Settlement and Metamorphosis of Coral Planulae Incubated with Commercial Peptide Hym-248 at Different Age

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Abstrak

Penempelan dan metamorfosis planula karang yang diinkubasi peptida komersial Hym 248 pada umur berbeda

Keberhasilan pemeliharaan planula karang membutuhkan dorongan pada planula untuk menempel pada substrat. Neuropeptida komersial Hym-248 diketahui mampu mendorong planula untuk menempel. Waktu pemberian peptida mempengaruhi penempelan dan metamorfosis planula. Penelitian ini bertujuan untuk mendeskripsikan perbedaan penempelan dan metamorfosis planula karang yang diberi peptida Hym-248 pada usia 1 hari dengan planula karang yang diberi peptida Hym-248 pada usia 6 hari. Penelitian ini menggunakan slick (kumpulan gamet yang dilepaskan koloni karang dari berbagai spesies yang melakukan spawning serentak) yang berasal dari perairan Pulau Sambangan, Kepulauan Karimunjawa. Planula diberi Hym-248 dengan konsentrasi 1×10^{-7} M dan 1×10^{-5} M untuk planula umur enam hari dan 1×10^{-7} M, 1×10^{-6} M, dan 1×10^{-5} M untuk planula umur satu hari. Planula yang diberi Hym-248 pada usia 1 hari sudah mulai menempel pada jam ke-4 dan mulai bermetamorfosis pada jam ke-120, sedangkan planula yang diberi Hym-248 pada usia 6 hari tidak mengalami penempelan dan metamorfosis sama sekali hingga akhir penelitian. Keberhasilan menempel tertinggi terjadi pada planula yang diberi Hym-248 dengan konsentrasi 1×10^{-6} M sebanyak 5.83% pada jam ke-192. Penempelan dan metamorfosis planula dari slick yang diberi Hym-248 pada usia 1 hari lebih baik dari planula yang diberi Hym-248 pada usia 6 hari. Penelitian ini memberi harapan untuk memproduksi benih karang secara masal menggunakan benah yang berasal dari reproduksi seksual.

Kata kunci: *penempelan, metamorfosis, planula karang, slick, Hym-248*

Abstract

The success of coral planulae rearing needs stimulant for planulae to attach onto substrate. Commercial neuropeptide Hym-248 is known to induce planulae to metamorphose and settle. The timing to add peptide affects planulae attachment and metamorphosis. This research was aimed to describe the difference of settlement and metamorphosis in coral planulae added Hym-248 at the age of 1 day and 6 days. This research used a slick, egg-spem bundles released from multi-specific spawning corals. The study conducted at Sambangan Island, Karimunjawa Islands. Planulae were added with Hym-248 at concentration of 1×10^{-7} M and 1×10^{-5} M for 6 days old planulae and 1×10^{-7} M, 1×10^{-6} M, and 1×10^{-5} M for 1 day old planulae. The highest settlement rate was observed on the planulae treated with 1×10-6 M dose of Hym-248 at 144^{th} hour with percentage of settled planulae was 32.08%. However, only 5.83% were metamorphosed after 60 hrs observation. One day old planulae incubated with Hym-248 started to attach at 4^{th} hour and metamorphosed at 120^{th} hour, whereas none of 6 days planulae attach or metamorphose until the end of the experiment. This study suggested that incubated planulae in Hym-248 media will give a better result when planulae are at one day old. This study highlight the possibility to produce mass-seedling colonies using sexually derived planulae.

Keywords: *settlement, metamorphosis, coral planulae, slick, Hym-248*

Introduction

Global declines of coral reef ecosystems continue in response to various stressors such as global warming, ocean acidification, increasing UV doses, raising sea level, anthropogenic eutrophication, pollution, overfishing and coastal development which lead to increase the spread of

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coral diseases. The higher intensity and frequency of coral bleaching devastated many reef areas and the irresible decline of some 70% of the world's reefs (Dubinsky and Stambler, 2011). For long, coral reefs represent a critical resource for millions of people. Coral reefs provide a source of food and livelihood for the people in the form of abundant marine resources to be harvested, including supplying compunds for pharmacutical and tourist attractions (Edward, 2010).

Corals has high productivity, hence larval rearing has the potential to produce a lot of coral juveniles (Omori, 2011). However, most coral rehabilitation efforts focused on asexual reproduction only. Rehabilitation with sexual reproduction methods can reduce the negative effects of transplant. It requires knowledge of coral larval development, particularly in settlement and metamorphosis phase of coral planulae. Coral planula settlement and metamorphosis process requires physical and chemical cues from the environment to induce planula to settled and began to metamorphosed (Negri *et al.,* 2001). Metamorphosis is a critical process in a coral life cycle. When planula larvae fail to settle and metamorphose, there will be no coral colonies. Metamorphosis occurs when larvae undergo physiological and morphological changes that are largely irreversible (Heyward and Negri, 1999). In most coral larvae, metamorphosis is started when larvae have developed into firmly attached disc-shaped structures with pronounced flattening of the oral-aboral axis and typically obvious septal mesenteries radiating from the central mouth region followed by rapid skeleton calcification(Harrison and Wallace 1990).

Currently, many studies focusing on the role of chemical cues to induces planulae larvae settlement and metamorphosed. Various compunds act as chemical cues were isolated from various sources. For example one family of neuropeptides, GLWamide was isolated from hydra, *Hydractinia echinata* (Leviev *et al.*, 1997), A bromotyrosine derivative, 11-deoxyfistularin-3 (10−7 M) isolated from crustose coralline red algae (Kitamura *et al.*, 2007), and tetrabromopyrrole (TBP) isolated from four bacterial *Pseudoalteromonas* strains obtained from the crustose coralline algae *Neogoniolithon fosliei* and *Hydrolithon onkodes* (Tebben *et al.,* 2011).

Bromotyrosine derivative was demonstrated to enhance metamorphosis of *Pseudosiderastrea tayamai* planula larvae by 27.5 ± 24.0% (Kitamura *et al*., 2007). Commercial neuropeptide Hym-248 was reported effectively induced metamorphosis and settlement of *Acropora tenuis* planulae larvae (Iwao et al. 2002); *A. nobilis* and *A. micropthalma* (Hirose *et al*., 2007). Erwin and Szmant (2010) showed that Hym-248 induced almost 100% metamorphosis in *A. palmata* planulae at concentrations $>1\times10^{-6}$ M. While Diah and Indrayanti (2011) showed that the same neuro-peptide induced settlement and metamorphosis of planulae larvae derived from slicks.

Planulae settlement and metamorphosis also affected by the time of peptide added. *A. palmata* planulae age 2-3 days post-fertilization did not respond to Hym-248 treatment, while 4 days post-fertilization planulae just begin to respond with 40% of metamorphosis (Erwin and Szmant, 2010). The effect of peptides varies by planulae age. Planulae attachment levels tend to be low if larvae age is too young (Iwao *et al.*, 2002). Experiment conducted by Erwin and Szmant (2010) found no attachment or metamorphosis occured on *A. palmata* planulae 2 days post-fertilization. Nearly 100% planulae metamorphosed in the added peptide at 5 days post-fertilization. This research was aimed to describe the difference of settlement and metamorphosis in Acroporids planulae larvae added Hym-248 at the age of 1 day and 6 days. The growth of settled planulae were also observed.

Materials and Methods

Site survey

Research was conducted at Sambangan Island (05°50' 39.2"S, 110°35' 12.4"E), Karimunjawa Archipelago. Prior the experiment, the site survey was conducted to determine the percentage of hard coral cover and coral reproductive status. Furthermore, the data were used as a reference to estimate mass spawning time to get the slick. Data collection was conducted on 26th to 27th March 2012 with LIT method at 3 stations in Sambangan Island waters. Reproductive status data was obtained by cutting a branch of coral colonies ecountered under the LIT line transect to reveal the eggs maturity (Baird *et al*., 2000). The number of mature colonies were calculated to obtained the percentage of total colony samples.

Gamete collection, fertilization, and larvae rearing

This study used coral planulae from slick (egg-sperm bundles released during multi-specific coral spawning event) collected from Sambangan Island, Karimunjawa, Jepara. Diah *et al*. (2012) reported that Karimunjawa corals released their gametes biannually, with the peak season on March-April and September-October. During this research, multi-specific coral spawning occurred on April 8th

2012. Slick was collected from the sea water and placed in a bucket. Gametes were then reared in an indoor tank supplied with running unfiltered seawater at ambient temperature. Planulae that formed was then washed, rinsed, and transferred into sterile containers containing filtered sea water.

Hym-248 induction

The experiments were conducted by adding peptide in media contained 10 planulae with different ages, i.e. one day and six days. Hym-248 was given in 2 different doses, which is 1×10^{-7} M dan 1×10^{-5} M for 6 days old planulae and 1×10^{-7} M, 1×10^{-6} M, and 1×10^{-5} M for one day old planulae. Experiment were conducted using wells plate 24. Every well was added 1 ml FSW with different peptide concentrations. At the base of each well was covered by tracing paper which act as substrate. Each dose of peptide concentration applied on a 24-wells plate. In total, five 24-well plates for treatment and two 24-well plate for control were used. Observation were conducted at 4 hours, 8 hours, 12 hours after treatment and then every 12 hours by using a light microscope.Planulae behaviour during experiment was described. The number of attached and metamorphosed planula were calculated using Excel. Planulae that successfully attached and metamorphosed then was photographed and digitalized using CPCe (Coral Point Count with Excel Extension; Kohler dan Gill, 2006) software to determine the changes of planulae's surface area (Figure 1).

Results and Discussion

Coral reefs condition and reproductive status

Coral reefs condition at Sambangan Island based on live coral cover was categorized as moderate to good (Gomes and Yap, 1988). The lowest coral cover was found at the first station (39.23%), followed by the second station (51.95%) and the third station (70.48%), respectively (Table 1).

Table 1. Percentage of Coral Cover (%) and Number of Live Coral Colonies ecountered during LIT sampling; number inside the brackets represent number of colonies encountered

No.	Genus	Percentage of Coral Cover (%) and Number of Colobies		
		Stasiun 1	Stasiun 2	Stasiun 3
1	*Acropora*	10,96 (15)	24,79 (42)	29,53 (43)
2	*Pocillopora*	1,98 (10)	0,29 (2)	0,68 (4)
3	*Porites*	15,27 (36)	11,74 (22)	17,12 (30)
4	*Montipora*	1,78 (3)	2,04 (5)	7,85 (16)
5	*Stylophora*	2,71 (10)	0,68 (4)	2,34 (4)
6	*Diploastrea*	0,84 (4)	0,39 (1)	0,48 (1)
7	*Pavona*	0,68(2)	0	0,72 (3)
8	*Goniastrea*	0,4 (3)	1,09 (3)	3,32 (11)
9	*Favites*	0,06 (2)	1,05 (4)	1,28 (6)
10	*Seriatopora*	0,25 (2)	0	0
11	*Goniopora*	0,32 (1)	1,44 (2)	1,19 (4)
12	*Galaxea*	0,03 (1)	0,09 (1)	0,6 (4)
13	*Fungia*	0,06 (1)	0	0
14	*Favia*	2,91 (4)	0,63 (3)	0,18 (2)
15	*Lobophyllia*	0,68 (1)	0,32 (1)	0
16	*Alveopora*	0,3 (1)	2,06 (4)	1,31 (1)
17	*Merulina*	0	0,38 (1)	2,69 (4)
18	*Leptoria*	0	0,43 (2)	0
19	*Millepora*	0	2,55 (4)	0
20	*Heliopora*	0	0,25 (1)	0
21	*Echinopora*	0	0,32 (1)	0
22	*Pachyseris*	0	0,35 (1)	0
23	*Caulastrea*	0	0,47 (2)	0
24	*Hydnophora*	0	0,16 (1)	0,36 (1)
25	*Astreopora*	0	0,43 (1)	0,73 (1)
26	*Anacropora*	0	0	0,1 (1)
27	DCA, Rubble, Sand	60,77	48,05	29,52
	Total Colonies	96	108	136

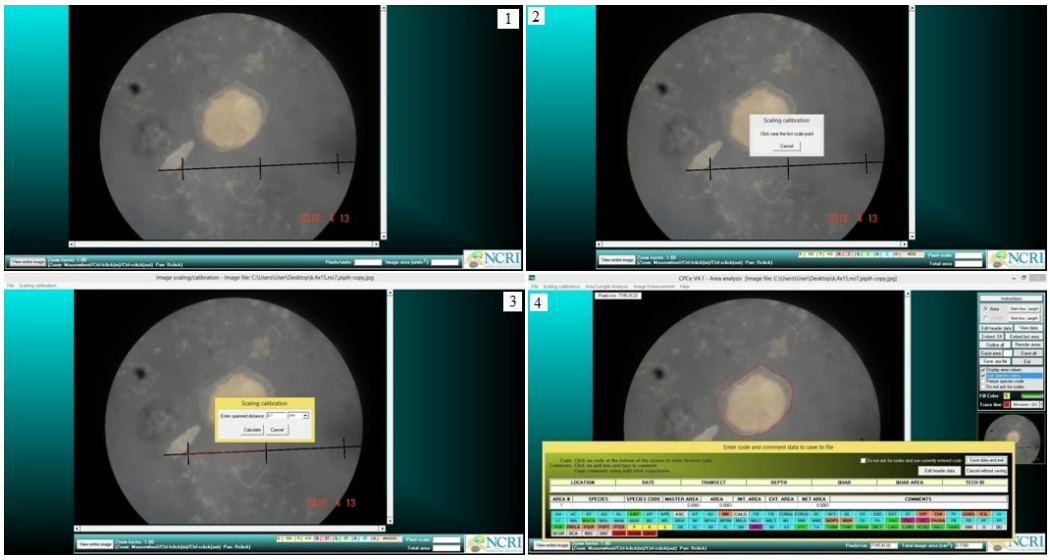

Figure 1. CPCe display when opening the image that will be processed (1). Scale calibration (2). Input scale value (3). Photo digitization process to know planulae's surface area (4)

Coral genus that dominating Sambangan Island waters is *Acropora* and *Porites*. In total 42 and 43 colonies were found at Station 2 and 3 while Station 1 is dominated by 36 colonies of *Porites* (Table 1). Sambangan Island is surrounded by transparrent waters. Though there are fish farm activities but it is likely yet affect the number of live coral cover. Moreover, Sambangan Island lies behind Genting Island which provide a barrier from ocean wave.

Table 2. Proportion of mature colonies in Each Genus; NC, number of Colonies; Pc, percentage

Genus	Station					
	1		2		3	
	NC	Pc	NC	Pc	NC	Pc
Acropora	1	6.67%	7	16.67%	6	13.95%
Porites	2	5.56%			1	3.33%
Lobophyllia	1	100%	1	100%		
Goniastrea					2	18.18%

Observations on March 26th and 27th, 2012 showed that from total 340 colonies observed, only 21 colonies contained mature eggs (6.18%). It was likely the coral colonies spawned their gametes prior the observation. There were Acropora, Porites, Lobophyllia and Goniastrea respectively (Table 2) with Acropora dominated the colonies contained mature eggs . Diah *et al*. (2012) observed that multi-specific spawning event on March-April was dominated by Acroporidae as well.

Attachment and metamorphosis of planulae incubated in media added with Hym-248

The shape of fully developed planulae were round., The larvae swam up and down or spinning inside the well. Planulae swimming speed were changing and sometimes stay still. While in free swimming stage, planulae began to tapered gradually into an oval shape, then started to shaped like a pear. Pear shaped planulae usually swimming while rotating on its axis and sometimes stop at the bottom of the substrat and then started to swim again. After found a suitable place to attach, planulae will begin to flattened like a plate. Settled planulae will continue changing shape until it looks like a flower, which will become a coral polyp. The observed shape changes can be seen in Figure 2.

Planulae already begun to tapered and pear shaped after the 4th hour in the whole experiment. Planulae shaped like a pear and begin to contract around the body axis at the 96th hour for 1×10^{-7} M dose of Hym-248, 108th hour for 1×10^{-6} M and 1×10^{-5} M dose of Hym-248. Planulae then flattened and shaped like a flower. Hirose *et al*. (2007) observed that during this stage coelenteron started to form and 6 mesenteries revealed. Similar shaped was observed when planulae attached (Figure 2). While untreated planula began to contract around body axis after 120 hrs observation. Diah and Indrayanti (2012) observed similar behaviour when conducted experiment on slick derived planulae induced with Hym-248.

Number of settled planulae in treated media showed different results. The highest rate of settled planulae treated with 1×10-7 dose of Hym-248 was observed at 120th hour. Percentage of settled planulae was 27.92%. When planulae was treated with 1×10-6 M dose, the highest settlement rate was observed at 144th hour with percentage of settled planulae was 32.08%. While 1×10-5 M dose

of Hym-248 made 11.25% planulae was settled after 168 hrs treatment. Untreated planulae showed different result. The planulae began to settled after 36 hrs observation but most of planulae began to detached and swim again. From the settled planulae, 5.83% planulae treated with 1×10-6 M dose of Hym-248 were metamorphosed after 60 hrs observation. While in 1×10-7 M dose and 1×10-5 M dose, planulae began to metamorphose after 120 hours of treatment (Figure 3). Control planulae began to metamorphosed at 24th hour, however, number of metamorphosed planulae was very small, only 0,42%.

The lower number of settled and metamorphosed planulae compared to previous studies (Iwao *et al*., 2002; Hirose *et al*., 2007; Erwin and Szmant, 2010) was likely related to the source of planulae. Planulae used for the experiments derived from slick or gametes collected from multi-specific spawning. Hym-248 was reported effective to induce settlement and metamorphosis of *Acropora* spp planulae. Hym-248 effectively induced settlemet and metamorphosis of *A. tenuis* ((Iwao *et al*., 2002), *A. nobilis* and *A. micropthalma* (Hirose *et al*., 2007) and *A. palmata* (Erwin and Szmant, 2010). Slick can be derived from various gametes released from different coral genera. In contrast, different results were obtained from study conducted by Diah and Indrayanti (2012). During this study, Hym-248 was effectively induced settlement and metamorphosed of planulae derived from slick. However, experiment was conducted one month earlier when the higher number of Acropora colonies released their gametes during multi-specific spawning event (Diah *et al*., 2012). Therefore, the probability of planulae derived from Acropora egg-sperm bundles was higher.

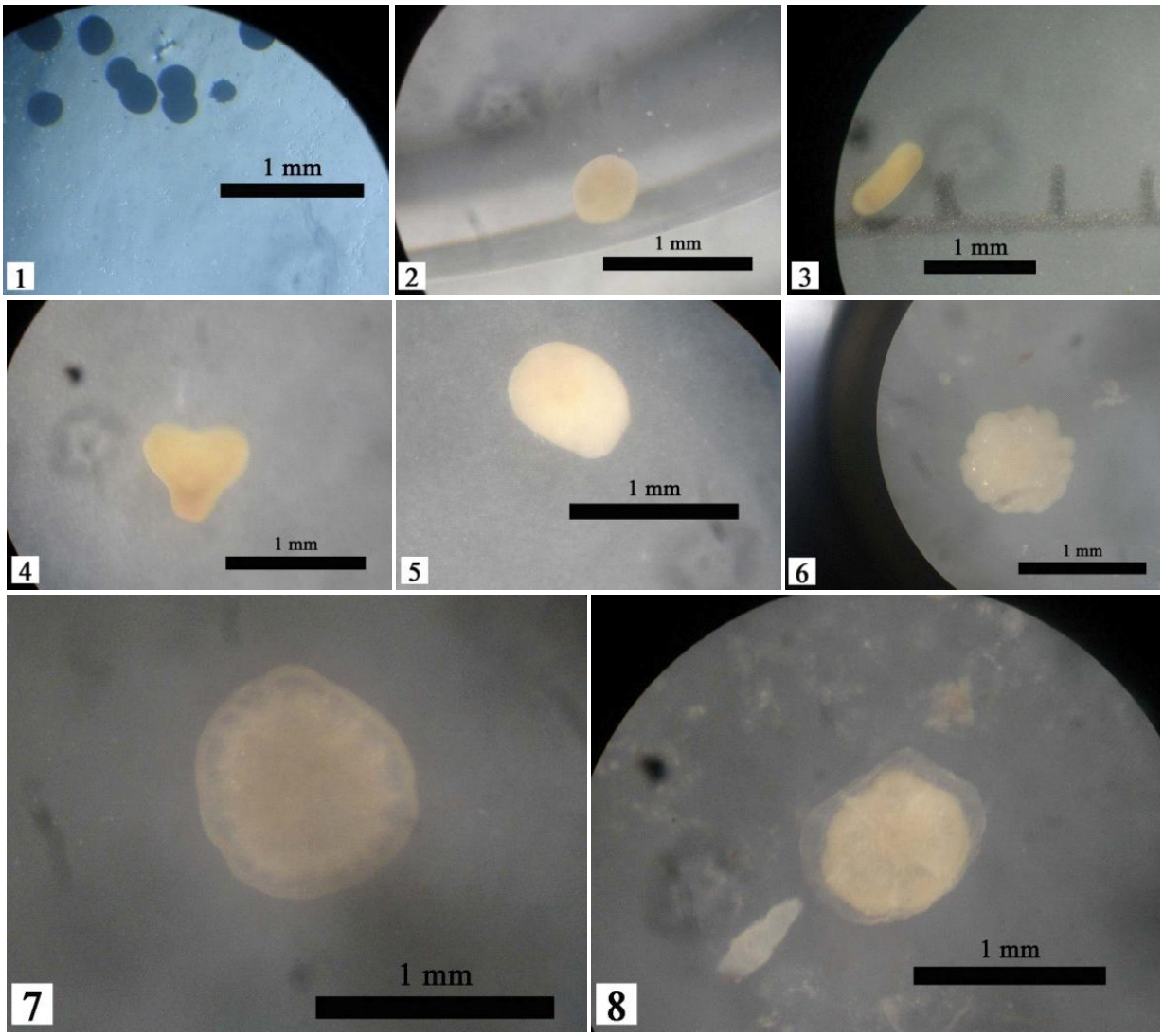

Figure 2. Sequence of planulae appearances before settle. (1) Egg (2) Rounded (3) Tapering to oval (4) Pear like shaped (5) Settled and flattened (6) Began to shaped like a flower (7) and (8) Skeleton takes shape

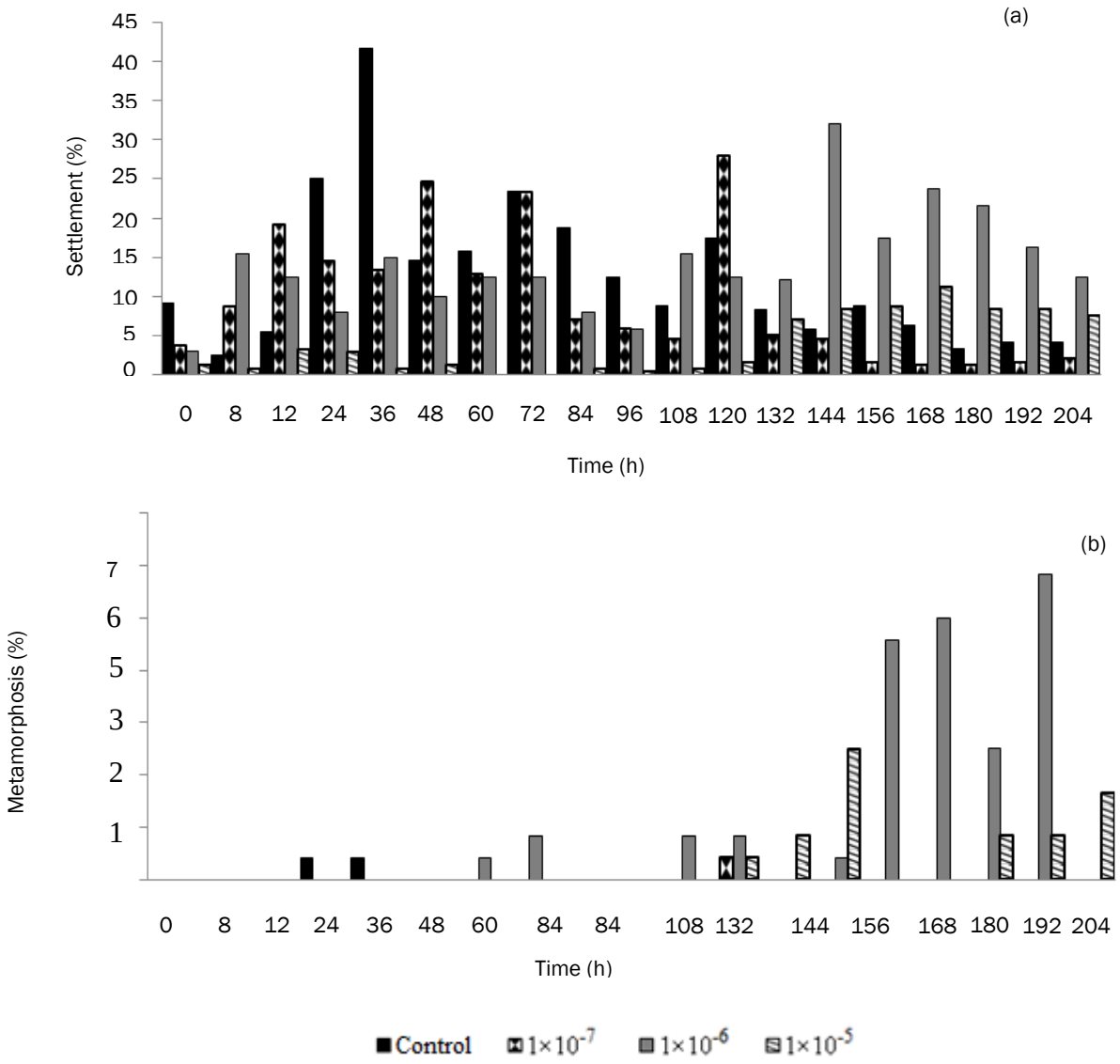

Figure 3. Average number of Planulae which Settle (a) and Metamorphosed (b)

Hym-248 experiment on planulae with different age

One day old planulae incubated with Hym-248 were started to attach at 4th hour and metamorphosed at 120th hour, while planulae with Hym-248 added at the age of 6 days did not attach nor metamorphose at all until the end of experiment (Figure 4). This suggests that Hym-248 could not induced settlement and metamorphosed when planulae are 6 days old. On the contratry, different results was demonstrated by Erwin and Szmant (2010) which observed that *Acropora palmata* planulae added Hym-248 at the age of 2 days didn't metamorphose and 6-7 days old planulae had 100% metamorphosis.

Planulae used for Hym-248 experiment at the age of 6 days came from brown colored slick. Upon further observed, allegedly planulae that were used is already contain zooxanthellae and from brooder type corals (Dai *et al.*, 1992). GLW-amide acts as internal mediator that released by neurons after getting an external signal and trigger metamorphosis in *Acropora* (Iwao *et al*., 2002). Hym-248 is known only able to induce settlement and metamorphosis of *Acropora* and doesn't affect *M. faveolata*, *F. fragum*, *Isopora brueggemanni*, *Montipora* sp., *Astreopora myriophthalma*, *Merulina ampliata*, and *Goniastrea retiformis* (Iwao *et al.,* 2002). This suggests that planulae responds to different types of peptides among coral species.

Surface area accretion on settled planulae

The highest surface accretion occurs on planulae with 1×10^{-6} M of Hym-248 (0.053 mm^2).

However, lowest surface area accretion occurs on planulae with highest concentration of Hym-248 (1×10^{-5} M),which is 0.01 mm^2 (Figure 5).

Planulae with 1×10^{-6} M of Hym-248 added has highest surface accretion rate, which is 0.021 $mm^2.day^{-1}$. The lowest surface area accretion occurs on control planulae with 0.003 $mm^2.day^{-1}$ (Figure 5). This suggests that Hym-248 not only induce attachment but also affect the surface area accretion of settled planula.

Conclusion

One day old planulae derived from slick which incubated in media added with Hym-248 were able to settle at 4th hour and metamorphose at 120th hour after treatment. Planulae are likely derived from Acropora colonies since the planulae used for the experiment has no zooxanthellae. However, 6 days old planulae which were incubated in the same media did not show similar results. Therefore, incubated planulae in Hym-248 media will give a better result when planulae are one day old. However, until now there is no report on how metamorphosed planulae induced with Hym-248 grow and calcify their skeleton. It is important to consider the source colonies of planulae before incubated the planulae with Hym-248. This study highlight the possibility to produce mass-seedling colonies using sexually derived planulae instead of asexually derived colonies produced through transplantation method.

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