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Nutritional Value of *Pseudodiaptomus annandalie* (Copepoda: Calanoida) and its Suitability as Feed to Milkfish Larvae

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ABSTRACT

The nutritional value of *Pseudodiaptomus annandalie* and its suitability as feed to milkfish larvae were determined. Pure isolates of *P. annandalie* was subjected to feeding in four different microalgal species (*Chlorella sp., Chaetoceros calcitrans, Isochrysis galbana and Tetraselmis chuii*) for 15 days of culture. Lipid extraction was done using Bligh and Dyer method and analyzed in a gas chromatograph for fatty acid profile. Results were analyzed using univariate ANOVA (p.0.05) and subsequent Tukey tests. Results of the first experiment showed that the number of fatty acids in *P. annandalie* varied significantly at different microalgal diets. DHA/EPA ratio was highest in *Chaetoceros calcitrans* (4.91%). This suggest that *Chaetoceros calcitrans* is the best microalgal food for *P. annandalie* because it promotes high DHA/EPA ratio in its fatty acid profile which is an important requirement for fish larvae. When live *P. annandalie* together with other natural foods were tested, results showed that treatment fed live copepods had significantly ($p<0.05$) higher average length after day 24 (14.86+0.78 mm)

over other treatments. Survival rate was significantly highest in treatment fed live *P. annandalie* (65.58+0.65%) compared with other treatments. Live copepod therefore is a suitable feed for milkfish larvae because it contains a high fatty acid profile.

Keywords **—** Aquatic Ecology, *Pseudodiaptomus annandalie*, nutritional value, suitability as feed, fatty acid profile, DHA/EPA milkfish larvae, Philippines

INTRODUCTION

Fish nutrition, feeds and feeding management plays an important role in increasing aquaculture production. Development of a well-balanced diet and adequate feeding are most important considerations for successful hatchery operations and management of milkfish larvae and other finfishes before it is grown in fishponds for marketable size. The practice of many hatchery operators now a day is to feed their larvae with commercial feeds that are very expensive although it meets the nutritional requirement of fish larvae. With this problem, however, several finfish hatchery operators shifted feeding commercial feeds to natural live food like *Artemia salina*, *Brachionus plicatilis* and copepod species particularly, *Pseduodiaptomus annandalie* (Sewell). Such copepod species is a newly identified and poorly known Indo-Pacific copepod (Calanoida) which occurs in North Queensland, Australia (Grigg, 1972) and from India to West China and South Australia (Walter, 1984; Walter, 1986a, 1986b, Walter 1987; Walter & Boxshall, 2002; Walter et al, 2006; Walter and Boxshall, 2012 ; and Shrinui, et al 2013;). Golez, Takashi, Ishimaru and Ohno (1999) have studied the post-embryonic development and reproduction of this species while Caturao (2014) studied the reproductive biology and mass production, and this can be scaled up and mass produce in larger tanks. This live food suits the mouths of finfish larvae during the larval rearing phase.

A well-documented critical factor during first feeding is the content of highly unsaturated fatty acids (HUFas) in the live food for the optimal growth of fish larvae (Kanazawa, 1985; Sargent, McEvoy, & Bell, 1997; Koven, Tandler, Kissil, & Sklan, 1992). Fish naturally require high levels of essential HUFAs in their diet for larval growth and survival particularly a high ratio of docosahexaenoic acid (22:6n-3; DHA) to eicosapentaenoic acid (20:5n-3; EPA). Furthermore, increased resistance to stress is related to the presence of adequate levels and ratios of n-3 to n-6 long-chain HUFAs (March, 1993; Sargent & Falk-Petersen, 1988).

Ohno and Okomura (1998) suggested the potential of *Pseudodiaptomus annandalie*, to replace or be added to *Artemia* nauplii, which is very expensive. *Brachionus plicatilis* and *Artemia salina*, are naturally deficient in several n-3 HUFAs (Leger, Bengston, Simpson, & Sorgeelos, 1986; Navarro et al., 1992). On the other hand, the fatty acid composition of copepods is markedly dependent on their microbial diet (Sargent & Falk-Petersen, 1988; Graeve, Kattner & Hagen, 1994). A study by Toledo, Golez, Masanori and Atsushi (1999), has shown further that *Epinephelus coioides* fish larvae fed with a high-density copepod displayed better feeding incidence, gut content, growth, and survival.

Billions of other species of fish such as salmon smolts, seabream fingerlings, and milkfish fry are being produced annually from the egg stage onwards (Lavens & Sorgeelos, 1996). The culture of these larvae is mostly done under controlled condition because they are very tiny, extremely fragile, and not well developed. Their mouth is practically small in size, the incomplete development of their perception organs, and their digestive system are limiting factors in proper feed selection and use during the start of feeding. As larvae further undergo several larval stages, eventually they changed their herbivorous filter-feeding behavior to a carnivorous hunter. The problem of the appropriate size of feed, high nutritive value, ease and cheap means of culture appears to be a major bottle-neck for the large-scale culture of marine fishes in the hatchery by hatchery operators worldwide. Thus, to determine the potential of this species as feed to milkfish larvae and other economically viable species of fish, the nutritional value and its suitability of *P. annandalie* are worth investigating.

OBJECTIVE OF THE STUDY

This study determined the nutritional value of *P. annandalie* by using its fatty acid profile and its suitability to milkfish larvae.

MATERIALS AND METHOS

Nutritional value of *P. annandalie*

The Culture of microalgal species. Pure isolates of microalgal species used in this study were obtained from the Phycology Laboratory of the Southeast Asian Fisheries Development Center, Aquaculture Department (SEAFDEC AQD) as starter for the culture of microalgae. Four 20-liter transparent plastic jars were used in the culture. Eighteen liters of filtered seawater was introduced to each

plastic jars and was subsequently fertilized with 1ml/liter each of Guillard and Ryther's Modified F medium for *Chaetoceros calcitrans* and *Isochrysis galbana* and Walne's Conway medium for *Chlorella sp*., and *Tetraselmis batan*. Two liters of algal starter for each of the four species of algae (*Chaetoceros calcitrans*, *Isochrysis galbana, Chlorella sp.,* and *Tetraselmis batan)* were added to each jar. The jars were then placed on the culture shelves 10-12 inches away from the 40-watt lamp, provided with aeration and cultured for 2-3 days and served as feed for the copepods.

The Culture of *Pseudodiaptomus annandalie*. Pure isolates of *Pseudodiaptomus annandalie* were obtained from the Natural Food Laboratory of SEAFDEC AQD and was stocked in four 1-ton fiberglass tanks. The stocking density in each tank was 100 ind/liter with at least 80% adult stage. Mild aeration was provided in each tank and cultured for 15 days. Salinity for all treatments was maintained at 20 ppt throughout the experiment and temperature was monitored twice daily. The volume of seawater containing the copepod was computed by the formula below:

$$T_{sd} = \frac{S_r}{D_c} \times V_c$$

where: T_{sd} = total stocking density in 1-ton fiberglass tank
S_r = stocking requirement
D_c = density of copepods from the stock culture
V_c = volume of culture container

After stocking, each treatment was fed initially with microalgal species assigned in a complete randomized design, namely; Treatment 1-*Chlorella sp*., Treatment II-*Chaetoceros calcitrans*, Treatment III-*Isochrysis galbana*, and Treatment IV- *Tetraselmis batan*. Each treatment was replicated three times. The feeding density requirement for all treatments was maintained at 100,000 cells/ml daily throughout the duration of the study. Counting for the densities of the microalgae was done by the use of the haemacytometer and cell counter by the formula of Martinez, Chackroff, and Pantastico (1975):

$$A_d = A_{ave} \times 10^6 \text{ cells/ml} \times 10$$

where: A_d = algal density
A_{ave} = average number of cells per haemacytometer block

The amount of feed introduced to the assigned treatments was calculated by the formula below:

$$A_f = \frac{F_r}{A_d} \times V_c$$

Where: A_f = Amount of feeds (in cells/ml)
F_r = feeding requirement
A_d = algal density from the stock
V_c = volume of culture container

Water change in all treatments was done every other day by siphoning 50% of the water volume and replacing the same with the use of a rubber hose attached to a filter net with a mesh size of 60 µm.

Harvesting and counting of *Pseudodiaptomus annandalie.* Before harvest of P. annandalie, sampling was done on each of the treatments to determine copepod density and stages composition**.** Modified Van Dorn sampler was used to sample copepods vertically in the water column by taking 3 liters of the water sample and filtered in plankton net with a mesh size of 60µm.

The collected samples were concentrated and placed in 20 ml test tubes and preserved in 10% buffered formalin solution. Counting of copepod density and identification of larval stages were done under the electric microscope. After which, percentage larval stage composition was determined.

Fatty acid analysis. The remaining copepods in every treatment were filtered separately by the use of a siphon directed towards a plankton box with a mesh size of 60 µm. The samples were washed with filtered seawater, concentrated and placed in properly labeled containers. It was immediately frozen and kept in a biofreezer at -20°C before freeze-drying and further analysis. Lipid extraction was carried out according to the method described by Bligh and Dyer (1959), adapted by SEAFDEC Centralized Analytical Laboratory.

Analysis of the fatty acid methyl esters (Eder, 1995) was done at three to six replicate samples with a Shimadzu GC-17A gas chromatography equipped with a 30 m x 0.32 mm (film thickness: 0.2µm) SPB PUFA capillary column using helium as the carrier gas and nitrogen as an auxiliary gas. Peaks were recorded in a Shimadzu-Chromatopac CR7A Plus recording integrator, identified by comparison with known standards, and quantified by means of the response factor of the internal standard.

Analysis of the Data. Fatty acid profiles were compared with concerning the four microalgal species using univariate analysis of variance utilizing a 0.05 level of significance. (Zar, 2010). When ANOVA P value is significant, subsequent Tukey tests were performed utilizing a significance value of 0.05. Before ANOVA, the normality of data was tested using Kolmogorov-Smirnov test with Lilliefors' correction, utilizing a P value for rejection of 0.05. Non-normal data were normalized using either logarithmic or reciprocal transformation.

Suitability of *P. annandalie* to Milkfish Larvae

Experiment on the suitability of *P. annandalie* to milkfish larvae was conducted in 250-liter fiberglass tanks in a complete randomized design with three replicates each at Natural Food Laboratory of SEAFDEC AQD. The following are the treatments and replicates:

Treatments	Replicates
Live *P. annandalie*	3
Live *Brachionus plicatilis*	3
Live *A. salina*	3
Frozen *P. annandalie*	3

Before the experiment started, the culture of live *A. Salina, B. plicatilis, and P. annandalie* was separately cultured in 1-ton tank and maintained throughout the experiment as a source of feed.

Milkfish fry at 5 days old was stocked in 250-liter fiberglass tanks at 20/liter. Each tank was provided mild aeration and cultured in 24 days. The fry of milkfish was fed with different feed (*A. salina*, *B. plicatilis*, *P. annandalie*, and frozen *P. annandalie*) based on the assigned treatment at 10 ind/ml. Water management followed the procedure practiced at SEAFDEC AQD (Gapasin & Marte, 1990; Parazo et al., 1990). Ambient salinity and temperature were used throughout the experiment.

Food density inside the experimental tank was monitored to adjust feeding by the use of the formula below:

Without previous feeding:

$$D_{cf} = \frac{(V_{wrt})\,(D_{cdrt})}{C_{dct\ (source)}}$$

Whereby:

D_{cf} = density of copepod as feed
V_{wrt} = volume of water in the rearing tank
D_{cdrt} = desired copepod density in the rearing tank
C_{dct} = copepod density in the culture tank (source)

Whereas, for the experimental tanks with previous feeding, the formula below was used:

$$D_{ctrt} = \frac{(V_{wrt})\,(D_{cdrt} - C_{drt})}{C_{dct\ (source)}}$$

Whereby:

D_{ctrt} = density of copepod to be added in the rearing tank
V_{wrt} = volume of water in the rearing tank
D_{cdrt} = desired copepod density in the rearing tank
C_{drt} = copepod density in the rearing tank
C_{dct} = copepod density in the culture tank (source)

Growth was monitored every week by taking samples from the experimental tanks and measured by using a weighing scale (10-gram scale). A sampling of the survival rate was done by taking 3 samples of 1-liter beaker from the two corners and at the center of the rearing tanks. Milkfish larvae from each beaker were counted and total count was averaged, and multiplied by the volume of water.

Dissolved oxygen, salinity and temperature were monitored daily by using a D.O. meter, refractometer and a thermometer, respectively. Water changed was done twice a week at about 50-80% of the total volume of water in the experimental tanks. Sediments, residues and any dirt at the bottom of the tank which may cause fouling and pollution of the water which led to the development of ammonia were siphoned.

After 24 days of culture, final growth (grams) and length (mm) were measured by using a weighing scale and a transparent ruler, respectively. Final survival rate of milkfish was calculated based on the number of fish survived over the initial stock, multiplied by 100.

Data were analyzed by One-Way analysis of Variance to determine the significant difference (at 0.05 level of significance) between treatments. Duncan's Multiple Range Test was used to test significant difference among treatment means.

RESULTS AND DISCUSSION

Nutritional value of *P. annandalie* by means of fatty acid profile

Fatty acid content of *P. annandalie* at different microalgal diets

Results of fatty acid content of *Pseudodiaptomus annandalie* at different microalgae *(Chlorella sp., Chaetoceros calcitrans, Isochrysis galbana and Tetraselmis batan*) is shown in Table 1.

Table 1. Fatty acid profile of *Pseudodiaptomus annandalie* at different microalgae

Fatty acid	CH	CC	ISO	TB
14:0	2.44 ± 0.38^{c}	3.81 ± 0.10^{a}	3.59 ± 0.10^{a}	2.62 ± 0.11^{b}
16:0	19.53 ± 1.15^{b}	20.21 ± 0.21^{b}	16.58 ± 0.38^{c}	31.00 ± 0.47^{a}
16:1	2.49 ± 0.55^{b}	2.42 ± 0.04^{b}	3.37 ± 0.13^{a}	1.45 ± 0.10^{c}
16:2	3.04 ± 0.25^{a}	3.39 ± 0.14^{a}	3.11 ± 0.11^{a}	1.49 ± 0.07^{b}
16:3	-	-	-	1.88 ± 0.14
18:0	11.40 ± 0.29^{b}	14.31 ± 0.16^{a}	11.49 ± 0.10^{b}	9.00 ± 0.37^{c}
18:1	5.52 ± 0.29^{d}	6.75 ± 0.11^{b}	6.06 ± 0.20^{c}	11.71 ± 0.06^{a}
18:2n-6	6.96 ± 0.98^{a}	2.30 ± 0.07^{b}	2.25 ± 0.14^{b}	6.23 ± 0.12^{a}
18:3n-3	-	-	1.53 ± 0.15^{b}	1.99 ± 0.16^{a}
18:4n-3	3.63 ± 1.56^{a}	2.24 ± 0.19^{b}	-	1.72 ± 0.19^{b}
20:1	-	2.84 ± 0.22^{b}	-	5.15 ± 0.26^{a}
20:2n-6	1.56 ± 0.13	-	-	1.71 ± 0.10
20:4n-6 (AA)	3.96 ± 0.51^{a}	3.44 ± 0.11^{ab}	3.04 ± 0.10^{b}	3.39 ± 0.16^{ab}
20:4n-3	-	-	2.88 ± 0.10^{a}	1.75 ± 0.17^{b}
20:5n-3 (EPA)	7.82 ± 0.06^{a}	5.18 ± 0.10^{c}	7.24 ± 0.09^{b}	4.02 ± 0.15^{d}

Fatty acid	CH	CC	ISO	TB
22:0	-	2.51 ± 0.25^{b}	6.90 ± 0.15^{a}	-
22:1	-	2.25 ± 0.10^{ab}	1.93 ± 0.27^{b}	2.66 ± 0.18^{a}
22:5n-3	2.60 ± 0.14^{a}	2.93 ± 0.10^{a}	2.07 ± 0.15^{b}	2.23 ± 0.13^{b}
22:6n-3 (DHA)	29.05 ± 0.23^{a}	25.42 ± 0.32^{c}	27.90 ± 0.33^{b}	9.98 ± 0.19^{d}
DHA/EPA	3.71 ± 0.04^{b}	4.91 ± 0.13^{a}	3.84 ± 0.07^{b}	2.48 ± 0.08^{c}

The values presented are means of three replicates and their standards deviations and presented as % of total fatty acids. CH-Chlorella sp; CC-Chaetoceros Calcitrans; ISO-Isochrysis galbana; TB-Tetraselmis batan, AA-Arachidonic acid; EPA-Eicosapentaenoic acid; DHA-Docosahexaenoic acid (-) Not detected; treatments which have the same superscripts are not significantly different from each other.

Generally, for all treatments, nineteen (19) fatty acids were identified, four (4) of which were saturated fatty acids (SFA) and four (4) were monoenoic fatty acids. Nine (9) highly unsaturated fatty acids (HUFAs) were identified and comprised of 18:2n-6, 18:3n-3,18:4n-3, 20:2n-6, 20:4n-3, 20:4n-6 (arachidonic acid or AA), 20:5n-3 (eicosapentaenoic acid or EPA) 22:5n-3, and 22:6n-3 (docosahexaenoic acid or DHA).

The number of fatty acids detected in each of the four treatments were as follows: thirteen (13) in *Chlorella spp*. fifteen (15) in *Chaetoceros calcitrans* and fourteen (14) in *Isochrysis galbana,* and nineteen (19) in *Tetraselmis batan.* Three fatty acids, 16:0, 18:0, and DHA (22:6n-3) occupy a large amount of the fatty acids profile of *P. annandalie.*

In all treatments other than the *Tetraselmis batan-fed* group, DHA (22:6n-3) accounts for at least 25% of the total fatty acids. In the *Tetraselmis batan* treatment, a higher amount of 16:0 fatty acid (31%), makes up the greatest portion of its total fatty acids, having 18:1 (11.71%) as the second most abundant fatty acids and DHA (22:6n-3=9.98%) falling as only the third in abundance. Conversely, the other three treatments, namely, *Chlorella sp, Chaetoceros calcitrans,* and *Isochrysis galbana* has the fatty acids DHA (22:6n-3=25.42-29.05%) as the most abundant, 16:0 (16.58-20.21%) as the second and 18:0 (11.40-14.31% as the third in abundance with respect to the other fatty acids. One fatty acid, 16:3, however, was present in the fatty acid profile of *Tetraselmis batan*-fed *P. annandalie* but was not found in any other three treatments. DHA/EPA ratio was highest in the *Chaetoceros calcitrans* treatment (4.91). This was followed by *Isochrysis galbana* fed *P. annandalie* (3.84). Notably lower levels of DHA (22:6n-3=9.98%) and EPA (20:5n-3=4.02%), and consequently, a low DHA/EPA ratio

(2.48), were found on the fatty acid profile of *P. annandalie* fed with *Tetraselmis batan.*

Levels of DHA (22:6n-3) were at least twice as high in the other treatments (*Chlorella sp., Chaetoceros calcitrans, Isochrysis galbana)* compared to the *Tetraselmis batan*-fed copepods. Levels of EPA (20:5n-3), although significantly different among treatments, did not show large variation, while arachidonic acid or AA (20:4n-6) show almost similar values.

The fatty acids 18:3n-3 and the 20:4n-3 were not found in the *Chlorella sp.* and *Chaetoceros calcitrans* treatments. In the treatments where *P. annandalie* fed *Chaetoceros calcitrans* and *Isochrysis galbana,* the fatty acid 20:2n-6 was not found. Similarly, in two treatments, *Chlorella sp.* and *Tetraselmis batan,* 22:0 was absent. This is the only fatty acid that was missing from the profile of the *Tetraselmis Batan* treatment that could be found in the fatty acid profile of the other treatment (*Chlorella sp., Chaetoceros calcitrans, Isochrysis galbana).* One fatty acid, 22:1 however, was absent in the *Chlorella sp.* treatment, which is otherwise found in the other three treatments (*Chaetoceros calcitrans, Isochrysis galbana and Tetraselmis batan).* Likewise, the fatty acid 18:n-3 is not found in the *Isochrysis galbana* treatment but was found in the other three treatments (*Chlorella sp., Chaetoceros calcitrans* and *Tetraselmis batan*).

Univariate analysis of variance on each of the fatty acids except one revealed a significant difference ($p<0.05$) on the treatment means. Only the fatty acid 20:2n-6 was not statistically significant ($p>0.05$) among treatments. Data on two fatty acids, 16:1 and 18:2n-6, failed to pass the Kolmogorov-Smirnoff normality test with Lilliefors' correction and were transformed using logarithmic transformation. Subsequent analysis of variance conducted on both 16:1 and 18:2n-6 fatty acids showed a significant result ($p<0.05$).

The results of Tukey test on significant ANOVAs of the different fatty acids reveal a highly significant difference ($p <0.05$) between *Chaetoceros calcitrans* and *Tetraselmis batan* treatments with an 85% difference according to the different fatty acids, followed by the results of Tukey test between *Chlorella sp.* and *Isochrysis galbana* with a difference of 83%. A 79% difference according to the detected fatty acids is indicated furthermore by Tukey test between *Isochrysis galbana and Tetraselmis batan* treatments while *Tetraselmis batan* and *Chlorella sp.* treatments differ by only 69.23%. A difference of 53% between *Chaetoceros calcitrans sp.* and between *Chaetoceros calcitrans* and *Isochrysis galbana* were also observed.

Fatty acids categories of *Pseudodiaptomus annandalie*

Some fatty acids categories in *Pseudodiaptomus annandalie* at different microalgal diets are shown in Table 2.

Table 2. Some fatty acids categories of *Pseudodiaptomus annandalie* at different microalgal diets

Fatty acid categories	CH	CC	ISO	TB
Saturated fatty acids	33.37 ± 1.21^{a}	40.84 ± 0.62^{b}	38.60 ± 0.59^{c}	42.62 ± 0.34^{b}
Monoenoic fatty acids	8.01 ± 0.73^{a}	14.26 ± 0.05^{b}	11.36 ± 0.23^{c}	20.97 ± 0.34^{d}
n-6 fatty acids	12.48 ± 1.45^{a}	5.74 ± 0.17^{b}	5.29 ± 0.04^{c}	11.35 ± 0.16^{a}
n-3 fatty acids	43.10 ± 0.65^{a}	35.77 ± 0.14^{b}	41.64 ± 0.36^{c}	21.69 ± 0.40^{d}
n-3 HUFAs	39.47 ± 0.40^{a}	33.53 ± 0.16^{b}	40.11 ± 0.29^{a}	17.98 ± 0.10^{c}

The values presented are means of three replicates and their standards deviation and presented as % of total fatty acids. CH-*Chlorella sp.,* CC-*Chaetoceros calcitrans;* ISO-*Isochrysis galbana;* TB-*Tetraselmis batan,* AA-*Arachidonic acid;* EPA-*Eicosapentaenoic acid;* DHA-*Docosahexaenoic acid;* treatments which have the same superscripts are not significantly different from each other.

The *Chlorella sp.* treatment showed the highest amount of n-3 fatty acids (43.10%) although the *Isochrysis galbana* treatment had the highest quantity of n-3 HUFAs (40.11%). *Chlorella sp. (*43.10%) and *Isochrysis galbana* (41.64%) treatments had n-3 fatty acids as their most abundant while the *Chaetoceros calcitrans* (40.84%) and *Tetraselmis batan* (42.62%) treatments have the higher saturated fatty acids. *Tetraselmis batan*-fed groups presented low amounts both in n-3 fatty acids (21.69%) and n-3 HUFAs (17.98%), which was almost half compared to the other three treatments. Saturated (42.62%) and monoenoic (20.97%) fatty acids, however, were highest in the *Tetraselmis batan* treatment group. On the other hand, *Chaetoceros calcitrans* (5.74%) and *Isochrysis galbana* (5.29%) treatments were low in n-6 fatty acids, while the Chlorella sp., fed *P. annandalie* shows the highest amounts of n-6 fatty acids (12.48%).

Univariate analysis of variance in each of the fatty acid categories showed a significant difference ($p<0.05$) in all of the treatment means. Normality analysis of data on the n-6 fatty acids, however, rendered it to be not normal and thus, the reciprocal transformation of the data was performed. Subsequently, it likewise showed a significant difference ($p<0.05$) among treatment means. Succeeding Tukey test illustrated that the treatments were different from each other (≥ 80% difference) according to the different fatty acid categories.

Average larval stage composition

The sampling of *P. annandalie* during harvest revealed a larval composition of at least 98% copepodite stage in all treatments (Table 3).

Table 3. Average larval stage composition of *Pseudodiaptomus* annandalie at different treatments

Larval stages	Treatments							
	CH		CC		ISO		TB	
	ind/I	% of pop	ind/I	% of pop	ind/I	% of pop	ind/I	% of pop
copepodite	544.33[b]	98.85	297.67[c]	98.13	842.33[a]	98.75	197.00[d]	98.01
nauplii	5.67[b]	1.03	4.33[c]	1.43	8.67[a]	1.02	2.67[d]	1.33
gravid	0.67	0.12	1.33	0.44	2.00	0.23	1.33	0.66
Average total abundance	**550.67**[b]	100.00	**303.33**[c]	100.00	**853.00**[a]	100.00	**201.00**[d]	100.00

Ch-*Chlorella sp*., CC-*Chaetoceros calcitrans*, ISO-*Isochrysis galbana*, TB-*Tetraselmis batan*; ind/l-Individuals per liter; % of pop-percent of total population: values which have the same letter superscripts are not significantly different among each other.

In all treatments, nauplii comprised more than 1% of the total population while gravid females were merely less than 0.66% of the population. *Isochrysis galbana* treatment (853 ind/l) was the highest in average total abundance followed by the *Chlorella sp*. treatment (550.67 ind/l). The lowest in total abundance was the *Tetraselmis batan* group (201 ind/l) which was the lowest in abundance.

Univariate analysis of variance shows that all treatments differ significantly ($p<0.05$) in total abundance and number of copepodites, and nauplii. They do not differ significantly in the number of gravid females ($p>0.05$).

Temperature and salinity

Average daily temperature values in all treatments ranged from 27°C to 30°C. No significant variation of temperature values observed in the morning and afternoon throughout the experiment. Salinity, however, was maintained at 20 ppt throughout the experiment because previous experiments revealed that 20 ppt is most suitable for the growth and reproduction of *P. annandalie*.

Suitability of *P. annandalie* to milkfish larvae

Average Length

Results on the suitability of *P. annandalie* to milkfish larvae (Table 5) showed that treatment fed live copepods had significantly ($p<0.05$) higher average length starting from day 12 (8.91+0.19 mm) until day 24 (14.80+0.78) over other treatments such as *Brachionus sp.* (9.39+0.29mm), *Artemia salina* (11.56+0.01 mm), and frozen copepods (9.378+0.081 mm). This means that live copepods could enhance the growth of milkfish larvae from fry stage up to fingerling size which is ready for stocking in the grow-out ponds.

Table 4. Average total length (mm) of milkfish larvae fed different natural live food (Trt 1, live *P. annandalie*; Trt 2, live *B. plicatilis*; Trt 3, live *A. salina* (control); and Trt 4 (frozen *B. plicatilis*)

Treatments	Days of Culture				
	Day 0	Day 6	Day 12	Day 18	Day 24
Trt 1 (Live *P. annandalie*)	3.81+.63	5.49+.19	8.91+.54	12.54+.78	14.80+0.78a
Trt 2 (Live *B. plicatilis*)	3.78+.16	4.65+.26	5.76+.45	7.11+.32	9.39+.29c
Trt 3 (Live *A. salina*) control	3.97+.53	5.53+.65	6.78+.16	8.82+.13	11.56+.01b
Trt 4 (Frozen *P. annandalie*)	4.03+.52	4.74+.43	5.87+.16	8.02+.48	9.38+.08bc

Values with different letter superscript are significantly different from each other

Survival rates

The result of the survival rate of milkfish for 24 days of culture is shown in Figure 1. Treatment fed live *P. annandalie* got the highest survival of 65.58+0.65%, followed by treatment fed live *A. salina* with 38.25+0.86%, then by treatment fed live *B. plicatilis* with 29.64+1.09%, and treatment fed frozen *P. annandalie* with a lowest survival rate of 10.53+0.25%. The significant difference was observed among treatments at 0.05 level of significance. This means that survival rate of milkfish larvae is better when fed with live copepods compared with other treatments.

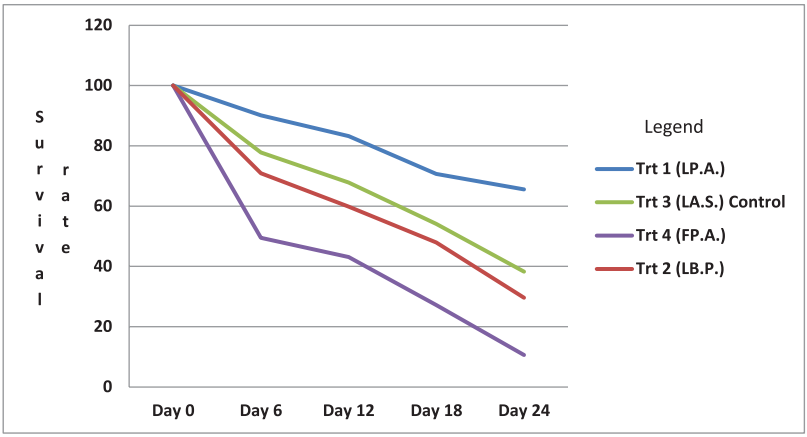

Figure 1. Average survival rate of milkfish larvae fed different live natural food (*P. annandalie, B. Brachionus, A. salina* and frozen *P. annandalie*) for 24 days of culture.

Physico-chemical parameters

Temperature ranged from 27.9-32ºC, while NH_3 N ranged from 0.05 – 0.065 ppm throughout the culture period without any fluctuations.

In this study, amounts of fatty acids in *P. annandalie* varied significantly at different microalgal diets. The amount of DHA (22:6n-3) in all algal diets was relatively high compared to DHA (22:6n-3) content of *Artemia salina* which was not even detected in his other study (Navarro & Amat, 1992). This result showed that n-3 HUFAs in the fatty acid profile of *P. annandalie* were four times higher than that found in *Artemia*. Likewise, a review by Watanabe, Kitajima, and Fujita (1983), revealed that *Artemia* nauplii had rather low amounts of n-3 HUFAs. It is thus evident that *Artemia* is naturally deficient in DHA (22:6n-3) (Sargent *et a*l.,1988). Levels of n-3 HUFAs on another species of copepod, *Acartia* (Watanabe *et al*., 1983), however, had almost similar n-3 HUFA content with that of *P. annandalie* in this study.

Compared with the fatty acid profile in the wild (Toledo *et al*., 1999) (Table 4), *P. annandalie* has remarkably low in n-3 fatty acids and n-3 HUFAs compared with this present study where DHA/EPA ratio was two times higher. This may probably be due to a mixture of phytoplankton species that copepod fed on in the wild and there was no strict feeding regimen applied to the copepods before harvest. The fatty acid profile of copepods depends primarily on the microalgae they feed upon (Sargent *et a*l., 1997). The fatty acid composition obtained by

Watanabe *et al*. (1983), on *Chlorella sp*. show a comparatively lower amount of n-3 HUFAs although EPA (20:5n-3) were abundant. In this present study, *P. annandalie* fed with *Chlorella sp*. showed high amounts of n-3 HUFAs (39.4%) as well as EPA (20:5n-3) (7.82%). In the study of Aujero, Millamena, Tech, and S. Javellana (1983) on the nutritional value of marine phytoplankton species, fatty acid composition of *Chlorella sp*. did not show any AA (20:4n-6) and EPA (20:5n-3). Whereas, in this study, these are present in the fatty acid profile of *P. annandalie* fed with *Chlorella sp*. Also, in the study of Alava *et al*. (2004), DHA was present only in *Chlorella*, and *Isochrysis*. These suggest that *P. annandalie* can convert certain fatty acids into another form such as DHA (22:6-3). In the same study by Aujero *et al.* (1983), *Chaetoceros calcitrans* and *Tetraselmis sp*. fatty acids were present in high amounts in the fatty acid profile indicated the presence of AA (20:4n: -6) and EPA (20:5n-3). These fatty acids were present in high amounts in the fatty acid profile of *P. annandalie* fed *Chaetoceros calcitrans* as well as *Chlorella sp*. in this study. In contrast, *Tetraselmis batan-fed P. annandalie* in this present study showed relatively lower amounts of n-3 HUFAS, particularly EPA (20:5n-3). Perhaps, other biochemical factors such as amino acid content or carbohydrate content of the microalgal feed may have affected their capacity for bioconversion. A lower average population density of the copepods was also found in this particular treatment. Effects of microalgal feed on survival and reproduction of the copepods may also account for the lower population. Moreover, algal preference of *P. annandalie* may not be discounted.

Chaetoceros calcitrans and *Isochrysis galbana* were classified as high HUFA feeds, where *Isochrysis* is abundant in DHA (22: n-3) and *Chaetoceros* is rich in EPA (20:5n-3) Fernandez-Reiriz *et al*., 1989; Napolitano, Ackman, & Ratnayaye, 1990; Thompson, Guo, & Harrison, 1993). The results of this study similarly show higher levels of EPA (20:5n-3) and DHA (22:6n-3) in the treatments fed with these two microalgae. *Isochrysis galbana*-grown *P. annandalie* in this present study demonstrated relatively high DHA (22:6n-3) probably due to the presence of high amounts of DHA (22:6n-3) in such microalgal species (Figaldo *et al*., 1998). Furthermore, the abundance of 16.0 in *Isochrysis galbana likewise* contributed to the high amounts of the same fatty acids in *P. annandalie.* In this present study, *P. annandalie* fed *Chaetoceros calcitrans* had the highest DHA/EPA ratio, which is suitable for fish (Sargent *et a*l., 1997), followed by those fed *Isochrysis galbana,* then *Chlorella sp.,* and lastly, those fed *Tetraselmis batan.* This suggests that *P. annandalie* fed *Chaetoceros calcitrans* is the most suitable as feed for fish larvae. Similarly, *Chlorella sp.* fed *P. annandalie* is also an excellent feed for fish larvae because of its high level of EPA (20:5n-3) and DHA (22:6N-3).

The levels of fatty acids of *P. annandalie* compared with that of their microalgal food (Aujero, *et al*., 1983; Fernandez-Reiriz *et al*., 1989; Napolitano *et al*., 1990; Thompson *et a*l., 1993) indicates that this species of copepod can convert certain fatty acids to HUFAs as well as certain HUFAs to other forms of HUFAs.

On the suitability of this copepods, results of this study have shown that *P. annandalie* is acceptable to milkfish larvae over other live feeds (Artemia*, Brachionus plicatilis*, and frozen copepods). *P. annandalie* had the highest DHA/EPA ratio which is 4.91% over other live feeds mentioned. This DHA/EPA is suitable for fish growth. Numerous studies had confirmed that copepods might have higher nutritional value than *Artemia salina*, as copepods have high nutritional requirements of marine fish larvae. Copepods have high protein content (44-52%) and have a good amino acid profile (Lavens and Sorgeloos, 1996). Particularly, in *P. annandalie*, the amount of DHA (22:6n-3) regardless of feeding them whatever microalgal diets, is relatively high (Caturao, 2010) compared to DHA (22:6n-3) content of *Artemia salina* (Navarro *et al*., 1992). Lavens and Sorgeloos (1996) further stated that they can be fed in different forms as nauplii, copepods at start feeding, and as on grown copepods until weaning. Their typical zigzag movement, followed by a short gliding phase, is an important visual stimulus for many fish, which they preferred over rotifer and other live feeds. Moreover, another advantage on the use of copepods especially the benthos-type species like *P. annandalie*, is that the non-predated copepods keep the walls of the fish larval rearing tanks clean by grazing the algae and debris.

Differences in biochemical composition, and more specific HUFA content, are not the only advantages over Artemia when offered as food to marine fish larvae, but also the qualitative and quantitative better content of digestive enzymes in copepodites and adults may play an important role as well. Early stages of marine fish larvae do not have a well-developed digestive system, and may benefit from the exogenous administration of enzymes. Evidence that copepods may be preferable to Artemia in this respect comes from Pederson (1984) who examined digestion in the first-feeding of herring larvae. Pedersen (1984) found that copepods passed more quickly through the gut and were better digested than *Artemia salina*. In the study of Rayner *et al*. (2015) the amino acid profile of *P. annandalie* reveals an abundance of essential amino acids above 53.8 ± 1.2%, combined with an estimated protein content of 57% of dry weight for adult females. Both the fatty and amino acid profiles show favorable nutritional values towards fish larvae rearing.

As to *Brachionus plicatilis* live feed, cultured copepods has been successfully used in various flatfish larvae. But in this experiment feeding of *B. plicatilis* to milkfish larvae is low (29.64+1.09) compared from feeding *P. annandalie* (65.58+0.65%). In hatchery operation, rotifers that cannot be fed immediately need to be stored at cold temperature (4°C) to prevent reduction of their nutritional quality. During the starvation period of one day at 25°C, rotifers can lose up to 26% of their body weight as a result of metabolic activity. Different culture and enrichment procedures will also starve the rotifer. Starvation of gut-enriched rotifers immediately before feeding to the larvae results in a very fast loss of their fatty acid content, as the animals start to empty their guts after 20 to 30 minutes. After about 6 hours in the larval rearing tanks, the HUFA content of rotifer has dropped to 1/3 of its original level. Moreover, Nellen, Quants, Witt, Kuhlmann, and Koske (1981) demonstrated that the larvae of turbot at start-feeding showed the preference for copepod nauplii over *Brachionus plicatilis*. After 14 days of culture, their feeding preference shifted towards adult copepods. Survival of the larvae was 50% and the fry reached 12 mg DW (17mm TL) at day 26.

On the other hand, frozen copepods were tried hoping that this becomes suitable as feed for marine fish larvae. However, it was found out that in this experiment, milkfish larvae fed with frozen *P. annandalie* has low survival rate (10.53+0.25%) because it is still thawed which become rapture in the water during feeding. Frozen copepods as in rotifers can lose up to 26% of their body weight and fatty acid profile content.

Physico-chemical parameters did not fluctuate significantly; all values are within the tolerable limits of milkfish larvae.

CONCLUSIONS

Nineteen (19) fatty acids were present in the fatty acid profile of *Pseudodiaptomus annandalie* in all treatments. High levels of n-3 HUFAs were detected (17.98% - 40.11%) Levels of n-6 fatty acids range from 5.29% - 12.48%. DHA (22:6n-3), 16:0 and 18:0 fatty acids were among the predominant fatty acids found in the fatty acid profile of *P. annandalie* in all treatments. Levels of DHA (22:6n-3) range from 9.98% - 29.0%, EPA(20:5n-3) range from 4.02% - 7.82% and AA (20:4n-6) range from 3.04% - 3.96%. Chlorella sp. fed groups had the highest amounts of DHA (22:6n-3) which has 29.05% and EPA (20:4n-3) which has 7.82%. *Chaetoceros calcitrans* fed groups had the highest DHA/EPA

ratio with 4.91%. I*sochrysis galbana* fed groups had the most n-3 HUFAs with 40.11%. *Tetraselmis batan* fed group had the most abundant saturated fatty acids with 42.62% and monoenoic fatty acids has 20.97%.

Compared from the wild, *P. annandalie* had remarkably low n-3 fatty acids and n-3 HUFAs as when they are fed with specific microalgal species.

These results suggest that *Chaetoceros calcitrans* is the best microalgal feed for *Pseudodiaptomus annandalie* because it promotes a high DHA/EPA ratio in the fatty acid profile of the copepod which is an important requirement in fish larvae feed. Likewise, *Chlorella sp.* is also excellent feed for *P. annandalie* since it enhances the amount of EPA (20:5n-3) and DHA (22:6n-3) in the fatty acid profile of the copepod which is necessary for the survival of fish larvae.

Moreover, milkfish larvae can grow well if fed with live *P. annandalie* because it has a higher nutritive value than *A. salina*, *B. plicatilis* and frozen *P. annandalie.* Such copepod species matched the nutritional requirements of marine fish larvae. Also, they can be given in different stages like nauplii, copepodites at start feeding, and as on-grown copepods until weaning. Moreover, their typical zigzag movement, followed by a short gliding phase, is an important visual stimulus for milkfish and any other fish larvae compared from among the feeds given.

TRANSLATIONAL RESEARCH

The findings of this study can be translated into training and extension manuals, and CDs for instructional purposes. Moreover, procedures in the culture of *Pseudodiaptomus annandalie* and feeding this into the milkfish larvae can be transformed into a hatchery operation guide for small-scale hatchery operators, and also for the students in one of their fisheries subject. Likewise, students who want to conduct further studies on this aspect can make a correlation study on the fatty acid profile of *P. annandalie* with the fatty acid profile of their microalgal diet to determine metabolic utilization of these fatty acids. Other species of microalgae should also be investigated as a potential food source for *P. annandalie.* Likewise, other strains of *P. annandalie* found in the different regions of the world should also be evaluated.

As an offshoot for this study, the fatty acid profile of the different larval stages of *P. annandalie* can also be determined to optimize the culture of this species as food for fish larvae. Mixed microalgae may also be used as experimental treatments for it may be possible that the combined effects of certain microalgae will enhance the fatty acid profile of the copepod. Also, the effects of *P. annandalie*

fed different microalgal diets on the growth of fish should also be studied as a practical application of the study.

Likewise, other physico-chemical factors, such as salinity, temperature, dissolved oxygen which could possibly affect the metabolic activities of *P. annandalie* should also be studied for its effects on the fatty acid profile of this particular copepod species.

Moreover, results of this study can be translated into another study about feeding of different stages of *P. annandalie* to milkfish larvae and other marine and freshwater species to determine which of the life stages will give high survival and growth. Also, a comparative cost and returns analysis of the different treatments undertaken from this study can be conducted to determine which among of the live feeds is economical.

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