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Research Article
Optimum Diet for Survival and Development Growth of Laboratory-scale Culturing Harpacticoid Species, *Stenhelia stephensoni* (Greenwood and Tucker 1984)

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Abstract

Objective: This study aims to introduce diets that are more affordable and applicable, compared to algal diet, which is time consuming and costly in maintenance. **Methodology:** The present study was designed for 15 days to observe the effects of different diets on population growth of marine tropical harpacticoid copepod, *Stenhelia stephensoni* under laboratory condition. The constant value of salinity was at 28 PSU and temperature at 27°C. **Results:** The diets tested were processed into juice form and are composed of single type diets and combination diets. Single type diets were made of carrot, banana, sago, *catappa* leaf, goat dung and seaweed, while combination diets were made with the mixture of goat dung and seaweed and goat dung with *catappa* leaf. Harpacticoid copepod which were fed with combination diet of goat dung and *catappa* leaf recorded significantly ($p<0.05$) higher population growth and survival (90%) compared to the rest of the treatments tested. **Conclusion:** The present study suggested that *Stenhelia stephensoni* could be a potential copepod for being commercially cultivated by as it is highly demand as food item for marine fish larvae in aquaculture industry.

Key words: Goat dung, *catappa* leaf, single diets, combination diets, *Stenhelia stephensonii*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Copepods had received much attention recently as they were proven to play an important role in aquaculture as live feed. They are naturally enriched with essential fatty acids, which are crucial for optimal growth of marine fish larvae[1-4]. Several studies have demonstrated that deficiency of these nutrients may cause general decrease of animal health such as retardation growth[5], abnormalities in visual and central nervous system development[6], in swimming and feeding activity[7] and also in the immune system[8].

Harpacticoid copepods are gaining popularity as potential live feed in marine larviculture[9-11]. Apart from nutritional factor, harpacticoids have a high reproductive potential, short turnover time, fast population growth and a wide range of environmental factors such as temperature and salinity[12-14]. Furthermore, harpacticoid copepods also offered a broad spectrum of sizes suitable for various fish larvae species to feed[15] and have the ability to clean tank walls of detritus by grazing[16]. Therefore, study on the best culture medium for harpacticoid copepods especially on its diets is a vital factor in its reproduction. According to Huys *et al.*[17] food availability is one of the factors that influence breeding frequency of the organism. Besides that, food types have been reported to affect harpacticoid reproduction and also modified the rate of development, longevity and fecundity[18]. Hence, this study was conducted to observe the effects of different diets using mostly terrestrial organic matter as alternative to algal feed, which were previously reported as an important aspect in obtaining maximum biomass yield in copepod culture[19,20]. This study aims to introduce diets that are more affordable and applicable, compared to algal diet, which is time consuming and costly in maintenance.

MATERIALS AND METHODS

Stock culture: Harpacticoid samples were collected from Buntal, Kuching, Sarawak coastal water area (1°41'36.1" N 110°22'22.5" E) on March, 2014 during low tide. Sediment samples were collected using perspex corer and sieved by 42 µm mesh size. Sediment retained on sieve were then transferred into container and immediately brought back to laboratory for further treatment. In the laboratory, harpacticoids were acclimatized for 30 days at salinity 28 PSU and temperature of 27°C with aeration. The most dominant harpacticoid species in the culture medium was selected by observed using compound microscope and identified down to the lowest possible taxa. Hundred individuals of harpacticoid species (*Stenhelia stephensoni*) were isolated into a new culture medium and further cultured prior to the diet experiment.

Diet experiment: List of different diets used in juice form:

- Carrot
- Banana
- Sago
- *catappa* leaf (CL)
- Goat Dung (GD)
- Seaweed (S)
- Mixed of GD and seaweed (GDS)
- Mixed of GD and *catappa* leaf (GDCL)

Diets preparation: All of the materials used in the diet were immersed in autoclaved seawater for 4 days before being air dried in laboratory. Each diets were then weighed and blended together with seawater to obtain juice form. For mixed diet, proportion of every single item is in ratio of 1:1.

The selected harpacticoid copepod species obtained from Buntal coastal area was identified as *Stenhelia stephensoni* and was further cultured for stock to be used in the different diet experiment. Twenty four pieces of 500 mL beakers were used for the different diet experiment. There were eight different diet treatments and each treatment consists of three beakers as replicates. Each beaker contains 15 gravid females, which were transferred from the stock culture. Autoclaved seawater with the same condition as stock culture (28 PSU and 27°C but without aeration) were used throughout the experiment. The eggs of the gravid females for each treatments were counted upon experimental setup. The number of hatched nauplii were used as the initial value to quantify growth performance and survival in tested treatments. Each replicates were fed daily and observed in 4-6 h interval in order to record survival rate and development time of nauplii to adult stages. Any debris or dead individual were removed from the experiment beaker. Observation was carried out until the termination of experiment at day 15.

Data analysis: At the end of the experiment, all the data obtained which included population density of *Stenhelia stephensoni* from different diets and development time of the species were analysed statistically using one-way analysis of variance (ANOVA). These data were tested at 0.05 probability and Tukey's multiple comparison test were used for further determination of specific difference if there were

significant differences among the different diets. The ANOVA was performed using GraphPad Prism 6.

RESULTS

The GDCL recorded 100% survival up to day 12 before declining to 90% on day 13 and remained constant until the end of the experiment. *Stenhelia stephensoni* survival in GDCL was significantly different among diet treatments except seaweed. Single diet treatment of goat dung and *catappa* leaf demonstrated 100% survival on day 8 before declining to 60 and 69%, respectively on day 15. Banana and sago resulted the poorest result, where survival was observed up to day 2 only. Harpacticoid fed with carrot recorded complete mortality on day 5 (Fig. 1).

The shortest embryonic development time was found in GDCL, which hatched within 0.44±0.51 days, followed by GDS which hatched within 0.53±0.71 days after the first appearance of eggs. The longest embryonic development time was 1.00±1.01 days in harpacticoids fed with banana (Table 1).

The development time from first stage of nauplii to first stage of copepodid (N1-C1) was longest in harpacticoids fed with seaweed, which took 6.0±1.0 days. On the other hand, the shortest development time was achieved by harpacticoids in GDCL treatment which only took 3.83±0.76 days.

The shortest time required to develop from copepodid to adult (C1-A) was 7.80±0.72 days, which was achieved by the GDCL treatment, while the longest development time was 12.6±0.53 days in harpacticoids fed with seaweed. The longevity of *Stenhelia stephensoni* in GDS treatment was significantly longer than the other treatments, while copepods fed with banana recorded the shortest population lifespan (Table 1).

Fifteen days culture of harpacticoids fed with different diets showed that the diet had a significant effect on the population density of *Stenhelia stephensoni*. Mean population density of *Stenhelia stephensoni* fed with combination of GDCL was significantly higher compared to other treatments with a final population density of 771±70.55 individual per 500 mL (Fig. 2). The second highest population density was observed in combination of GDS with

Table 1: Development time and longevity of *Stenhelia stephensoni* fed with different diets

Diets	Embryonic development (days)	N1-C1 (days)	C1-adult (days)	Longevity (days)
Carrot	0.74±0.76^{a}	0.00±0.00^{a}	0.00±0.00	5.00±0.00^{a}
catappa leaf	0.56±0.50^{ab}	4.50±0.50^{b}	11.80±0.44^{a}	31.33±1.53^{bcd}
Sago	0.90±1.36^{a}	0.00±0.00^{c}	0.00±0.00	2.43±0.51^{ae}
Banana	1.00±1.01^{a}	0.00±0.00^{d}	0.00±0.00	2.00±0.00^{e}
Goat dung	0.59±0.36^{abc}	5.83±0.76^{be}	11.90±1.01^{ac}	34.33±2.08^{bfgh}
Seaweed	0.65±1.15^{abc}	6.57±0.51^{ef}	12.60±0.53^{a}	26.90±1.65^{cf}
GD+seaweed	0.53±0.71^{abd}	6.00±1.00^{bfg}	9.07±0.90^{abc}	41.10±1.01^{gi}
GD+catappa leaf	0.44±0.51^{ad}	3.83±0.76^{abcdg}	7.80±0.72^{b}	36.73±1.55^{dhi}

Appearance of egg and hatching (embryonic development time), development time from nauplii to copepodid (N1-C1), copepodid to adult (C1-adult) and longevity of *Stenhelia stephensoni*. Values are Mean±Standard Deviation (n = 3). Means in the column with the same superscript are not significantly different (p>0.05)

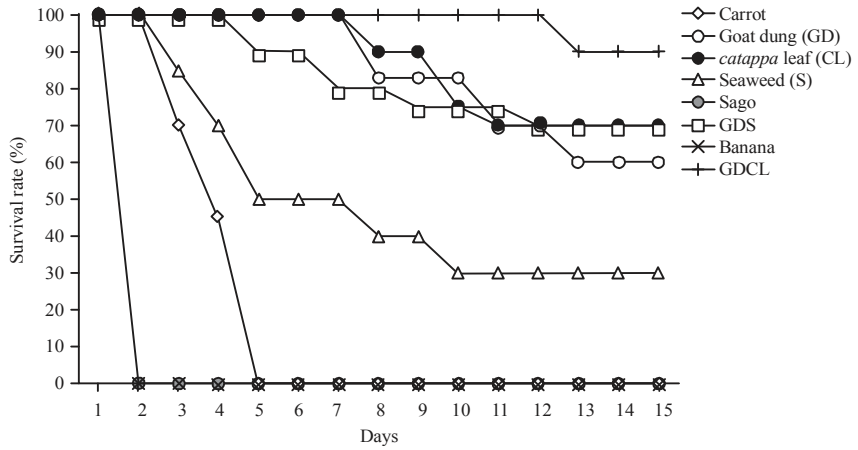

Fig. 1: Survival rate (%) of *Stenhelia stephensoni* cultured for 15 days with different diets, GDS: Mix single diet of goat dung and seaweed and GDCL: Mix single diet of goat dung and *catappa* leaf

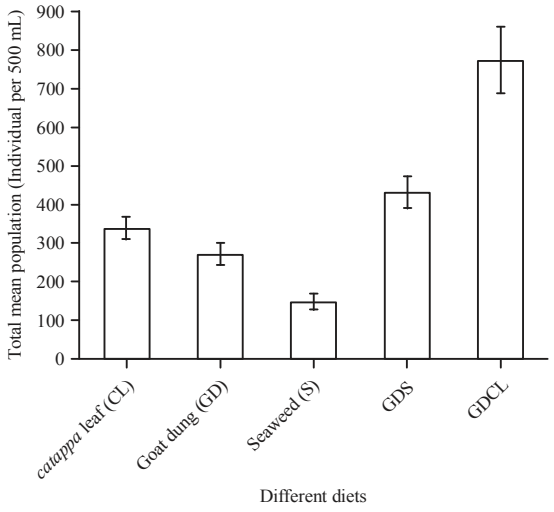

Fig. 2: Mean population of *Stenhelia stephensoni* fed with different diets at day 15

the final number of 431±34.39 individual per 500 mL. The lowest population density was recorded in harpacticoids fed with seaweed.

DISCUSSION

The present study shows that single type diet resulted in low population growth compared to combination diets. Banana and sago diets were unsuitable for *Stenhelia stephensoni* culture as the species recorded 100% mortality in just a few days. Findings of this study showed that mixed diets promote and enhance harpacticoids population which is in agreement with several studies[21-23]. Gaudy *et al.*[24] stated that combination of several foods item are better as it provide sufficient nutrients, trace elements, minerals and vitamins. Hicks and Coull[25] also added that reproductive performance and health of copepod populations in the laboratory require mixed foods, which considered as a balanced diet, while single diet resulted lower population density maybe due to lack some of the key nutrients[26].

Harpacticoids fed with goat dung, *catappa* leaf and seaweed in combination and single diet performed well in this study. All three material are quite common and can be related with wild population of copepod and copepod culture. Dungs and manures are commonly used in aquaculture practises especially in zooplankton culture. Wurts[27] reported that manure could provide essential nutrients for zooplankton growth. Manure contains rich source of organic matters and has several type of microbes, which converts them into carbohydrates, proteins, pigments, oils, alcohol and aldehydes etc., used by the plankton for their higher population rate[28]. In fact, some studies had shown that reproduction of copepods fed with dung or manure are comparable to those fed with microalgal[14,23]. Habitats associated with leaf litters such as *catappa* and terrestrial forest are commonly inhabited by harpacticoids[29].

The *catappa* leaf contribute to the high leaf litter, a form of decaying matter or detritus, which were utilized by harpacticoids as food. According to Meyer and Bell[30] harpacticoids prefer feed leaf litter since detrital forms of organic material were more palatable and more accessible than fresh material for consumers. Decaying macrophyte or macrophytodetritus from seagrass meadow also had been reported to support diverse species of harpacticoids by sheltering, housing and feeding[31].

Findings in this study also indicated that food type influenced the embryonic development time of *Stenhelia stephensoni*. Overall, harpacticoid eggs took only 0.44-1.00 days to hatch, nearly 50% faster than the time recorded[32,33]. *Stenhelia stephensoni* fed with GDCL have the shortest time development, higher fecundity and hatching rate but those fed with GDS have the longest longevity. This result was probably due to the rapid population growth in GDCL culture media, which causes the population to collapse earlier than those with lower population such as in the GDS culture media.

CONCLUSION

The objective of the present study was accomplished as the results showed positive response in *Stenhelia stephensoni* growth and development under culture condition. Ultimately, findings showed that *Stenhelia stephensoni* was best fed with combination diet of goat dung and *catappa* leaf rather than single type diets. This study also showcases that cheap materials such as goat dung and *catappa* leaf can be used in aquaculture especially in culturing live feed such as harpacticoid copepods. We strongly encourage the use of this material because it is of natural origin and environmental friendly since waste products can be converted to protein.

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