Comparative Studies on the Effect of Copepoda (*Cyclops* sp.) and *Artemia* (*Artemia salina*) Diet on Growth of Golden Trevally (*Gnathodon speciosus*) Larvae

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Abstract: The dietary effects of copepods (*Cylops* sp.) and *Artemia* nauplii were investigated on the growth of 15-30 day old golden trevally (*Gnathodon speciosus*) larvae. The highest specific growth rate (19.3%/day) was attained in larvae fed with copepods and was significantly different ($p<0.05$) from fish fed with only *Artemia* nauplii, but not significantly different ($p>0.05$) from fish fed with combination of *Artemia* and copepods (1:1). Larvae markedly preferred diet of copepods and this suggest the possible use of copepods as an alternative or supplement to *Artemia* as a food source for fish larvae.

Keywords: effect, diet, *Artemia*, copepod, larvae growth

Abstrak: Kajian ke atas kesan diet kopepod (*Cylops* sp.) dan nauplii *Artemia* ke atas rega ikan gerong (*Gnathodon speciosus*) yang berumur 15-30 hari telah dijalankan. Kadar pertumbuhan spesifik yang tinggi (19.3%/hari) didapati pada rega yang diberi diet kopepod dan terdapat perbezaan yang signifikan ($p<0.05$) daripada rega ikan yang hanya diberi diet nauplii *Artemia*, tetapi tidak mempunyai perbezaan yang signifikan ($p<0.05$) dari diet kombinasi antara nauplii *Artemia* dan kopepod (1:1). Rega menunjukkan keutamaan kepada diet kopepod dan justeru itu dicadangkan penggunaan kopepod sebagai makanan pengganti kepada *Artemia* ataupun sebagai makanan tambahan kepada *Artemia*.

Introduction

The success of aquaculture as a bio-industry stems from the breakthrough in larviculture of the species currently farmed. A major obstacle in the initiation of aquaculture program involving a number of marine fish species is the unsustainability of small sized larvae culture with currently available artificial food. The ideal solution to the problem would be the development of suitable artificial feed for these small larvae but, such an achievement seems unlikely to occur in the very near future. Therefore, efforts on the interim solution in developing a reliable live food source for marine fish larvae could be developed.

Among the live diets used in the larviculture of marine fish, nauplii of the brine shrimp *Artemia* constitute the most widely used food item. However, with the expansion of aquaculture production, the demand for the *Artemia* cysts soon exceeded the offer and prices rose rapidly, turning *Artemia* into a bottleneck for the expansion of the hatchery production of marine fishes. Especially the third world countries could hardly afford to import the very expensive cysts (Gilbert Van Stappen, 1996).

Copepod show promise as a *Artemia* replacement in larval diets. Where problems have occurred in the development of larviculture practises for new aquaculture species, copepods have often provided significant benefits (Witt *et al.*, 1984; Doi *et al.*, 1997). These benefits include an increased feeding response by larvae (Kuhlmann *et al.*, 1981; Doi *et al.*, 1997) and a higher nutritional content (Watanabe *et al.*, 1983; Kraul *et al.*, 1992).

Apparently, the nutritionally value of *Artemia* nauplii decreases rapidly after hatching because of high metabolism rates and growth result in rapid utilization of yolk material (Maddox and Manzi, 1976). On the other hand, each copepod gut is usually filled with bacteria and algae which could provide additional nutrition for the cultured larvae. Thus, one might predict that copepod could provide a continuously high level of nutrition for cultured larvae (Gronkjaer *et al.*, 1995; Naess *et al.*, 1992; Sargent *et al.*, 1986). Based on our preliminary observations on copepod *Cyclops* sp., perhaps it could be another potential copepod species used as food for fish larvae. Furthermore, this species is naturally available and grows in rotifer tanks almost all year round. Therefore, the objective of this study is to ascertain the suitability of copepod *Cyclops* sp. which is also possible to be mass propagated in culture tanks to serve as live food organism as substitution of *Artemia* for the purpose of mass fry production.

Materials and Methods

Source of copepod Cyclops sp.

Initially, copepod *Cyclops* sp. were obtained from the 300 tonnes rotifer culture ponds. The copepod culture method were adopted from Ohno and Okamura (1988). Preliminarily, the copepod culture tank will be fertilized with chemical fertilizers to encourage diatom bloom for a few days prior to introduction of adult copepods. The copepods were harvested according to daily requirement. Based on the early observations, a single matured female copepod *Cyclops* sp. can produce 24 to 32 nauplii daily for 3-5 consecutive days. The same female will reproduce again after 3 days of lag period even without male. The newly hatched nauplii will grow and attain maturity within 5-6 days. More than half of the newly matured copepods were observed as female. Species identification of the copepod were based on figures illustrated by Dakin and Colefax (1940), Newel and Newel (1963) and Shirota (1966). Copepods were concentrated in a 100 μm mesh size plankton net and rinsed with clean sea water before being fed to larvae of golden trevally.

Source of Artemia nauplii

The *Artemia* cysts were incubated for 24 hours in seawater in a conical tank. The temperature of the seawater are in the range of 25-28°C and salinity of 28-30 ppt. The newly hatched nauplii were harvested and rinsed prior to offer them to the fish larvae

Source of golden trevally larvae

For the experimental purposes the golden trevally eggs were bought from Johore and were reared until 15 days before starting the experiment.

Experimental protocols

Larval culture experiment were carried out indoor using nine 30 L fiberglass tanks. Filtered seawater were used to fill up the larval rearing tanks throughout the experimental period. The following are the feeding regimes that were tested:

Treatment 1: Feeding the larvae with *Artemia* nauplii by adopting the rearing technique of seabass larvae. Whereby, on the second day of rearing, the larvae were initially fed on rotifers with 15-20 ind/mL/day and the number of ingested prey increased with the size of the larvae until day ten where the rotifer given will be reduced to 5 ind/mL. *Artemia* were introduced starting on the tenth day of rearing with 0.5-2 nauplii/mL/day and increased to 5-10 nauplii/mL/day two times daily starting on the 15th day until day 30.

Treatment II: Feeding the larvae with copepod *Cyclops* sp. by adopting the rearing technique of seabass larvae. Whereby, on the second day of rearing, the larvae were initially fed on rotifers with 15-20 ind/mL/day and the number of ingested prey increased with the size of the larvae until day ten where the rotifer given will be reduced to 5 ind/mL. Copepod were introduced starting on the tenth day of rearing with 5 nauplii/mL/day and increased to 5-10 nauplii/mL/day two times daily starting on the fifteenth day until day 30.

Treatment III: Combination of *Artemia* and copepod *Cyclops* sp. by adopting the rearing technique of seabass larvae. Whereby, the density will be 1:1.

Each treatment have three replicates and arranged in random following the Completely Randomized Design (CRD) (Villegas, 1989). Due to the unavailability of larvae, the initial stocking density of golden trevally larvae were lowered to 15 larvae/L. Water management for larval rearing tanks were based on semi-static system where the water change were carried out in the morning between 0800-0900 hour for 100% everyday. Live foods were introduced into larvae rearing tanks at a known density twice daily upon completion of water change. The remaining of food organisms in each culture tank were estimated prior to water change in the next morning. The number for each type of live food organisms were counted by using the microscope.

Three larvae, were sampled daily from each tank from day 15 to day 30 and fixed with 4-5% formalin in seawater for gut study, as well as for total length. The guts were opened and food organism were separated from the esophagus to the rectum and were examined under the microscope. Prey organisms were counted and identified. Water temperature, pH, dissolved oxygen and total ammonia were observed daily in the morning before water change. The final growth rate of the larvae upon termination of the experiment after 16 days were estimated. Statistical analysis for larval wet weight and total length were carried out using Statistix 7.0 software.

Results and Discussion

A major problem facing hatchery operations for marine species is dependent upon nauplii of *Artemia* as the primary food for early staged larvae. Other feeds have been used for rearing marine finfish larvae. However, none of these can readily replace *Artemia*. Either these alternative feeds can be used only as supplements to *Artemia* nauplii or else availability of the alternative feeds is limited. The primary disadvantage to the use of *Artemia* as a larval food for marine finfish is the high expense of brine shrimp cysts, particularly in developing countries.

Copepods *Cylops* sp. (Fig. 1) have a short life cycle, simple dietary requirements, can be cultured in high densities and have favourable nutritional content. Because it is small in size, it can be ingested completely by marine larvae.

Figure 1: Photograph of *Cyclops* sp.

Results on the growth performance of fish larvae fed with the treatment diets for 15 days are shown in Table 1. The highest body weight and total length was attained in larvae restricted to a diet of copepods but it was not significantly different ($p>0.05$) from fish fed with combination of *Artemia* and copepod. Statistical analyses indicated that larvae fed with *Artemia* were significantly higher ($p<0.05$) from the larvae fed with copepod or combination of copepod and *Artemia*. Larvae body weight in all treatment began to differ significantly starting on day 6 as shown in Table 1 while total length started to show significantly in day 5. The graph for body weight and total length, larvae fed with the three treatment diets are also shown in Fig. 2 and Fig. 3. These results are in line with studies where an *Artemia* diet has been supplemented with wild net collected plankton which have often led to better results in terms of growth and survival (Witt *et al.*, 1984; Nellen, 1985). Such results raise doubts in concerning the nutritional suitability of *Artemia* for fish larvae. Watanabe *et al.* (1983), pointed out that the nutrient content of *Artemia* is extremely variable perhaps more importantly, that *Artemia* is low in the C20 (*n*-3) and C22 essential fatty acids of the linolenic family (*n*-3). These authors suggest that the fatty acid composition of *Artemia* nauplii might be more suitable for fish larvae food by prefeeding the *Artemia* on a diet high in (*n*-3) highly unsaturated fatty acids (HUFA). Copepods, on the other hand, to be naturally rich in these fatty acids (Watanabe *et al.*, 1983).

Table 1: Comparison of means for growth performance

	Treatment 1 ***Artemia***	**Treatment 2** **Copepod**	**Treatment 3** ***Artemia* + Copepod**	**LSD**
Mean BW (g)	0.0942[b]	0.1607[a]	0.1586[a]	$p<0.05$
Mean TL (cm)	1.5240[b]	1.8822[a]	1.8447[a]	$p<0.05$
Mean BW Day 6 (g)	0.0553[b]	0.0988[a]	0.0683[a]	$p<0.05$
Mean TL Day 5 (cm)	1.1333[b]	1.4667[a]	1.2833[a]	$p<0.05$
Mean Survival Rate (%)	22[b]	37.9[a]	32[ab]	$p<0.05$
SGR (%/day)	19.3	21.7	21.2	$p>0.05$
Equations	$Y=-0.05+1.35/(1+\exp(-(x-3.41)/0.82))$	$Y=-0.07+2.81/(1+\exp(-x-4.51)/1.03))$	$Y=-0.03+0.85/(1+\exp(-(x-2.84)/0.64))$	-

There is a significant difference ($p<0.05$) in mean comparison of survival rate with larvae fed a diet of copepod (22%) and with larvae fed artemia (37.9%) but not significant ($p>0.05$) to combination of *Artemia* and copepod (32%). There are no significance difference ($p>0.05$) in larvae fed with artemia alone and combination of *Artemia* and copepod. The mortalities in larvae fed with the *Artemia* are extremely high. This might be due to *Artemia* based diets exucia and shed cysts capsules and accumulate in larval culture vessels. Bacterial degradation of these materials fouls the water, accumulated debris and larval mortalities will increase and also by using *Artemia* as a food organism the problem of indigestability and the danger of ingestion of empty cysts by the fish larvae arise (Nellen, 1985).

Fig. 4 indicates the exponential relationship between total length and body weight of all the treatment diets. The body weight and total length differed significantly among treatments toward the end of the experiment. The result also suggests that the total length and body weight can be differentiated among treatments when the total length reached 3 cm and 0.4 g for the latter. These trends were further accentuated when growth rates was expressed.

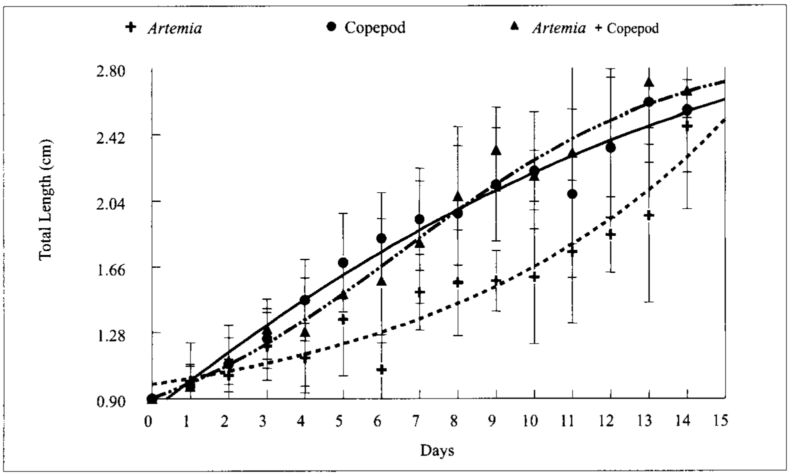

Figure 2: Growth curve of total length for three treatment diets

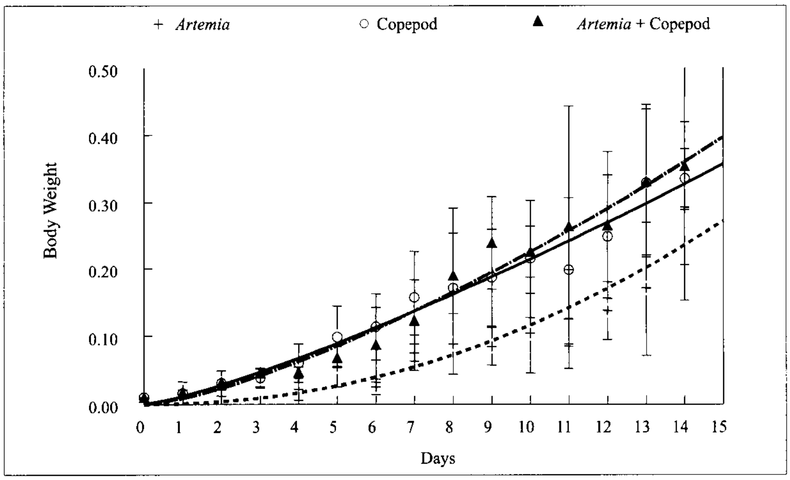

Figure 3: Growth curve of body weight for three treatment

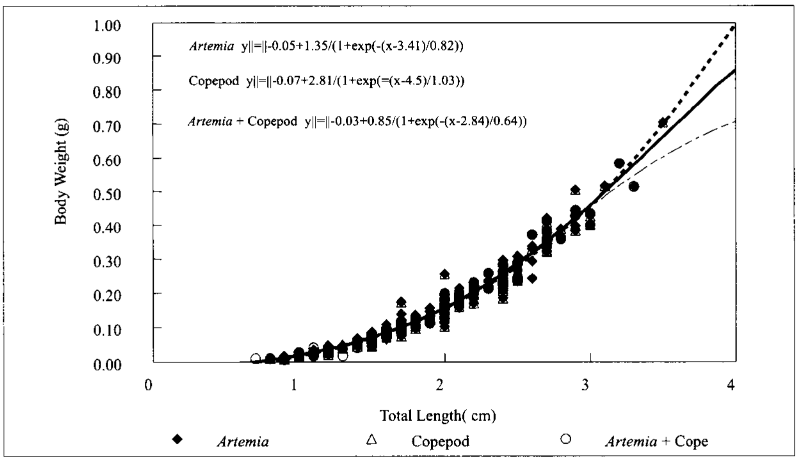

Figure 4: Relationship between body weight and total length

Fig. 5 indicates the relationship between number of live feed consumed based on the body weight. Golden trevally larvae fed with *Artemia* alone gives the highest number of individuals (*Artemia*) consumed and larvae fed with the copepod gives lower number of individuals consumed for the same amount of body weight. This is in line with the results of low growth rates for larvae fed with *Artemia*, whereby feed consumption and body growth is affected by the quality of ingested feed which is known to affect the larval development. The result also suggested that larvae fed with copepod gives higher body weight with low feed consumption. The investigations on the stomach content (Fig. 6) also indicated the preferable amount of individuals taken by the goden trevally larvae. Fig. 7 indicates that *Artemia* was highly consumed by the larvae until day 11 where the number of *Artemia* consumed decrease rapidly. The *Artemia* graph pattern for the larvae fed with combination of copepod and *Artemia* also showed similar pattern in the amount of individuals consumed. On the other hand, for the larvae fed with copepods, the number of individuals taken were linear with the culture period. Larvae fed with combination of copepod and *Artemia*, indicate that copepod consumed were more prominent in the stomach of golden trevally larvae starting on day 10.

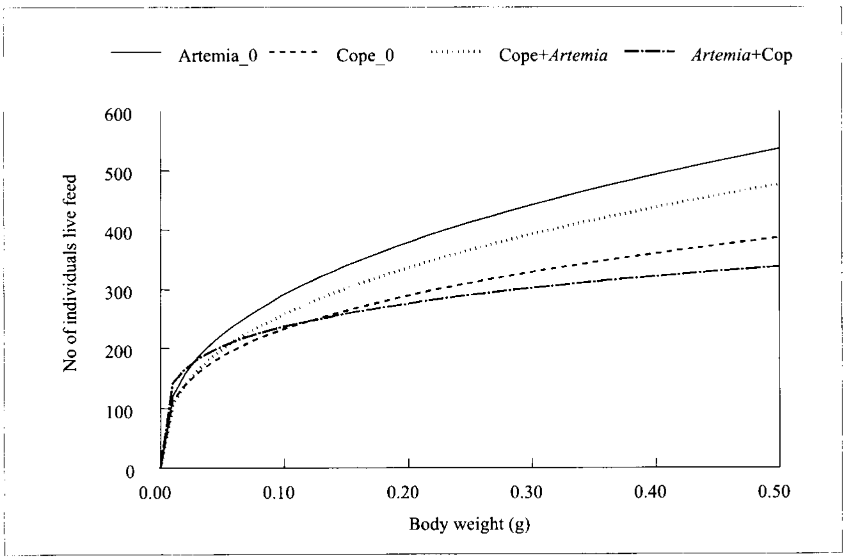

Figure 5: Relationship between number of individual live feed organisconsumed based on the body weight of golden trevally larvae

Figure 6: Photograph of *Artemia* and copepod in the stomach content of Golden trevally larvae

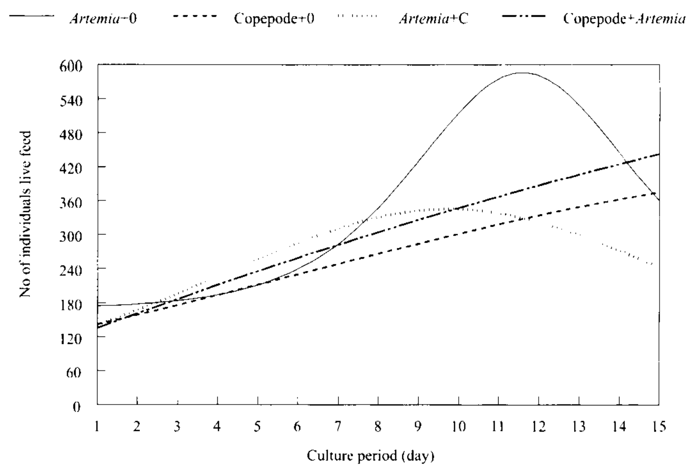

Figure 7: Preferable amount of live feed taken by golden trevally larvae

These research findings are supported by ealier studies of other fish species. Evidence that copepods may be preferable to *Artemia* as food for marine fish larvae also comes from the work of Pedersen (1984) who examined digestion in first-feeding herring (*Clupea harengus*) larvae hatched in the laboratory and found that copepods from wild zooplankton samples passed more quickly through the gut and were more thoroughly digested than *Artemia*. Futhermore, Pedersen noted that copepods were stopped at the entrance to the gut whereas *Artemia* passed straight through. She hypothesized that the pause prior to entering the gut may somehow be related to the release of enzymes necessary to digestive processes. This result suggest that the possibility of using copepods as an alternative to *Artemia* as a food source for fish larvae to improve growth rates, which is supported by the work of Watanabe *et al.* (1983).

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