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STUDY ON SURVIVABILITY OF NEWLY HATCHED LARVAE OF TWO SPECIES OF PEPPERMINT SHRIMP FED WITH DIFFERENT COMBINATIONS AND DENSITIES OF LIVE FOODS

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ABSTRACT

This study was aimed to evaluate different types and combinations of live foods in relation to the survivability of newly hatched *Lysmata vittata* and *Lysmata intermedia* larvae. The experiment consisted of three trials (different species, combinations, and densities of live foods) arranged in a completely randomized design. The first and second trials were subjected to *L. vittata* with three treatments for each trial (1A, 1B, 1C for trial-1; 1D, 1E, 1F for trial-2). The third trial consisted of two treatments (2A and 2B) tested on *L. intermedia*. Each treatment had three replicates. The results showed that the survival rates were low in all treatments. However, each treatment showed a significant effect ($P<0.05$) on the average survival rate of *L. vittata* and *L. intermedia* larvae. In the first trial, treatment 1C was the only one that had survived larvae after day 35 with 4.44% of final average survival rate (FASR). Four of the larvae reached the post-larval stage. In the second trial, treatment 1F showed a better condition than the other treatments with 5.56% FASR. Nevertheless, no larvae in the second trial had transformed to post-larval stage before the experiment ended at day 46. In the third trial, no larvae survived to reach the post-larval stage. In spite of this, treatment 2B had better daily average survival rate (DASR) than treatment 2A. This research concludes that the use of copepods as live food at an early larval stage and *Artemia* at a later stage is relatively more effective to improve the survival rate of peppermint shrimp larvae.

KEYWORDS: larvae; live foods; *Lysmata vittata*; *Lysmata intermedia*; survivability

INTRODUCTION

The peppermint shrimp is one of several groups of marine ornamental shrimps from the genus *Lysmata* that are highly favoured by marine ornamental fish enthusiasts. Unfortunately, their availability on the market depends mostly on wild stocks. Thus, the shrimps have suffered high exploitation which will render its future sustainability uncertain (Calado, 2008; Cato & Brown, 2008). The efforts to successfully produce the shrimps in a culture environment to meet the market demands both in number and continuity have been generally slow.

Recently, through a series of aquaculture application helped by science and technology, some species of marine ornamental shrimp are in a great progress in producing commercially cultured marine ornamental shrimps, for example, skunk cleaner shrimp *Lysmata amboinensis*, fire shrimp *L. debelius*, and peppermint shrimp *L. seticaudata* (Palmtag & Holt, 2001; Rocha, 2007; Calado, 2008). However, it is still necessary to explore and optimize the existing techniques as well as potential future practices and methods in order to improve the quality, quantity, and diversity in the commercial aquaculture of marine ornamental shrimp (Holt, 2003; Rocha, 2007; Calado, 2008). In fact, there are some other species of marine ornamental shrimp that are not quite well known recently but have a high potential for future market, for example, peppermint shrimp *L. vittata* and *L. intermedia*. These two species of marine ornamental shrimp are deemed as the new favorite species among marine ornamental fish enthusiasts. Unfortunately, there was limited information regarding the best possible techniques, methods, and diets in the cul-

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ture technique and survival rate, particularly at larval stage of the shrimp (D'Acoz, 2000; Calado, 2008; Baeza, 2008; Marin *et al*., 2012; Marine Fish Direct, 2014). A successful aquaculture of marine ornamental shrimp strongly correlates with a reliable production of eggs as well as good development and growth of larvae and juveniles (Holt, 2003). Early life stages, especially larval stage, are a very critical period in the aquaculture of marine ornamental shrimp. At this stage, high-quality foods are essential to support an optimum larval development and increase the survival rate (Olivotto *et al*., 2011; Holt, 2003). Therefore, finding the best types and optimum combinations beyond the existing diets is the most important priorities. For example, despite the facts provided by some studies that young juvenile and adult shrimp from genus *Lysmata* are considered to be a strict carnivorous species (Le Vay *et al*., 2001), adding microalgae into their carnivorous diets at larval stage enhanced their larvae survival as a consequences of beneficial effects to their nourishment (Simoes *et al*., 2002). Many studies such as those conducted by Buskey *et al*. (1993), Zhang *et al*. (1998), and Cunha *et al*. (2008) have confirmed the effective use of one or a combination of microalgae, copepods, rotifers, and *Artemia* to feed larvae of marine ornamental fish. The aim of this study was to evaluate the survivability of newly hatched *L. vittata* and *L. intermedia* larvae using different types and combination of live foods.

MATERIALS AND METHODS

Feeding Trial Designs

The experiment consisted of three trials and was arranged in a complete randomized design. The first and second trial had three different treatments each (1A, 1B, 1C for trial-1; 1D, 1E, 1F for trial-2) and were subjected to *L. vittata* (Figure 1A). The third trial (trial-3) consisted of two treatments (2A and 2B) and was tested for *L. intermedia* (Figure 1B). Each treatment had three replicates. The first trial was carried out from 20 March to 07 May 2014 followed by the second trial from 17 April to 01 June 2014. The third trial was conducted from 08 April to 17 May 2014. The experiments were done in two laboratory rooms (Room 11 and Bld. 170) located at Marine and Aquaculture Research Facilities Unit (MARFU), James Cook University, Australia. The seawater supply was obtained from MARFU water circulation system with salinity maintained at 29-31 ppt.

Research Procedures

The sources of larvae of peppermint shrimp *L. vittata* and *L. intermedia* used in this experiment were obtained from the selected berried female broodstock nurtured in an aquarium at MARFU laboratory. Two weeks after spawning, the selected females were isolated into another container until the eggs hatched. The hatching container was filled with seawater, gently aerated without circulation and filtration. The water temperature was maintained at 27°C-29°C. After the eggs hatched, the females were placed back to the aquarium.

In each trial, on the first day after hatch (DAH 1) the newly hatched larvae of the two shrimp species were distributed into the treatment media consisting of three 500 mL beakers in triplicates. Each beaker was filled with seawater, aerated very gently through a glass pipette, and stocked with 30 larvae. The water exchange was done at 100% every day by gently transferring each survived larva into a new clean beaker filled with fresh seawater by using a modified 1 mL plastic pipette. The larvae were maintained in an optimum culture environment within the recommended temperature and feeding regime (Zhang *et al*., 1998; Calado *et al*., 2008, 2009; Figueiredo & Narciso, 2006). The temperature was monitored and maintained at 27°C-29°C with a heater placed whenever necessary.

The dietary treatments implemented in the first trial were 1A, 1B, and 1C. Microalgae *Isochrysis* sp. at a concentration equivalent to 100,000 cells/mL was used as treatment 1A, copepod *Parvocalanus crassirostris* at a density of 10 ind./mL was used as treatment 1B, and rotifer *Brachionus rotundiformis* at a density of 15 ind./mL was used as treatment 1C. In trial-2, the dietary treatments implemented were treatments 1D, 1E, and 1F. A combination of rotifer *B. rotundiformis* at a density of 10 ind./mL and microalgae *Isochrysis* sp. at a concentration equivalent to 50,000 cells/mL was used as treatment 1D. A mixed of copepod *P. crassirostris* at 5 ind./mL density and microalgae *Isochrysis* sp. at a concentration equivalent to 50,000 cells/mL was used as treatment 1E. Newly hatched *Artemia nauplii* at a density of 10 ind./mL was used as treatment 1F. In the third trial, the treatments implemented were 2A and 2B. A combination of rotifer *B. rotundiformis* at a density of 10 ind./mL and microalgae *Isochrysis* sp. at a concentration equivalent to 50,000 cells/mL was used as treatment 2A. Treatment 2B was divided into two phases, $2B^1$ and $2B^2$. Copepod *P. crassirostris* at the first two weeks (day 1 to 14) at a density of 10 ind./mL was used as treatment $2B^1$. After that period, newly hatched *Artemia* (day 14 to end) at a density of 10 ind./mL was used as treatment $2B^2$.

All the microalgae, copepod, rotifer, and *Artemia* used in these experiments were live foods. The microalgae, copepod, and rotifer were taken from

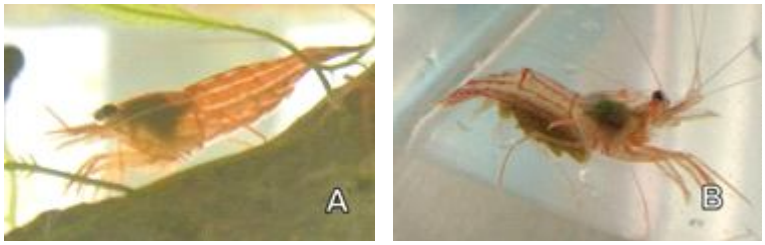

Figure 1. Peppermint shrimp at MARFU, JCU: (A) *Lysmata vittata*; (B) *Lysmata intermedia*, carrying eggs.

cultured tanks while the *Artemia* were produced daily. Each larva that successfully reached the post-larval stage was transferred into separate tanks and was included in final counting of survivability after the treatments ended. The treatments were considered complete when there were no survived larvae left in each beaker or when the last larvae in a beaker successfully transformed to the post-larval stage. One exception was for trial2, which all the treatments had to be stopped at day after hatch (DAH) 46 due to the limited resources and time.

Calculation and Statistical Analysis

The daily average survival rates (DASR) and temperature variations were assessed for variance (Levene's test and boxplot) and then analysed with one way ANOVA. Significant differences ($P<0.05$) in all treatments were identified using the T-method multiple comparison test (Sokal & Rohlf, 1995). The number of survived larvae at the end of each treatment was counted as the final average survival rate (FASR).

RESULTS AND DISCUSSION

In trial-1, the overall survival rates were low in all treatments (Figure 2). At day 23, the last larva in treatment 1A had died before reaching post-larval stage. The larvae fed with copepod (treatment 1B) had a similar condition where the last larva had died on day 34 without being able to transform to post-larval stage. The larvae fed with rotifer (treatment 1C) had the best performance compared to the other treatments in trial-1 where four larvae survived and successfully reached post-larval stage. This study found that after DAH 5, rotifer was more effective to support larvae survivability than microalgae or copepod (Figure 2). The final average survival rate (FASR) for *L. vittata* larvae fed with rotifer was 4.44%. Statistically, there were significant differences ($P<0.05$) in the survival rates between the larvae fed with microalgae, copepod, and rotifer.

Trial-2 has shown similar results compared to the first one where the overall survival rates were low in all treatments (Figure 3). However, in the three treatments of trial-2, some larvae had survived at the end of the experiment (day 46). Despite that, none of the survived larvae had transformed to post-larval stage at the end of the experiment (day 46). In treatment 1D, three larvae were still survived at day 46 with 3.33% FASR. In treatment 1E, there was only one larva survived at day 46 with 1.11% FASR. The larvae in treatment 1F showed a better condition where five still survived at day 46 with 5.56% FASR. However, regardless of these seemingly similar situations, there were significant differences ($P<0.05$) on the survival rates among these three different treatments.

In all treatments of trial-3 with shrimp species *L. intermedia*, no larvae survived and reached the post-larval stage (Figure 4). In treatment 2A, no larvae survived at day 39. In treatment 2B, at the first two weeks (day 1 to 14; 2B1) and after that (day 14 to end; 2B2), the feed was changed with *Artemia*. The FASR of treatment 2B was better than that of treatment 2A where one larva had survived in treatment 2B two days after the last larva in treatment 2A died. Despite none of the larvae in both treatments survived or transformed to post-larval stage, the graph (Figure 4) noticeably shows that treatment 2B was better than treatment 2A based on the daily average survival rate (DASR). Furthermore, there were significant differences ($P<0.05$) in the survival rates between larvae fed with treatments 2A and 2B.

The average daily temperature during trial-1 was the highest (28.84 ± 0.17°C) and statistically significant ($P<0.05$) compared to trial-2 (27.63 ± 0.16°C) and trial-3 (27.76 ± 0.14°C) (Figure 5). Unfortunately, the ambient temperature, as well as the water temperature at the experiment sites, started to decrease by the end of April 2014. In order to maintain water temperature between 28°C-29°C, a heater was placed in the containers starting on 8 May 2014, which was day 22 of trial-2 and day 31 of trial-3, until the experi-

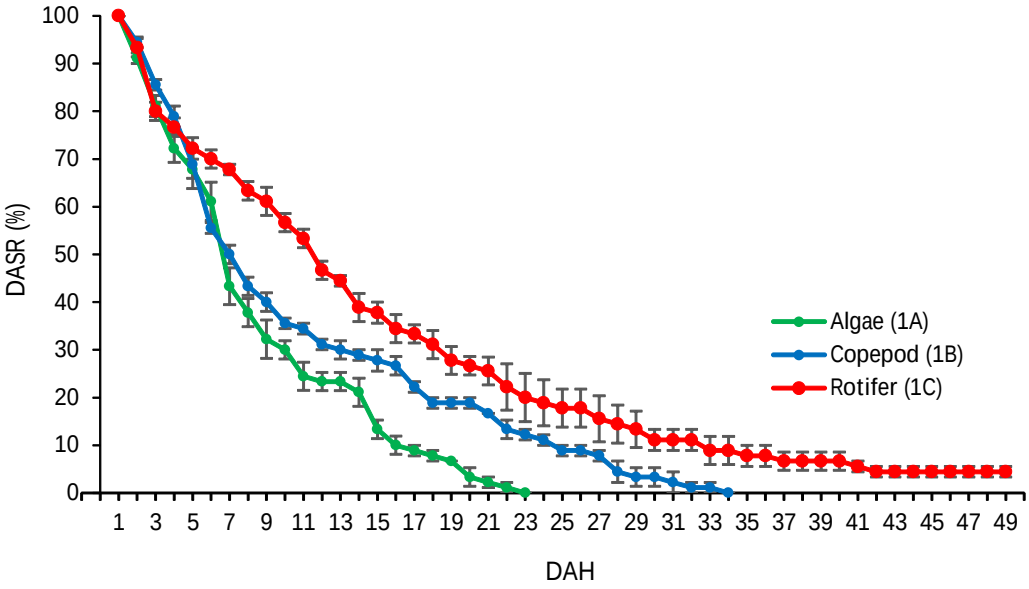

Figure 2. Daily average survival rates (%) of *Lysmata vittata* fed with microalgae *Isochrysis* sp. (treatment 1A), copepod *Parvocalanus crassirostris* (treatment 1B), and rotifer *Brachionus rotundiformis* (treatment 1C). Error bars represent ± SE.

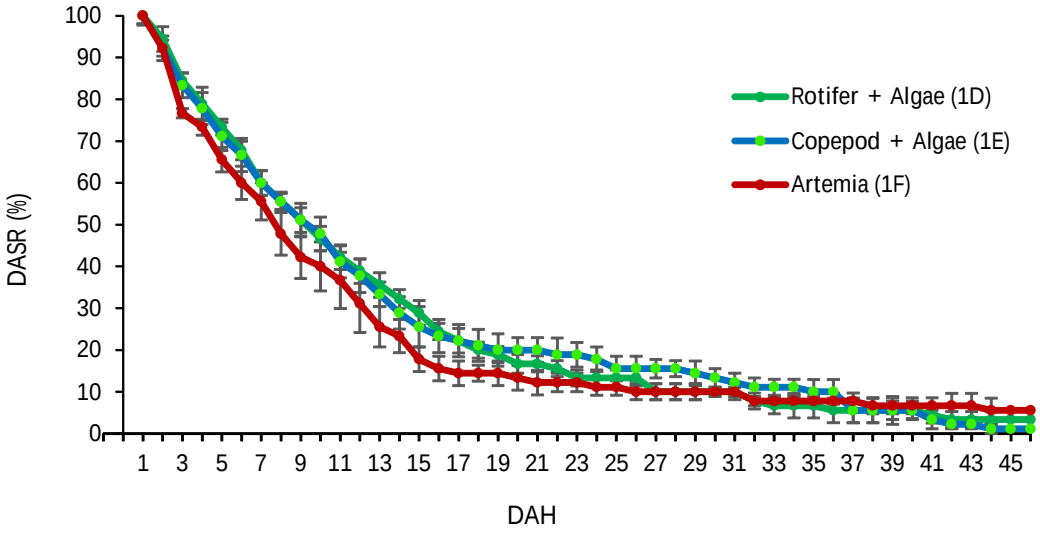

Figure 3. Daily average survival rates (%) of *Lysmata vittata* fed with combination of rotifer *Brachionus rotundiformis* and microalgae *Isochrysis* sp. (treatment 1D), copepod *Parvocalanus crassirostris* and microalgae *Isochrysis* sp. (treatment 1E), and *Artemia* only (treatment 1F). Error bars represent ± SE.

ments ended. The beakers used in these trials were proportionately placed inside two containers filled with water with the same water height as the beakers. Then the heater was placed between the beakers. Therefore, the ambient temperature data on and after 8 May 2014 were excluded from this report.

Overall, these experiments have resulted in low survival rates in all treatments for *L. vittata* and *L. intermedia* even though the temperature, salinity, and feeding regimes were maintained at recommended ranges. This result might relate to the high initial larval stocking density of 60 ind./L. Cunha *et al*. (2008) reported that the survival rate of cleaner shrimp, *L. amboinensis* was significantly higher at the initial stocking density of 10 ind./L rather than at the density of 20 ind./L, with the highest survival rate reach 48% for larvae fed with rotifer at 35 ind./mL. However, these findings by Cunha *et al*. (2008) observed the average

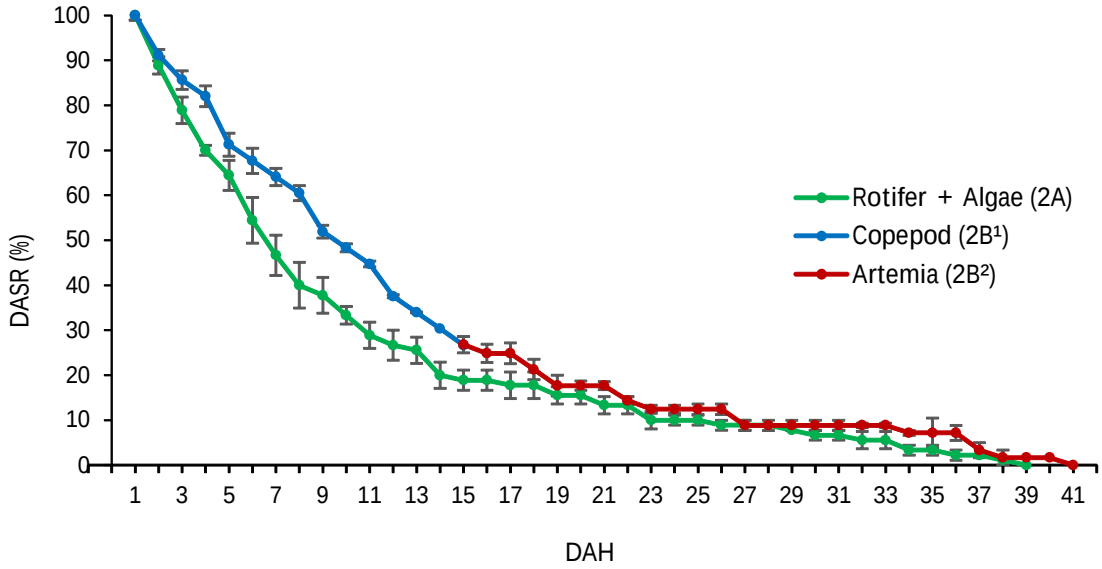

Figure 4. Daily average survival rates (%) of *Lysmata intermedia* fed with treatment 2A which was a combination of rotifer *Brachionus rotundiformis* and microalgae *Isochrysis* sp. and larvae fed with treatment 2B which these larvae were fed with copepod *Parvocalanus crassirostris* at the first two weeks (day 1 to 14; 2B1) and then after that change with *Artemia* nauplii (day 14 to end; 2B2). Error bars represent ± SE.

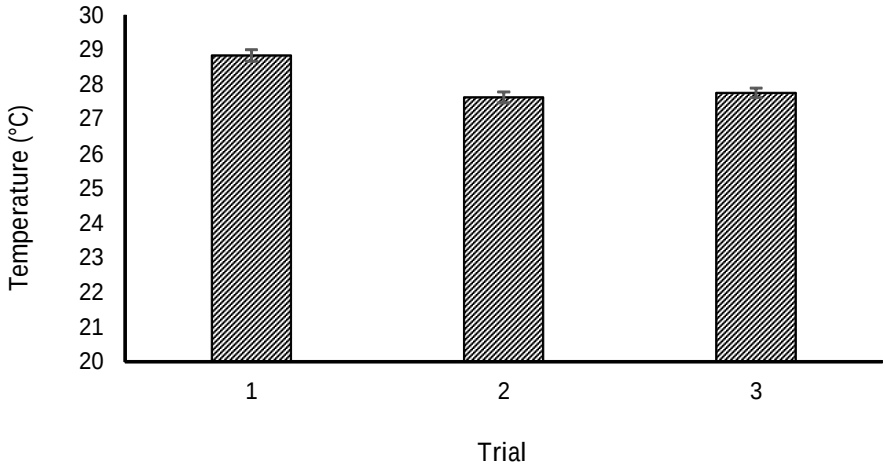

Figure 5. The average daily temperature during the experiments. Error bars represent ± SE.

survival rate of *L. amboinensis* fed with different amount of rotifer at three density: 20, 35, and 50 ind./mL, and only during the first six days of larval stage. Compared to present research results in which larvae of *L. vittata* stocked at a higher initial density of 60 ind./L and fed with a lower amount of rotifer at 15 ind./mL (treatment 1C), at day-6 this present experiments achieved an average survival rate of 70.0%. The effects of such densities are most probably related to stress, physical damage, or discomfort as a consequence of higher encounter rates between individuals (Cunha *et al*., 2008).

All treatments for both *L. vittata* and *L. intermedia* larvae had a significant effect ($P<0.05$) to the DASR of both larvae. Larvae with treatments 1A and 1B had the lowest survival rates with none of them survived after day 22 for treatment 1A and day 33 for treatment 1B. Zhang *et al*. (1998) reported that the use of microalgae is only effective to support the early stages of larvae and none of them survived to later stages or even transformed to post-larval stages. Nevertheless, using copepod as one of the diets for the early larval stages of *Lysmata* spp. is highly potential due to a better survival rate compared to using

microalgae. In spite of this, the treatments with copepod in this experiment have similar results to the treatment with microalgae where they are effective to support the early stages of the larvae. These results probably related to the swimming behavior and body size of calanoid copepod, *P. crassirostris* used in this experiment. Calanoid copepod is a planktonic species that spends all its life cycle in the water column (Støttrup, 2006). Contrastingly, at the later larval stages, peppermint shrimp *Lysmata* spp. tend to settle at the bottom rather than swimming (Zhang *et al*., 1998; D'Acoz, 2000; Marin *et al*., 2012). In addition, their swimming patterns are significantly different than rotifer. Calanoid copepod has faster swimming speed and higher rates of change of direction and accelerations compared to rotifer. These abilities resulted in higher escape responses of the copepod from predation compared to rotifer (Buskey *et al*., 1993). Furthermore, the size of calanoid copepod has also influenced the capturing ability of *Lysmata* spp. larvae. Calanoid copepod has a smaller body size compared to rotifer (Buskey *et al*., 1993). Although larvae of *Lysmata* spp. are not a visual predator, they primarily rely on their olfactory and mechanical sensory to capture preys (Rhyne & Lin, 2004). These resulted in lower success rates of *L. vittata* and *L. intermedia* at a later larval stage to capture copepod compared to rotifer

In contrast, the experiment with treatment 1C has four survived larvae, all of which successfully reached post-larval stage. The experiment also has shown that the use of rotifer resulted in a better larval survival rate at a later stage compared to microalgae or copepod. Many studies such as Buskey *et al*. (1993); Zhang *et al*. (1998); and Cunha *et al*. (2008), have confirmed the effective use of rotifer to feed larvae of *Lysmata* spp. A study by Zhang *et al*. (1998) has also confirmed that the use of rotifer led to a better survival rate along with a successful transformation of some of the larvae to reach post-larval stage at around day 32-36. Zhang *et al*. (1998) findings were equivalent to trial-2 which in these three treatments (1D, 1E, and 1F), some larvae still survived until the last day of the experiment (day 46). However, none of the survived larvae in trial-2 were transformed to post-larval stage until the end of the experiment at day 46. The reason for a longer period of larval stages might be associated with the lower average temperature in this second trial compared to the first trial (Figure 5). In these experiments, all treatments were conducted within the range of recommended temperatures of 24°C-30°C (Zhang *et al*., 1998; Figueiredo & Narciso, 2006; Calado *et al*., 2008, Calado *et al*., 2009). However, one exception was that the temperature in trial-2 before the installation of the heater at day 22 was lower (27.63 ± 0.16°C) than in trial-1 (28.84 ± 0.17°C). According to Zhang *et al*. (1998) and Calado *et al*. (2008), a lower temperature will significantly prolong the duration of the larval stage of *Lysmata* spp.

In treatment 1D, three larvae still survived at day 46 with 3.33% FASR. This survival rate was lower than in treatment 1C where none of the larvae had reached post-larval stage. However, it is hard to make a comparison between them since treatment 1D was conducted in a lower temperature range than treatment 1C which might affect the survival rate as the consequence of longer larval development at such high density. In treatment 1E, there was only one larva survived at day 46 with 1.11% FASR. This result is better than treatments 1A and 1B. On the contrary, the use of only *Artemia* in the diet (treatment 1F) has produced a lower survival rate at the early larval stage than the other treatments (1D and 1E). In spite of this, *Artemia* diets resulted in a better survival at the later larval stage. Several studies have also indicated that the use of *Artemia* as a diet for larvae of *Lysmata* spp. resulted in a better growth, development, and survival rate compared to other diets such as microalgae and rotifer (Zhang *et al*., 1998; Lin *et al*., 2002).

In trial-3, no larvae survived and reached post-larval stage (Figure 4). Although closely associated with *L. vittata* in size, appearance, color pattern, and habitat, *L. intermedia* has quite different characteristics. This species tends to be more vulnerable than other peppermint shrimp species, especially *L. vittata*. D'Acoz (2000) discovered that the geographical distribution of *L. intermedia* is limited to tropical/subtropical region range from western Atlantic to the western Indian Ocean. On the other hand, *L. vittata* has a wider geographical distribution ranging from the eastern Atlantic to the western Pacific. It can be found along the eastern part of the African continent and Russian water of Sea of Japan to the northern coast of New Zealand (Marin *et al*., 2012). This wide-ranging distribution indicates that *L. vittata* is a more robust species than *L. intermedia*. Therefore, the culture and nursery of larvae *L. intermedia* are more difficult and complicated which probably responsible for the higher mortality rate of this species. In this trial, treatment 2B had a better average survival rate from the early stage until the end of the experiment. This result indicates that the use of copepod at an early stage was more effective to improve the survival rate of peppermint shrimp larvae rather than only fed them with *Artemia* as achieved in the previous experiment (treatment 1F). However, it is arguably difficult to make a comparison between both studied species as

they have different responses to given feed and sensitivity to rearing environment.

CONCLUSION

The survival rates of *L. vittata* and *L. intermedia* larvae were low in all different combinations and feeding practices of live foods. However, each treatment showed a significant effect ($P < 0.05$) on the average survival rate of both species larvae. The use of copepod as live feed at an early larval stage and *Artemia* at a later stage is relatively more effective to improve the survival rate of peppermint shrimp larvae compared to the other treatments.

Both of the species are sensitive to low temperature which leads to a prolonged larval stage. A stable temperature at 28°C-29°C is advised in rearing *Lysmata*s spp. larvae to ensure the normal transformation to post-larval stage.

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