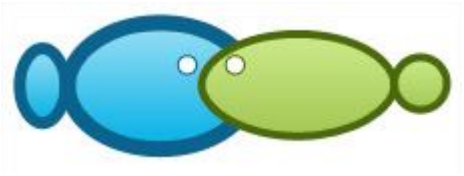

Amphiascoides neglectus (Copepoda: Harpacticoida) as diet for aquarium corals cultured under laboratory condition

[1]Hazwani H. Hasnan, [2,3]Wan Mohd. A. Wan Sulaiman, [1]Zuhairi Ahmad, [4]Nadiah Wan Rasdi, [1,3]Zaleha Kassim

[1] Kulliyyah of Science, International Islamic University Malaysia, Kuantan, Pahang, Malaysia; [2] Kulliyyah of Pharmacy, International Islamic University Malaysia, Kuantan, Pahang, Malaysia; [3] INHART, International Islamic University Malaysia, Gombak, Selangor, Malaysia; [4] School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, 23010, Kuala Terengganu, Terengganu, Malaysia. Corresponding author: Z. Kassim, drzack@iium.edu.my

Abstract. The main issue in coral aquaculture is the identification of suitable food that can speed up the growth and supply enough nutrients. While copepods can be found in coral ecosystem, the study of its contribution in coral growth is far from mature. A series of feeding experiment was therefore carried out to investigate the effect of copepod diet (*Amphiascoides neglectus*) on coral growth under laboratory conditions. The feeding of coral (*Protopalythoa* sp., a soft coral and *Acropora* sp.) on *A. neglectus* nauplii was examined for seven days to investigate the feeding preference time. Overall, there was no significant effect of feeding times between groups (am and pm) as shown by the F-test result where the *p*-value was $p = 0.260$ ($p > 0.05$). From tests of between-subjects effects, there was no significant difference of mean feeding on corals between two different feeding times ($p = 0.312$) regardless of day. The coral growth was measured (increase of size) for five weeks. Overall comparisons between two groups on different types of feeds (*A. neglectus* nauplii and mixed copepods) were analyzed over coral growth. There was no significant difference in growth as shown by the F-test where the *p*-value was $p = 0.794$ ($p > 0.05$). From tests of between-subjects effects, there was no significant difference in mean coral growth between two different types of copepods ($p = 0.579$) regardless of week. Results indicate the suitability of cultured copepod *A. neglectus* to maintain corals in captivity with less procedure and more cost effective.

Key Words: *Amphiascoides neglectus,* aquarium coral, harpacticoid copepod, live feed.

Introduction. Coral ecosystem is an important bottom substrate for the existence of harpacticoids (Gheerardyn et al 2009; Kramer et al 2014). It has been reported to house cyclopoids as well as some other harpacticoid species (Cheng et al 2016). As one of the main groups in meiobenthos, harpacticoids crawl along the sea bottom while some other benthic harpacticoid copepods swim vertically as their strategy of dispersal in shallow water column, acting as zooplanktons (Shimode et al 2006). Harpacticoids act as a source of dietary supplement for most coral reef fish larvae (Llopiz & Cowen 2009). For instance, zooplankton is an important dietary component for corals that are related to anthozoans; *Stylophora pistillata* (Ferrier-Pagès et al 2003), *Galaxea fascicularis*, and *Tubastrea aurea* (Houlbrèque et al 2004).

One of the greatest challenges in intensive coral aquaculture is to find the optimal source of nutrition to achieve the fastest growth rate (van Os et al 2012). The common practice in aquaculture is the use of *Artemia* as live feed. However, studies (e.g. Cutts 2003; Drillet et al 2011) have proven its deficiency of nutrient supply if compared to copepods. Moreover, nauplii of *Artemia* are bigger than those of the copepods, thus limit the ingestion performance (Barros & Valenti 2003).

Harpacticoid copepods have shown their potential use as live feed for aquaculture due to their smaller sizes of nauplii and adults in addition to their good nutritional profile,

particularly their fatty acid contents (Rhodes 2003a; Ladhar et al 2014). When cultured under the right conditions, harpacticoids could produce a large number of nauplii as live feed supply (Zaleha & Busra 2012).

The use of harpacticoids as live feed for aquarium corals is still new, the basic information on the harpacticoid ingestion by corals living in captivity is still scarce, and the effect of harpacticoid feeding on the captive coral growth is yet to be known. Thus, this study addressed these gaps by analysing the suitability of harpacticoids as live feed for corals.

Material and Method

Preparation for A. neglectus. The study was carried out between January 2016 until January 2017. Harpacticoid *Amphiascoides neglectus* was cultured since February 2016 in the marine laboratory at Kulliyyah of Science, International Islamic University Malaysia. The salinity, the temperature and the light exposure were controlled at 27-35 ppt, 25-32°C and 12L: 12D respectively. After one month, the culture was then up-scaled as to control the density in which Bakers's yeast was used as their diet. Culture procedure was adapted from Zaleha & Farahiyah Ilyana (2010) and Zaleha et al (2016).

Preparation for corals. Samples of corals obtained from a coral aquarium shop were button corals *Protopalythoa* sp. and branching coral *Acropora* sp., which were later maintained in an incubation tank for two weeks for acclimation and healing of any wounds. The samples were observed daily to ensure all the polyps having fully opened, indicating their good health condition. No feed was offered during this phase.

Experiment 1: feeding time for coral. This experiment was carried out in the duration of three months. Both species of corals as samples were fragmented prior to the experiment and each fragment were glued on a piece of bio-rock. During the experiment, all samples were placed in a 40L tank filled with 35L artificial saltwater. The artificial saltwater was prepared by a dilution of artificial sea-salt in Reverse-Osmosis (RO) water. The water quality was maintained at the optimum condition (< 4 µM ammonium, < 1 µM nitrate, < 0.3 µM nitrite and < 0.5 µM phosphorus) to reduce stress of the samples as recommended by Ferrier-Pagès et al (2000).

Nauplii of *A. neglectus* were collected from a population stock by sieving through a 62 microns sieve and then through a 20 microns sieve. Those retained on the 20 microns sieve were used for the experiment. The feeding ration was offered at 60 ind mL^{-1} in each tank. The feedings were carried out two times a day, morning (7:00 am) and evening (6:00 pm), for seven days, up to eight weeks. The samples were fed for two hours at every feeding time. After two hours, the uneaten copepods were collected back by sieving using a 20 µ mesh net and calculated under a stereo microscope.

Experiment 2: coral feeds on different copepods. This experiment was done right after the completion of Experiment 1 and took another two months to complete. The samples were divided into two groups based on feeding with two different treatments, as presented in Table 1. The replicates of corals as samples were transferred into independent vials of 100 mL for one hour before commencing the feeding experiment. This was to ensure all the polyps have expended well. The water used was taken from the incubation tank to reduce the samples' stress.

Table 1

Experiment summary for copepod diets offered to the coral species

Coral species	*Treatment*	
	Amphiascoides neglectus	*Wild mixed copepod*
Protopalythoa sp.	√	√
Acropora sp.	√	√

The growth of the samples was measured in correspondence to the increase of weight and size (diameter for *Protopalythoa* sp. and length for *Acropora* sp.).

Results

The population of A. neglectus as stock of live feed. *A. neglectus* showed good growth performance by producing high number of nauplii and copepodids under the culture condition in the laboratory (Table 2). They have increased in density after the 6th day of culture and needed subsequent two weeks (14 days) to reach their maximum density (Figure 1). The numbers of gravid females have increased gradually throughout the culture period, indicating good supply of stock for future nauplii.

Table 2

Mean density (ind mL^{-1}) of different stage of *A. neglectus* in 21 days of culture

Growth stage	*Density*
Nauplii	26.29±16.84
Copepodids	23.19±16.82
Non-gravid females	11.27±6.93
Gravid females	8.52±6.17

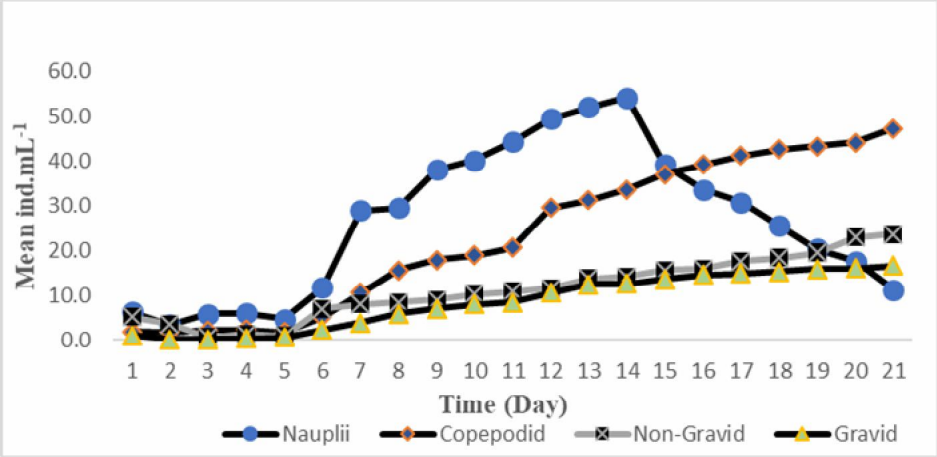

Figure 1. Population of *A. neglectus* cultured as stock of live feed under the laboratory condition.

Effect of feeding time on A. neglectus ingestion. Data on the feeding of corals were recorded each day for seven days: Day 1, Day 2, Day 3, Day 4, Day 5, Day 6 and Day 7, and the overall comparisons between the two groups of time (am and pm) were analyzed. F-test was carried out at the *p*-value of less than 0.05 to indicate the significant difference between the groups of time (Table 3).

Table 3

Test of between subjects effects, time of feeding and day of experiment

Variables	*Mean square*	*F*	*p-value*
Day	64.746	2.467	0.22
Time	1139.532	1.272	0.260
Day*Time	31.081	1.184	0.312

This test was then followed by the post-hoc multiple comparisons to identify which pairs of groups were significant if there were more than two levels of groups (Table 4). Post-hoc multiple comparisons result showed that there was no significant difference in the mean feeding time (p = 0.260) between morning (am) and evening (pm). It was concluded that there was no significant difference in the ingestion of harpacticoid nauplii when fed between in the morning and in the evening.

Table 4

Mean difference of feeding test on corals between two different times (am and pm)

Comparison	*Mean difference (95% CI)*	*p-value*
am-pm	-1.595 (-4.379, 1.190)	0.260

There was also no significant difference if compared by day (Table 5), indicating a consistent result from the daily data collection.

Table 5

Comparisons of mean feeding of corals between two different time groups based on day

Day	*Feeding time*	*Mean feeding test on coral (SD)*	*95% CI*	
			Lower	*Upper*
Day 1	AM	28.61 (10.865)	26.71	30.51
	PM	31.38 (12.575)	29.18	33.57
Day 2	AM	28.08 (11.201)	26.12	30.04
	PM	30.27 (12.497)	28.08	32.45
Day 3	AM	28.16 (12.197)	26.03	30.30
	PM	29.32 (12.627)	27.11	31.53
Day 4	AM	27.73 (11.653)	25.69	29.76
	PM	29.49 (12.861)	27.24	31.74
Day 5	AM	28.68 (11.859)	26.61	30.75
	PM	30.04 (12.702)	27.82	32.26
Day 6	AM	28.31 (12.052)	26.20	30.42
	PM	29.03 (12.817)	26.79	31.27
Day 7	AM	28.15 (12.078)	26.04	30.26
	PM	29.36 (13.478)	27.00	31.72

Repeated measure of ANOVA between time analyzed with regard to day was applied with the assumptions that the normality, the homogeneity of variance and the compound symmetry were checked and were fulfilled. Based on Table 5, there was no significant difference in the mean feeding test of corals between am and pm in Day 1 to Day 7. It can be concluded that there was no difference in feeding of corals between in the morning and in the evening throughout the experimental period.

Effect of types of copepod diet on coral growth. Both types of diets; the nauplii of *A. neglectus* and mixed copepods from the wild, were offered to both types of corals. There was no significant difference in weight increment (Figure 2), length increment (Figure 3) and diameter increment (Figure 4) between corals fed with different copepods for both types of corals.

The growth data was then pooled for both species and was analyzed for five weeks: Week 1, Week 2, Week 4, Week 6 and Week 8. The overall comparison between two groups with different types of nauplii diet (*A. neglectus* and mixed copepod) were also analyzed over coral growth (week). The *p*-value based on F-test was more than 0.07 ($p = 0.794$), indicating that there was no significant difference in diet types on the corals (Table 6). From the tests of between-subjects effects, there was no significant difference of mean of coral growth between two different types of feeding ($p = 0.579$) regardless of week.

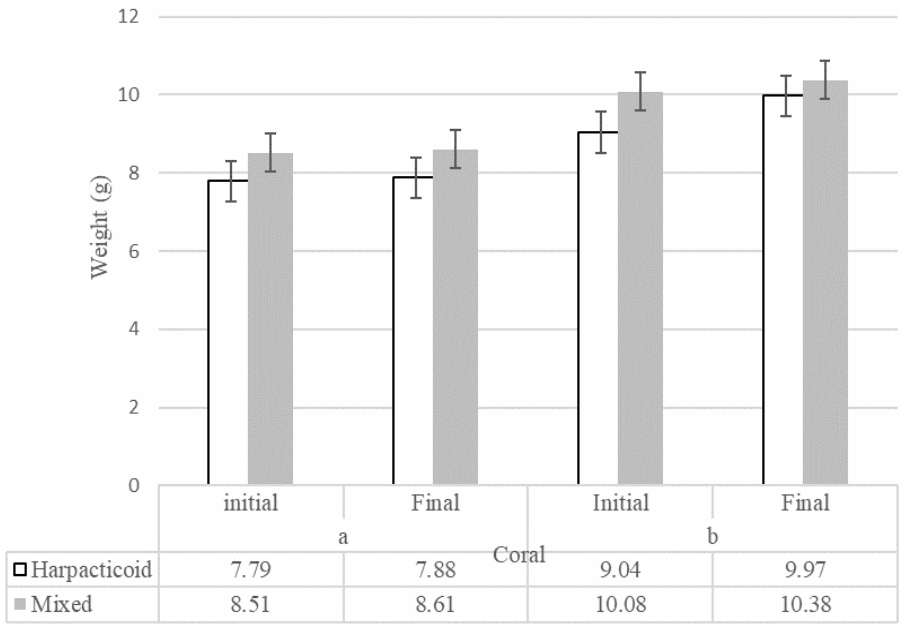

Figure 2. Weight increment for *Protopalythoa* sp. (a) and *Acropora* sp. (b) fed with different copepod diet for 8 weeks of experiment.

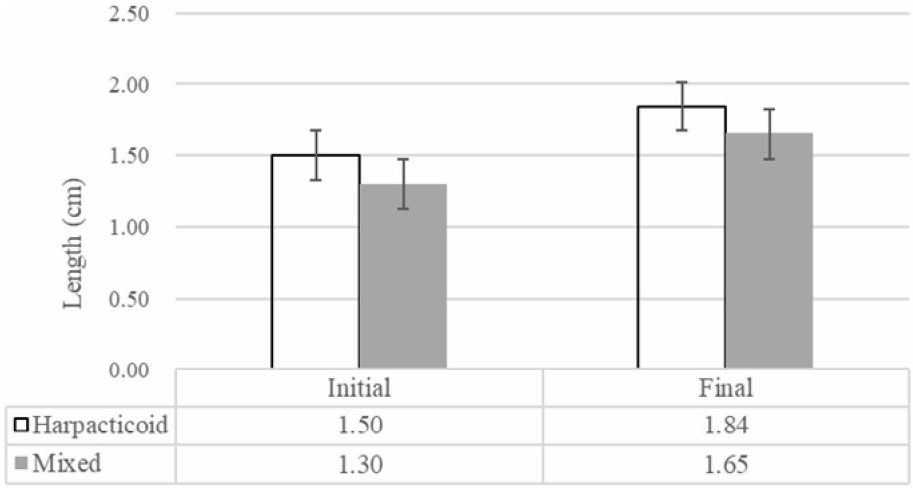

Figure 3. Length increment for *Acropora* sp. fed with different copepod diet for 8 weeks of experiment.

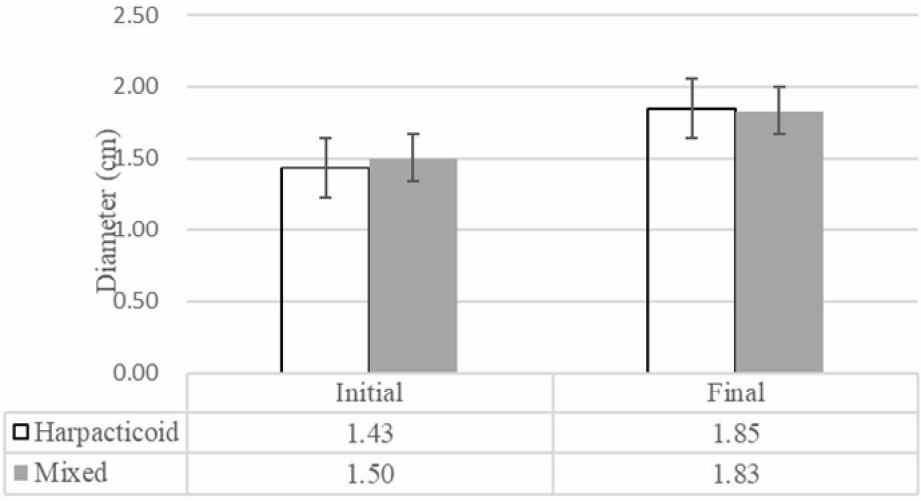

Figure 4. Diameter increment for *Protopalythoa* sp. fed with different copepod diet in 8 weeks of experiment.

Table 6

Test of between subjects effects different diet on growth of corals according to week

Variables	*Mean square*	*F*	*p-value*
Week	0.619	9.920	0.000
Feed	5.293	0.070	0.794
Week*Feed	0.045	0.721	0.579

Based on Table 7, post-hoc multiple comparisons result showed that there was no significant difference of mean types of diet (*A. neglectus* and mixed copepod) on the growth of corals (p = 0.794).

Table 7

Mean difference of feeding test on corals between two different copepod types

Comparison	*Mean difference (95% CI)*	*p-value*
Harpacticoid – Mixed copepod	-0.364 (-3.178, 2.450)	0.794

Repeated measure ANOVA between feed analyses with regard to week was applied with the assumptions that the normality, the homogeneity of variance and the compound symmetry were checked and were fulfilled. Based on Table 8, in Week 1, there was no significant difference in mean feeding types between coral growths. For Week 2 to Week 8, there was also no significant difference in mean feeding types between coral growths. It can be concluded that there was no significant difference in feeding types between coral growths.

Table 8

Comparison of mean of coral growth between two different feeds on coral groups

Coral growth (week)	*Types of feed on coral*	*Mean (SD)*	*95% CI*	
			Lower	*Upper*
Week 1	Harpacticoid	4.938 (3.66)	2.987	6.888
	Mixed copepod	5.347 (4.208)	3.105	7.589
Week 2	Harpacticoid	4.988 (3.622)	3.058	6.918
	Mixed copepod	5.389 (4.180)	3.162	7.617
Week 4	Harpacticoid	5.053 (3.590)	3.140	6.966
	Mixed copepod	5.459 (4.134)	3.256	7.662
Week 6	Harpacticoid	5.124 (3.534)	3.241	7.001
	Mixed copepod	5.494 (4.109)	3.304	7.683
Week 8	Harpacticoid	5.384 (3.766)	3.378	7.391
	Mixed copepod	5.617 (4.144)	3.408	7.825

Discussion. Several species of benthic harpacticoid copepods were successfully tested for their suitability as live feeds in aquaculture (Pinto et al 2001; Cutts 2003; Rhodes 2003b; Olivotto et al 2008). In this study, *A. neglectus* proved to be a potential candidate due to the rapid increase in population, density of nauplii produced and stable population growth in the culture container. Nauplii production is an important aspect of live feed as it is always very small in size yet highly nutritious (Karlsen et al 2015).

Some commercial corals grew in captivity were reported to ingest *Artemia* without significant difference in the feeding times (Tagliafico et al 2018). Corals in captivity were also reported to showing better health conditions when fed with fatty acid enriched *Artemia* (Lim et al 2017). Cultured harpacticoid was reported as highly rich in essential fatty acid (Zaleha et al 2014; Zaleha et al 2016) thus suitable as live feed for corals. The ingestion had no difference between morning and evening, which could not be the important factor in terms of nutrient supply. This is possibly true as corals might just digest the prey externally as extracoelenteric digestion to get the nutrient and that this takes up to six hours (Wijgerde et al 2011). Thus, the calculation of the leftover

copepods in the culture vessel would positively relate to the number of copepods not digested by the corals externally.

The dependency on symbiotic microalgae alone might not support the growth and corals could switch from autotroph to the heterotroph feeding for survival (Hoogenboom et al 2010). Heterotrophic feeding is definitely an important strategy for nutrient and energy supply in corals (Houlbrèque & Ferrier-Pagès 2009). Thus, heterotroph feeding on zooplankton such as harpacticoid copepod could compensate the autotrophic stress and at the same time, could provide essential fatty acids (Rhodes 2003a; Ladhar et al 2014) required by the corals. Heterotrophic compensation is a possible mechanism for corals to manage stress in their natural habitat (Hughes & Grottoli 2013). Reports from these studies indicated the acceptability of corals in captivity towards offered diet and the importance of nutritious diet to their growth performance.

The non-preference diet offered to the captive corals could be due to their adaptability to the culture condition. Aquarium corals are known to be maintained by various single diets for the purpose of survival. On the other hand, wild copepods were reported to supply high fatty acid for aquaculture purposes (Rocha et al 2017). Nonetheless, it was also found that cultured zooplankton might be a viable and cost-effective strategy for increasing the growth of the intensively cultured marine species (Katan et al 2016). Domestication of wild copepods would be a better strategy than to solely depend on the wild. It was reported that domestication would improve the fatty acid contents and reproduction capacity in copepods (Alajmi et al 2015) which will then reduce the operation cost. On another note, the ingestion of food could be slowed down if the corals were cultured beyond the optimal condition (Ferrier-Pagès et al 2010) or in an elevated temperature since water condition would affect the autotrophic efficiency and the polyp expansion for external feeding (Pacherres et al 2013). Thus, maintenance of the culture condition at the optimal level would be the main priority in coral aquaculture.

Conclusions. Marine harpacticoid, *A. neglectus,* cultured under optimal conditions in the laboratory has successfully produced nauplii as a diet for aquarium corals *Protopalythoa* sp. and *Acropora* sp. The feeding times did not affect copepod ingestion by these corals. These corals feeding on harpacticoid have also shown growth without significant difference from the wild mixed copepod fed on corals, indicating the acceptability and adaptability of aquarium corals to the offered diet. The results would improve our knowledge on the feeding procedures for coral culture.

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Authors:
Hazwani Hanim Hasnan, Department of Marine Science, Kulliyyah of Science, International Islamic Univeristy Malaysia, P.O Box 141, 25710 Kuantan, Pahang Darul Makmur, Malaysia, e-mail: waniehanimhasnan253@gmail.com
Wan Mohd. Azizi Wan Sulaiman, Kulliyyah of Pharmacy, International Islamic University Malaysia, P.O.Box 141, 25710 Kuantan, Pahang Darul Makmur, Malaysia ; International Institute for Halal Research and Training (INHART), Level 3, KICT Building, International Islamic University Malaysia, P.O Box 10, 50728 Kuala Lumpur, Malaysia, e-mail: drwanazizi@iium.edu.my
Zuhairi Ahmad, Department of Marine Science, Kulliyyah of Science, International Islamic Univeristy Malaysia, P.O Box 141, 25710 Kuantan, Pahang Darul Makmur, Malaysia, e-mail: zuhairiahmad@iium.edu.my
Nadiah Wan Rasdi, School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, 21030, Kuala Terengganu, Terengganu, Malaysia, e-mail:nadiahrasdi@gmail.com
Zaleha Kassim, Department of Marine Science, Kulliyyah of Science, International Islamic Univeristy Malaysia, P.O Box 141, 25710 Kuantan, Pahang Darul Makmur, Malaysia; International Institute for Halal Research and Training (INHART), Level 3, KICT Building, International Islamic University Malaysia, P.O Box 10, 50728 Kuala Lumpur, Malaysia, e-mail: drzack@iium.edu.my
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