Fisheries Science (2024) 90:281–294
https://doi.org/10.1007/s12562-024-01751-8

ORIGINAL ARTICLE

Aquaculture

Euryhaline copepod *Pseudodiaptomus inopinus* changed the prey preference of red sea bream *Pagrus major* larvae

Taku Sasaki[1] · Hideaki Matsui[2] · Yusuke Kuwabara[1] · Saichiro Yokoyama[1] · Manabu Ishikawa[1] · Tomonari Kotani[1,3]

Received: 23 July 2023 / Accepted: 14 December 2023 / Published online: 22 January 2024

Abstract

We evaluated the potential of the euryhaline copepod *Pseudodiaptomus inopinus* as a prey to enhance the feeding activity of red sea bream *Pagrus major* larvae. *Pseudodiaptomus inopinus* was used to evaluate free amino acid composition and dietary effects on the rearing performance of fish larvae, in comparison to rotifers (control prey). Among free amino acids, alanine, arginine, and glycine were markedly higher in *P. inopinus* than in rotifers. Larvae were reared for 20 days post-hatching under three feeding treatments: rotifers (control), rotifers supplemented with copepods, and copepods only. Larvae fed copepods alone had a higher growth rate than those in the other treatments. However, the survival rates of these larvae were lower than those under control or copepod supplementation. While equivalent stocking densities of rotifers were employed in the rearing water for both larvae, whether supplemented with copepods or not, the supplemented treatment yielded diminished larval survival and failed to enhance growth rates. According to gut content analysis, larvae receiving supplemented treatment preferentially fed on copepods, and the number of ingested rotifers was reduced. As a result, total ingested mass was lower in the supplemented larvae. This study suggests that copepods containing free amino acid species negatively affect prey acceptance of fish larvae.

Keywords Feeding stimulant · Free amino acids · Gut contents · Larviculture · *Pseudodiaptomus inopinus*

✉ Tomonari Kotani
kotani@fish.kagoshima-u.ac.jp

Taku Sasaki
k4190281@kadai.jp

Hideaki Matsui
Matsui@fish-u.ac.jp

Yusuke Kuwabara
kuromu36@yahoo.co.jp

Saichiro Yokoyama
yokoyama@fish.kagoshima-u.ac.jp

Manabu Ishikawa
ishikawa@fish.kagoshima-u.ac.jp

[1] Faculty of Fisheries, Kagoshima University, Shimoarata 4-50-20, Kagoshima 890-0056, Japan

[2] Department of Applied Aquabiology, National Fisheries University, Nagata-Honmachi 2-7-1, Yamaguchi 759-6595, Japan

[3] The United Graduate School of Agricultural Sciences, Kagoshima University, Korimoto 1-21-24, Kagoshima 890-0065, Japan

Introduction

Copepods are a major live prey for marine fish larvae in natural waters, and are also considered an effective initial live prey for larviculture (Kuwahara and Suzuki 1983; Hagiwara et al. 2001). Copepods contain high levels of *n*-3 highly unsaturated fatty acids (*n*-3 HUFA) and taurine, which are essential for the survival and growth of fish larvae (Sargent et al. 1999; Bell et al. 2003; van der Meeren et al. 2008). Commonly used live prey, such as rotifers and *Artemia* nauplii, do not contain enough *n*-3 HUFA to meet the requirement for larvae (Watanabe et al. 1989).

Copepods have still not been implemented as a practical prey for marine finfish larvae, due to their low productivity, difficult maintenance, and high culture cost (Alajmi and Zeng 2015; Takayama et al. 2021). Densities of copepods belonging to the genus *Acartia* (Sarkisian et al. 2019; Takayama et al. 2021) and *Pseudodiaptomus* (Puello-Cruz et al. 2009) are reported to be ≤ 2 individuals/mL/day, which is very limited compared to the productivity of continuous cultures of rotifers (approximately 750 ind./mL/day; Kotani et al. 2009). However,

Øie et al. (2015) succeeded in enhancing the rearing performance of finfish larvae, even in short periods after hatching, by feeding cultured copepod nauplii to these larvae. Also, Barroso et al. (2013) reported that marine finfish larvae fed on a mixture of cultured marine copepods *Acartia tonsa* and rotifers demonstrated improved growth and survival rates compared to those that were on an exclusively rotifer-based diet. Toledo et al. (1999) similarly reported that co-feeding several marine copepod and rotifer species improved the growth and survival rates of orange-spotted grouper *Epinephelus coioides* larvae compared with those that were fed only rotifers. Accordingly, the insufficiency of copepod biomass can be mitigated by circumscribing the temporal extent of copepod feeding to a concise interval or by effecting co-feeding of copepods alongside rotifers.

Furthermore, copepods contain large amounts of free amino acids (FAAs) such as glycine, alanine, and arginine which function as a feeding stimulant for marine fish (Goh and Tamura 1980; Kolkovski et al. 1997; Helland et al. 2003; van der Meeren et al. 2008). Kolkovski et al. (1997) also reported that when artificial feed and *Artemia* were fed to fish larvae, FAAs released from the *Artemia* stimulated feeding of the larvae and increased intake of commercial feed. Therefore, the utilisation of copepods as an additive to routine diets for larval fish could promote active feeding and thus enhance rearing performance such as larval growth and survival rate.

We aimed to evaluate the significance of the euryhaline copepod *Pseudodiaptomus inopinus* as a dietary supplement to rotifers for rearing of marine finfish larvae in this study. *Pseudodiaptomus inopinus* is a common copepod species in East Asia and the most abundant copepod in euryhaline waters of the mainland in Japan (Sakaguchi and Ueda 2011). This species was the only copepod successfully cultured, and other species were difficult to obtain. It was difficult to artificially culture copepod, especially to produce it at a level where it could be fed in quantity, and there was no other option but to use this species. Here, we analysed the FAAs of rotifers and *P. inopinus*, a key stimulant for larval feeding. We selected red sea bream as an experimental fish species, since this fish is among the most widely cultivated species in Japan and other Asian countries (Nam et al. 2019; Bu et al. 2023). To evaluate the effect of copepod supplementation, the rearing performance of the larvae was compared with those fed rotifers only, and those fed copepods only.

Materials and methods

Ethical treatment of the animals

All animal studies were performed in accordance with the regulations of the Animal Experimental Committee, Kagoshima University.

Culture of microalgae

Three microalgae, *Tisochrysis lutea*, *Pavlova lutheri*, and *Phaeodactylum tricornutum*, were cultured for the copepods. The seawater used for culturing each microalgae was collected from Kagoshima Bay (15 m from the shore at a depth of 5 m) and filtered using a sand filter in a closed filtration system. The seawater was filtered through 100 µm, 25 µm, and 10 µm mesh cartridge filters (Micro-Cilia; 250-EX-100, 250-EX25, 250-EX10, Roki Techno Co., Ltd., Fukuoka, Japan) and glass fibre filter paper (GF/C, GE Healthcare, CT, USA). The seawater was sterilised with ultraviolet irradiance (UVF-1000, Iwaki Co., Ltd., Tokyo, Japan). The culture medium used was diluted at 17 practical salinity units (psu) and enriched with nutrients (KW21, Daiichi Seimo Co., Ltd., Kumamoto, Japan), at water temperature of 20 °C and photoperiod of 24L/0D with gentle aeration at 0.5 L/min. Each culture was maintained in a 3-L glass triangular flask or 5-L polycarbonate bottle. Fresh medium for each species was prepared weekly, and the culture was started by inoculating cells.

Culture of copepods

Pseudodiaptomus inopinus was collected using a plankton net (mesh 63 µm) from the Yakugachi River near the confluence of the Sumiyou River (28°25′12.029″ N, 129°24′39.426″ E) and was incubated in the laboratory at the Faculty of Fisheries, Kagoshima University (Kagoshima, Japan). Seawater for the copepod culture medium was collected from Kagoshima Bay (Kagoshima, Japan) and sterilised using ultraviolet irradiance (UVF-1000, Iwaki Co., Ltd., Tokyo, Japan). It was then filtered through a graded series of 100, 25, and 10 µm mesh cartridges (Micro-Cilia; 250-EX-100, 250-EX25, 250-EX10, Roki Techno Co., Ltd., Fukuoka, Japan) and diluted with tap water that had been aerated for at least 1 day to adjust the salinity to approximately 17 psu. Copepods were incubated in a 5-L polypropylene beaker or 50-L and 200-L polycarbonate tanks filled with 4 L, 40 L, and 100 L of diluted seawater, respectively. Water temperature was adjusted to 20 °C. Aeration rate was increased with up-scaled volume of the copepod culture at 1 mL/min, 3 mL/min, and 6 mL/min for 4 L, 40 L, and 100 L, respectively. Three species of microalgae were supplied to copepods as a mixture every 2 days at a concentration of 4×10^5 cells/mL of *T. lutea*, 2×10^5 cells/mL of *P. lutheri*, and 4×10^5 cells/mL of *P. tricornutum*. The culture medium was changed three times a week by harvesting copepods in a 63 µm mesh plankton net (NYTAL; 25XX, Tanaka Sanjiro Co., Ltd., Fukuoka, Japan) and then transferring them to a fresh medium.

Culture of rotifer and hatching *Artemia*

Euryhaline rotifer *Brachionus plicatilis* (Obama strain, L-type) was used as a live prey for red sea bream and was cultured in 200-L polycarbonate incubation tanks using the continuous culture method described by Kotani et al. (2009). The cultivation conditions were maintained at salinity of 20 psu and water temperature of 25 °C, and the detritus was removed using a hanging filter (Vilene Mat, Tanaka Sanjiro Co., Ltd., Fukuoka, Japan) put in the culture tank, and the filter was changed daily. *Chlorella vulgaris* (Super Fresh Chlorella V-12, Chlorella Industry Co., Ltd., Tokyo, Japan) was continuously supplied to the culture tank at a rate of 40 mL/min, 15×10^9 cells/mL, using an electromagnetic metering pump (EHN-B16VC1R, Iwaki Co., Ltd., Tokyo, Japan). A seawater-based medium filtered through 100 µm cartridge filters and diluted to 20 psu was supplied simultaneously. For nutritional enrichment of rotifers, *n*-3 highly unsaturated fatty acid (*n*-3 HUFA)-enriched *C. vulgaris* (Super Fresh Chlorella V-12) was fed at 46,000 cells/ind. and was harvested after 24 h. Taurine, an essential nutrient for red sea bream larvae (Chen et al. 2004), was also enriched in rotifers using a commercial taurine enrichment product (Aquaplus ET, Marubeni Nisshin Feed Co., Ltd., Tokyo, Japan) following the manufacturer's instructions (1.2 g/L per day).

Artemia cysts (Great Salt Lake Artemia, Ogden, UT, USA) were incubated in *Artemia* incubation tanks filled with seawater filtered through a 100 µm mesh cartridge filter, and the incubation temperature was set at 28 °C using a thermostat and ceramic heater. The eggs were exposed to 24 h electric light and aeration for their hatching. Hatched nauplii were enriched with *n*-3 HUFA for 12 h using salmon roe oil (MarineTech Co., Ltd., Aichi, Japan) before being fed to the larvae.

Larval rearing of red sea bream

Treatments

Three feeding treatments were prepared: rotifers (control), rotifers supplemented with copepods (supplement), and copepods alone (copepod). Fish larvae have a point of no return (PNR) at early periods after hatching, during which they are more likely to die if they do not successfully feed (Blaxter and Hempel 1963). For successful first feeding of larvae, in the present study, larvae at 2–3 days post-hatching (dph) were fed at specifically higher densities compared to those at later age. In the control treatment, larvae were fed on rotifers twice a day from the day of mouth opening (2–3 dph) at 10 ind./mL and 4–19 dph at 5 ind./mL. For later-developed larvae, rotifers were supplemented with a small quantity of *Artemia* nauplii (0.1 ind./mL) from 15 to 19 dph, as no supplementation often leads to unsuccessful larviculture (Furuita et al. 1996). In the supplement treatment (copepod supplementation with rotifers), copepods were additionally fed at 0.24 ind./mL of nauplii and 0.47 ind./mL of copepodites from 2 to 3 dph, and at 0.07 ind./mL of nauplii and 0.14 ind./mL of copepodites from 4 to 19 dph. Rotifers were fed the same amount in both control and supplementation treatment. In the copepod treatment, the amount of nauplii and copepodites for larvae at PNR periods (i.e. 2–3 dph) was approximately 3.2 ind./mL and 5.7 ind./mL, respectively. For later periods (4–19 dph), the nauplii and copepodites fed to larvae were reduced to 0.13 ind./mL and 0.22 ind./mL, respectively, to save on prey.

Rearing conditions

Naturally fertilised and spawned red sea bream eggs were obtained from Ogata Suisan, Inc. (Kumamoto, Japan). Eggs were transported to the Kinko Bay Onshore Laboratory, Faculty of Fisheries, Kagoshima University. Three 30-L black polyethylene circular tanks (Summit Tanks, Morymer Sum Plastics Co., Ltd., Osaka, Japan) were prepared for each treatment. Each tank was filled with seawater and maintained at 22.6 ± 1.5 °C, 32.0 ± 1.2 psu salinity, 7.9 ± 0.1 pH, and 7.1 ± 1.2 mg/L dissolved oxygen. The larvae were reared in a photoperiod of 12L:12D. Approximately 900 eggs were placed in each tank and incubated under aeration that was initially supplied at 15 mL /min, and this gradually increased with larval growth. The hatching rate of each treatment was $50.3 \pm 17.7\%$. High water quality of the culture medium was maintained by filtration through a 100 µm cartridge filter and sterilisation by ultraviolet irradiance (UVF-1000, Iwaki Co., Ltd., Tokyo, Japan). The rearing period was set to 20 days. During the rearing period, the fish were fed twice daily at 8:00 am and 4:00 pm. The water circulation rate for larval rearing was set at 3.5 mL/s. A 63 µm mesh was attached to the drainage pipe to prevent live prey from being released outside the tank. As a buffer for the larval rearing medium, up to 1 million cells/mL of *Nannochloropsis oculata* (K2 Frozen *Nannochloropsis*, Chlorella Industry Co., Ltd., Tokyo, Japan) was added twice a day at the same time as feeding live prey to each tank.

Evaluation of growth and survival rates of red sea bream larvae and observation of their gut contents

The larvae at 0, 2, 3 dph (the time of mouth opening), and at 5, 10, 15, and 20 dph were sampled 2 h after feeding and then put in a 1.5 mL tube on ice. After the larvae were euthanised using ice, they were fixed in 10% formalin. Ten fish were sampled from each tank on each dph. Samples were not collected from the supplement and copepod treatment tanks in which all larvae died during rearing. The larvae and micro-ruler (MR-2,

Kenis Co., Ltd., Tokyo, Japan) were photographed using a digital camera (D5200, Nikon Co., Ltd., Tokyo, Japan) attached to a stereomicroscope (SMZ800, Nikon Co., Ltd., Tokyo, Japan), and the standard lengths of the larvae were measured using image analysis software (ImageJ ver. 1.50, National Institutes of Health, MD, USA). The growth rate was evaluated from the regression coefficients of regression curves generated from the raw data of standard larval lengths. Survival rates were calculated based on Kotani et al. (2011). We determined survival rates corrected for errors resulting from individuals who died due to sampling through the larval rearing using the following equation:

$$N_t = e^{-mt}\left(N_0 - \sum N_{Sn} e^{mdn}\right)$$

where N_t indicates the population size at t-dph, m indicates the mortality coefficient, T indicates the total rearing period, dn indicates the rearing period until the nth sampling event, and N_{Sn} indicates the sampled size in the nth sampling event. The survival rate was determined by solving the equation and calculating m using previously known values other than m.

To observe the feeding selectivity of larval fish in supplement treatment, the larvae of 5, 10, and 15 dph from each treatment were dissected using a needle, and the contents of the digestive tract were observed under a stereomicroscope. The number of copepods and rotifers in the gut contents was counted under a microscope. To evaluate whether the feeding of copepods by the larvae stimulates feeding of rotifers as well, the number of rotifers in the gut contents in the supplement was compared with that in the control. The dry mass of the gut contents of the larvae was measured as described by Matsui et al. (2021). The median dry weight of rotifers, copepod nauplii, copepodites, male adults, and female adults was 0.11 µg/ind., 0.18 µg/ind., 1.15 µg/ind., 3.52 µg/ind., and 6.24 µg/ind., respectively. To analyse the feeding selectivity of the larvae by 5, 10, and 15 dph, Ivlev's selectivity index was calculated (Ivlev 1961). To evaluate which prey species larvae preferentially fed on, the selectivity index E was calculated using the following equation:

$$E = \left(\mathrm{r}_i - \mathrm{p}_i\right) / \left(\mathrm{r}_i + \mathrm{p}_i\right)$$

where r_i and p_i are the population percentages of prey species in the stomach and rearing water, respectively. The selectivity ranged from −1 to 1, and a value between −1 and 0 suggested avoidance, and 0 to 1 suggested preference for live prey.

Free amino acids analysis for larval prey

Free amino acids (FAAs) in prey were determined by high-performance liquid chromatography (HPLC; CMB20A, LC20AB, RF20A, CTO20A, SIL20A, LC10AD, DGU-20A3, Shimadzu Co., Ltd., Kyoto, Japan) according to the method described by Teshima et al. (1986). Quantitative analysis was performed using the LC workstation software (LC-solution, Shimadzu Co., Ltd., Kyoto, Japan). The biomass of the rotifers, *Artemia*, or cultured copepods was harvested using a 63 µm plankton net, washed with distilled water, and preserved at −80 °C until measurement. After freeze-drying, a portion of the dry mass (approximately 0.1 g) was distributed in a test tube. A solution of norleucine (0.06 mg/100 µL) was used as the internal standard. Subsequently, each sample was mixed with 100 µL of norleucine solution, 4 mL of 8% trichloroacetic acid solution, and 900 µL of distilled water, followed by homogenisation (2 min) on ice, and then centrifugation (4 °C, 815×g, 15 min). A diethyl ether was mixed in each sample solution, and the organic layer containing trichloroacetic acid was discarded. The process of trichloroacetic acid removal was repeated 10 times. The pH of each sample solution was adjusted to 2.20 ± 0.05 by adding a small amount of 30% perchloric acid and 4 N sodium hydroxide. The solutions were filtered through a 0.45 µm membrane filter (Minisart® NML syringe filter, Sartorius, Göttingen, Germany) using a syringe, and the filtered solutions were analysed by HPLC. To evaluate the difference in FAAs between copepod sources, wild copepods harvested in China (Chinese frozen copepods, I.S.C Co., Ltd., Fukuoka, Japan) and those in the Arctic (Deep-frozen CYCLOP-EEZE®, Argent Chemicals Laboratories Inc., WA, USA) were also analysed with the same methodology described above.

Statistical processing

Statistical processing was performed using R version 4.3.1 (https://www.r-project.org/). The growth rate of the larvae was analysed using a two-way analysis of variance (ANOVA) based on the standard lengths of all larvae, and the slopes of the growth curves were compared. The significance of the regression of the growth curves was confirmed by regression analysis. The standard length of larvae at 20 dph was compared using the Kruskal–Wallis test, followed by Dunn's test. Survival rates were compared using the chi-squared test after a Bonferroni correction. The Wilcoxon rank-sum test was applied to compare the number of gut contents of red sea bream larvae every 5 days. For Ivlev's selectivity index, the Wilcoxon rank-sum test was applied to compare the indices between rotifers and copepods. Comparisons of the amount of amino acids were performed using one-way ANOVA, followed by Tukey's honestly significant difference (HSD) test. Significant differences were considered at $p < 0.05$.

Results

Rearing performance of red sea bream larvae

The regression exponential curve was generated from the relationship between each dph and the standard length of each sampled larvae (Fig. 1, regression analysis, $p < 0.05$). The growth rate of the copepod-fed larvae was significantly higher than that of the control fed on rotifers and supplement-fed larvae (two-way ANOVA; control-supplement: $df = 5$, $F = 1.664$, $p > 0.05$; control-copepod: $df = 5$, $F = 50.04$, $p < 0.05$; supplement-copepod: $df = 5$, $F = 36.81$, $p < 0.05$). However, there was no significant difference in larval growth between the control and the supplement. According to histograms of larval standard lengths at 20 dph (Fig. 2), those in the control and supplement treatments ranged approximately 6–8 mm, while larval standard lengths in the copepod treatment (approximately 8–10 mm) were significantly higher than that of the control and supplement (Kruskal–Wallis test, $H = 26.69$, $df = 2$, $p < 0.05$; Dunn's test, $p < 0.05$). However, the larval survival rates in the copepod treatment were lower than that in the other treatments (Fig. 3; treatment of copepods: $2.4 \pm 2.1\%$, supplement: $8.2 \pm 14.2\%$, control: $38.9 \pm 20.6\%$; chi-squared test, $p < 0.017$).

Gut contents of larvae in supplement and control treatments

The gut contents of the larvae in the treatment of supplement were examined (Figs. 4 and 5) to evaluate why their larvae showed low survival in spite of rotifers given at the same ration as control. In 5-dph larvae under the treatment of supplement, rotifers and copepods were ingested in similar numbers (Wilcoxon rank-sum test, $p > 0.05$) (Fig. 4a). The number of ingested rotifers or copepods was consistent for larvae at 10 or 15 dph (Wilcoxon rank-sum test, $p > 0.05$). Such trends were also observed on the basis of dry mass (Fig. 4b). However, the dry mass of gut contents for each individual sample at 15 dph in the supplement treatment showed that more than 80% of the larvae tended to preferentially feed on copepods (Fig. 5). In comparison to the larvae of the control, the larvae of copepod supplementation ingested significantly lower numbers (Fig. 6a) and dry mass (Fig. 6b) of rotifers at 5 and 15 dph. The results for total gut contents on a dry mass basis showed that the larvae at 15 dph in the supplement group did not feed on prey compared to the control (Fig. 7). Analysis of Ivlev's selectivity index for the larvae in the supplement showed that larvae fed preferentially on copepods during larviculture (Fig. 8). The indices for copepods at 5, 10, and 15 dph were 0.09 ± 0.94, 0.48 ± 0.84, and 0.85 ± 0.16, respectively, while those for rotifers were -0.51 ± 0.51, -0.52 ± 0.42, and -0.75 ± 0.00, respectively. Significant differences were detected in the indices of selective preference during the culture period (Wilcoxon rank-sum test, $p < 0.05$).

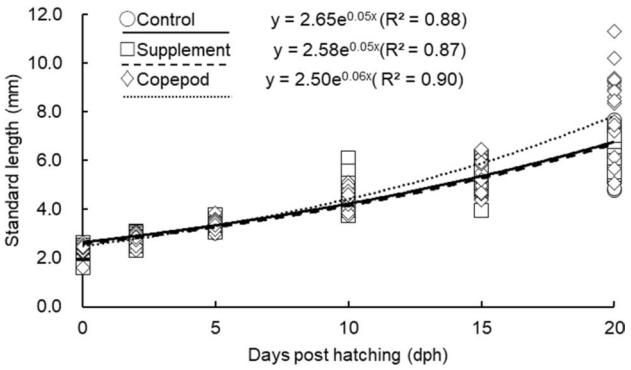

Fig. 1 Growth curve of red sea bream *Pagrus major* larvae. Plots indicate the standard length of all measured larvae each day. The sample numbers of the control were as follows: 0 days post-hatching (dph) = 41, 2 dph = 49, 5 dph = 65, 10 dph = 33, 15 dph = 30, and 20 dph = 57. The numbers of the supplement were as follows: 0 dph = 41, 2 dph = 49, 5 dph = 41, 10 dph = 14, 15 dph = 11, and 20 dph = 15. The numbers of the copepods were as follows: 0 dph = 41, 2 dph = 49, 5 dph = 60, 10 dph = 35, 15 dph = 20, and 20 dph = 19

Free amino acids analysis

Except for cysteine, all free amino acids (FAAs) were detected in the live prey (Table 1). The content of total amino acid (TAA) or essential amino acid (EAA) in copepods was higher than that in rotifers and *Artemia* (Table 2, one-way ANOVA, $p < 0.05$; Tukey's HSD test, $p < 0.05$). The content of 10 of these FAAs (alanine, arginine, glycine, histidine, isoleucine, leucine, methionine, proline, threonine, and valine) was more abundant in cultured copepods than in rotifers and *Artemia* (Table 2, one-way ANOVA, $p < 0.05$; Tukey's HSD test, $p < 0.05$). Among copepod sources, the content of alanine, arginine, glycine, methionine, and taurine was higher in *P. inopinus* than in Chinese or Arctic copepods.

Discussion

It has been reported in several studies conducted at laboratory scale that copepods stimulate the feeding of fish larvae by their unique movements, and several FAAs contained in large amounts in live prey also stimulate the feeding of fish (Kolkovski et al. 1997; Buskey 2005). This is also the case in the wild, where various fish larvae such as black sea bream *Acanthopagrus schlegelii*, Japanese flounder *Paralichthys olivaceus*, Japanese whiting *Sillago japonica*, and red sea bream *Pagrus major* are known to preferentially feed on

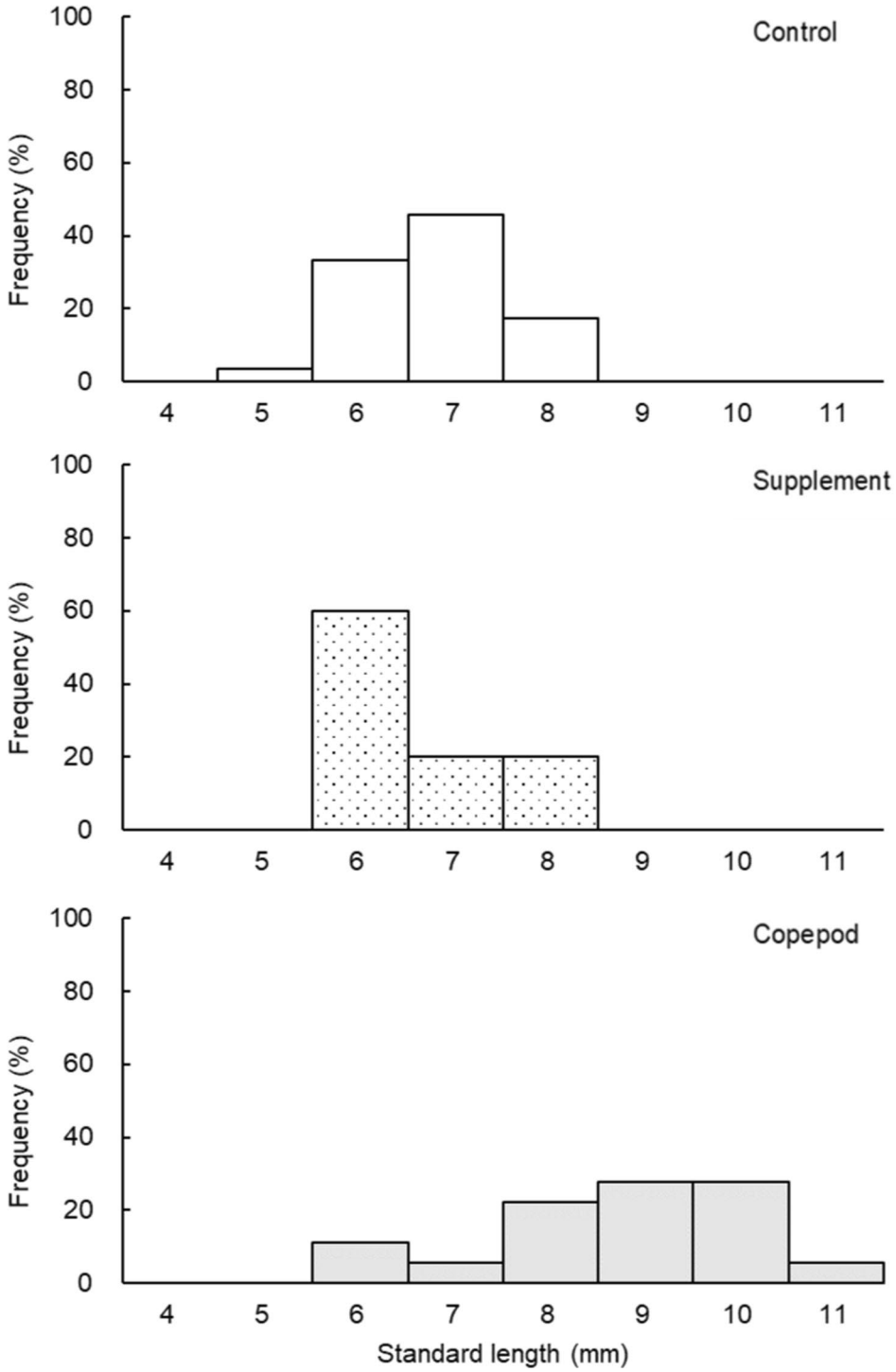

Fig. 2 Frequency of each treatment of standard length of the larvae at 20 days post-hatching (dph). The histogram shows the frequency of the standard length of 20-dph larvae for each treatment. The sample numbers of the control (single diet of rotifer), supplement, and copepods were 57, 15, and 19, respectively

copepods (Kuwahara and Suzuki 1983). Similar to the natural copepods (Arctic, China), *P. inopinus* contained more FAAs such as alanine and glycine, which are involved in stimulating the feeding activity of red sea bream, than did rotifers and *Artemia* (Goh and Tamura 1980). We hypothesised that the presence of copepods, known for their high levels of FAAs, would enhance the feeding activity of larvae up to 20 dph. Furthermore, since previous studies have reported that FAAs emitted by prey increased feeding in larval fish, it was considered that the larvae would exhibit increased consumption of rotifers when fed in conjunction with copepods (supplement group), in comparison to when fed on rotifers only (control) (Kolkovski et al. 1997). However, the trend of a selective preference of the larvae for copepods was observed and resulted in a decrease in rotifer consumption (Fig. 5). Consistent with the findings of Matsui

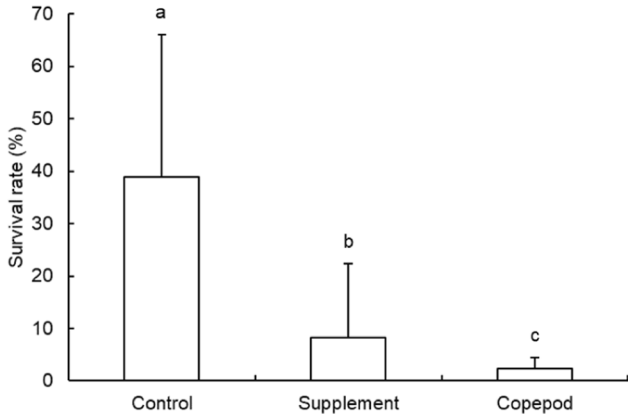

Fig. 3 Survival rate of red sea bream *Pagrus major* larvae after 20 days post-hatching. The columns indicate the survival rates in each treatment, and the error ranges indicate standard deviations (chi-square test, Bonferroni correction, a > b > c, $p < 0.017$)

et al. (2021), where red sea bream larvae were exclusively fed *P. inopinus*, this study observed a significant increase in larval growth when *P. inopinus* were fed alone compared to control feeding (combination of rotifers and *Artemia*). A histogram of larval size showed that some fish were 10 mm or larger in the copepod treatment compared to the other treatments. However, it cannot be ruled out that the copepod treatment may have affected the growth performance, and as a result, only large larvae that successfully fed on copepod survived. This phenomenon could occur, for example, if there were insufficient numbers of rotifers, and this would result in the larger larvae surviving. However, since the nutritional value of the rotifers would be the same as if there were sufficient numbers, the sizes of larvae would be normal as if they were in a larger population. If taking into account that larger larvae were present when being fed with copepods than with rotifers, it is likely that the copepods were sufficient to meet the nutritional requirements of the larvae, and hence their prey value was high. Furthermore, the abundant presence of total FAAs—particularly essential FAAs—found in *P. inopinus* may significantly contribute to larval growth by virtue of their facile digestibility and high absorption rates, as corroborated by prior research (Rønnestad et al. 1992; Conceição et al. 1997). The lower density of *P. inopinus* than rotifers may have caused starvation, and thus a decline in larval survival was observed. The amount of copepod nauplii that could be fed to the copepod treatment group was approximately 0.5 ind./mL/day on average in this study. Although we do not have information on the optimal prey density for red sea bream larvae, we suggest that if survival rates are to be increased in copepod treatment, larvae in copepod treatment should ideally be fed at the same population density as rotifers in the control treatment to increase prey encounter. Although we hypothesised that both survival and growth would experience enhancements when the larvae were fed a supplement with rotifers due to the growth-promoting properties of *P. inopinus*, unexpectedly, neither parameter exhibited improvement in this study. The underlying factor contributing to this lack of improvement is considered to be the significantly lower amount of rotifers consumed by the larvae at 5 and 15 dph compared to the control treatment. The outcomes might differ should rotifers, with their population density, be employed as the primary dietary source, and copepods relegated to a supplementary role.

The composition of FAAs varies among copepod species, and in some cases its concentration is lower than that of rotifers (Drillet et al. 2006; van der Meeren et al. 2008). In this study, the content of several FAAs in rotifers was higher than that in copepods, and the content of some FAAs was lower than that in natural commercial copepods obtained from China and the Arctic, possibly due to fluctuations between species (van der Meeren et al. 2008). For taurine, rotifers can directly consume substances dissolved in water, and thus rotifers possess a high value of taurine compared to *Artemia* and copepods (Takahashi et al. 2005). According to Kolkovski et al. (1997), the amino acids that stimulate the feeding of gilthead sea bream larvae are alanine, arginine, and glycine. Goh and Tamura (1980) reported that red sea bream adults prefer alanine, arginine, glycine, glutamine, serine, and valine. It is not known which amino acids act as feeding stimulants in the larval stages of red sea bream; however, the amounts of these FAAs, especially alanine and glycine, were higher in *P. inopinus* than in rotifers and *Artemia*. Gut contents also showed that the number of rotifers in the gut of the supplement treatment was not as high as that in the control throughout the period, even though both treatments were presented with an equal number of rotifers (Fig. 7). The high alanine, arginine, and glycine content of *P. inopinus* may have induced a preference in the larvae for copepods, but at 5–10 dph, the nostrils of the red sea bream larvae are immature (Anraku et al. 1999).

Although the olfactory bulb forms immediately after hatching, whether it functions as an olfactory organ by 10 dph is unknown (Miyazaki et al. 1991). Like olfaction, gustation is a sensation associated with feeding, but taste buds do not develop until after 30 dph (Miyazaki et al. 1991). However, the visual organs, namely the eyes and retina, develop just after hatching, and thus visual feeding begins after 6 dph (Hirano 1963; Miyazaki et al. 1991). A characteristic of the feeding behaviour of red sea bream is the habit of feeding on objects that exhibit specific movements (Koo et al. 2013). Copepods, including *P. inopinus*, are known to engage in characteristic movement such as hopping and jerking, and are thus reported to be easily recognised as prey by fish larvae (Buskey 2005). If the larval stage of fish had the same habit as adults, *P. inopinus* movement could have

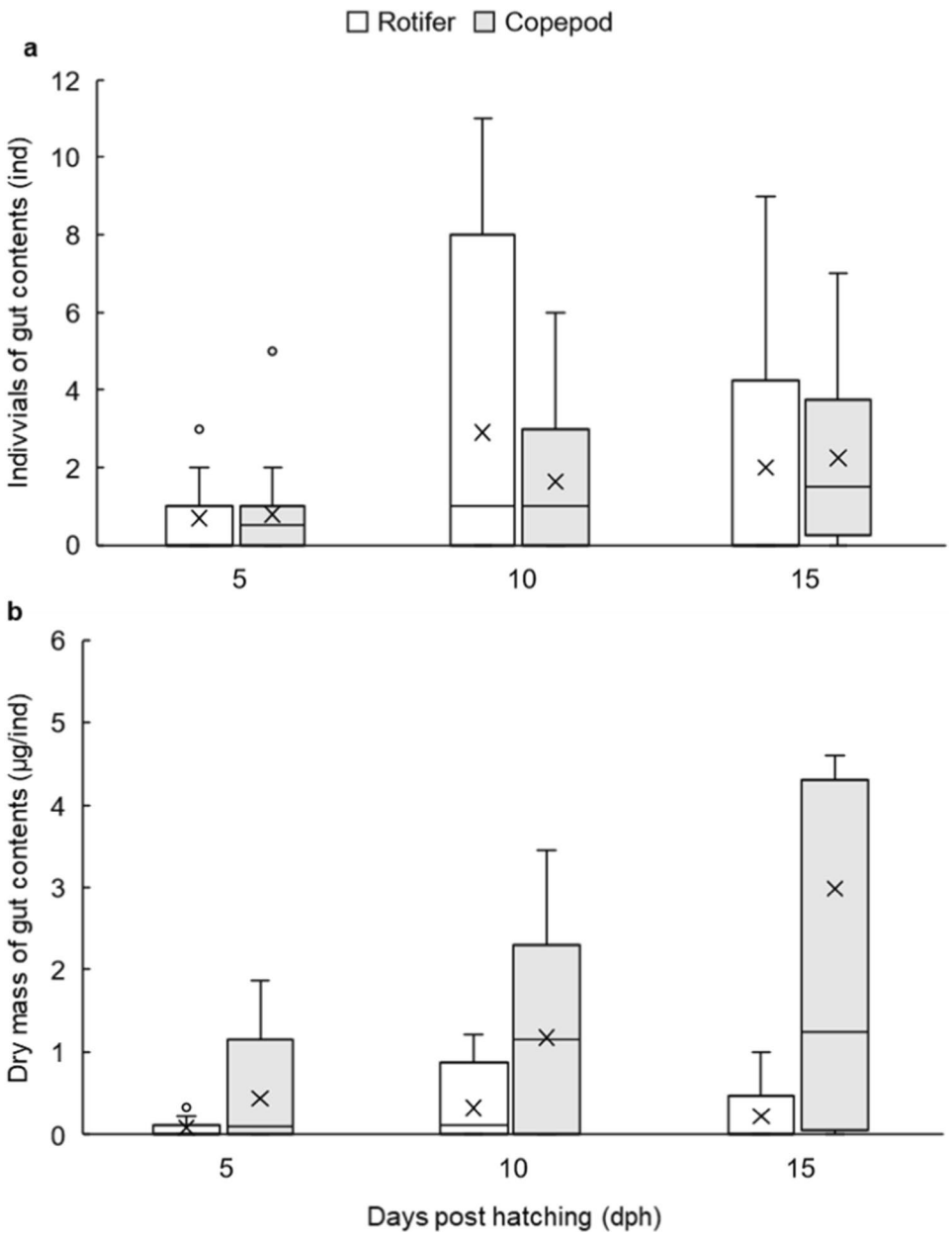

Fig. 4 Individuals of the rotifers and copepods (**a**) and their dry mass (**b**) in the gut of red sea bream *Pagrus major* larvae in supplement treatment. Box plots represent average value (cross mark), median value (central line), 25% and 75% quantiles (box limits), 5% and 95% quantiles (whiskers), and outliers (circles). The sample numbers were as follows: 5 days post-hatching (dph): $n=20$, 10 dph: $n=11$, 15 dph: $n=8$

induced feeding behaviour in red sea bream larvae. Previous studies have reported that biological movement stimulates feeding in finfish larvae (Matsunaga and Watanabe 2012; Nakayasu and Watanabe 2014). It is proposed that the movement of copepods stimulates the feeding behaviour of red sea bream larvae, resulting in their selective feeding on copepods as much as or even more than on rotifers, despite the high stocking density of rotifers. In particular, larvae at 15 dph tend to preferably feed on copepods, which is expected to be the result of the development of chemoreceptors and a change in feeding preference. Ivlev's selectivity index showed that the feeding selectivity of copepods was positive at all dph examined and that of rotifers was negative, indicating that red sea bream larvae have a higher preference for copepods than rotifers. Furthermore, the selectivity indices of Ivlev revealed that larvae selectively fed on copepods as they grew.

Although copepods were selectively fed to the larvae in the supplement treatment, their survival rate was very low, which may be due to the low feeding density of the copepods and low encounter rate for the prey in this study. However, it seems reasonable that they would feed on rotifers, which were immediately in front of them, and still stocked higher than copepods. Mayer and Wahl (1997) reported that the larvae of walleye *Stizostedion vitreum* exhibited improved survival when the size of prey was suitable. In other words, it is possible that if the prey size distribution is not suitable for the larvae, larval survival decreases. The copepod *P.*

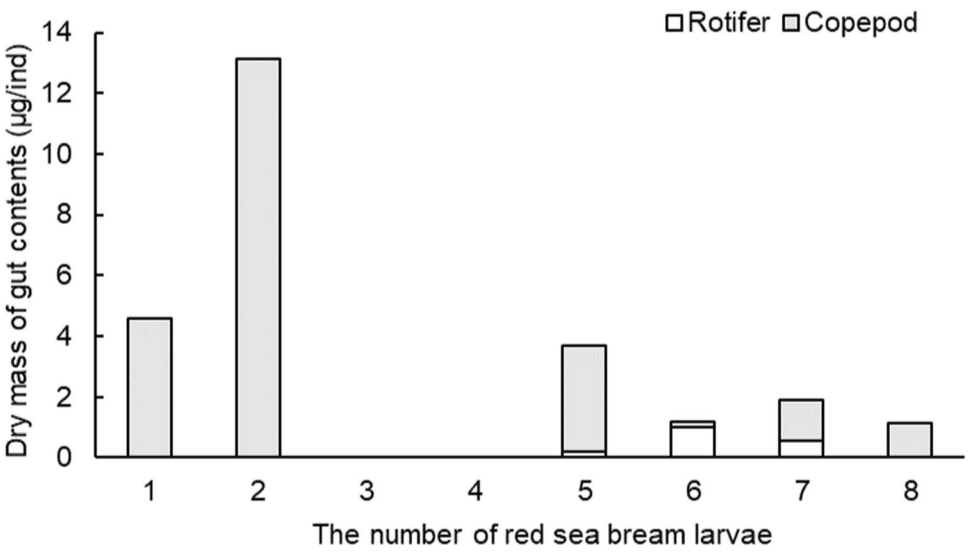

Fig. 5 Dry mass of gut contents for each individual sample at 15 dph in the supplement treatment. The columns indicate dry mass of rotifers and copepods in the gut content of each individual sample ($n = 8$)

inopinus, especially nauplii, is similar to rotifers (Matsui et al. 2021) and is not inferior to them in size. Owing to their visual feeding stimulation, copepods are the more preferred and suitable prey of fish larvae (Buskey 2005). Therefore, a decline in the copepod population would decrease the survival rate of these larvae due to lack of appropriate prey. However, the number of samples in gut contents per developmental stage of larval fish in this study was not sufficient to prove this phenomenon, and further research is needed. Moreover, apart from visual stimulation, other factors such as chemical responses may contribute to preferential feeding and the aforementioned induced movement. The low copepod biomass due to predation and disappearance of copepods from the rearing water could results in larval mortality due to starvation, despite rotifer availability. To clarify the phenomenon of larval mortality, it is necessary to confirm the gut contents of dead individuals or fish after enough time has elapsed since feeding. However, we did not examine this aspect, as it was beyond the scope of this study.

Copepods are known to have high nutritional value and are associated with various positive aspects of rearing performance in experimentally fed fish larvae (Toledo et al. 1999; Barroso et al. 2013). Moreover, copepods are a main larval live prey in nature (Kuwahara and Suzuki 1983). Therefore, copepods are expected to be used as live prey for larviculture (Støttrup and Norsker 1997). However, the cultivation of copepods is difficult, and there are limited examples of their practical usage (Engell-Sørensen et al. 2004). Contrary to expectations, practical applications are far from being achieved. Although copepods were used as a supplementary prey with rotifers, the survival rate of the larvae was very low in this study. According to the observed phenomenon, co-feeding of *P. inopinus* and rotifers affects the preference of red sea bream larvae, and they tend not to feed actively on rotifers. In fact, it is clear that Ivlev's selectivity index for rotifers decreased with each dph. Although the impact of FAAs on larval feeding preference has not been previously reported, it is well known that FAAs can exert a significant influence on the feeding preference of red sea bream and other fish species (Goh and Tamura 1980; Levina et al. 2021). If FAAs indeed affect the feeding preference of the larvae, this study represents pioneering research on larval preference affecting survival. Furthermore, this study is the first to present the novel finding that, despite the recognition of copepods as a promising live prey, the occurrence of this phenomenon has a detrimental impact on the survival rates of fish larvae. Although further research is needed to determine which FAAs are involved in stimulation of feeding in fish larvae, the results of this study suggest that if copepods are to be fed to larval fish in a practical manner, it is necessary to select feeding methods that match the feeding preferences of each growth stage of the larvae.

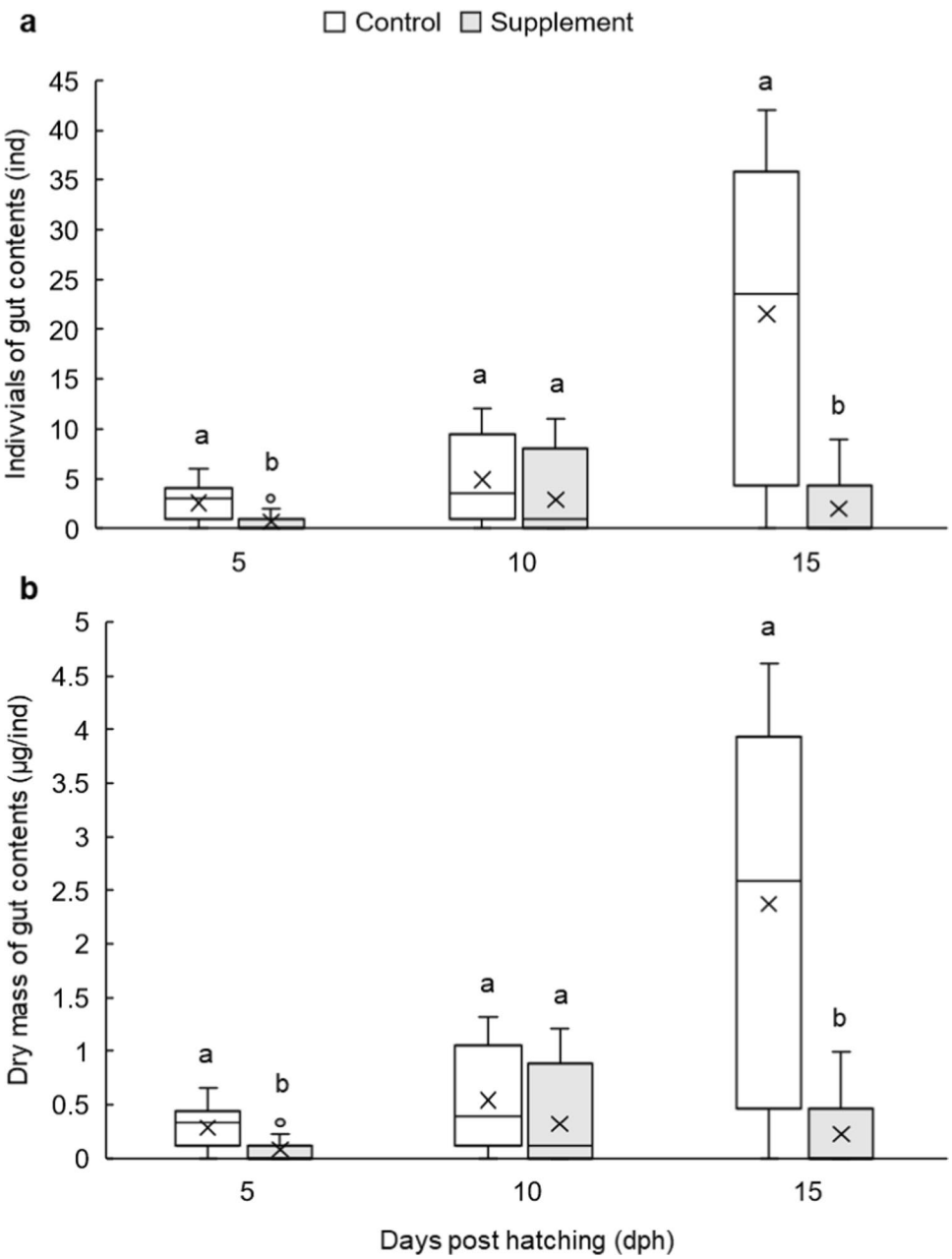

Fig. 6 Individuals of the rotifer group (**a**) and their dry mass (**b**) in the gut contents of red sea bream *Pagrus major* larvae in control and supplement treatment. Box plots represent the average value (cross mark), median value (central line), 25% and 75% quantiles (box limits), 5% and 95% quantiles (whiskers), and outliers (circles). The control sample numbers were as follows: 5 days post-hatching (dph): $n=20$, 10 dph: $n=20$, 15 dph: $n=20$. The supplement sample numbers were as follows: 5 dph: $n=20$, 10 dph: $n=11$, 15 dph: $n=8$ (Wilcoxon rank-sum test, a > b, $p<0.05$)

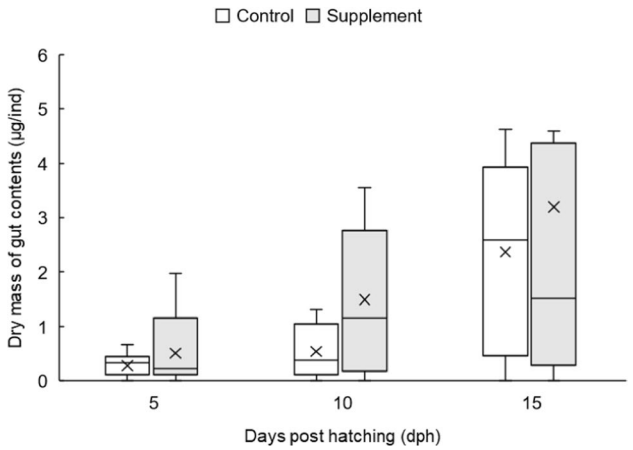

Fig.7 Total dry mass in the gut contents of red sea bream *Pagrus major* larvae in control and supplement treatments. Box plots represent the average value (cross mark), median value (central line), 25% and 75% quantiles (box limits), and 5% and 95% quantiles (whiskers). The control sample numbers were as follows: 5 days post-hatching (dph): $n=20$, 10 dph: $n=20$, 15 dph: $n=20$. The supplement sample numbers were as follows: 5 dph: $n=20$, 10 dph: $n=11$, 15 dph: $n=8$

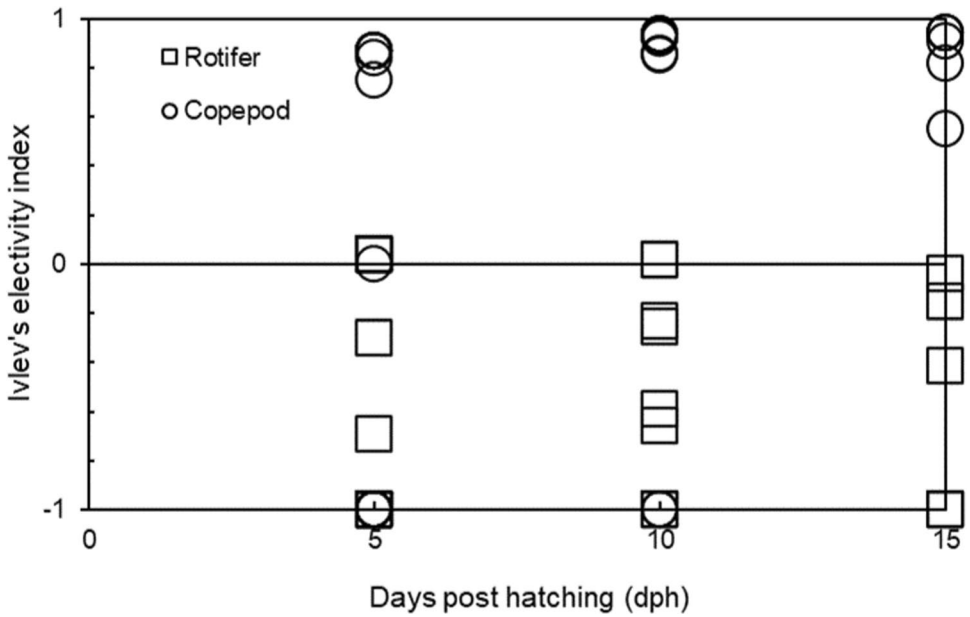

Fig. 8 Ivlev's selectivity index of red sea bream *Pagrus major* larvae in supplement treatment at 5, 10, and 15 dph. The square and circle plots show the feeding selectivity indices of larvae with respect to rotifers and copepods, respectively. The sample numbers were as follows: 5 dph: $n=20$, 10 dph: $n=11$, 15 dph: $n=8$

Table 1 Free amino acid composition in prey for marine finfish larvae

Amino acid (mg/g)	Rotifer	*Artemia*	*Pseudodiaptomus*	Chinese copepod	Arctic copepod
Ala	$^{c}1.80 \pm 0.15$	$^{b}3.95 \pm 0.29$	$^{a}10.19 \pm 0.55$	4.01 ± 0.35	6.88 ± 0.31
Arg	$^{b}2.81 \pm 0.22$	$^{b}2.99 \pm 0.33$	$^{a}12.75 \pm 1.51$	8.91 ± 0.61	11.41 ± 0.39
Asp	$^{a}0.53 \pm 0.13$	$^{b}0.19 \pm 0.02$	$^{a}0.71 \pm 0.04$	0.73 ± 0.07	2.90 ± 0.16
Cys	ND	ND	ND	ND	ND
Glu	$^{a}4.20 \pm 0.33$	$^{a}3.66 \pm 0.43$	$^{b}2.62 \pm 0.07$	3.33 ± 0.29	8.06 ± 0.40
Gly	$^{b}1.06 \pm 0.08$	$^{b}0.79 \pm 0.05$	$^{a}3.70 \pm 0.19$	0.98 ± 0.10	3.16 ± 0.16
His	$^{b}1.36 \pm 0.02$	$^{b}1.40 \pm 0.20$	$^{a}1.95 \pm 0.03$	2.74 ± 0.23	4.26 ± 0.15
Hxpro	ND	0.09 ± 0.01	ND	ND	ND
Ile	$^{b}0.47 \pm 0.07$	$^{b}0.59 \pm 0.08$	$^{a}1.30 \pm 0.06$	1.41 ± 0.13	2.69 ± 0.12
Leu	$^{b}0.74 \pm 0.14$	$^{b}0.87 \pm 0.19$	$^{a}2.24 \pm 0.21$	2.53 ± 0.24	5.59 ± 0.25
Lys	$^{b}1.78 \pm 0.05$	$^{a}2.35 \pm 0.32$	$^{a}2.53 \pm 0.12$	4.20 ± 0.36	6.62 ± 0.25
Met	ND	$^{b}1.15 \pm 0.06$	$^{a}6.24 \pm 0.10$	1.46 ± 0.11	2.50 ± 0.11
Phe	$^{a}5.46 \pm 0.21$	$^{c}3.34 \pm 0.34$	$^{b}3.98 \pm 0.05$	4.72 ± 0.39	4.94 ± 0.16
Pro	$^{b}0.79 \pm 0.06$	$^{b}1.73 \pm 0.13$	$^{a}4.04 \pm 0.03$	1.64 ± 0.16	8.76 ± 0.39
Ser	$^{a}1.19 \pm 0.19$	$^{b}0.96 \pm 0.11$	$^{c}0.73 \pm 0.05$	1.39 ± 0.15	3.46 ± 0.16
Tau	$^{a}13.22 \pm 0.75$	$^{b}4.62 \pm 0.29$	$^{b}5.62 \pm 0.22$	0.20 ± 0.03	2.88 ± 0.15
Thr	$^{b}1.60 \pm 0.13$	$^{b}1.65 \pm 0.18$	$^{a}2.31 \pm 0.05$	2.69 ± 0.26	4.99 ± 0.20
Trp	$^{b}0.10 \pm 0.01$	$^{a}0.22 \pm 0.03$	$^{a}0.22 \pm 0.02$	0.41 ± 0.02	0.12 ± 0.00
Tyr	$^{a}3.03 \pm 0.43$	$^{a}2.37 \pm 0.26$	$^{b}1.48 \pm 0.08$	3.48 ± 0.30	3.91 ± 0.11
Val	$^{b}0.70 \pm 0.10$	$^{b}0.84 \pm 0.10$	$^{a}2.61 \pm 0.06$	1.92 ± 0.19	3.33 ± 0.14
EAA	$^{b}15.81 \pm 0.83$	$^{b}15.42 \pm 1.79$	$^{a}36.14 \pm 2.01$	30.99 ± 2.47	46.46 ± 1.75
NEAA	$^{a}25.82 \pm 1.53$	$^{b}18.37 \pm 1.51$	$^{a}29.08 \pm 1.02$	15.76 ± 1.44	40.01 ± 1.80
TAA	$^{b}40.85 \pm 2.32$	$^{b}33.78 \pm 3.27$	$^{a}65.22 \pm 3.02$	46.76 ± 3.90	86.46 ± 3.55

ND not detected

The numbers in the table indicate the mean ± standard deviation. The table shows the amounts of each free amino acids, essential amino acids (EAA), non-essential amino acids (NEAA), and total amino acids (TAA). FAAs of frozen copepods harvested in China and the Arctic were also analysed in order to evaluate differences in FAAs among copepod sources ($n = 3$, one-way analysis of variance, $p < 0.05$, Tukey's HSD test, a > b > c, $p < 0.05$)

Table 2 Results of one-way ANOVA for free amino acid analysis

Amino acid	Degrees of freedom	*F*-value	*P*-value
Ala	2	423.99	0.35×10^{-6}
Arg	2	120.16	14.45×10^{-6}
Asp	2	29.91	$0.76.\times10^{-3}$
Cys	2	NaN	NaN
Glu	2	19.39	2.41×10^{-3}
Gly	2	502.52	0.21×10^{-6}
His	2	24.35	1.32×10^{-3}
Hxpro	2	162.78	5.93×10^{-6}
Ile	2	127.74	12.08×10^{-6}
Leu	2	62.17	97.57×10^{-6}
Lys	2	12.15	7.77×10^{-3}
Met	2	7279.80	0.07×10^{-9}
Phe	2	67.10	0.08×10^{-3}
Pro	2	60.97	0.10×10^{-3}
Ser	2	21.68	1.80×10^{-3}
Tau	2	285.97	1.12×10^{-6}
Thr	2	26.96	1.00×10^{-3}
Trp	2	28.36	0.88×10^{-3}
Tyr	2	21.27	1.89×10^{-3}
Val	2	427.84	0.34×10^{-6}
EAA	2	166.53	5.54×10^{-6}
NEAA	2	48.04	0.20×10^{-3}
TAA	2	97.19	26.85×10^{-6}

NaN not a number

Acknowledgements We thank the graduate students from the Laboratory of Aquatic Animal Nutrition and Larval Rearing Management at Kagoshima University. This study was supported by JSPS KAKENHI (grant number 24580275).

Author contributions TS, YK, and TK designed the study and performed experiments. TS and YK conducted the experiments and collected data. TS prepared the draft of the manuscript and revised it with HM and TK. SY and MI provided HPLC and advice on the methodologies of free amino acid analysis. TS and HM performed statistical analyses. TK supervised this study. All authors contributed to the experiments and read and approved the final version of the manuscript.

Declarations

Conflict of interest The authors declare that they have no competing or financial interests.

References

Alajmi F, Zeng C (2015) Evaluation of microalgal diets for the intensive cultivation of the tropical calanoid copepod Parvocalanus Crassirostris. Aquacult Res 46:1025–1038. https://doi.org/10.1111/are.12254

Anraku K, Masuda T, Kawamura G, Mana R-R (1999) Development of the inter-nostril epidermis in hatchery reared red sea bream, *Pagrus major*. Nippon Suisan Gakkaishi 65:501–502. https://doi.org/10.2331/suisan.65.501

Barroso MV, de Carvalho CVA, Antoniassi R, Cerqueira VR (2013) Use of the copepod *Acartia tonsa* as the first live food for larvae of the fat snook *Centropomus parallelus*. Aquaculture 388–391:153–158. https://doi.org/10.1016/j.aquaculture.2013.01.022

Bell JG, McEvoy LA, Estevez A, Shields RJ, Sargent JR (2003) Optimizing lipid nutrition in first-feeding flatfish larvae. Aquaculture 227:211–220. https://doi.org/10.1016/S0044-8486(03)00504-0

Blaxter JHS, Hempel G (1963) The influence of egg size on herring larvae (*Clupea harengus* L.). J Cons Perm Int Explor Mer 28:211–240. https://doi.org/10.1093/icesjms/28.2.211

Bu Y, Zhao Y, Zhou Y, Zhu W, Li J, Li X (2023) Quality and flavor characteristics evaluation of red sea bream surimi powder by different drying techniques. Food Chem. https://doi.org/10.1016/j.foodchem.2023.136714

Buskey EJ (2005) Behavioral characteristics of copepods that affect their suitability as food for larval fishes. In: Lee CS, O'Bryen PJ et al (eds) Copepods in aquaculture. Wiley-Blackwell, Hoboken, NJ, USA, pp 91–105. https://doi.org/10.1002/9780470277522.ch8

Chen J-N, Takeuchi T, Takahashi T, Tomoda T, Koiso M, Kuwada H (2004) Effect of rotifers enriched with taurine on growth and survival activity of red sea bream *Pagrus major* larvae. Nippon Suisan Gakkaishi 70:542–547. https://doi.org/10.2331/suisan.70.542

Conceição LEC, van der Meeren T, Verreth JAJ, Evjen MS, Houlihan DF, Fyhn HJ (1997) Amino acid metabolism and protein turnover in larval turbot (*Scophthalmus maximus*) fed natural zooplankton or *Artemia*. Mar Biol 129:255–265. https://doi.org/10.1007/s002270050166

Drillet G, Jorgensen NOG, Sorensen TF, Ramlov H, Hansen BW (2006) Biochemical and technical observations supporting the use of copepods as live feed organisms in marine larviculture. Aquacult Res 37:756–772. https://doi.org/10.1111/j.1365-2109.2006.01489.x

Engell-Sørensen K, Støttrup JG, Holmstrup M (2004) Rearing of flounder (*Platichthys flesus*) juveniles in semiextensive systems. Aquaculture 230:475–491. https://doi.org/10.1016/S0044-8486(03)00437-X

Furuita H, Takeuchi T, Toyota M, Watanabe T (1996) EPA and DHA requirements in early juvenile red sea bream using HUFA enriched *Artemia* nauplii. Fish Sci 62:246–251. https://doi.org/10.2331/fishsci.62.246

Goh Y, Tamura T (1980) Effect of amino acids on the feeding behaviour in red sea bream. Comp Biochem Physiol C Comp Pharmacol 66:225–229. https://doi.org/10.1016/0306-4492(80)90131-8

Hagiwara A, Gallardo WG, Assavaaree M, Kotani T, Araujo A-B (2001) Live food production in Japan: recent progress and future aspects. Aquaculture 200:111–127. https://doi.org/10.1016/S0044-8486(01)00696-2

Helland S, Terjesen BF, Berg L (2003) Free amino acid and protein content in the planktonic copepod *Temora longicornis* compared to *Artemia franciscana*. Aquaculture 215:213–228. https://doi.org/10.1016/S0044-8486(02)00043-1

Hirano R (1963) Problems of feed with fish larvicultureII. Suisan-Zoshoku 2:93–99. https://doi.org/10.11233/aquaculturesci1953.11.Special2_93

Ivlev VS (1961) Experimental ecology of the feeding of fishes. Yale University Press. New Haven, CT, USA, pp 41–115

Kolkovski S, Arieli A, Tandler A (1997) Visual and chemical cues stimulate microdiet ingestion in sea bream larvae. Aquacult Int 5:527–536. https://doi.org/10.1023/A:1018305416501

Koo MS, Ishizaki M, Kim SJ, Fuwa S, Archdale MV (2013) Characteristics of periodical movement of live squid (*Todarodes pacificus*) hooked on the branch line of a red sea bream long line. J Korean

Wood Sci 49:227–237. https://doi.org/10.3796/KSFT.2012.49.3.227
Kotani T, Genka T, Fushimi H, Hayashi M, Dierckens K, Sorgeloos P (2009) Effect of cultivation methods on nutritional enrichment of euryhaline rotifer *Brachionus plicatilis*. Fish Sci 75:975–984. https://doi.org/10.1007/s12562-009-0105-1
Kotani T, Yokota M, Fushimi H, Watanabe S (2011) How to determine the appropriate mortality in experimental larval rearing? Fish Sci 77:255–261. https://doi.org/10.1007/s12562-011-0329-8
Kuwahara A, Suzuki S (1983) Relationship between feeding habits of fish larvae and their food organisms in the western Wakasa Bay. Nippon Suisan Gakkaishi 49:1507–1513. https://doi.org/10.2331/suisan.49.1507
Levina AD, Mikhailova ES, Kasumyan AO (2021) Taste preferences and feeding behaviour in the facultative herbivorous fish, Nile tilapia *Oreochromis niloticus*. J Fish Biol 98:1385–1400. https://doi.org/10.1111/jfb.14675
Matsui H, Sasaki T, Kobari T, Waqalevu V, Kikuchi K, Ishikawa M, Kotani T (2021) DHA accumulation in the polar lipids of the euryhaline copepod *Pseudodiaptomus inopinus* and its transfer to red sea bream *Pagrus major* Larvae. Front Mar Sci 8:632876. https://doi.org/10.3389/fmars.2021.632876
Matsunaga W, Watanabe E (2012) Visual motion with pink noise induces predation behaviour. Sci Rep 2:1–7. https://doi.org/10.1038/srep00219
Mayer CM, Wahl DH (1997) The relationship between prey selectivity and growth and survival in a larval fish. Can J Fish Aquat Sci 54:1504–1512. https://doi.org/10.1139/f97-056
Miyazaki T, Fujiwara K, Oka H, Yoshikawa M (1991) Histological studies on development of tissues and organs of larval and juvenile red sea bream. Bull. Fac. Bioresource, Mie Univ 5: 97–116. Retrieved from https://mie-u.repo.nii.ac.jp/?action=pages_view_main&active_action=repository_view_main_item_detail&item_id=5717&item_no=1&page_id=13&block_id=21
Nakayasu T, Watanabe E (2014) Biological motion stimuli are attractive to medaka fish. Anim Cognit 17:559–575. https://doi.org/10.1007/s10071-013-0687-y
Nam B-H, Yoo D, Kim Y-O, Park JY, Shin Y, G-hee S, C-ll P, Kim H, Kwak W (2019) Whole genome sequencing reveals the impact of recent artificial selection on red sea bream reared in fish farms. Sci Rep 9:6487. https://doi.org/10.1038/s41598-019-42988-z
Øie G, Galloway T, Sørøy M, Holmvaag Hansen M, Norheim IA, Halseth CK, Almli M, Berg M, Gagnat MR, Wold PA, Attramadal K, Hagemann A, Evjemo JO, Kjørsvik E (2015) Effect of cultivated copepods (*Acartia tonsa*) in first-feeding of Atlantic cod (*Gadus morhua*) and Ballan wrasse (*Labrus bergylta*) larvae. Aquacult Nutr 23:3–17. https://doi.org/10.1111/anu.12352
Puello-Cruz AC, Mezo-Villalobos S, González-Rodríguez B, Voltolina D (2009) Culture of the calanoid copepod *Pseudodiaptomus euryhalinus* (Johnson 1939) with different microalgal diets. Aquaculture 290:317–319. https://doi.org/10.1016/j.aquaculture.2009.02.016
Rønnestad I, Fyhn HJ, Gravningen K (1992) The importance of free amino acids to the energy metabolism of eggs and larvae of turbot (*Scophthalmus maximus*). Mar Biol 114:517–525. https://doi.org/10.1007/BF00357249
Sakaguchi SO, Ueda H (2011) Morphological divergence of *Pseudodiaptomus inopinus* Burckhardt, 1913 (Copepoda: Calanoida) between the Japan sea and Pacific coasts of western Japan. Plankton Benthos Res 6:124–128. https://doi.org/10.3800/pbr.6.124
Sargent J, McEvoy L, Estevez A, Bell G, Bell M, Henderson J, Tocher D (1999) Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179:217–229. https://doi.org/10.1016/S0044-8486(99)00191-X
Sarkisian BL, Lemus JT, Apeitos A, Blaylock BR, Saillant AE (2019) An intensive, large-scale batch culture system to produce the calanoid copepod, *Acartia tonsa*. Aquaculture 501:272–278. https://doi.org/10.1016/j.aquaculture.2018.11.042
Støttrup JG, Norsker NH (1997) Production and use of copepods in marine fish larviculture. Aquaculture 55:231–247. https://doi.org/10.1016/S0044-8486(97)00120-8
Takahashi T, Amano T, Takeuchi T (2005) Establishment of direct enrichment method of taurine to rotifer. Aquacult Sci 53:121–126. https://doi.org/10.11233/aquaculturesci1953.53.121
Takayama Y, Hirahara M, Toda T (2021) Bioreactor cultivation of the planktonic copepod *Acartia steueri* Smirnov for egg collection. Aquacult Res 52:5912–5917. https://doi.org/10.1111/are.15421
Teshima SI, Kanazawa A, Yamashita M (1986) Dietary value of several proteins and supplemental amino acids for larvae of the prawn *Penaeus japonicus*. Aquaculture 51:225–235. https://doi.org/10.1016/0044-8486(86)90314-5
Toledo JD, Golez MS, Doi M, Ohno A (1999) Use of copepod nauplius during early feeding stage of grouper *Epinephelus coioides*. Fish Sci 65:390–397. https://doi.org/10.2331/fishsci.65.390
Van der Meeren T, Olsen RE, Hamre K, Fyhn HJ (2008) Biochemical composition of copepods for evaluation of feed quality in production of juvenile marine fish. Aquaculture 274:375–397. https://doi.org/10.1016/j.aquaculture.2007.11.041
Watanabe T, Izquierdo MS, Takeuchi T, Satoh S, Kitajima T (1989) Comparison between eicosapentaenoic and docosahexaenoic acids in terms of essential fatty acid efficacy in larval red seabream. Nippon Suisan Gakkaishi 55:1635–1640. https://doi.org/10.2331/suisan.55.1635