Effects of feeding copepod and *Artemia* on early growth and behaviour of the self-fertilizing fish, *Rivulus marmoratus,* under laboratory conditions

Maria Vivian Camacho Grageda[a,d,*], Tomonari Kotani[b,e], Yoshitaka Sakakura[c], Atsushi Hagiwara[a]

[a]Graduate School of Science and Technology, Nagasaki University, Nagasaki 852-8521, Japan

[b]Nagasaki Industrial Promotion Foundation, Collaboration of Regional Entities for the Advancement of Technological Excellence, Nagasaki, Japan

[c]Faculty of Fisheries, Nagasaki University, Nagasaki 852-8521, Japan

[d]Animal Biology Division, Institute of Biological Sciences, University of the Philippines at Los Baños, Laguna, Philippines

[e]Present address: Faculty of Life Science, Fukuyama University, Hiroshima 729-0292, Japan

* Corresponding author. Animal Biology Division, Institute of Biological Sciences, University of the Philippines at Los Banos, College, Laguna. Tel. and Fax No. (+62-049-5362843)

E-mail address: mavivs@yahoo.com

Abstract

Growth and survival have often been used as parameters to assess the effects of live feeds on marine finfish, however, behavioural effects, which entail energy cost and may have consequences on fish growth have been given less emphasis. Thus, a 20-day feeding experiment was conducted to determine the effects of copepod *Acartia tsuensis* (104-732 μm), unenriched, and docosahexaenoic acid, DHA-enriched, first instar *Artemia franciscana* nauplii (656-906 μm) on growth and behaviour of the mangrove killifish *Rivulus marmoratus*. Growth was significantly higher in *Acartia*-fed larvae compared with larvae fed *Artemia* (unenriched and DHA-enriched) until day 10. On day 20, *Acartia*-fed larvae had significantly lower growth than fish fed DHA-enriched *Artemia*. Feeding success was highest in larvae fed *Acartia* on day 1. Ingestion rate and satiation time did not differ among fish fed different types of feeds until day 20. Swimming activity before feeding was significantly lower in larvae fed *Acartia* compared with larvae fed *Artemia* (unenriched and DHA-enriched) until day 10. Higher growth in *Acartia*-fed fish on day 10 is probably due to the suitable size and high DHA content of *A. tsuensis*, and lower swimming activity of the larvae. However, on day 20, lower growth observed in *Acartia*-fed fish may be attributed to the shift in the food size preference of the fish. The present study was able to demonstrate the effects of copepods on growth and behavioural development of marine finfish using *R marmoratus* as a model animal.

Keywords: Feeding behaviour; Growth; Mangrove killifish; Swimming activity; n-3 HUFA

1. Introduction

Copepods have been recognized as the most suitable feed for early stages of fish larvae because of their nutritional advantage (high essential fatty acids) compared with other live feeds such as rotifers and *Artemia* (Nanton and Castell, 1998; Evjemo et al., 2003; Støttrup and McEvoy, 2003). Interest on copepod culture started as a result of the discovery that they have better nutritional value compared with commonly used rotifers and *Artemia* (Støttrup and McEvoy, 2003). Copepods have been mass-cultured as early as 1970s and 1980s in Japan and have been used as feed for Pacific cod larvae in rearing trials in fisheries stations (Hagiwara et al., 2001). In the early 1990s, intensive culture of different species of copepods expanded due to the increasing diversity in the marine finfish culture, particularly those with small larvae, such as grouper and red snapper, and the shortage of commonly used live feed *Artemia* (Doi et al., 1997; Støttrup and McEvoy, 2003). Since then, research on copepod culture (Barthel, 1983; Berggreen et al., 1988; Ohno and Okamura, 1988; Davis, 1993; Abu-Rezq et al., 1997; Hernandez Molejon and Alvarez-Lajonchere, 2003) for the main purpose of utilizing them as feed for commercially important marine fish species, led to their widespread use in larval rearing trials (Kraul et al., 1992; Nanton and Castell, 1998; Shields et al., 1999; Evjemo et al., 2003) as well as in European hatcheries (Støttrup, 2000). Although copepod culture in intensive indoor systems has been successful, its mass production at a commercial scale has not been attained due to technical constraints, and is still in progress (Støttrup, 2000; Hagiwara et al., 2001).

Positive nutritional effects of copepods on marine finfish have been reported such as increased growth and survival (Doi et al., 1997; Nanton and Castell, 1998; Støttrup et al., 1998; Copeman et al., 2002; Skalli and Robin, 2004), improved pigmentation (Bell

et al., 2003), retinal morphology (Shields et al., 1999), broodstock reproductive performance, and egg and larval quality (Mazorra et al., 2003). However, less emphasis has been given on the effects of copepods on the behavioural development of fish, particularly on their feeding and swimming behaviour (Hunt Von Herbing and Gallager , 2000). Behavioural observations are useful in understanding patterns of prey selection and have important implications on metabolic energy costs. Increased efficiency in foraging has been shown to increase net energy gain and consequently growth and survival (Dill, 1983; Wahl et al., 1995). In this study, three types of diets using *Acartia tsuensis* and *Artemia franciscana*, with different nutritional composition were used to determine their dietary effects on growth and behavioural development using mangrove killifish, *Rivulus marmoratus*, as a model animal.

R. marmoratus, recently recognized as a synonym of *Kryptolebias marmoratus* (Costa, 2004), has been used as an experimental animal because it is capable of self-fertilization (produce clones) and it is easy to culture (Kallman and Harrington, 1964; Harrington and Kallman, 1968; Koenig and Chasar, 1984). *R. marmoratus* will be highly useful in investigating the effects of different types of diets since individuals are homozygous clones, thus, any observed variations in traits or characters can be attributed to nutritional effects and not to individual variations. Also, the early life history of this species has been studied in detail (Grageda et al., 2004) but the nutritional requirements during the early stages of its development have never been investigated.

With the aim to compare the effects of feeding different types of live feeds on early growth and behavioural development in *R. marmoratus*, a 20-day feeding

97 experiment was conducted using three types of diets namely: *A. tsuensis* (D1),
98 unenriched (D2), and DHA-enriched (D3) first instar *A. franciscana* nauplii.
99
100 **2. Materials and methods**
101
102 *2.1 Culture and size measurement of live feeds*
103 *A. tsuensis* were collected from the Yukinoura River of Ooseto in Nagasaki,
104 Japan using a plankton net (45 μm mesh size). They were cultured in 5 l plastic
105 containers with 4 l of 17 g l^{-1} brackish water (prepared by mixing 2 l of distilled water
106 and 2 l of natural seawater, filtered in 47 mm glass microfibre filter) with mild aeration,
107 at a density of 2-10 ind. ml^{-1}. Copepods were fed daily with 1 x 10^5 cells ml^{-1} of
108 *Chaetoceros* sp and 2 x 10^5 cells ml^{-1} of *Isochrysis* sp. The amount of feed left was
109 checked daily and the amount of food fed was adjusted accordingly. Culture water was
110 totally replaced every 2-3 days.
111 About 1 g of *A. franciscana* cyst was incubated in 3 l white plastic container
112 with about 1.5 l of 17 g l^{-1} brackish water with strong aeration. After 1 day, newly
113 hatched nauplii were collected using a 100 μm sieve and distributed equally to two, 2 l
114 plastic containers (for D2 and D3) half-filled with 17 g l^{-1} artificial brackish water
115 (Marine Art Hi, Tomita Pharmaceutical Co. Ltd., Naruto, Japan), provided with strong
116 aeration and placed in a water bath maintained at 25 ± 1 °C. For D3, *A. franciscana*
117 were enriched (0.3 g l^{-1}, Aquaran plus, BASF, Japan) for 12-16 h prior to feeding.
118 *A. tsuensis* (n = 95) and *A. franciscana* (n = 30) were randomly sampled and
119 preserved in 5 % formaldehyde. Size (expressed in μm) was measured using a digital
120 microscope (VH 6300, Keyence Corp., Japan). Body sizes of nauplii, copepodites and

adult copepods were measured as body length and prosome length (from the anterior end of the prosome to the posterior lateral end of the prosome), respectively (Mauchline, 1998).

2.2 Dietary treatments

Three types of diets (D1: *A. tsuensis*, D2: unenriched first instar *A. franciscana* nauplii, and D3: DHA-enriched first instar *A. franciscana* nauplii) were used in the feeding experiment. Details of the nutritional composition of each of the diet, with emphasis on their highly unsaturated fatty acid (HUFA) components are shown in Table 1.

2.3 Experimental fish and general rearing conditions

The killifish (*Rivulus marmoratus*) used in this study were the PAN-RS strain derived from reared broodstock, which originated from Bocas del Toro, Panama and obtained from W.P. Davis (U.S. Environmental Protection Agency, Florida). This strain was collected in 1994 and has been reared in our laboratory for over 5 generations since 1998.

Ten fish for each dietary treatment were individually reared from hatching (day 0) in 1 l aquarium filled with 700 ml of 17 g l^{-1} artificial brackish water, with mild aeration and under natural photoperiod for 20 days. Aquaria were arranged randomly in 150 l water bath maintained at 25 ± 1 °C using a cooling thermo pump (CTP 201, Eyela, Japan). The daily feed for each tank was as follows: 100-1500 individuals of mixed stages (nauplius, copepodite, and adult) of *A. tsuensis* for D1, and 12 to 365 individuals of 2-day old *A. franciscana* nauplii for D2 and D3, depending on the age of fish. In

order to feed the fish to satiation and to minimize the remaining feed in each tank, the amount of feed remaining was counted daily for each aquarium and the amount of feed fed was adjusted accordingly. *Chaetoceros* sp and *Isochrysis* sp were added at a density of 1 x 10^5 cells ml^{-1} and 2 x 10^5 cells ml^{-1}, respectively to each aquarium every 2 days. The amount of microalgae left was checked and the amount fed was adjusted accordingly to maintain the same density. Culture water was not replaced throughout the experiment.

2.4 Growth and behavioural experiments

Growth measurements and behavioural observations were made on fish on days 1, 10, and 20. All fish were starved 24 h prior to observation. The observation container (7.5 cm x 10 cm) was placed in a water bath, which was maintained at 25 ± 1 °C. Fish were transferred to the observation container with a depth of 2 cm of 17 g l^{-1} artificial brackish water. Fish were acclimated for 10 min prior to observation. Behaviour was recorded 10 min before and 10 min after feeding from above using a video camera (TRV 50, Sony Corp., Japan). At each observation period, the same amount of feed was fed to all individuals of the same age. For D1, fish were fed 100-185 individuals of *A. tsuensis*, and for D2 and D3, 11-35 individuals of 2-day old *A. franciscana,* depending on the age of fish. After each observation, fish were anaesthetized with 100 mg l^{-1} of MS 222 (3-aminobenzoic acid ethyl ester, Sigma Chemical Co., St. Louis, MO) for a few seconds. Then, growth (standard length, SL) was measured to the nearest 0.01 mm using a digital microscope. Immediately after measurement, fish were allowed to recover in 1 l of aerated 17 g l^{-1} artificial brackish water for 10 min, before being returned to the rearing aquaria.

The behaviours observed were as follows: focus (fish turns and orients toward the prey), attack (movement of fish towards the prey prior to ingestion), ingest (fish eats the prey), and fail (fish is unable to ingest prey). These data were used to calculate the following indices: feeding success (number of prey ingested over the number of attacks) and ingestion rate (number of prey ingested min^{-1}). Satiation time (min), the time the fish fed until satiation, was also recorded. The total time the fish spent at rest and swimming, 10 min before and 10 min after feeding were also observed (Almazan-Rueda et al., 2004).

Condition factor (CF), which is based on the final weight and length of fish fed the different diets was calculated using the formula: [wet weight (g)/standard length (cm)] X 10^3.

2.5 Fatty acid analysis

Samples of *A. franciscana* (unenriched and enriched) and *A. tsuensis* were concentrated separately in a sieve (100 μm) and washed with distilled water. All fish fed the different live feeds were starved 24 h prior to sampling and were also washed with distilled water. All samples were weighed (mg), pooled for each treatment, immediately frozen and stored at –80 ˚C for fatty acid analysis. Samples were analyzed for fatty acid composition by a commercial laboratory (Chlorella Industry Co., Ltd, Fukuoka, Japan), and for total lipid content by the method of Folch et al. (1957).

2.6 Statistical analysis

Comparison of growth and behavioural parameters among fish fed the different diets at each age group was done using one-way ANOVA and further analyzed using Fisher's PLSD posthoc test. Analysis was done using a statistical software program

193 (StatView ver. 5, SAS Inst. Inc.). Size of live feeds was compared using Student's t-test
194 (Minitab, release 13.31, Minitab, Inc.). Final wet weight (mg) and CF of fish fed the
195 different diets were compared using one-way ANOVA and further analyzed using
196 Fisher's PLSD posthoc test.
197
198 **3. Results**
199 The size frequency of mixed stages of *A. tsuensis* used in this study is shown in
200 Fig. 1.The sizes (mean ± S.D.) of *A. tsuensis* (454 ± 228 μm) and *A. franciscana* (762 ±
201 59 μm) differed significantly (Student's t-test, $t = -11.93$, $P < 0.001$). Size ranges of *A.*
202 *tsuensis* and *A. franciscana* were 104-732 μm and 656-906 μm, respectively.
203 Early growth in D1-fed larvae was significantly higher than D2- and D3-fed
204 larvae on day 10 (Fisher's PLSD, $P < 0.05$). On day 20, D3-fed larvae had a
205 significantly higher growth than the D1- and D2-fed larvae (Fisher's PLSD, $P < 0.0001$;
206 Fig. 2). Similarly, final weight of D3-fed fish was significantly higher than fish fed D1
207 and D2 ($P < 0.01$). However, condition factor did not differ among fish fed the different
208 diets.
209 One-day old larvae had significantly higher feeding success with D1 than D3
210 (Fisher's PLSD, $P < 0.05$). Feeding success among larvae fed the different diets did not
211 differ on days 10 and 20 (Fig. 3). Both ingestion rate and satiation time did not differ
212 among larvae fed the different diets at all age groups.
213 Swimming activity before feeding was significantly higher in larvae fed D2 and
214 D3 compared with D1 on day 10 (Fisher's PLSD, $P < 0.05$). However, swimming
215 activity among larvae fed the different diets was of the same level on day 20 (Fig. 4a).

With food present, swimming activity did not differ among larvae fed the different diets at all age groups (Fig. 4b).

Eicosapentaenoic acid, EPA (mg 100 g wet wt^{-1}) levels in fish fed all dietary treatments increased from 0.2 - 3 fold compared to the diets. Despite the absence of DHA in the diets, an increase of 120.9 mg 100 g wet wt^{-1} was detected in fish. DHA (mg 100 g wet wt^{-1}) in D1- and D3-fed fish increased from 3 - 8 fold compared with the diet (Table 1 and 2).

4. Discussion

The present study was able to demonstrate the successful culture and use of copepods in a small-scale experiment to investigate their effects on early growth and behaviour of *R. marmoratus.* Most studies on the effect of copepods on marine finfish have reported improvement in larval growth and survival in yellowtail flounder, (Copeman et al., 2002) and red-spotted grouper (Doi et al., 1997), growth of European sea bass (Skalli and Robin, 2004), larval haddock and American plaice (Nanton and Castell, 1998), dorsal pigmentation of turbot and halibut (Bell et al., 2003), and broodstock reproductive performance and egg and larval quality of Atlantic halibut (Mazorra et al., 2003), which have been attributed to its nutritional effects. Copepods have been reported to contain higher amounts of highly unsaturated fatty acids (n-3 HUFA) content, particularly EPA (20:5n-3) and DHA (Watanabe et al., 1983; Evjemo et al., 2003; Hernandez Molejon and Alvarez-Lajonchere, 2003). The present study did not only confirm the positive effects of copepods on growth similar with previous reports, but also reports its effect on behavioural development in marine finfish using *R.*

239 *marmoratus*. It is also the first attempt to correlate growth with behavioural
240 observations.

241 Higher growth observed in *Acartia*-fed fish on day 10 may be due to lower
242 swimming activity of the larvae. Swimming activity is energy-costly especially for
243 larvae with poor energy-saving mechanisms (Kamler, 1992). This activity involves
244 consumption of high amounts of oxygen, ranging from 2 to 15 times above the resting
245 level in some species of fish larvae, such as brown trout, Pacific sardine, whitefish, and
246 in some cyprinids (Kamler, 1992).

247 Our results showed that the effects of the live feeds were mainly due to the type
248 and size of live feed species rather than their nutritional composition. Despite the
249 absence of DHA in the feed, DHA was detected in *R. marmoratus* indicating that they
250 are capable of synthesizing DHA. Thus, higher growth in *Acartia*-fed fish on day 10
251 may be related to the suitable size (composed mainly of 55 % of 500-600 µm
252 copepodites and adults and 34 % of 100-200 µm nauplii) of *A. tsuensis* rather than their
253 DHA content. On the other hand, the positive nutritional effect of *Acartia* containing
254 high amounts of DHA on larval growth still remains a possibility. Lower growth
255 observed on day 20 may be due to the shift in food size preference of the fish. Also, it
256 may be possible that EPA exerted a positive effect on the growth of fish fed DHA-
257 enriched *Artemia.*

258 The decrease in growth from day 10 to 20 in *Acartia*-fed fish and conversely, the
259 increase in growth in *Artemia*-fed fish may be attributed to the shift in food size
260 preference. The shift in size preference can be related to morphological, physiological,
261 and behavioural changes occurring at these phases, which were previously described
262 (Grageda et al., 2004; unpublished observations). Based on the size, larvae fed *Acartia*

on day 10 can be classified under the shift to exogenous feeding phase while the fish fed enriched *Artemia* on day 20, under the juvenile phase. During the shift to exogenous feeding, higher growth was observed in larvae feeding on smaller-sized prey (*A. tsuensis*; 104-732 μm), however, as it approached the juvenile phase, higher growth was observed in fish feeding on larger-sized prey (*A. franciscana*; 656-906 μm). During the shift to exogenous feeding, *R. marmoratus* have been reported to possess complete fin-ray counts in the majority of the fins, and to undergo increased ossification in the skull, vertebrae and fin rays (Grageda et al., 2004), which coincided with increased swimming activity similar with observations in sea breams (Faustino and Power, 2004) . These features may have contributed to increased efficiency in catching *A. tsuensis*, which has been reported to swim in an irregular and zigzag motion (Shuvayev, 1978 as cited in Govoni et al., 1986). However, digestive enzyme activities in the digestive tract (such as esterase and alkaline phosphatase) at this phase are still low (Kolkovski, 2001), indicating that the larva has limited absorptive capacity, thus, it prefers smaller-sized and easily digestible prey. As the fish approached the juvenile phase, it shifted to a prey with a more regular swimming movement, complementing with the unchanged swimming activity of the fish at this phase. Since a positive effect in growth was observed among fish feeding on *A. franciscana*, larger-sized prey may be preferred at the juvenile phase. This indicates that the fish is physiologically capable of digesting and absorbing larger prey, as evidenced by efficient transformation of food to somatic growth. This could be attributed to increased digestive and absorptive efficiency, as evidenced by a significant increase in digestive enzyme activities such as alkaline phosphatase and esterase, and increased mucosal folds and goblet cells in the digestive tract at the juvenile phase, as observed in developing seabream larvae (Moyano et al.,

1996). Also, zymogen granules, known precursors of proteolytic enzymes (Gisbert et al., 2004), are distinctly visible at this phase (unpublished observations), indicating active pancreatic secretions. Positive correlation between gape size and body size of fish and the size of prey has been reported (De Vries, 1998). In *R. marmoratus*, gape size increases with age, however, gape size relative to standard length decreases with age at the early stage of development (Grageda et al., 2004). A similar observation has been reported in both field-caught and laboratory-reared red drum larvae and juveniles, showing that the size of prey consumed was not constrained by gape size (Krebs and Turingan, 2003). This suggests that other prey-capture mechanisms such as the development of feeding apparatus (such as hyoid apparatus) may influence a shift in prey size preference (Krebs and Turingan, 2003). Moreover, this observed increase in consumption of larger prey with growth is consistent with previous findings on greenback flounder, long-snouted flounder and red drum (Jenkins, 1987; Krebs and Turingan, 2003). Apart from prey size, characteristics of prey have also been identified as an important factor in prey selectivity (Checkely, 1982). Other factors affecting prey selection have been identified related to the characteristics of the larvae. Meng and Orsi (1991) demonstrated that learning and swimming behaviour of striped bass larvae and their interaction with their prey strongly affects prey selectivity. Moreover, the importance of learning behaviour and innate preference by the percid and flounder larvae has been suggested (Jenkins, 1987; Wahl et al., 1995). Similarly, a positive preference for familiar prey has been observed in greenback flounder larvae (Cox and Pankhurst, 2000).

The importance of copepods in the early larval nutrition of *R. marmoratus* was also demonstrated in this study. This species performed better when fed with *A. tsuensis*

during the early larval stage as evidenced by higher feeding success compared with *A. franciscana* on day 1, and a positive effect on larval growth on day 10. Higher density of *A. tsuensis* compared with *A. franciscana*, may have contributed to this effect, however, these densities had to be maintained to be able to feed the fish to satiation and to reduce the remaining feed in each tank. Higher feeding preference for *Acartia* may be indicative of the innate preference of the larvae for copepod prey as previously suggested by Jenkins (1987). Also, our study confirmed previous observations regarding food preference of *R. marmoratus*, revealing the presence of an unidentified harpacticoid copepod based on indirect observation through gut analysis of specimens collected from the field (Taylor, 1992). Copepods are commonly present in mangrove estuaries, thus, calanoids, another order of copepods to which *Acartia* belongs, may play an important role in early larval feeding of *R. marmoratus*. Previous studies on food habits of this species have reported gastropods, insects, amphipods, isopods, crustacean parts, fragments of annelid worms, and fish scales as their food (Harrington and Rivas, 1958; Huehner et al., 1985; Taylor 1992). It is possible that calanoids would constitute a significant part of their diet during the larval stage in their natural habitat, although this needs further confirmation in the field. *A. tsuensis* belongs to the family Acartiidae, a group composed of species found in estuarine and neritic environments throughout the world (Mauchline, 1998).

Acknowledgements

The first author is grateful to The Ministry of Education, Culture, Sports, Science and Technology (Monbukagakusho) for the scholarship awarded to her. This study was financially supported by the Japanese Grants-in-Aid for Promotion of

335 Scientific Research (13760145 and 15780135) to the third author and Nagasaki
336 Prefecture Collaboration of Regional Entities for Advancement of Technological
337 Excellence, JST to the second, third, and fourth authors.

338

339 **References**

340 Abu-Rezq, T.S., Yule, A.B., Teng, S.K., 1997. Ingestion, fecundity, growth rates and
341 culture of the harpacticoid copepod, *Tisbe furcata*, in the laboratory.
342 Hydrobiologia 347, 109-118.
343 Almazan-Rueda, P., Schrama, J.W., Verreth, J., 2004. Behavioural responses under
344 different feeding methods and light regimes of the African catfish (*Clarias*
345 *gariepinus*) juveniles. Aquaculture 231, 347-359.
346 Barthel, K.G., 1983. Food uptake and growth efficiency of *Eurytemora affinis*
347 (Copepoda: Calanoida). Mar. Biol. 74, 269-274.
348 Bell, J.G., McEvoy, L.A., Estevez, A., Shields, R.J., Sargent, J.R., 2003. Optimising
349 lipid nutrition in first-feeding flatfish larvae. Aquaculture 227, 211-220.
350 Berggreen, U., Hansen, B., Kiørboe, T., 1988. Food size spectra, ingestion, and growth
351 in *Acartia tonsa* during development: implications for determination of copepod
352 production. Mar. Biol. 99, 341-352.
353 Checkley, D.M. Jr., 1982. Selective feeding by Atlantic herring *(Clupea harengus*)
354 larvae on zooplankton in natural assemblages. Mar. Ecol. Prog. Ser. 9, 245-253.
355 Copeman, L.A., Parrish, C.C., Brown, J.A., Harel, M., 2002. Effects of
356 docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth,
357 survival, lipid composition and pigmentation of yellowtail flounder (*Limanda*
358 *ferruginea*): a live food enrichment experiment. Aquaculture 210, 285-304.

359 Costa, W.J.E.M., 2004. A new killifish genus and species from the coastal plains of
360 north-eastern Brazil (Teleostei: Cyprinodontiformes: Rivulidae). Zootaxa 642, 1-10.
361 Cox, E.S., Pankhurst, P.M., 2000. Feeding behaviour of greenback flounder larvae,
362 *Rhombosolea tapirina* (Gunther) with differing exposure histories to live prey.
363 Aquaculture 183, 285-297.
364 Davis, C.S., 1993. Laboratory rearing of marine calanoid copepods. J. Exp. Mar. Biol.
365 Ecol. 71, 119-133.
366 De Vries, D.R., Bremigan, M.T., Stein, R.A., 1998. Prey selection by larval fishes as
367 influenced by available zooplankton and gape limitation. Trans. Amer. Fish. Soc.
368 127, 1040-1050.
369 Dill, L.M., 1983. Adaptive flexibility in the foraging behaviour of fishes. Can. J. Fish.
370 Aquat. Sci. 40, 398-408.
371 Doi, M., Toledo, J.D., Golez, S., de los Santos, M., Ohno, A., 1997. Preliminary
372 investigation of feeding performance of larvae of early red-spotted grouper,
373 *Epinephelus coioides*, reared with mixed zooplankton. Live Food in Aquaculture.
374 In: Hagiwara, A., Snell, T.W., Lubzens, E., Tamaru, C.S. (Eds) Proceedings of
375 the Live Food and Marine Larviculture Symposium held in Nagasaki, Japan,
376 September 1-4, 1996. Hydrobiologia 358, 259-263.
377 Evjemo, J.O., Reitan, K.I., Olsen, Y., 2003. Copepods as live food organisms in the
378 larval rearing of halibut larvae (*Hipoglossus hipoglossus* L.) with special
379 emphasis on the nutritional value. Aquaculture 227, 191-210.
380 Faustino, M, Power D.M., 1998. Development of osteological structures in the sea
381 bream: vertebral column and caudal fin complex J. Fish Biol. 52, 11–22.

382 Folch, J., Lees, M., Stanley, H.S., 1957. A simple method for the isolation and
383 purification of total lipids from animal tissue. J. Biol. Chem. 226, 497-506.
384 Gisbert, E., Piedrahita, R., Conklin, D., 2004. Ontogenetic development of the digestive
385 system in California halibut (*Paralichthys californicus*) with notes on feeding
386 practices. Aquaculture 232, 455-470.
387 Govoni, J., Ortner, P., Al-Yamani, F., Hill, L., 1986. Selective feeding of spot,
388 *Leiostomus xanthurus*, and Atlantic croaker, *Micropogonias undulatus*, larvae in
389 the Northern Gulf of Mexico. Mar. Ecol. Prog. Ser. 28, 175-183.
390 Grageda, M.V.C., Sakakura, Y., Hagiwara, A., 2004. Early development of the self-
391 fertilizing mangrove killifish, *Rivulus marmoratus*, reared in the laboratory.
392 Ichthyol. Res. 51, 309-315.
393 Hagiwara, A., Gallardo, W.G., Assavaaree, M., Kotani, T., de Araujo, A.B., 2001. Live
394 food production in Japan: recent progress and future aspects. Aquaculture 200,
395 111-127.
396 Harrington, R.W. Jr., Kallman, K., 1968. The homozygosity of clones of the self-
397 fertilizing hermaphroditic fish *Rivulus marmoratus* Poey (Cyprinodontidae,
398 Atheriniformes). Am. Nat. 102, 337-343.
399 Harrington, R.W. Jr., Rivas, L.R., 1958. The discovery in Florida of the cyprinodontid
400 fish *Rivulus marmoratus*: with a redescription and ecological notes. Copeia 1958,
401 125-130.
402 Hernandez Molejon, O.G., Alvarez-Lajonchere, L., 2003. Culture experiments with
403 *Oithona oculata* Farran, 1913 (Copepoda: Cyclopoida), and its advantages as
404 food for marine fish larvae. Aquaculture 219, 471-483.

405 Huehner, M.K., Schramm, M.E., Hens, M.D., 1985. Notes on the behaviour and
406 ecology of the killifish *Rivulus marmoratus* Poey 1880 (Cyprinodontidae). Fla. Sci.
407 48, 1-6.
408 Hunt von Herbing, I., Gallager, S. M., 2000. Foraging behavior in early Atlantic cod
409 larvae (*Gadus morhua*) feeding on a protozoan (*Balanion* sp.) and a copepod
410 nauplius (*Pseudodiaptomus* sp.) Mar. Biol. 136, 591-602.
411 Jenkins, G.P., 1987. Comparative diets, prey selection, and predatory impact of co-
412 occurring larvae of two flounder species. J. Exp. Mar. Biol. Ecol. 110, 147-170.
413 Kallman, K., Harrington, R.W. Jr., 1964. Evidence for the existence of homozygous
414 clones in the self-fertilizing hermaphroditic teleost *Rivulus marmoratus* (Poey). Biol.
415 Bull. 26, 101-114.
416 Kamler, E., 1992. Early Life History of Fish: An Energetic Approach. Chapman and
417 Hall, London. 288 pp.
418 Koenig, C., Chasar, M., 1984. Usefulness of the hermaphroditic marine fish, *Rivulus*
419 *marmoratus*, in carcinogenicity testing. Natl. Cancer Inst. Monogr. 65, 15-33.
420 Kolkovski, S., 2001. Digestive enzymes in fish larvae and juveniles—implications and
421 applications to formulated diets. Aquaculture 200, 181-201.
422 Kraul, S., Ako, H., Nelson, A., Brittain, K., Ogasawara, A., 1992. Evaluation of live
423 feeds for larval and postlarval mahimahi, *Coryphaena hippurus*. J. World Aquacult.
424 Soc. 23, 299-306.
425 Krebs, J.M., Turingan, R.J., 2003. Intraspecific variation in gape-prey size relationships
426 and feeding success during early ontogeny in red drum, *Sciaenops ocellatus*.
427 Environ. Biol. Fishes 66, 75-84.

428 Mauchline, J., 1998. The Biology of Calanoid Copepods. Academic Press, San Diego.
429 710 pp.
430 Mazorra, C., Bruce, M., Bell, J.G., Davie, A., Alorend, E., Jordan, N., Rees, J.,
431 Papanikos, N., Porter, M., Bromage, N., 2003. Dietary lipid enhancement of
432 broodstock reproductive performance and egg larval quality in Atlantic halibut
433 (*Hipoglossus hipoglossus*). Aquaculture 227, 21-33.
434 Meng, L., Orsi, J.J., 1991. Selective predation by larval striped bass on native and
435 introduced copepods. Trans. Am. Fish. Soc. 120, 187-192.
436 Moyano, F.J., Diaz, M., Alarcon, F.J., Sarasquete, M.C., 1996. Characterization of
437 digestive enzyme activity during larval development of gilthead seabream
438 (*Sparus aurata*) Fish Physiol. Biochem. 15, 121-130.
439
440 Nanton, D.A., Castell, J.D., 1998. Preliminary trials using a harpacticoid copepod, *Tisbe*
441 sp., as a diet for larval haddock and American plaice. Bull. Aquacul. Assoc. Canada.
442 4, 38-40.
443 Ohno, A., Okamura, Y., 1988. Propagation of the calanoid copepod, *Acartia tsuensis*, in
444 outdoor tanks. Aquaculture 70, 39-51.
445 Shields, R.J., Bell, J.G., Luizi, F.S., Gara, B., Bromage, N.R., Sargent, J.R., 1999.
446 Natural copepods are superior to enriched *Artemia* nauplii as feed for larvae
447 (*Hipoglossus hipoglossus*) in terms of survival, pigmentation, retinal
448 morphology: relation to dietary essential fatty acids. J. Nutr. 129, 1186-1194.
449 Skalli, A., Robin, J.H., 2004. Requirement of n-3 long chain polyunsaturated fatty acids
450 for European sea bass (*Dicentrarchus labrax*) juveniles: growth and fatty acid
451 composition. Aquaculture 240, 399-415.

452 Støttrup, J.G., 2000. The elusive copepods: their production and suitability in marine
453 aquaculture. Aquacult. Res. 31, 703-711.
454 Støttrup, J.G., McEvoy, L.A., 2003. Live Feeds in Marine Aquaculture. Blackwell
455 Science Ltd., Oxford 318 pp.
456 Støttrup, J.G., Shields, R., Gillespie, M., Gara, M.B., Sargent, J.R., Bell, J.G.,
457 Henderson, R.J., Tocher, D.R., Sutherland, R., Naess, T., Mangor Jensen, A.,
458 Naas, K., Van der Meeren, T., Harboe, T., Sanchez, F.J., Sorgeloos, P., Dhert, P.,
459 Fitzgerald, R., 1998. The production and use of copepods in larval rearing of
460 halibut, turbot, and cod. Bull. Aquacult. Assoc. Canada 4, 41-45.
461 Taylor, D.S., 1992. Diet of the killifish *Rivulus marmoratus* collected from land crab
462 burrows, with further ecological notes. Environ. Biol. Fishes 33, 389-393.
463 Wahl, D.H., Einfalt, L.M., Hooe, M.L., 1995. Effect of experience with piscivory on
464 foraging behaviour and growth in walleyes. Trans. Am. Fish. Soc. 124, 756-763.
465 Watanabe, T., Kitajima, C., Fujita, S., 1983. Nutritional values of live organisms used in
466 Japan for mass propagation of fish: a review. Aquaculture 34, 115-143.
467
468
469
470
471
472
473
474
475

476 Table 1. Highly unsaturated fatty acid (HUFA) composition (mg 100g wet wt^{-1}) of
477 different diets (D1: *Acartia tsuensis*; D2: unenriched *Artemia franciscana* and D3:
478 DHA- enriched *A. franciscana*).
479

HUFA	D1	D2	D3
Eicosapentaenoic acid	14.2 (4.7)	17.3 (2.1)	71.4 (5.3)
Docosahexaenoic acid	26.1 (8.6)	0 (0)	57.9 (4.3)
Arachidonic acid	1.5 (0.5)	5.8 (0.7)	14.8 (1.1)
DHA/EPA	1.8	-	0.8
Σ n-3 HUFA	41.8 (13.8)	23.1 (2.8)	144.1(10.7)

480
481 Number in parenthesis indicates % of total fatty acids.
482
483
484
485
486
487
488
489

Table 2. Highly unsaturated fatty acid (HUFA) composition (mg 100g wet wt^{-1}) of mangrove killifish fed different diets (D1: *Acartia tsuensis*; D2: unenriched *Artemia franciscana* and D3: DHA- enriched *A. franciscana*) at 22 days after hatching.

HUFA	D1	D2	D3
Eicosapentaenoic acid	20.4 (1.7)	69.5 (2.3)	87.7 (2.1)
Docosahexaenoic acid	233.2 (19.4)	120.9 (4.0)	213.0 (5.1)
Arachidonic acid	19.2 (1.6)	33.2 (1.1)	66.8 (1.6)
DHA/EPA	11.4	1.7	2.4
Σ n-3 HUFA	272.8 (22.7)	223.6 (7.4)	367.5 (8.8)

Number in parenthesis indicates % of total fatty acids.

505 Figure captions

506

507 Fig. 1. Size frequency (%) of mixed stages (nauplius, copepodite, and adult) of *Acartia*
508 *tsuensis* used in the feeding experiment.

509

510 Fig. 2. Growth expressed as standard length (mean mm ± S.D.) of mangrove killifish
511 fed different diets (D1: *Acartia tsuensis*, triangle with short broken lines; D2:
512 unenriched first instar *Artemia franciscana* nauplii, circle with solid line; D3: DHA-
513 enriched first instar *A. franciscana* nauplii, square with long broken lines) for 20 days.
514 Different letters indicate significant difference among fish fed different diets at each age
515 group (Fisher's PLSD, $\underline{P} < 0.05$, a > b).

516

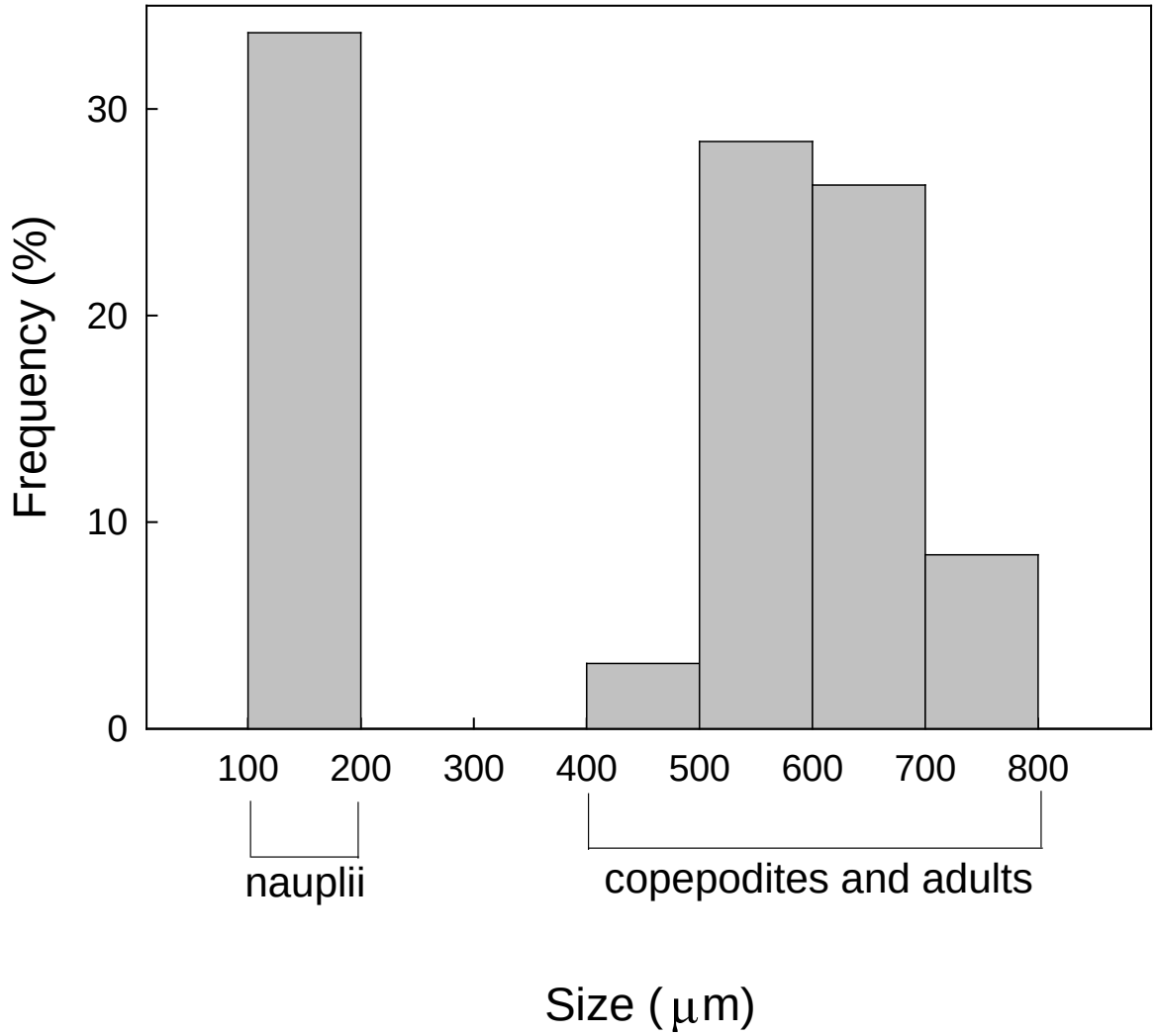
517 Fig. 3. Feeding success (mean % ± S.D.) of mangrove killifish fed different diets (D1:
518 *Acartia tsuensis*, solid bars; D2: unenriched first instar *Artemia franciscana* nauplii,
519 open bars; D3: DHA-enriched first instar *A. franciscana* nauplii, bars with diagonal
520 lines) for 20 days. Different letters indicate significant difference among fish fed
521 different diets at each age group (Fisher's PLSD, $\underline{P} < 0.05$, a > b).

522

523 Fig. 4. Swimming activity (mean % ± S.D.) of mangrove killifish fed different diets
524 (D1: *Acartia tsuensis*, solid bars; D2: unenriched first instar *Artemia franciscana* nauplii,
525 open bars; D3: DHA-enriched first instar *A. franciscana* nauplii, bars with diagonal
526 lines) for 20 days at (a) 10 min before and (b) 10 min after feeding. Different letters
527 indicate significant difference among fish fed different diets at each age group (Fisher's
528 PLSD, $\underline{P} < 0.05$, a > b).

529

530 Fig. 1.



531

532

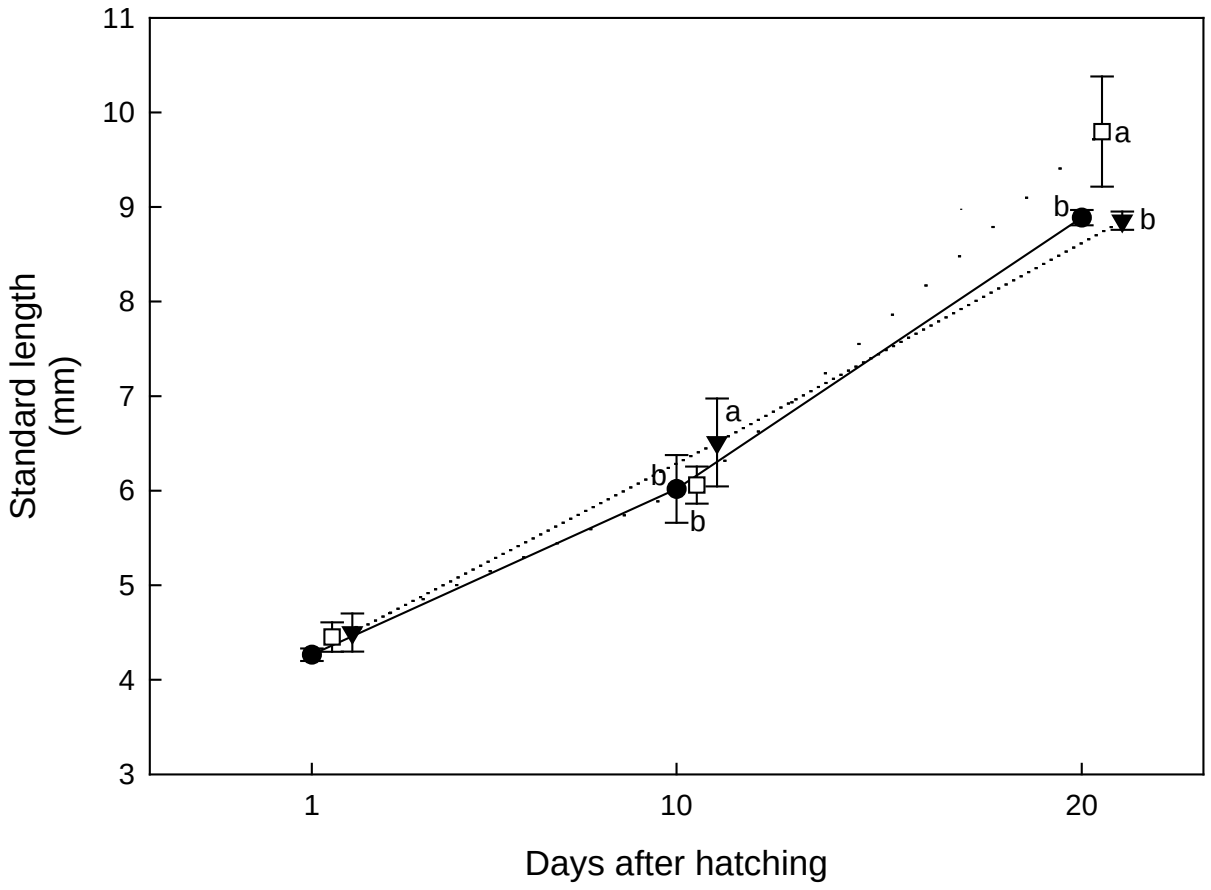
533

534

535

536

Fig. 2.


11
10
9
8
7
6
5
4
3
Standard length (mm)
1
10
20
Days after hatching
a
b
b
a
b
b

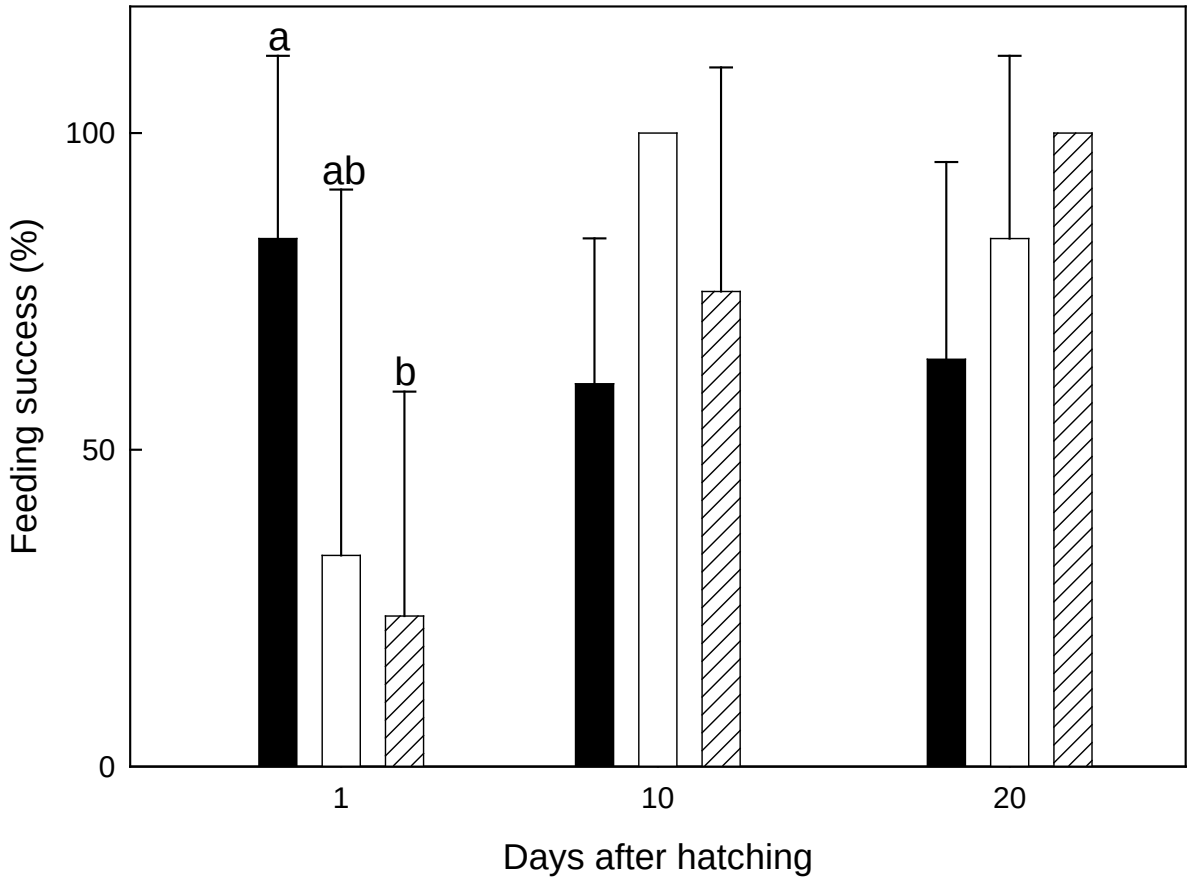
548

549

550

551

552 Fig. 3.



553

554

555

556

557

558

559 Fig. 4.