Larval Rearing of the Giant Trevally, *Caranx ignobilis* (Forsskål, 1775), Fed Live Food Combinations of Rotifers, Copepods and *Artemia salina*

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Abstract

The giant trevally, *Caranx ignobilis*, is a high value fish which has been studied for spawning in captivity and domestication in the Philippines in view of its aquaculture potential. Initial larval rearing of the species encountered mortality issues, and this study attempts to address the problem to improve their survival. Hatchery-bred *C. ignobilis* larvae were reared for 40 days in nine units of 100 L conical fiberglass tanks at a stocking density of 500 larvae.tank^{-1} under three feed treatments with different combinations of rotifers (*Brachionus plicatilis*), brine shrimp (*Artemia*) and copepods (*Pseudodiaptomus* spp.): T1- *Brachionus plicatilis* + *Artemia*, T2- *Brachionus plicatilis* + *Pseudodiaptomus* spp., and T3- *Brachionus plicatilis* + *Pseudodiaptomus* spp. + *Artemia* fed at 0800 h and 1600 h. The results showed that the inclusion of copepods in the feeding regimen had positive effects on survival. Survival rate was significantly higher ($P < 0.05$) in T2 (23.47 ± 3.18 %) than T1 (11.67 ± 1.90 %) but was not statistically different from T3 (14.37 ± 3.88 %). Length increment was significantly different ($P < 0.05$) among treatments but with no statistical difference in specific growth rate ($P > 0.05$). The erratic swimming behaviour leading to mortality associated with insufficient levels of highly unsaturated fatty acids in *Artemia* was not observed in T2, thus, validating the assumption that the feeding with copepods will increase survival during larval rearing. The use of copepods for *C. ignobilis* larviculture is seen to play an important role in the success of the seed production protocol for this species. However, a sustainable supply of copepods through appropriate mass propagation techniques must be ensured to achieve higher production of *C. ignobilis* seeds in the hatcheries.

Keywords: larviculture, *Caranx*, copepod, rotifer, *Artemia*

Introduction

The giant trevally, *Caranx ignobilis* (Forsskål, 1775), locally known as "maliputo", is a candidate species for high value finfish aquaculture in the Philippines due to its wide market acceptability, high market price and suitability for rearing in brackish, fresh and marine waters (Mutia et al., 2017). The National Fisheries Research and Development Institute (NFRDI), the government fisheries research agency in the Philippines, has identified *C. ignobilis* as a potential aquaculture species hence is implementing its giant trevally aquaculture development program. The first recorded induced spawning of this species in the Philippines was successfully conducted by NFRDI (Mutia et al., 2020) using human chorionic gonadotropin (HCG) and luteinising hormone-releasing hormone analogue (LHRHa) with initial reports of successful larval rearing of the species.

The preliminary larval rearing of *C. ignobilis* as reported by Mutia et al. (2020) encountered mortalities in the initial phase of the larval rearing cycle and a few days after the introduction of *Artemia*, where the larvae exhibited erratic, swirling swimming behaviour prior to sinking into the tank bottom where they spin down and eventually die. Similar mortalities have also been reported in other carangid species, striped jack *Caranx delicatissimus* Döderlein, 1884 (Arakawa et al., 1987), yellowtail amberjack *Seriola lalandi* Valenciennes, 1833 (Stuart and Drawbridge, 2012), and longfin yellowtail *Seriola rivoliana* Valenciennes, 1833 (Roo et al., 2012),

with mortalities associated with n-3 highly unsaturated fatty acid (HUFA) and docosahexaenoic acid (DHA) deficiencies during *Artemia* feeding stage. Research studies have shown that marine fish require high levels of HUFA (Sargent et al., 1999) and DHA:EPA ratio of at least 2.0 (Bell et al., 2003). Although *Artemia* is widely used as live feed for several marine finfish that are produced commercially worldwide, its use has certain limitations, primarily due to its insufficient levels of HUFA which is required by marine finfish larvae (Ohs et al., 2019) such as *C. ignobilis*. The lack of essential fatty acids can result in retarded physiological development and altered behaviour, such as the swirling, erratic swimming exhibited by *C. ignobilis* and other carangid species mentioned earlier.

Alternative live feed such as copepods have been used in the larval rearing of some marine finfish species and several studies have been reported on the important role of copepod as feeds for marine fish larviculture (Bell et al., 1997; Støttrup, 2000; Lee et al., 2005). Copepods which have superior nutritional value (Watanabe et al., 1983; Støttrup and Jensen, 1990); high level of HUFAs (Ohs et al., 2019) and natural sources of antioxidants (Van der Meeren, 2003); are easily digestible (Pederson, 1984); elicit feeding response to larvae (Buskey, 2005; Marcus, 2005); and are small, which makes them suitable for first feeding (McKinnon et al., 2003). These characteristics of the copepods makes them ideal live feed for marine finfish including *C. ignobilis*. In this study, the use of copepod in the larviculture of *C. ignobilis* was investigated to increase growth and survival under different larval feeding regimens and minimise the mortality issues encountered in the initial larval rearing studies.

Materials and Methods

Ethical approval

Prior approval was obtained from the National Fisheries Research and Development Institute, the fisheries research agency of the Philippines, for the appropriate handling, use and care of aquatic animals used for research purposes for this particular study. No animals were sacrificed during the course of the study (10.09.2023).

Experimental set up

The study was conducted at the indoor marine finfish hatchery of the Department of Agriculture-National Fisheries Research and Development Institute-Freshwater Fisheries Research and Development Center (DA-NFRDI-FFRDC), Taal, Batangas, Philippines. The larval fish were set up in nine units of 100 L conical fiberglass tanks (45 cm diameter, 90 cm height) with black painted sides and clear bottom. Each fiberglass tanks were provided with moderate aeration to maintain optimum dissolved oxygen levels. The experiment followed a completely randomised design comparing three treatments with three replicates in each treatment (Table 1). Rotifers were fed in all treatments from day 2 to day 25 after hatching and then different combinations of copepods and *Artemia* (only *Artemia*, only copepods and *Artemia* and copepods) were fed (Table 1).

Experimental live food and preparation

Rotifer and *Artemia* fed to the *C. ignobilis* larvae in this study were sourced from the natural food production tanks of the marine finfish hatchery of NFRDI-FFRDC while copepods were collected from Balayan Bay and/or the pond of the Center composed mainly of *Pseudodiaptomus* sp. and *Acartia* sp. Rotifers (*Brachionus plicatilis*) were mass produced in 10-ton concrete tanks using the batch culture method where the rotifers were fed *Nannochloropsis* sp. which were harvested daily and enriched with DHA Culture Selco (INVE Aquaculture, USA), vitamin C and cod liver oil four hours before feeding time. The copepods were collected early in the morning from the beach shore of Balayan Bay adjacent to NFRDI-FFRDC using a 200 µm plankton net. They were then rinsed with filtered, chlorine-treated water. The copepods were stocked in 100 L culture tanks with 30 ppt seawater and maintained at temperatures ranging from 26 °C to 30 °C. The copepods were harvested daily from the culture tanks and fed to the larvae. *Artemia* were prepared by incubating *Artemia* cysts for 48 to 72 h in 20 L fiberglass tanks with 30 ppt to 35 ppt water at 26 °C to 30 °C. *Artemia* was enriched with DHA Easy Dry Selco (INVE Aquaculture, USA), vitamin C and cod liver oil for 4 h before feeding to larvae. All live foods were rinsed thoroughly with filtered, chlorine-treated seawater prior to feeding these to the fish larvae.

Experimental larvae and larval rearing

Newly hatched *C. ignobilis* larvae were produced from a set of *C. ignobilis* broodstock (one female:two males) which were induced to spawn using luteinising hormone-releasing hormone analogue (LHRHa). The female breeder then spawned spontaneously in a 40-ton capacity circular concrete tank (5 m diameter, 2.5 m depth) after which the eggs were incubated and hatched in 100 L incubation tanks. A total of 6,000 day-old larvae were randomly distributed into the nine units of 100 L fiberglass tanks at a density of 5 larvae.L^{-1}.

The initial total length (TL) of the larvae was measured at the start of the study. After complete yolk sac absorption at 2 days after hatching (DAH 2), larvae were fed the different feeding regimens for a period of 40 days. *C. ignobilis* larvae were fed twice a day at 0800 h and 1600 h with rotifer at a density of 2 to 20 ind.mL^{-1} (DAH 2 to 25), copepod at 0.003–1.6 ind.mL^{-1} (DAH 5 to 40) and *Artemia* at 0.1–0.25 ind.mL^{-1} (DAH 21 to 40) (Fig. 1). *Nannochloropsis* sp. was inoculated into the fiberglass tanks and maintained at a density of 100,000 to 300,000 cell.mL^{-1} from DAH 2 to DAH 25. The live natural food was maintained at these densities in the rearing tanks. Counting of the densities of rotifer,

Table 1. Live natural food regimens used in feeding experiment for the larval rearing of *Caranx ignobilis*.

Treatment	Live food combination	Feeding regimen		
		Rotifer	Copepod	*Artemia*
1	Rotifer + *Artemia*	DAH 2 to DAH 25		DAH 21 to DAH 40
2	Rotifer + Copepod	DAH 2 to DAH 25	DAH 5 to DAH 40	
3	Rotifer + Copepod + *Artemia*	DAH 2 to DAH 25	DAH 5 to DAH 40	DAH 21 to DAH 40

DAH = days after hatching.

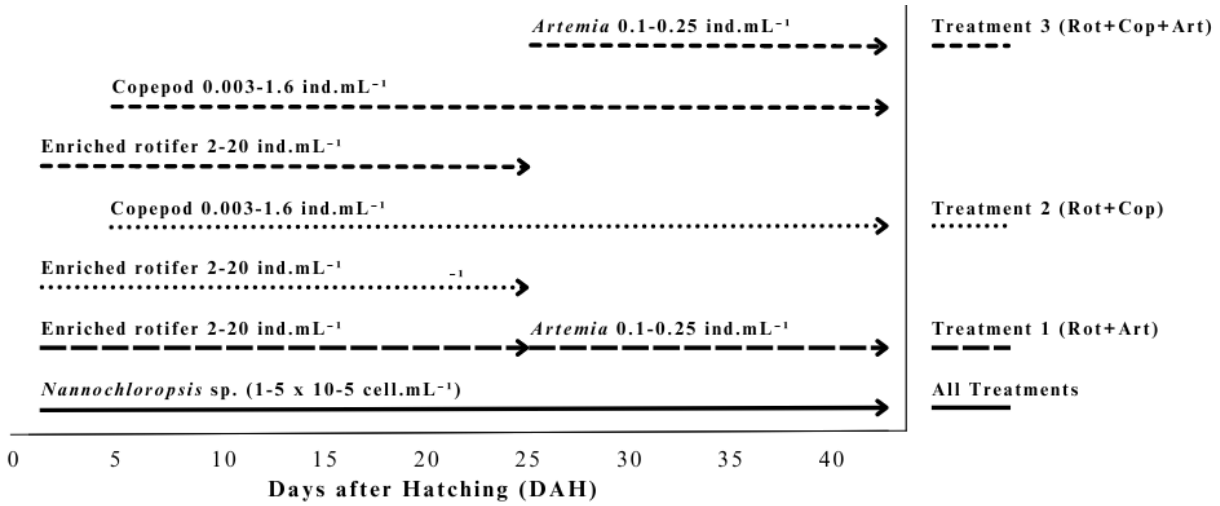

Fig. 1. The three different feeding regimens used in the experiment of larval *Caranx ignobilis* growth. Treatment 1 (Rotifer + *Artemia*); Treatment 2 (Rotifer + Copepod); Treatment 3 (Rotifer + Copepod + *Artemia*) - compared in the larval rearing of *C. ignobilis* from DAH 0 to DAH 40.

copepod and *Artemia* in the fiberglass tanks were done daily to ensure that the desired density of live food was maintained in the culture tanks. The density of rotifer, copepod and *Artemia* in the larval tanks was estimated using volumetric method taking three sub-samples of known volume taken from different points in the larval tank. One mL of the live food sample is placed in Sedgewick-Rafter counting chamber (Graticules S50, USA), viewed and counted under a compound microscope (BA310 Elite, Motic, China). The number of individuals per mL of rotifer or copepod or *Artemia* that was counted in the Sedgewick-Rafter is the density of the live food in the larval tank water.

Water management and water quality monitoring

Water quality in the rearing tanks was maintained at its optimum through daily water change (30 % to 50 % exchange rate) and siphoning of the tank bottom (Fig. 2). Salinity of the tank water was maintained at 30 ppt. Water quality monitoring was done daily at 0800 h and 1500 h for the following parameters: temperature, salinity and pH. Dissolved oxygen and temperature were measured using Pro 20 dissolved oxygen meter (YSI, USA) and pH was measured using pH pen pocket tester (LaMotte, USA). Salinity was measured using refractometer (Atago, Japan). Moderate aeration was provided to the rearing tanks using air stones attached to air hose which were connected to 1/2 HP ring blower.

Growth and survival monitoring

Sampling of larvae was done at DAH 10, 20, 30 and 40 by randomly taking 10 pcs of larvae per tank. The total length and body depth were carefully measured and recorded. Larval samples were then returned back to the rearing tanks right after the sampling. Total length (TL) was measured to the nearest 0.01 mm under a stereo microscope (SMZ800N C-LEDS, Nikon, Japan) with micrometer eyepiece at 10× magnification. The length increment and specific growth rates were determined using the following formula:

$$\text{Length increment (LI) (mm)} = \text{Final length} - \text{Initial length}$$

$$\text{Specific growth rate (SGR) (\%/day)} = (\ln L2 - \ln L1)/ (T2 - T1) \times 100$$

where L1 = Initial live body length at time T1, L2 = Final live body length at time T2.

The behaviour of the larvae was monitored closely on a daily basis especially during feeding periods. Dead larvae were counted, retrieved and recorded daily immediately upon observation. At DAH 10, 20 and 30 larvae were counted visually from the rearing tanks and recorded to calculate survival rate. At the final 40th day of rearing, all larvae were counted individually. Survival rate was calculated by the following formula:

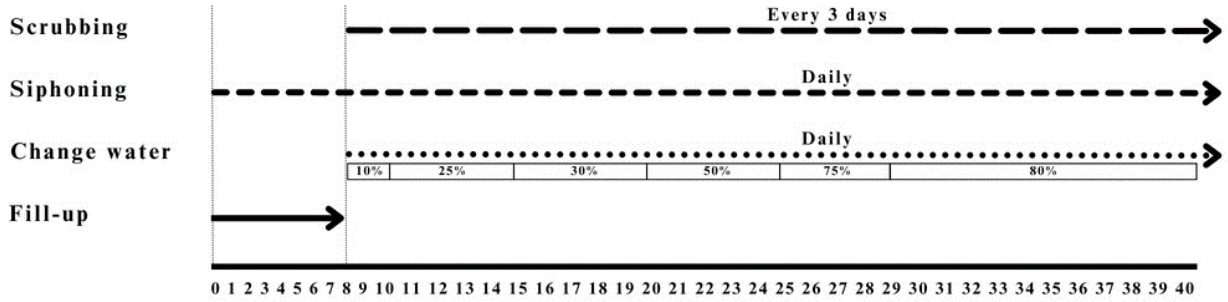

Fig. 2. Water management protocol used in the larval rearing of *Caranx ignobilis* from DAH 0 to DAH 40.

Survival rate (%) = Number of remaining larvae / Total number of larvae stocked × 100

Data analysis

At the end of the 40-d rearing period, all larvae were counted and measured to estimate survival and growth performance (final total length, body depth, length increment and specific growth rate). Leven's test (F-max test) was used to test for homogeneity of variance. After confirming homogeneity of variance, one-way analysis of variance (ANOVA) was employed using a univariate general linear model to determine significant difference among treatment effects. This was followed by Duncan's Multiple Range Test to test for specific treatment mean difference. Percentage data were arc sine transformed prior to ANOVA. ANOVA showed significant difference among treatment means in the survival rate, thus, the survival rate may have an effect on the growth performance parameters. To account for the effects of this parameter, analysis of covariance (ANCOVA) was done for growth parameters (total length, body depth, length increment and specific growth rate) to determine the significant difference among treatment means using survival rate as covariate. Sidak test was then done to test for specific treatment mean difference. All statistical significance was compared at 5 % probability level. All computations and analyses were carried out using SPSS version 20. The data were presented as treatment means ± standard error of the mean.

Results

Survival of Caranx ignobilis *larvae*

Larvae in T2 had significantly higher survival rate (23.47 ± 3.18 %) than larvae in T1 (11.67 ± 1.90 %), but survival rate did not differ significantly between T1 and T3 (14.37 ± 3.88 %), nor between T2 and T3 (Table 2).

At the onset of first exogenous feeding between DAH 3 to DAH 5, the larval density was observed to significantly decrease in density in all larval tanks. The significant decrease in the number of larvae was again observed in T1 and T3 rearing tanks at DAH 25 to DAH 30, a few days after the introduction of *Artemia* as live food, with the mortality still occurring gradually until DAH 40. Dead larvae were observed early in the morning scattered throughout the tank bottom with the larvae having stomach full of ingested *Artemia*. At this period, several larvae-fed T1 and T3 were also observed to exhibit erratic, swirling swimming behaviour at the water surface wherein they sank to the tank bottom and then eventually died. In T2 rearing tanks, gradual decrease in the population were also observed over time from DAH 27 to 40 but larvae in T2 did not exhibit the erratic swimming behaviour that was observed in T1 and T3-fed larvae. Dead larvae in T2 tanks were characterised by a thin body as a result of shortage in live food due to difficulty in collecting copepods from the wild at certain periods during the larval rearing study which occurred four times between DAH 25 to DAH 40. The copepods provided to the larvae in T2 and T3 were limited at these times.

Growth performance of Caranx ignobilis *larvae*

The initial mean total length (TL) and body depth (BD) of the larvae in all treatments was 2.55 ± 0.06 mm and 0.30 ± 0.01 mm, respectively. At the end of larval rearing, larvae fed-T3 (rotifer + copepod + *Artemia*) (13.08 ± 0.98 mm TL) were significantly longer than larvae fed-T1 (rotifer + *Artemia*) (10.55 ± 0.88 mm TL) but did not differ significantly from the length of T2-fed larvae (rotifer + copepod) (10.27 ± 1.02 mm TL) (Table 3). The TL of larvae in T1 and T2 was not significantly different. The TL of T2 larvae was also not statistically different from T3 larvae. The BD of T3-fed larvae (rotifer + copepod + *Artemia*) (5.94 ± 0.38 mm)

Table 2. Mean survival rate (± SE) of *Caranx ignobilis* larvae fed different feeding regimens at 40 days after hatching (DAH).

Treatment	Survival rate (%)
Treatment 1 (Rotifers + *Artemia*)	11.67 ± 1.90^{b}
Treatment 2 (Rotifers + Copepods)	23.47 ± 3.18^{a}
Treatment 3 (Rotifers + Copepods + *Artemia*)	14.37 ± 3.88^{ab}

Note: In a column, means superscripted by a common letter are not significantly different at 5 % level.

Table 3. Growth performance (mean ± SE) of *Caranx ignobilis* larvae fed different feeding regimens at 40 days after hatching.

	Treatment 1 (Rotifers + *Artemia*)	Treatment 2 (Rotifers + Copepods)	Treatment 3 (Rotifers + Copepods + *Artemia*)
Initial total length (mm)	2.55 ± 0.06^{a}	2.55 ± 0.06^{a}	2.55 ± 0.06^{a}
Final total length (mm)	10.55 ± 0.88^{ab}	10.27 ± 1.02^{ab}	13.08 ± 0.98^{a}
Initial body depth (mm)	0.30 ± 0.01^{a}	0.30 ± 0.01^{a}	0.30 ± 0.01^{a}
Final body depth (mm)	4.96 ± 0.35^{b}	4.97 ± 0.50^{ab}	5.94 ± 0.38^{a}
Length increment (mm)	8.0 ± 1.90^{b}	7.72 ± 1.02^{bc}	10.53 ± 0.98^{a}
SGR (% day^{-1})	21.06 ± 1.96^{a}	20.32 ± 0.58^{a}	27.72 ± 2.25^{a}

Note: In a row, means superscripted by a common letter are not significantly different at 5 % level.

was significantly higher than larvae fed-T1 (rotifer + *Artemia*) (4.96 ± 0.35 mm) but was not statistically different from T2 larvae (rotifer + copepod) (4.97 ± 0.50 mm) (Table 3). The mean BD was not significantly different between T1 and T2 fed larvae. Similarly, the mean length increment of T3-fed larvae (rotifer + copepod + *Artemia*) (10.53 ± 0.98 mm) was significantly higher than that of larvae fed-T1 (rotifer + *Artemia*) (8.0 ± 1.90 mm) and T2 larvae (rotifer + copepod) (7.72 ± 1.02 mm). The mean length increment was not significantly different between T1 and T2 fed larvae. On the other hand, the specific growth rates were not significantly different among treatment means ($P > 0.05$). Mean specific growth rate was 21.06 ± 2.36 % day^{-1}, 20.32 ± 2.58 % day^{-1} and 27.72 ± 2.59 % day^{-1} for T1, T2 and T3-fed larvae, respectively (Table 3). The overall mean specific growth rate across all three treatments was 23.21 % per day. The ANCOVA results to test for differences in specific growth rate and length increment of larval *Caranx ignobilis* among feeding regimens with survival rate as a covariate are presented in Tables 4 and 5.

Table 4. Analysis of covariance to test for differences in specific growth rate of larval *Caranx ignobilis* among feeding regimens with survival rate as a covariate.

Source	SS	df	MS	F	Sig.	Partial eta squared
Correct model	105.546	3	35.515	2.366	.187	.587
Intercept	230.110	1	230.110	15.329	.011	.754
Survival rate (Covariate)	6.922	1	6.922	.461	.527	.084
Feeding regimen	105.159	2	52.579	3.503	.112	.584
Error	75.056	5	15.011			
Total	4956.873	9				
Corrected total	181.602	8				
a. R squared = .587 (Adjusted R squared = .339)						
b. Computed using alpha = 0.05						

SS: sum of squares; df: degrees of freedom; MS: mean sum of squares; F: F-statistic; Sig.: significance.

Table 5. Analysis of covariance to test for differences for length increment of larval *Caranx ignobilis* among feeding regimens with survival rate as a covariate.

Source	SS	df	MS	F	Sig.	Partial eta squared
Correct model	19.763	3	6.588	7.837	.025	.825
Intercept	80.567	1	80.567	95.843	.000	.950
Survival rate (Covariate)	5.402	1	5.402	6.427	.052	.562
Feeding regimen	12.874	2	6.437	7.657	.030	.754
Error	4.203	5	.841			
Total	713.203	9				
Corrected total	23.966	8				
a. R squared = .599 (Adjusted R squared = .466)						
b. Computed using alpha = 0.05						

SS: sum of squares; df: degrees of freedom; MS: mean sum of squares; F: F-statistic; Sig.: significance.

At DAH 10, there was no significant difference in the mean TL, BD, LI and SGR of the larvae among treatments ($P > 0.05$) (Fig. 3). However, at DAH 20 the mean TL, BD, LI and SGR becomes significantly different among treatments ($P < 0.05$) with T3-fed larvae having significantly higher mean TL, BD, LI and SGR than T1-fed and T2-fed larvae but was not significantly different from T1 larvae (Fig. 3). At DAH 30 and DAH 40, mean growth parameters were not significantly different among treatments ($P > 0.05$).

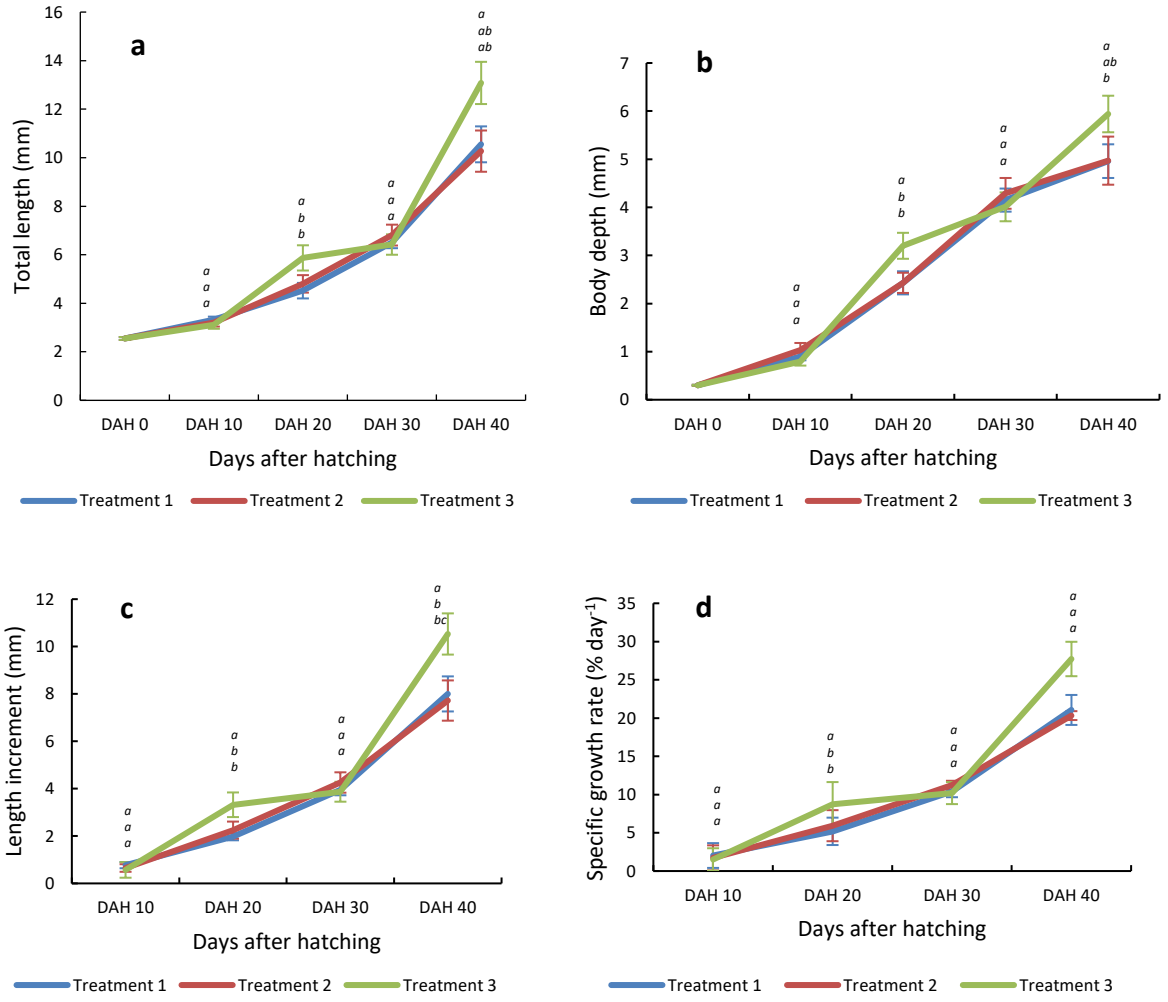

Fig. 3. Mean (a) total length, (b) body depth, (c) length increment and (d) specific growth rate (± SE) of *Caranx ignobilis* larvae fed different feeding regimens at 10, 20, 30 and 40 days after hatching.

Water quality

None of the mean values for the water parameters were statistically different among treatments. The AM and PM water parameter readings ranged from 23.0 °C to 28.9 °C for temperature, 6.3 ppm to 10.6 ppm for dissolved oxygen and 30 ppt for salinity. The parameters were within the optimum levels for larviculture of *C. ignobilis*.

Discussion

The first successful induced spawning of *C. ignobilis* in the Philippines was reported by Mutia et al. (2020) using human chorionic gonadotropin and luteinising hormone-releasing hormone analogue with a short description of successful larval rearing of hatchery bred *C. ignobilis* larvae fed with rotifer and *Artemia*. This present study is part of the *C. ignobilis* seed production program of NFRDI-Philippines to refine the hatchery protocols with emphasis on the use of live feed for larval rearing.

The success of the seed production in marine finfish hatchery depends primarily on the use of live natural food for larviculture. The utilisation of an appropriate live feed during the first feeding phase of the larval cycle is one of the major challenges in the commercial production of currently grown species and success with candidate species and is extremely crucial for the optimal development of marine fish larvae. A live feed with the proper nutritional composition, constituting a suitable size range and stimulating a feeding response is necessary to expand the number of species of marine

fish produced (Ohs et al., 2019). Current protocols for marine finfish hatchery utilise various species of rotifer and copepods and the commercially available *Artemia salina*, whose mass propagation techniques have been well established (Dhert, 1996; Lavens and Sorgeloos, 1996; Lubzens and Zmora, 2003; Schipp, 2006).

Results of the study showed that the survival rate of the larvae was affected by the different combinations of live food. In comparison with traditional hatchery feeding regimen for high value species of rotifer + *Artemia* (T1), the substitution of copepod instead of *Artemia* (T2) resulted in significantly higher survival rate which indicated suitability of this live food for *C. ignobilis* larval requirements. Previous initial work on *C. ignobilis* by Mutia et al. (2020) also employed rotifer and *Artemia* as live feed but encountered larval mortalities during the early phase of the larval cycles (days after hatching [DAH] 3 to 5) and a few days after the introduction of *Artemia* as live food (DAH 12 to 16). Physical manifestation of some of the larvae prior to mortality involved erratic, swirling swimming behaviour. These symptoms are related to a deficiency in highly unsaturated fatty acid (HUFA), particularly docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) (Watanabe, 1993; Parrish et al, 1994) and arachidonic acid (ARA) (Bessonart et al., 1999; Estevez et al., 1999). Similar mortalities were observed in other carangid species, e.g., striped jack *Caranx delicatissimus* (Arakawa et al., 1987), yellowtail amberjack *Seriola lalandi* (Stuart and Drawbridge, 2012) and longfin yellowtail *Seriola rivoliana* (Roo et al., 2012), with the mortalities being associated with n-3 HUFA and DHA deficiencies during *Artemia* feeding stage. In the larviculture of several marine aquaculture species, growth is negatively correlated with survival rate such as in *Sander lucioperca* (Szkudlarek and Zakes, 2007), *Archosargus rhomboidalis* (Houde, 1975), *Paralabrax maculatofasciatus* (Alvarez-González et al., 2007), and *Rachycentron canadum* (Hitzfelder et al., 2006). In the current study, statistical analysis showed that the survival rate affects the length increment of the larvae. The ANCOVA analysis considered survival rate as covariate to account for its effect on growth and the statistical results show that the feeding regimen affected the length increment. This demonstrates the positive effects of the availability of natural live food with copepod inclusion in increasing the length increment, as exhibited by the higher length increment of T3-fed larvae which included copepods as natural food compared to T1-fed larvae without copepods.

The larval mortality in *C. ignobilis* reported by Mutia et al. (2020) identified an issue with diet deficiency, hence the conduct of this present study on the potential use of copepods as alternative live feed for *C. ignobilis* larviculture. The aim is basically to partially or completely replace *Artemia*, which was the possible reason for the aforementioned high mortality. This present study has shown that feeding *C. ignobilis* larvae with copepods significantly increased the survival during larval rearing. Feeding with rotifers and copepods alone increased the survival rate by more than 200 % and the survival rate increased by 61 % when copepods were added to the feeding regimen of rotifer + *Artemia*. The use of copepods in marine finfish larviculture has been known to be more efficient than *Artemia* in terms of survival and growth (Toledo et al., 1999; Olivotto et al., 2008; Barroso et al., 2013; Rasdi and Qin, 2018; Zeng et al., 2018). Copepods have superior nutritional value containing high levels of highly unsaturated fatty acids (HUFAs) without the need for enrichment (Schipp, 2006). Copepods also contain HUFAs in their structural or polar lipid protein for easier assimilation by the larvae (Bell et al., 2003), are more easily digested than rotifers and *Artemia* (Pederson, 1984), and are natural sources of antioxidants, astaxanthin and vitamins C and E (Van der Meer, 2003). DHA and EPA weight by % total fatty acids of copepods range from 24.7 % to 30.3 % and 11.2 % to 6.8 %, respectively. In contrast, enriched rotifer has 6.5 % and 13.1 % and enriched *Artemia* with 3.0 % and 11.6 %, respectively (Bell et al., 2003). Moreover, the "jerking" movement of copepod and their nauplii triggers the larval feeding response (Barroso et al. 2013) resulting in higher feeding activity. The present study depended primarily on wild collected copepods and insufficient number of copepods could be collected at some periods during the larval rearing cycle. Although this occurred on just four occasions between DAH 25 and DAH 40, some of the gradual mortalities that were recorded in the rotifer + copepod fed treatment could be attributed to this limitation. Survival rates at DAH 40 could have been higher had copepod supply not been a problem.

Similar to the initial larval rearing research of *C. ignobilis* by Mutia et al. (2020), the present study observed mortalities a few days after the introduction of *Artemia* as live feed for the larvae in rotifer + *Artemia* and rotifer + copepod + *Artemia* treatments at DAH 22 to 25, with larvae exhibiting the same erratic, swirling swimming behaviour. In contrast, the rotifer + copepod fed larvae did not exhibit such erratic swimming behaviour. Although *Artemia* is the most widely used live feed for larval fish culture globally due to ease of mass production and long storage of cysts, its use has many disadvantages, most notable of which are its insufficient levels of HUFAs- DHA, EPA and ARA (Ohs et al., 2019). Marine fish contains large amounts of DHA and EPA in the phospholipids of their cellular membranes, especially in the neural and visual membranes (Sargent et al., 1999). The lack of these essential fatty acids can result in retarded physiological development and altered behaviour, such as impaired pigmentation and poor vision in low light intensities, resulting in increased predation and reduced hunting capability (Bell et al., 1995; Estevez et al., 1999; Sargent et al., 1999). ARA is also essential to marine fish, a precursor to the eicosanoids which are immunological compounds that are important for the fish immune system (Ohs et al., 2019). Moreover, embryonic and larval development of many marine fish requires a DHA:EPA ratio of at least 2.0 (Parrish et al.,

1994) and unenriched and enriched *Artemia* has relatively low DHA:EPA ratio of only 0.0 and 0.3, respectively (Bell et al., 2003). Although the low concentrations of HUFAs of *Artemia* could be augmented with enrichment of essential fatty acids and nutrients as in the present study, such enrichment has limitations. For example, brine shrimp do not uniformly consume the enrichments and some of the ingested enrichments will be metabolised by the brine shrimp before they are eaten by fish larvae, and brine shrimp has the tendency to catabolise DHA to EPA, thus the ability to increase the DHA:EPA ratio with enrichment may be limited (Ohs et al., 2019). In the present study, the negative effects of the *Artemia* on the survival of the *C. ignobilis* larvae at certain periods of the larval rearing at DAH 25 to DAH 40 was evident, not only in rotifer + *Artemia* treatment but also in rotifer + copepod + *Artemia* fed larvae where erratic, swirling swimming were observed prior to mortality, which is symptomatic of the low levels of HUFAs.

Although copepods were included in the feeding regimen of the rotifer + copepod + *Artemia* treatment, larvae were observed to exhibit erratic swimming behaviour prior to mortality which may be attributed to the feeding of larvae with *Artemia*, which has low levels of HUFA. It is therefore inferred that *C. ignobilis*, like other carangid species, requires higher levels of HUFAs than most of the marine finfish species that have been commercially reared in the hatcheries using enriched rotifer and *Artemia*. Feeding *C. ignobilis* with copepods is therefore important to increase larval survival. The nonsignificant difference in the survival rate between T2 (rotifer + copepod feed) and T3 (rotifer + copepod + *Artemia*) feed treatments at DAH 40 indicates the positive role of brine shrimp in the growth of the fish larvae. This may be attributed to the relatively higher protein content of *Artemia* (52.2–56.4 %) (Leger et al., 1987) than those of copepod (44–52 %). However, to reduce the incidence of erratic swimming behaviour associated with low levels of HUFAs, brine shrimp may be introduced to *C. ignobilis* at a later stage of larval rearing, after larvae have completed metamorphosis and when their requirement for HUFAs has decreased to the fatty acid profile of *Artemia*. Larvae would then not be susceptible to erratic swimming behaviour mortality.

The results of the present study indicate that the growth parameters were affected by the different live food combinations at DAH 20 but not at DAH 10, DAH 30 and DAH 40. Feeding with rotifer alone (T1) or in combination with copepod (T2 and T3) between DAH 2 to DAH 10, is apparently suitable for *C. ignobilis* larvae as shown in the nonsignificant difference among treatments in the SGR at DAH 10. Although the digestive tract is just starting to develop at this stage, the available digestive enzymes of the larvae were able to digest and assimilate the nutrients found in the two live food that were essential for its growth. Between DAH 11 to DAH 20 when live food that were fed to larvae were rotifer alone (T1) or rotifer + copepod (T2 and T3), both rotifer and copepod were essential to *C. ignobilis* larvae and feeding with rotifer alone significantly decreased the growth, as indicated by the significantly lower specific growth rate (SGR) of T1 larvae compared to T2 and T3 larvae at DAH 20. At this stage, essential nutrients found in the rotifer were not enough for its growth, and additional nutrients from copepods were needed to satisfy the requirements for larval growth. By DAH 21 to DAH 30 and DAH 31 to DAH 40, the three feeding regimens of rotifer + *Artemia*, rotifer +copepod and rotifer + copepod + *Artemia* were all suitable for *C. ignobilis* as reflected in the non-significant difference in treatment means for growth at DAH 30 and DAH 40. At this point, the larvae had already undergone metamorphosis, with their digestive tracts reaching full development. In the present study, the use of enriched rotifers as live feed in the initial larval cycle in the rearing of *C. ignobilis* was found to be suitable, as the mouth gape of the larvae was large enough to receive the live feed. Two marine rotifer species, *Brachionus plicatilis* and *B. rotundiformis* have been used to culture over 60 species of marine fish larvae and 18 species of crustacean larvae (Dhert, 1996). Like *Artemia*, rotifers do not have the full nutritional profile required by fish larvae and lack DHA, EPA and ARA, and therefore must be enriched before they are fed to marine fish larvae (Sargent et al., 1997). Some studies suggest that rotifers are not easily digested (Schipp et al., 1999) and their steady random motion, controlled by their ciliated crown, does not induce a feeding response in all marine fish larvae (Chesney, 2005). Feeding with live feed other than rotifers, such as copepods, in the early stage of larval rearing period is therefore important (Toledo et al., 1999), as in the case of *C. ignobilis* larviculture. A combination of rotifers and copepods would be more advantageous, and this is clearly shown by the higher growth of *C. ignobilis* larvae at DAH 20 in Treatment 3 when fed rotifers and copepods, than those of Treatment 1 when fed on rotifers alone during the initial 20 days larval rearing period.

Results of this study suggest that the use of copepod for *C. ignobilis* larviculture plays a crucial role in its survival and growth and may partially replace the use of *Artemia*. However, large scale production of copepods is needed to sustain the needs of the larvae throughout the larval rearing period. The study is limited to smaller tank volumes and needs to be verified in larger culture tanks. Further studies are also needed to determine the requirements for other aspects of larviculture including suitable copepod species, size of live food and food density in order to improve survival and growth. Culture techniques for the mass production of copepods for larviculture is also important to address the need for a sustainable source of copepods for the hatcheries. Furthermore, studies on the digestive enzyme development in *C. ignobilis* larvae are needed to explain the possible role of the enzyme development on the digestibility of essential nutrients of the different live food, as well as its effect on survival and growth of the larvae at

different larval rearing periods.

The water management protocols employed in the study, which was derived from the previous study of the same authors on *C. ignobilis* spawning and larval rearing (Mutia et al., 2020), showed that it was suitable for the larviculture of the species. No detectable issues with the larvae were associated with water quality. Water quality parameters were within the optimum levels for larviculture of most marine finfish (Hoff et al., 1978; Berlinsky et al., 1997; Toledo et al., 1999; Emata, 2003; Roo et al., 2012; Stuart and Drawbridge, 2012; Mutia et al., 2020).

Conclusion

The study has shown that the feeding *C. ignobilis* larvae with copepods in combination with rotifers and *Artemia* significantly increases its survival. The use of copepods for *C. ignobilis* larviculture could partially replace the use of *Artemia*. Mass seed production of *C. ignobilis* is therefore foreseeable in the next few years, which will support the development of this candidate species into a commercial species for high value aquaculture. However, the requirements of larvae for adequate supply of copepods must be satisfied through appropriate culture methods in mass production of copepods to achieve success in hatchery seed production.

Based on the results of this study, improvement in the survival of *C. ignobilis* larvae was associated with the improvement of larval rearing protocols and the introduction of copepods in the diet, reaching the highest recorded survival rate for this species. Although some issues need to be addressed such as production of copepods for routine use and weaning of *Artemia*, the current increase in larval survival rates because of the improvements in breeding and larval rearing protocol is a remarkable accomplishment in the culture of this species. Hatchery-reared fingerlings can soon augment the limited supply of *C. ignobilis* fingerlings collected from the wild. With a sustainable supply of seeds for aquaculture readily available, the high-value *C. ignobilis* is targeted as an alternative commodity for aquaculture, which can provide livelihood opportunities, increase food security, and enhance economic growth.

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