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## USE OF JUVENILE INSTAR *DIAPHANOSOMA CELEBENSIS* (STINGELIN) IN HATCHERY REARING OF ASIAN SEA BASS *LATES CALCARIFER* (BLOCH)

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### Abstract

The effects of size, dry mass intake and nutritional value of the brackishwater cladoceran, *Diaphanosoma celebensis*, on the growth and survival of 15-30 day sea bass (*Lates calcarifer*) larvae reared in a static green water system were determined. The highest specific growth rate (29.4%/day) was attained in larvae fed a 1:1 combination of *Artemia* nauplii and adult *Diaphanosoma* but it was not significantly different ( $p > 0.05$ ) from fish fed only adult *Diaphanosoma* (28.8%/day) or only juvenile instar *Diaphanosoma* (28.6%/day). Survival rates of larvae (92.4-99.0%) fed the different live diets did not significantly differ ( $p > 0.05$ ). Larvae markedly preferred juvenile instar *Diaphanosoma* over *Artemia* nauplii and adult *Diaphanosoma*. The crude protein contents of juvenile *Diaphanosoma* (58.7%), adult *Diaphanosoma* (58.3%) and *Artemia* (56.7%) were substantially high and satisfied the dietary protein requirements of larvae. The fatty acid profile of the sea bass fry reflected the lipid composition of the live diet. Improved growth, survival and dry mass intake in larvae indicate the potential of juvenile *Diaphanosoma* in the hatchery rearing of sea bass larvae.

### Introduction

In the standard scheme for rearing Asian sea bass, *L. calcarifer* (Bloch, Centropomidae), larvae are fed nauplii of the brine shrimp *Artemia* (Great Salt Lake strain) from the age of 15 days until metamorphosis (Duray and Juario, 1988; Parazo et al., 1991). Several studies were conducted to find other zooplankton species to min-

imize or totally replace the use of *Artemia* due to its high cost and infrequent supply (Fermin, 1991; Fermin and Bolivar, 1994; Ganzon-Naret and Fermin, 1994; Peña de la et al., 1998). The use of nauplii in sea bass hatcheries produced inconsistent growth and survival results, maybe because of variations in nutritional value of

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*Artemia* from different strains and batches (Watanabe et al., 1980). Besides the biochemical composition of a diet, the efficiency of its acquisition by the organism is a consideration in improving feeding strategy and, thus, can have a significant economic impact on the production of finfish larvae.

The importance of food size on the growth of fishes has been cited in various studies (Hyatt, 1979; Wankowski, 1979; Wankowski and Thorpe, 1979; Dabrowski and Bardega, 1984; Khadka and Rao, 1986; Dhert et al., 1991; Hasan and Macintosh, 1992). The size of the mouth of the fish determines the largest prey that can be ingested (Wong and Ward, 1972; Wankowski, 1979; Knights, 1983; Dabrowski and Bardega, 1984). The freshwater cladoceran *Moina macrocopa*, due to its large size, can be used after *Artemia* and prior to minced fish as a food for sea bass larvae (NICA, 1986; Fermin and Bolivar, 1994; Ganzon-Naret and Fermin, 1994). The body widths of *Moina* neonates, small adults and large adults range 200-480  $\mu\text{m}$ , 620-900  $\mu\text{m}$  and >950  $\mu\text{m}$ , respectively (Fermin, 1991). The width of the mouth of sea bass larvae with a total length of  $5.48 \pm 0.1$  mm is  $531.03 \pm 11.3$   $\mu\text{m}$ . Sea bass can ingest organisms which are 80% their mouth size (Duray and Kohno, 1988). Recently, it was found that juvenile instar *Diaphanosoma celebensis* with a body width of 170-320  $\mu\text{m}$  can partially replace *Artemia* nauplii (Peña de la et al., 1998). In a preliminary experiment, sea bass larvae fed a 1:1 ratio of juvenile instar *Diaphanosoma* and *Artemia* had growth and survival comparable to those of larvae fed a complete *Artemia* ration (Peña de la et al., 1998).

This study examined in greater detail the use of *Diaphanosoma* to establish the possible relationship between the preferred food particle size and growth and survival of larvae. Prey-size selection was determined by dry mass ingestion (mg/fish) of the fish in a 15-day feeding experiment using juvenile and adult instar *Diaphanosoma* as individual treatments. *Diaphanosoma* is a promising live food organism for mass culture because of its high reproduction rate (1.11-1.63 times/day; Segawa and Yang, 1988). Beside the effect of food size, the

nutritive value of juvenile and adult instar *Diaphanosoma* was evaluated in relation to the growth and survival of the sea bass larvae.

### Materials and Methods

**Zooplankton culture.** Staggered batches of the cladoceran *D. celebensis* were cultured in eight 1.5 m<sup>3</sup> fiberglass tanks (Peña de la et al., 1998) to ensure availability of food throughout the rearing period. Juvenile and adult *Diaphanosoma* were harvested daily by siphoning them into a wooden box lined with a  $324 \pm 3$   $\mu\text{m}$  mesh. Juvenile *Diaphanosoma* (JD) with a body length (BL) of 527-734  $\mu\text{m}$  and body width (BW) of 170-320  $\mu\text{m}$  passed through the mesh while those that remained on the net was classified as adult *Diaphanosoma* (AD) with a BL of 765-1,600  $\mu\text{m}$  and BW of 410-604  $\mu\text{m}$ . *Artemia* nauplii (AR) with a BL of 310-550  $\mu\text{m}$  and BW of 110-200  $\mu\text{m}$  came from cysts (Sanders Brine Shrimp Co., USA) hatched 18 hours in strongly aerated sea water. Unhatched cysts were separated from newly hatched nauplii by covering the hatching tank with a black cloth. The cysts that floated on top of the hatching tank were siphoned out and discarded. Before feeding to the fish, all live food treatments were washed well with sea water that passed through a sand filter and a 5  $\mu\text{m}$  filter bag.

**Experimental treatments and design.** Before stocking, larvae in treatments to be fed *Diaphanosoma* (ARJD, ARAD, JD and AD) were gradually acclimated from 32‰ to 15‰ salinity in 0.3 m<sup>3</sup> fiberglass tanks for eight hours since *D. celebensis* is a brackishwater species. The larvae in treatments to be fed *Artemia* (AR32 and AR15) were kept at 32‰ salinity in a separate 0.3 m<sup>3</sup> fiberglass tank. Six feeding treatments with three replicates each were assigned in a completely randomized design. There were two *Artemia* control treatments (groups fed *Artemia* only), one was maintained at 32‰ (AR32) and one at 15‰ (AR15) while the other treatments (ARJD, ARAD, JD, AD) were maintained at 15‰ salinity throughout the 15-day feeding experiment.

**Fish and feeding experiment.** Sea bass eggs were obtained from captive female broodstock which spawned naturally in floating net cages at

the SEAFDEC Aquaculture Department in Igang Marine Station, Guimaras, Philippines, and transported to Tigbauan, Iloilo, for incubation and hatching. The larvae were reared in 1.5 m<sup>3</sup> fiberglass tanks and fed with s-type rotifers (*Brachionus rotundiformis*) for the first 10 days, followed by *Artemia* nauplii on days 11-15 (Parazo et al., 1991). *Chlorella*, at a density of  $1-3 \times 10^5$ , were added as food for the rotifers and as a water conditioner. Fifteen-day old larvae (4.6 mm SL, 1.8 mg wet BW) were stocked at 30 individuals per ml in 10 l polyethylene cylindrical tanks. A single airstone near the bottom of each tank provided continuous aeration at a flow rate of 1.43-4.76 l/min. *Artemia* were fed at a rate of 5 individuals per ml throughout the rearing period (Parazo et al., 1991). The zooplankton differed in size and therefore the feeding rate was determined according to average dry weights. The average dry weight of the *Artemia* was  $17.7 \pm 0.6$   $\mu$ g while that of juvenile *Diaphanosoma* was  $8.2 \pm 0.1$   $\mu$ g and of adult *Diaphanosoma* was  $9.8 \pm 0.8$   $\mu$ g. Hence, to feed the same amount of dry mass in all treatments, 11.5 juvenile or 9.5 adult *Diaphanosoma* per ml was considered equivalent to 5 *Artemia* nauplii per ml. In the treatments where both *Artemia* and *Diaphanosoma* were given, the feed ration was 2.5 *Artemia* and 5.75 juvenile (ARJD) or 4.75 adult (ARAD) *Diaphanosoma*. The live foods were not enriched with essential fatty acids and were fed directly to the fish. Fish were fed once a day at 09:00 for 15 days. Before feeding, uneaten food and fish excreta were siphoned out and 30% of the rearing water was replaced daily with either 15‰ or 32‰ salinity sea water. *Tetraselmis* sp. at  $10-15 \times 10^3$  cells per ml were added to the rearing tanks after the water change to serve as food for the zooplankton and as a water conditioner.

**Water quality.** Water quality was monitored prior to water change and feeding. Temperature and salinity (ATAGO S/Mill 8613 hand refractometer) were monitored daily while dissolved oxygen (DO) and pH were measured twice weekly using a YSI oxygen meter (model 51B) and a Perkin Elmer pH meter (model 5996), respectively. Ammonia and nitrite were also measured twice a week (Strickland and Parsons, 1972). During the rearing period, the

DO concentration (5.0-7.6 ppm), temperature (26-29°C) and pH (7.0-8.0) were within desirable levels for optimum fish growth (Kungvankij et al., 1988). Nitrite (0.08-3.01 ppm) and ammonia (0.59-2.34 ppm) were also within tolerable levels for fish (Meade, 1985).

**Fish sampling and statistical analysis.** Every five days during the 15-day growth and survival study, ten fish from each tank were collected and sacrificed for standard length and weight measurements. Fish were washed with distilled water and blotted dry on absorbent paper. Standard length (SL) was measured to the nearest 0.1 mm using a profile projector (Nikon Model 6C, Japan). Weight was determined to the nearest 0.1 mg using an analytical balance (Mettler, AE 160, USA). Sacrificed fish were dissected under a Swift binocular stereoscope and the ingested zooplankton were counted. Partially ingested zooplankton with an intact head were considered one individual. The number of zooplankton ingested by each fish (ind/fish) was converted to dry mass (mg/fish) by multiplying the number of ingested individuals by their corresponding dry weight. Dead fish were counted daily while unaccounted fish were assumed to have been lost due to cannibalism (Katavic et al., 1989). At the end of the experiment, surviving fish were counted.

Growth and survival data were analyzed using one-way analysis of variance. Differences among treatment means were analyzed for significance by Duncan's Multiple Range Test (DMRT) at the 5% level using SAS linear model procedures (SAS, 1991). Dry mass ingestion data within each age (20, 25 and 30 days) were analyzed by *t* test using Sigma Plot Scientific Graphing Software (Jandell, 1995).

**Proximate and fatty acid analyses.** Proximate analyses of the live feeds were carried out using the standard procedures described by the Association of Official Analytical Chemists (AOAC, 1975). Total lipids were extracted from live feed and fish carcass samples following the method of Bligh and Dyer (1959). The lipid materials were saponified and transesterified into fatty acid methyl esters (FAME) using methanolic sodium hydroxide and BF<sub>3</sub> methanol reagent (Metcalf et al., 1966). Component fatty acids were separated

and identified using a gas-liquid chromatograph (Shimadzu GC-9) equipped with a flame ionization detector. An FW-WCOT capillary column with a stabilized cyanopropyl silicon coating (P-Sil 90, RESCOM, Belgium) was used. Fatty acids were identified using known standards and cod liver oil. Fatty acids were quantified by Shimadzu C-R7A plus Chromatopac.

### Results

**Growth and survival.** The size increment, growth and survival of the 15-day old sea bass fed the treatment diets for 15 days are shown in Table 1. Standard length (SL) increments in fish fed juvenile (JD,  $5.6 \pm 0.0$  mm) or adult (AD,  $5.8 \pm 0.4$  mm) *Diaphanosoma* and adult *Diaphanosoma* together with *Artemia* (ARAD,  $5.9 \pm 0.3$  mm) were significantly higher ( $p < 0.05$ ) than in fish fed juvenile *Diaphanosoma* together with *Artemia* (ARJD,  $4.6 \pm 0.2$  mm). The fish fed *Artemia* alone, reared in either 32‰ (AR32,  $3.4 \pm 0.1$  mm) or 15‰ salinity (AR15,  $3.6 \pm 0.2$  mm) had the lowest SL increments ( $p < 0.05$ ). Similarly, the SGRs in fish fed juvenile (JD,  $28.6 \pm 0.1\%/day$ ) or adult (AD,  $28.8 \pm 1.1\%/day$ ) *Diaphanosoma* and adult *Diaphanosoma* together with *Artemia* (ARAD,  $29.4 \pm 0.3\%/day$ ) were remarkably higher ( $p < 0.05$ ) than in fish fed juvenile *Diaphanosoma* together with *Artemia* (ARJD,  $25.9 \pm 0.6\%/day$ ). The fish fed *Artemia* alone, reared in either 32‰ (AR32,  $20.8 \pm 0.5\%/day$ ) or 15‰ salinity (AR15,  $21.4 \pm 0.5\%/day$ ), had the lowest SGR ( $p < 0.05$ ).

Survival after 15 days ranged from 99.0% (AR15) to 92.4% (ARAD) and did not differ significantly among treatments ( $p > 0.05$ ). Mortality (0.4-2.4%) and apparent loss due to cannibalism (0.6-5.2%) were generally low and did not differ significantly among treatments ( $p > 0.05$ ). Larvae growth, expressed as mean SL and mean wet BW, on days 20, 25 and 30 (day 15 was initial) is shown in Fig. 1. On day 20, the growths of fish in all treatments were similar ( $p > 0.05$ ) however, on day 25, fish fed juvenile (JD) or adult (AD) *Diaphanosoma* alone or with *Artemia* (ARAD, ARJD) grew faster ( $p < 0.05$ ) than larvae fed *Artemia* alone (AR32, AR15). At day 30, the fish in treatments JD, AD and ARAD were significantly longer and heavier ( $p < 0.05$ ) than the fish in treatment ARJD. The

fish in treatment AR32 and AR15 were significantly smaller than those in other treatments ( $p < 0.05$ ).

**Ingestion.** The mean zooplankton dry mass ingested by 20 to 30-day old sea bass larvae offered a single feed is shown in Table 2. Larvae fed juvenile *Diaphanosoma* significantly ( $p > 0.05$ ) consumed the highest dry mass on day 20 ( $0.21 \pm 0.03$  mg/fish) and day 30 ( $3.26 \pm 0.28$  mg/fish). However, on day 25, larvae in the AR15 treatment ingested a significantly higher ( $p < 0.05$ ) dry mass ( $1.47 \pm 0.27$  mg/fish) than those in other treatments. On day 20, the dry mass intake of the larvae in treatment AD ( $0.07 \pm 0.01$  mg/fish) was comparable with those of larvae in the AR treatments (AR32,  $0.01 \pm 0.01$  mg/fish; AR15,  $0.10 \pm 0.05$  mg/fish). On day 25, larvae in the AD treatment ( $0.30 \pm 0.04$  mg/fish) had a lower consumption than larvae in the JD treatment ( $0.98 \pm 0.07$  mg/fish) but it was not significantly different ( $p > 0.05$ ) than consumption among larvae in the AR32 treatment ( $0.37 \pm 0.03$  mg/fish). The lowest dry mass intake recorded on day 30 was in the AD treatment ( $0.74 \pm 0.24$  mg/fish) and then in the AR treatments (AR32,  $1.72 \pm 0.04$  mg/fish; AR15,  $1.97 \pm 0.09$  mg/fish;  $p < 0.05$ ).

Table 3 shows the mean zooplankton dry mass ingested by fish fed both *Artemia* and *Diaphanosoma* (ARJD, ARAD). A significantly higher ( $p < 0.05$ ) dry mass intake of juvenile *Diaphanosoma* was recorded in the ARJD treatment on day 20 ( $0.12 \pm 0.01$  mg/fish), day 25 ( $0.43 \pm 0.05$  mg/fish) and day 30 ( $2.10 \pm 0.08$  mg/fish) than of *Artemia* on the same days ( $0.01 \pm 0.01$ ,  $18 \pm 0.06$  and  $0.41 \pm 0.03$  mg/fish, respectively). Similarly, the dry mass intake of adult *Diaphanosoma* in the ARAD treatment was significantly higher on day 20 ( $0.06 \pm 0.01$  mg/fish), day 25 ( $0.12 \pm 0.02$  mg/fish) and day 30 ( $1.07 \pm 0.10$  mg/fish) than of *Artemia* on the same days ( $0.01 \pm 0.00$ ,  $0.05 \pm 0.02$ ,  $0.15 \pm 0.02$  mg/fish, respectively).

**Proximate and fatty acid composition.** The proximate compositions and fatty acid profiles of the live foods and the sea bass larvae are presented in Table 4. The crude protein content of juvenile (58.7%) and adult (58.3%) *Diaphanosoma* and *Artemia* (56.7%) were comparably high and satisfied the dietary pro-

Table 1. Mortality, survival, apparent loss due to cannibalism, specific growth rate (SGR) and standard length increment of sea bass larvae fed different zooplankton feeds for 15 days, n=31.

Treatment	Observed mortality <sup>1</sup> (%)	Survival <sup>2</sup> (%)	Apparent loss due to cannibalism <sup>3</sup> (%)	SGR <sup>4</sup> (%/day)	Standard length increment <sup>5</sup> (mm)
AR32	0.8±0.2 <sup>a</sup>	97.8±0.8 <sup>a</sup>	1.4±0.7 <sup>a</sup>	20.8±0.5 <sup>c</sup>	3.4±0.1 <sup>c</sup>
AR15	0.4±0.2 <sup>a</sup>	99.0±0.7 <sup>a</sup>	0.6±0.6 <sup>a</sup>	21.4±0.5 <sup>c</sup>	3.6±0.2 <sup>c</sup>
ARJD	1.0±0.5 <sup>a</sup>	95.9±1.6 <sup>a</sup>	3.1±1.7 <sup>a</sup>	25.9±0.6 <sup>b</sup>	4.6±0.2 <sup>b</sup>
ARAD	2.4±1.5 <sup>a</sup>	92.4±4.1 <sup>a</sup>	5.2±4.7 <sup>a</sup>	29.4±0.3 <sup>a</sup>	5.9±0.3 <sup>a</sup>
JD	1.6±0.2 <sup>a</sup>	95.5±1.5 <sup>a</sup>	2.9±1.7 <sup>a</sup>	28.6±0.1 <sup>a</sup>	5.6±0.0 <sup>a</sup>
AD	1.9±0.4 <sup>a</sup>	96.9±1.1 <sup>a</sup>	1.2±0.9 <sup>a</sup>	28.8±1.1 <sup>a</sup>	5.8±0.4 <sup>a</sup>

Values within columns ±SEM having the same superscript are not significantly different (p>0.05).

1 Observed mortality = total number of dead fish/initial stock x 100. Ten fish per tank were sacrificed every five days for length and weight measurements and were excluded from mortality computations.

2 Survival = total number of survivors/initial stock x 100. Ten fish per tank were sacrificed every five days for length and weight measurements and were excluded from survival computations.

3 Apparent loss due to cannibalism = 100 % initial stock - (% observed mortality + % survival).

4 SGR =  $\ln W_{i(mg)} - \ln W_{f(mg)} / T$  x 100.

5 SL increment = Final SL<sub>(mm)</sub> - Initial SL<sub>(mm)</sub>. Initial standard length and wet body weight of 15 day old larvae were 4.7 mm and 1.8 mg, respectively.

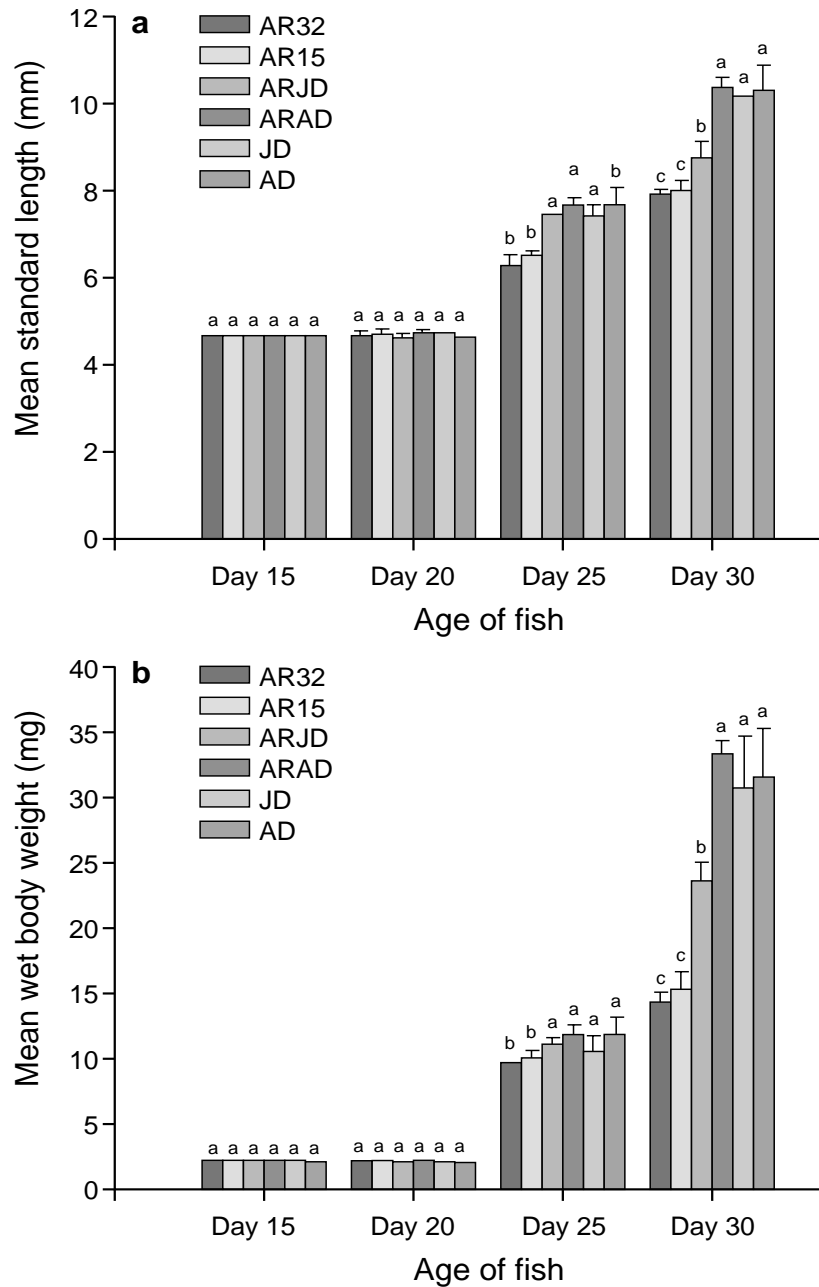


Fig. 1. (a) Mean standard length (mm) and (b) mean wet body weight (mg) of sea bass *Lates calcarifer* larvae reared in 32‰ or 15‰ salinity under various feeding regimes. The vertical line above each bar represents the standard error of the mean (SEM). Among fish of the same age, bars having the same superscripts are not significantly different ( $p > 0.05$ ).

Table 2. Mean zooplankton dry mass ingested by sea bass larvae in 32‰ and 15‰ salinity fed a single species.

Treatment	Zooplankton	Age of fish		
		Day 20 (mg/fish)	Day 25 (mg/fish)	Day 30 (mg/fish)
AR 32	<i>Artemia</i>	0.01±0.01 <sup>b</sup>	0.37±0.03 <sup>c</sup>	1.72±0.04 <sup>b</sup>
AR15	<i>Artemia</i>	0.10±0.05 <sup>b</sup>	1.47±0.27 <sup>a</sup>	1.97±0.09 <sup>b</sup>
JD	Juvenile <i>Diaphanosoma</i>	0.21±0.03 <sup>a</sup>	0.98±0.07 <sup>b</sup>	3.26±0.28 <sup>a</sup>
AD	Adult <i>Diaphanosoma</i>	0.07±0.01 <sup>b</sup>	0.30±0.04 <sup>c</sup>	0.74±0.24 <sup>c</sup>

Values within columns ± SEM having the same superscript are not significantly different ( $p>0.05$ ).

Table 3. Mean zooplankton dry mass ingested by sea bass larvae in 15‰ salinity fed a combination of *Artemia* and *Diaphanosoma*.

Treatment	Zooplankton	Age of Fish		
		Day 20 (mg/fish)	Day 25 (mg/fish)	Day 30 (mg/fish)
ARJD	<i>Artemia</i>	0.01±0.01 <sup>b</sup>	0.18±0.06 <sup>b</sup>	0.41±0.03 <sup>b</sup>
	Juvenile <i>Diaphanosoma</i>	0.12±0.01 <sup>a</sup>	0.43±0.05 <sup>a</sup>	2.10±0.08 <sup>a</sup>
ARAD	<i>Artemia</i>	0.01±0.00 <sup>b</sup>	0.05±0.02 <sup>b</sup>	0.15±0.02 <sup>b</sup>
	Adult <i>Diaphanosoma</i>	0.06±0.01 <sup>a</sup>	0.12±0.02 <sup>a</sup>	1.07±0.10 <sup>a</sup>

Within ARJD or ARAD, values within columns ± SEM having the same superscript are not significantly different ( $p>0.05$ ).

tein requirements of the larvae (Catacutan and Coloso, 1995). Juvenile *Diaphanosoma* contained the highest level ( $p<0.05$ ) of crude fat (20.3%) compared to *Artemia* (18.7%) and adult *Diaphanosoma* (12.7%). *Artemia* had a higher concentration of n-3 fatty acids (10.05 mg/g DW) than juvenile (6.55 mg/g DW) and adult (3.15 mg/g DW) *Diaphanosoma*. The n-3 fatty acids were higher in larvae fed *Artemia* (7.29 mg/g DW) than in those fed juvenile (1.69 mg/g DW) or adult (1.65 mg/g DW) *Diaphanosoma*. The three live foods and the

sea bass did not contain docosahexaenoic acid (22:6n-3). The fatty acid profiles of the sea bass fry were strongly influenced by the dietary lipid composition of the live foods.

#### Discussion

The high SGR (25.9-29.4%/day) and survival (92.4-96.9%) of sea bass larvae in the JD, AD, ARJD and ARAD treatments show that juvenile and adult *Diaphanosoma* have potential as a live food for the hatchery rearing of 15-30 day old sea bass. This is unlike *Moina* wherein



Table 4. Proximate (% dry matter) and fatty acid (mg/g dry weight) composition of juvenile and adult *Diaphanosoma celebensis*, *Artemia* nauplii and the sea bass larvae fed these live foods.

	Juvenile <i>D. celebensis</i>	Larvae	Adult <i>D. celebensis</i>	Larvae	<i>Artemia nauplii</i>	Larvae
Crude protein	58.7 <sup>a*</sup>		58.3 <sup>a</sup>		56.7 <sup>a</sup>	
Crude fat	20.3 <sup>a*</sup>		12.7 <sup>c</sup>		18.7 <sup>b</sup>	
Crude fiber	2.1 <sup>a*</sup>		2.8 <sup>a</sup>		2.6 <sup>a</sup>	
Nitrogen Free Extract	11.4 <sup>c*</sup>		22.9 <sup>a</sup>		16.3 <sup>b</sup>	
Ash	7.5 <sup>a*</sup>		3.3 <sup>c</sup>		5.7 <sup>b</sup>	
Total Lipids	13.30 <sup>*</sup>	10.15	15.60	11.56	20.10	8.07
Fatty Acids						
14:0	0.33	0.40	1.12	0.25	0.17	0.95
16:0	2.83	5.47	4.90	3.63	4.13	10.79
16:1	3.70	-	4.32	2.31	2.03	-
18:0	0.75	1.86	1.50	1.26	0.86	2.89
18:1	5.76	4.79	6.37	5.26	10.93	11.56
18:2n-6	3.57	1.64	3.30	1.96	2.44	3.26
18:3n-3	5.32	0.78	2.92	0.40	9.08	5.66
20:4n-6	0.05	0.72	-	-	-	0.91
20:5n-3	1.23	0.91	0.20	0.54	0.14	1.63
22:4n-6	-	-	0.33	-	0.07	-
22:4n-3	-	-	0.03	0.71	0.83	-
22:6n-3	-	-	-	-	-	-
Total saturated FAs	3.91	7.73	7.52	5.14	5.16	14.63
Total monoenoic FAs	9.46	4.79	10.69	7.57	12.96	11.56
Total n-6 FAs	3.62	2.36	3.63	1.96	2.51	4.17
Total n-3 FAs	6.55	1.69	3.15	1.65	10.05	7.29
n-3/n-6 FAs	1.80	0.72	0.90	0.84	4.00	2.07
DHA/EPA	-	-	-	-	-	-

Values within rows having the same superscripts are not significantly different (p>0.05).

\* Peña de la et al., 1998.

young specimens are preferred by 15-day sea bass and adult *Moina* can only be given starting day 25 (Fermin, 1991; Fermin and Bolivar, 1994). The SGR and survival in this study are better than our preliminary findings in sea bass larvae of the same age which also showed that juvenile instar *Diaphanosoma* can partially replace *Artemia* (SGR 18.7%/day; survival 96%; Peña de la et al., 1998). In that study (Peña de la et al., 1998), the growth of sea bass larvae fed *Artemia* was better than that of larvae fed juvenile *Diaphanosoma* due to the remarkably high content of n-3 HUFA (40.4%) in the *Artemia*. However, the present study showed that either juvenile or adult instar *Diaphanosoma*, alone or in combination with *Artemia*, resulted in better growth than *Artemia* alone. In this study, the n-3 HUFA content of the *Artemia* was not as high as it was in the previous experiment (Peña de la et al., 1998). The marked difference between the growth of the sea bass larvae in the earlier study (Peña de la et al., 1998) and in the present study may be due to the difference in the nutritional value of the *Artemia*. The variation of nutritional value of *Artemia* nauplii as food for marine fish has been well documented (Schauer et al., 1980; Watanabe et al., 1980; Leger et al., 1988). Nutrients vary not only among different sources of *Artemia* (Fujita et al., 1980), but even among batches of cysts of the same geographical origin (Watanabe et al., 1980; Vanhaecke and Sorgeloos, 1983). The fatty acid profiles of the sea bass in this study clearly reflected the fatty acid composition of the live foods. This result confirms the findings of Borlongan and Parazo (1991) that the total fatty acid content of sea bass carcass is determined by the dietary fatty acids.

The significant differences in stomach content between larvae fed a single food and those fed a combined diet indicate a marked preference for juvenile *Diaphanosoma* over adult *Diaphanosoma* and *Artemia*. Although sea bass larvae are able to ingest *Artemia* and adult *Diaphanosoma*, the fish preferred the intermediate-sized *Diaphanosoma*. In this study, the estimated mouth width of the sea bass ranged from  $531.02 \pm 11.4 \mu\text{m}$  on day 15 to  $1268 \pm 14.8 \mu\text{m}$  on day 30. The body width of the three live

foods is within the food size recommended by Duray and Kohno (1988) for sea bass larvae. Similar results were observed by Wong and Ward (1972) on yellow perch *Perca flavescens*, Khadka and Rao (1986) on *Cyprinus carpio* var. *communis* and Hasan and Macintosh (1992) on *Cyprinus carpio* L. Further, the prey size selectivity of the sea bass larvae in this study was probably influenced by the differences in feeding density of the live foods although the dry weights and feeding levels were uniform. Capture of *Diaphanosoma* by sea bass larvae was probably easier than that of *Artemia* since the former were given at a higher density (one *Artemia* nauplii was equivalent to 2.3 juvenile or 1.9 adult *Diaphanosoma*). Prey density markedly affects the encounter rates between prey and predator (Werner and Hall, 1974). Fish become opportunistic when they are exposed to abundant prey (Moore and Moore, 1976; Khadka and Rao, 1986). Ponton and Muller (1990) observed a high ingestion rate in white fish *Coregonos* sp. when a prey of suitable size was abundant. Larger sea bass were attained when fish were fed a higher density of *Artemia* (10 ind/ml; Parazo et al., 1991).

The use of this cladoceran in its early form improves the feeding efficiency of larvae and thus can have a significant contribution in the hatchery production of sea bass. *Diaphanosoma* has the advantage of having a smaller body than *Artemia*, one which complies with the size of the zooplankton required by fast growing larvae. From the culturists' point of view, harvest of juvenile *Diaphanosoma* is more efficient than of adult *Diaphanosoma* since the adult cladoceran reproduces in the culture system. Future studies on increasing the potential of the cladoceran by enrichment with essential fatty acids are recommended.

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