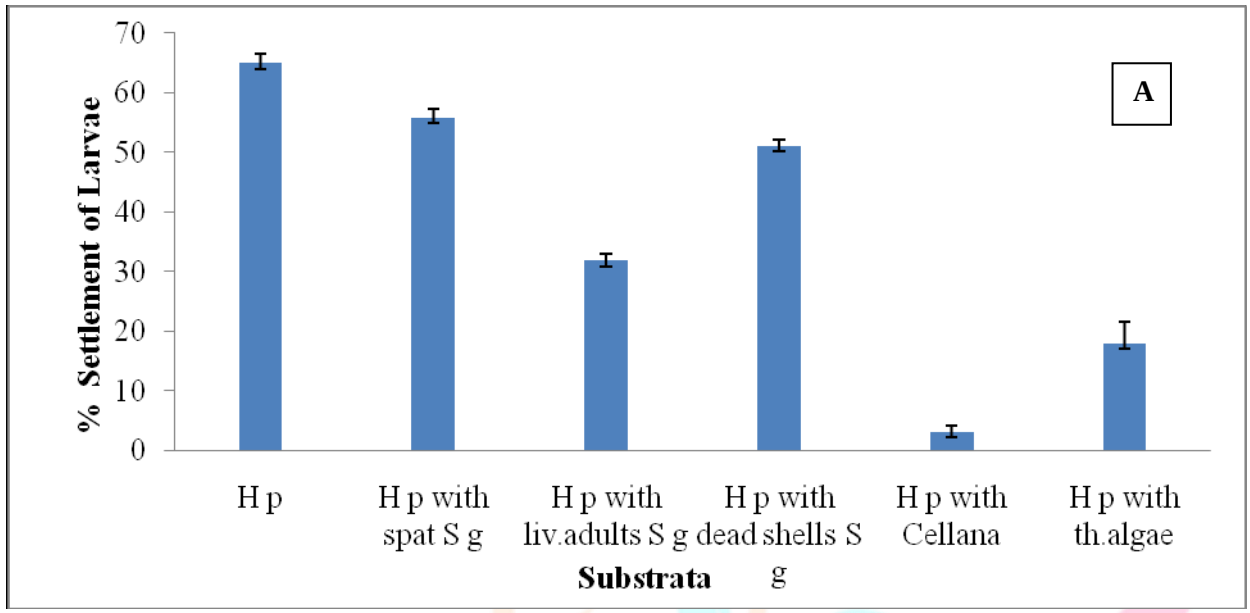


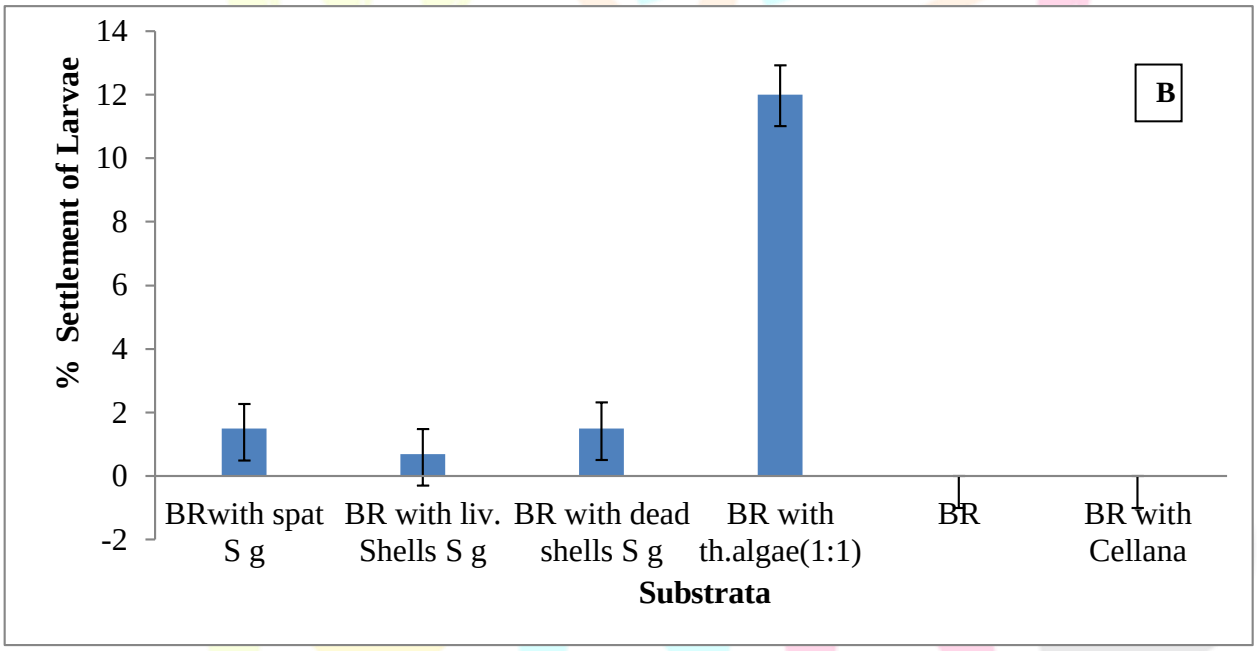
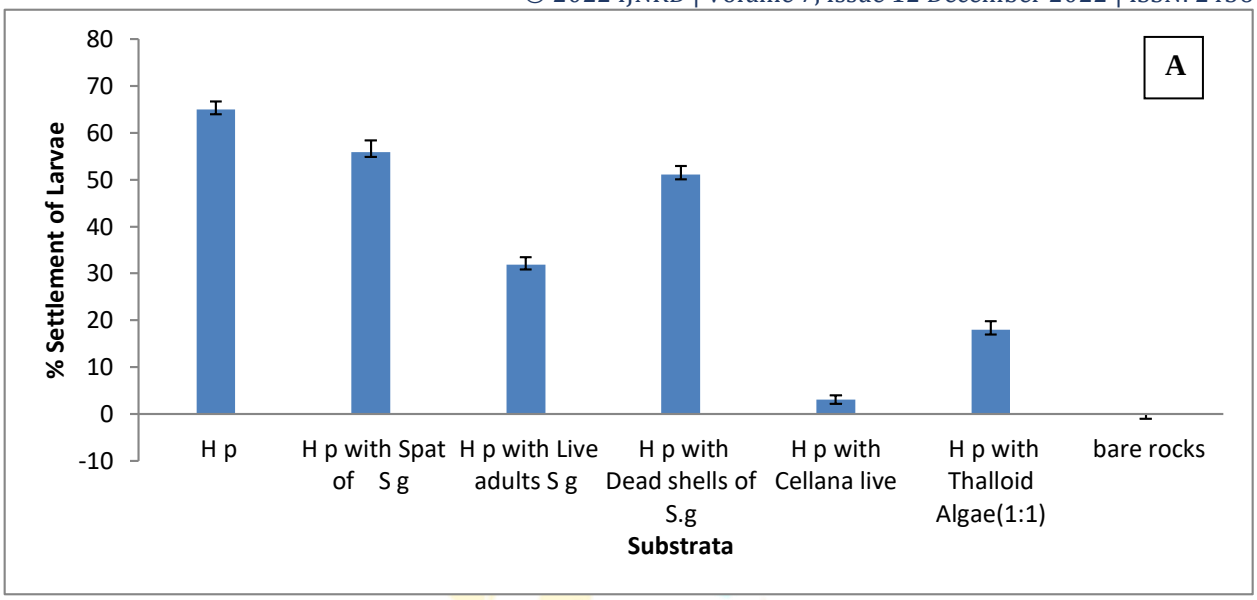


Fig.1. *S. guamensis* (*S.g*). Mean (±SE) percent settlement of larvae on the substrata placed all in one aquarium (7- way experiments). A- on rocks covered by *Hildenbrandia prototypus* (*H.p*), rocks covered by *H.p* & spat of *S.g*, rocks covered by *H.p* & live adults of *S.g*, rocks covered by *H.p* & dead shells of *S.g*, rocks covered by *H.p* & live *Cellana*, rocks covered by *H.p* & thalloid algae(1:1), bare rocks (BR), side walls of aquarium (aq. walls), water column (wat. Col.). B- BR with spat of *S.g*, BR with live adults of *S.g*, BR with dead shells of *S.g*, BR with thalloid algae(1:1), BR and BR with live *Cellana*



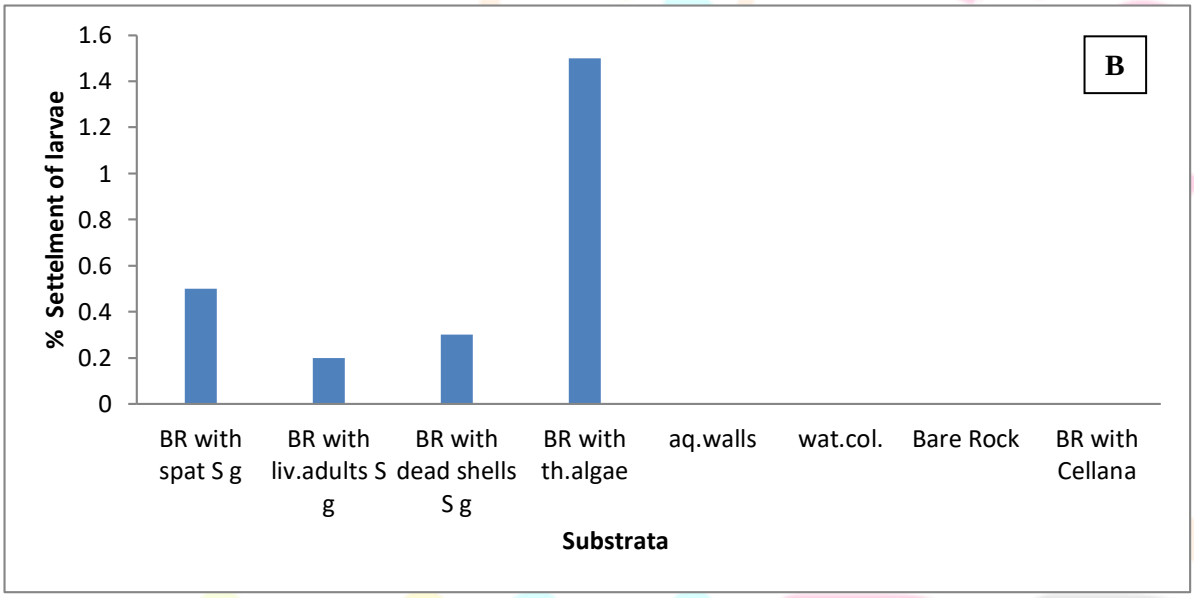


Fig. 2. *S. guamensis* (*S.g*). Mean (±SE) percent settlement of larvae on the substrata placed, each in individual aquarium. A- on rocks covered by *Hildenbrandia prototypus* (*H.p*), rocks covered by *H.p* & spat of *S.g*, rocks covered by *H.p* & live adults of *S.g*, rocks covered by *H.p* & dead shells of *S.g*, rocks covered by *H.p* & live *Cellana*, rocks covered by *H.p* & thalloid algae(1:1), bare rocks (BR), side walls of aquarium (aq. walls), water column (wat. Col.). B- BR with spat of *S.g*, BR with live adults of *S.g*, BR with dead shells of *S.g*, BR with thalloid algae(1:1), aq. walls, wat. Col. and BR with live *Cellana.*

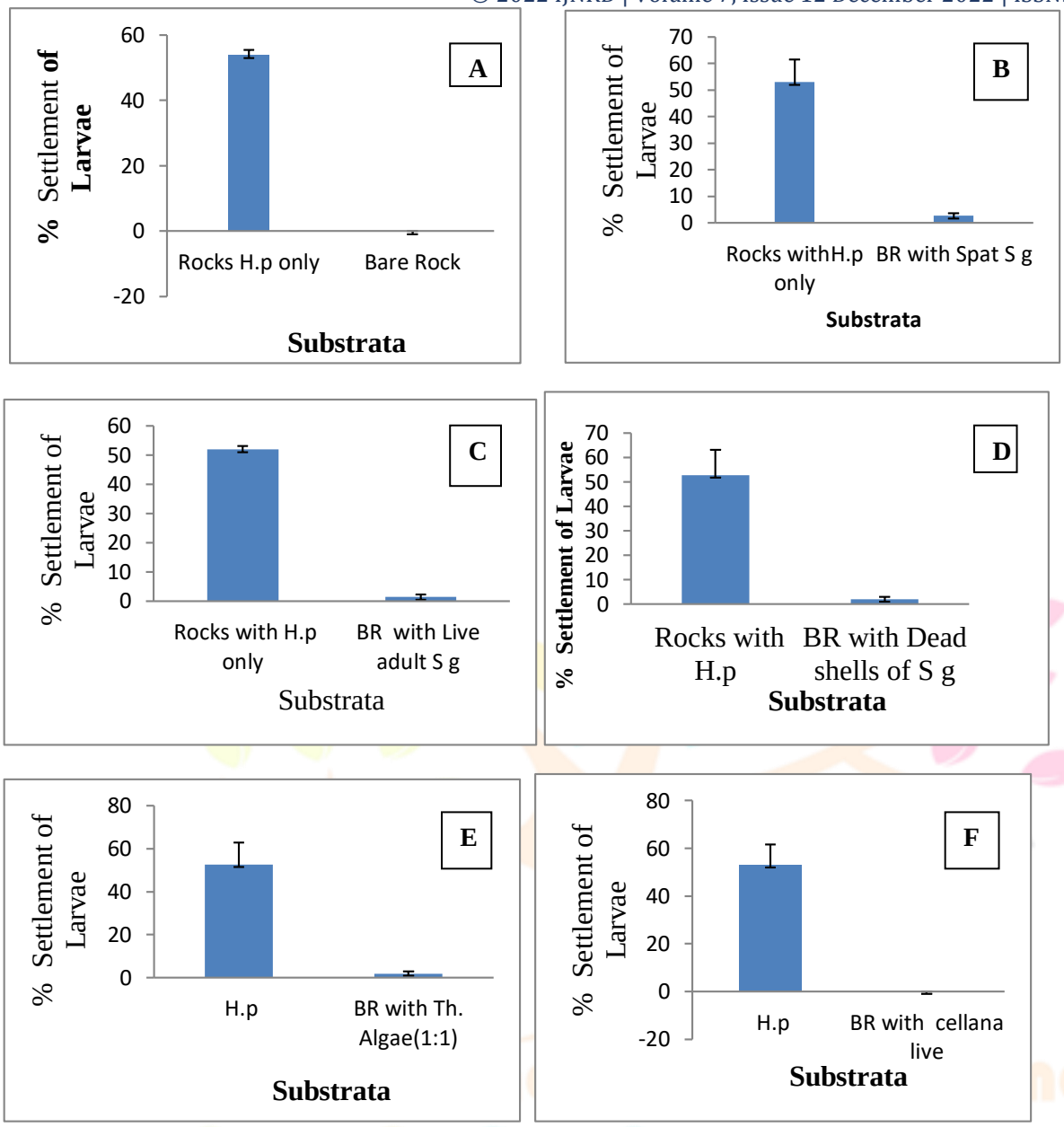


Fig. 3. *S. guamensis* (*S.g*). Mean (±SE) percent settlement of larvae on the paired substrata placed in individual aquarium. A- on rocks covered by *Hildenbrandia prototypus* (*H.p*) & bare rocks, B- rocks covered by *H.p* & spat of *S.g*, C- rocks covered by *H.p* & BR with live adults of *S.g*, D- rocks covered by *H.p* & BR dead shells of *S.g*, E- rocks covered by *H.p* & thalloid algae(1:1), F- rocks covered by *H.p* & live *Cellana* bare rocks (BR), side walls of aquarium (aq. walls), water column (wat. Col.).

Table. 1. *S.guamensis*. Number of larvae settled in each aquarium with specific substrata.
a. all substrata covered by *H. p* ;
b. all substrata bare rocks.
H.p = Hildenbrandia prototypus, Aq = Aquarium, *S.g = Siphonaria guamensis*

a

Exptl. Aq.No.	No. of larvae released	Aq. with rocks covered by *H.p*	Aq. with rocks covered by *Hp*& Spat of *S.g* (<3mm)	Aq. with rocks covered by *Hp*& Live adults of *S. g* (6mm)	Aq. with rocks covered by *Hp*& Dead shells of *S.g*	Aq. with rocks covered by *H.p* & Cellana live	Aq. with rocks covered by *H. p* & thalloid algae (1:1)	Aq. with bare rocks
1	1800	1164	1010	575	914	56	320	0
2	1800	1162	1018	569	918	54	324	0
3	1800	1167	998	567	927	58	332	0
4	1800	1176	1004	578	915	60	330	0
5	1800	1170	1015	580	924	53	315	0
6	1800	1178	999	576	918	55	328	0
7	1800	1173	1000	569	912	60	324	0
8	1800	1168	997	580	925	54	318	0
9	1800	1172	1005	575	922	58	326	0
10	1800	1165	1012	578	926	53	320	0
Total	18000	11695	10058	5747	9201	561	3237	0
Mean	1800	1169.5	1005.8	574.7	920.1	56.1	323.7	0
%		65	55.87	31.9	51.1	3.11	17.98	0
SE		1.751543	2.513175	1.587373	1.801577	0.908805	1.819307	0

b

Exptl. Aq. No.	No. of larvae released	Bare rock	Aq. With bare rocks on which spat of *S.g* (<3mm)	Aq. With bare rocks on which live adults of *S.g* (6mm)	Aq. With bare rocks on which dead shells of *S. g*	Aq. With bare rocks on which *Cellana* live	Aq. With bare rocks covered by Thalloid Algae(1:1)	Side walls of aq.	Water column
1	1800	0	7	5	7	0	34	0	0
2	1800	0	12	4	4	0	24	0	0
3	1800	0	10	5	9	0	27	0	0
4	1800	0	8	4	4	0	22	0	0
5	1800	0	8	4	5	0	26	0	0
6	1800	0	9	3	5	0	33	0	0
7	1800	0	11	4	6	0	23	0	0
8	1800	0	8	5	6	0	28	0	0
9	1800	0	9	4	5	0	30	0	0
10	1800	0	7	3	7	0	23	0	0
Total	18000	0	89	41	58	0	270	0	0
Mean	1800	0	8.9	4.1	5.8	0	27	0	0
Percentage		0	0.5	0.2	0.3	0	1.5	0	0
SE		0	0.5259	0.2333	0.4898	0	1.3416	0	0

Table. 2: *S.guamensis*. Number of larvae settled on each type of substratum in the same Aquarium
a.7 way settlement on rocks with H p & other organisms;
b. 7 way settlement on bare rocks with other organisms.
H.p = Hildenbrandia prototypus, Aq = Aquarium, *S.g = Siphonaria guamensis*

a

Exp tl. Aq. No	No. of larvae released	Aq. with rocks covered by *H.p*& spat of *S.g* (<3mm)	Aq. with rocks covered by *H.p* & live adults of *S.g*	Aq. with rocks covered by *H.p* & dead shells of *S.g*	Aq. with rocks covered by *H.p* & *Cellana* live	Aq. with rocks covered by *H .p*& Th. algae	Aq. with bare rocks	Side walls of aq.	Water column	Bottom of Aq.
1	1800	434	324	361	40	145	0	0	0	0
2	1800	430	319	357	31	148	0	0	0	0
3	1800	432	326	365	34	140	0	0	0	0
4	1800	428	324	355	39	144	0	0	0	0
5	1800	432	325	363	40	146	0	0	0	0
6	1800	430	320	362	33	143	0	0	0	0
7	1800	435	328	364	38	147	0	0	0	0
8	1800	433	321	360	36	140	0	0	0	0
9	1800	431	327	359	38	146	0	0	0	0
10	1800	436	324	356	33	143	0	0	0	0
Total	1800	4321	3238	3601	362	1442	0	0	0	0
Mean		432.1	324	360	36	144	0	0	0	0
%		24	18	20	2	8	0	0	0	0

b

Exp tl. Aq. No	No. of larvae released	Bare rocks with spat of *S.g* (<3mm)	Bare rocks with live adults of *S.g*	Bare rocks with dead shells of *S.g*	Bare rocks with *Cellana* live	Bare rocks with Th. algae(1:1)	Aq. with bare rocks	Side walls of aq.	Water column	Dead Larvae on Bottom of Aq.
1	1800	28	12	24	0	27	0	0	0	725
2	1800	29	14	16	0	24	0	0	0	716
3	1800	29	14	25	0	20	0	0	0	714
4	1800	25	15	17	0	28	0	0	0	726
5	1800	26	10	21	0	27	0	0	0	720
6	1800	24	16	22	0	22	0	0	0	723
7	1800	23	18	19	0	29	0	0	0	718
8	1800	27	16	23	0	28	0	0	0	728
9	1800	28	13	15	0	23	0	0	0	725
10	1800	30	12	18	0	24	0	0	0	723
Total	18000	269	140	200	0	252	0	0	0	7200
Mean	1800	27	14	20	0	25.2	0	0	0	720
%		1.5	0.8	1.1	0	1.4	0	0	0	40

Table 3*: S.guamensis*. Number of larvae settled on each type of substratum in the same aquarium (7 way settlement with H p & bare rocks;
H.p = Hildenbrandia prototypus, Aq = Aquarium, *S.g = Siphonaria guamensis*

Exptl. Aq. No	No. of larvae released	Aq. with rocks covered by *H.p*	Bare rocks	bare rocks on which spat of *S.g* (<3mm)	bare rocks on which dead shell of *S.g*	bare rocks on which live adults (6mm)	bare rocks on which *Cellan a* live	bare rocks covered by thalloid Algae (1:1)	Side walls of aq.	Water colum n	Dead shells of larvae on the bottom of aq.
1	1800	990	0	28	24	12	0	217	0	0	725
2	1800	995	0	29	24	14	0	214	0	0	716
3	1800	987	0	29	25	14	0	220	0	0	714
4	1800	985	0	25	27	15	0	218	0	0	726
5	1800	994	0	26	26	10	0	217	0	0	720
6	1800	990	0	24	30	16	0	212	0	0	723
7	1800	986	0	23	28	18	0	219	0	0	718
8	1800	996	0	27	31	16	0	218	0	0	728

9	1800	984	0	28	28	13	0	213	0	0	725
10	1800	993	0	30	29	12	0	214	0	0	723
Total		990	0	269	272	140	0	2162	0	0	7218
Mean		990	0	26.9	27.2	14	0	216.2	0	0	721.8
%		55	0	1.49	1.5	0.7	0	12	0	0	40.1
SE		1.4572	0	0.7769	0.8134	0.7856	0	0.9135	0	0	

Table. 4: *S.guamensis*. Number of larvae settled on each type of substrata in the pair - wise placement of rocks in the individual aquaria. (Rocks with *H.p* vs bare rocks). *H.p* = *Hildenbrandia prototypus*, Aq = Aquarium, *S.g* = *Siphonaria guamensis*

Exptl. Aq. No	No. of larvae released into each aquarium (aq.)	No. of larvae settled in pair wise placement of rocks in the same aq.		No. of larvae settled in pair wise placement of rocks in the same aq.		No. of larvae settled in pair wise placement of rocks in the same aq.		No. of larvae settled in pair wise placement of rocks in the same aq.		No. of larvae settled in pair wise placement of rocks in the same aq.	
		Rocks covered by *H.p* only	Bare rocks	Rocks covered by *H.p* only	Bare rocks on which spat of *S.g* (<3mm)	Rocks covered by *H.p* only	Bare rocks on which live adults (6mm) of *S.g*	Rocks covere d by *H .p*	Bare rocks on which dead shells of *S.g*	Rocks covered by *H .p*	Rocks 50% covered by thalloid algae
1	1800	972	0	1000	46	938	32	920	36	910	31
2	1800	965	0	960	47	932	24	1000	39	900	28
3	1800	979	0	940	49	929	26	980	37	940	36
4	1800	968	0	920	50	940	27	930	41	920	34
5	1800	975	0	980	48	940	25	960	33	950	28
6	1800	964	0	960	52	937	28	940	31	930	30
7	1800	968	0	920	50	935	30	920	35	990	35
8	1800	972	0	950	49	934	25	900	36	950	32
9	1800	975	0	980	43	936	27	980	40	990	36
10	1800	973	0	930	52	935	26	970	32	980	34
Total		9711	0	9540	486	9356	270	9500	360	9460	324
Mean		971.1	0	954	48.6	935.6	27	950	36	946	32.4
%		53.95	0	53	2.7	51.977	1.5	52.777	2	52.555	1.8
SE		1.5088	0	8.5893	0.8717	1.0873	0.7745	10.327	1.0645	10.241	0.9683

Table 5: *S.guamensis*. Number of dead shells collected from the bottom of different experimental aquaria in the laboratory.
H.p = Hildenbrandia prototypus Aq = Aquarium * Number of larvae settled *S.g = Siphonaria guamensis*

Expt Aq. No	No. of larvae released in to each exptl. aq.	Aq. with rocks covered by *H.p* *	Dead shells larvae collected from the bottom of Aq.	Aq. with rocks covered by *H.p*& spat of *S.g* (<3mm) *	Dead shells of larvae collected from the bottom if Aq.	Aq. with rocks covered by *Hp* & dead shells of *.S.g* *	Dead shells of larvae collected from the bottom of Aq.	Aq. with rocks covered by *H p* & Live adult (6mm) *	Dead shells of larvae collected from the bottom of Aq.	Aq. with rocks covered by *H.p* & Cellana live *	Dead shells of larvae collected from the bottom of Aq.	Aq. with rocks covered by *H.p* & thalloid algae(1: 1) *	Aq. with bare rocks	Dead shells of larvae collected from the bottom of Aq. *	Only water without substrata	Dead shells of larvae collected from the bottom of Aq. *
1	1800	1164	636	1010	790	914	886	575	1225	56	1744	320	0	1800	0	1800
2	1800	1162	638	1018	782	918	882	569	1231	54	1746	324	0	1800	0	1800
3	1800	1167	633	998	802	927	873	567	1233	58	1742	332	0	1800	0	1800
4	1800	1176	624	1004	796	915	885	578	1222	60	1740	330	0	1800	0	1800
5	1800	1170	630	1015	785	924	876	580	1220	53	1747	315	0	1800	0	1800
6	1800	1178	622	999	802	918	882	576	1224	55	1745	328	0	1800	0	1800
7	1800	1173	627	1000	800	912	888	569	1231	60	1740	324	0	1800	0	1800
8	1800	1168	632	997	803	925	875	580	1220	54	1746	318	0	1800	0	1800
9	1800	1172	628	1005	795	922	878	575	1225	58	1742	326	0	1800	0	1800
10	18000	1165	635	1012	788	926	874	578	1222	53	1747	320	0	1800	0	1800
Total		11695	6305	10058	7943	9201	8799	5747	12253	561	17439	3237	0	18000		
Mean		1169.5	630.5	1005.8	794.3	920.1	879.9	574.7	1225.3	56.1	1743.9	323.7	0	1800		
%		65	35.02	55.87	44.13	51.1	48.88	31.9	68	3.11	96.88	17.98	0	100		