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Effects of Live Food enrichment on Yellowtail Amberjack *Seriola lalandi dorsalis* (Gill 1863) Larvae

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Keywords: Yellowtail amberjack *Seriola lalandi dorsalis*; live food enrichment; S.presso; growth and survival; fatty acids

Abstract

Our study examined the effect of live food enrichment on the rearing performance of yellowtail amberjack *Seriola lalandi dorsalis* from day 3 post-hatch (DPH) to 12 DPH. Three rotifer enriched treatments, 1) *Nannochloropsis* sp, 2) S.presso® (INVE Aquaculture) half dose (Sp1/2), and 3) S.presso® full dose (Sp1) were administered. Growth of yellowtail amberjack larvae was not significantly affected by these enrichments ($P>0.05$) but final survival rates were significantly affected ($P<0.05$). Jaw malformation of fish fed with only *Nannochloropsis* sp. enriched rotifers was significantly higher than those fed S.presso enriched rotifers. Fatty acid composition of rotifers was significantly affected by the enrichment treatments ($P<0.05$). Arachidonic acid (ARA, 20:4n-6) and Eicosapentaenoic acid (EPA, 20:5n-3) were highest in rotifers enriched with *Nannochloropsis*. Docosahexaenoic acid (DHA, 22:6n-3) level was highest in Sp1 treatment. Total saturated fatty acids in rotifers was not significantly affected ($P>0.05$), but total polyunsaturated fatty acid content was significantly affected ($P<0.05$) by enrichment. DHA/EPA ratio in rotifers was significantly affected by the enrichment ($P<0.05$). Highest total polyunsaturated fatty acids were observed in rotifers enriched with Sp1. On 12 DPH, fatty acid composition in the larvae was significantly affected by the nutrient enhancement ($P<0.05$). ARA and EPA levels were highest in the larvae fed with *Nannochloropsis* sp. enriched rotifers; highest DHA/EPA ratio in fish larvae was found in Sp1 treatment. Total polyunsaturated fatty acid in the larvae enriched with Sp1/2 and Sp1 was similar to the amount observed in fertilized eggs of yellowtail amberjack ($P>0.05$). Results indicate that enriched live feed can significantly affect rearing performance of yellowtail amberjack larvae; S.presso full dose enrichment is suitable for their initial rearing stages.

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Introduction

A major prerequisite for marine fish larvae culture is knowledge about the nutritional requirements of fish larvae during their early developmental stages. The success of the larval stages of growth and survival are greatly influenced by first feeding regimes and the nutritional quality of feeds with dietary lipids (Ma et al.,2012) which are recognized as amongst the most important nutritional factors. Food available in the wild is high in energy value, proteins, minerals and vitamins. Among the nutrients fatty acids have important and diverse functions for normal development and are required in different amounts by vertebrates (Sargent, et al., 1995). The majority of marine organisms have a very limited ability to synthesize essential fatty acids such as EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid), through the elongation and desaturation of shorter chain polyunsaturated fatty acids (Bell, et al., 2007) or by retro conversion (Watanabe, 1993). Hence, it is important to provide live feed rich in fatty acids to fish larvae (Øie & Olsen, 1997; Palmtag, et al., 2006).

Yellowtail amberjack (*Seriola lalandi dorsalis*) belongs to the Carangidae family and is widely distributed throughout warm-temperate waters of the northern hemisphere. It is a prime species for aquaculture due to its fast growth, high flesh quality, and suitability for offshore cage culture. However, low survival and unreliable fingerling supply and quality have greatly hindered production in China (Yu, et al., 2017). A previous study of *Seriola lalandi lalandi* (Valenciennes, 1833) indicated that S.presso enrichment can significantly affect the rearing performance of fish larvae. In this study, yellowtail amberjack larvae were fed live feeds enriched with microalgae and S.presso to quantify its effects on rearing performances of fish larvae. The aim of this study was to improve our understanding of fatty acid requirements of yellowtail amberjack larvae.

Materials and Methods

Fertilized eggs were produced by broodstock in Tropical Aquaculture Research and Development Centre, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. All eggs hatched in 500 L fiberglass incubators at 24.0ºC. On day 2 post-hatch (DPH), fish larvae were stocked into 1000 L fiberglass rearing tanks at a density of 30 larvae/L. All the rearing tanks were supplied with filtered seawater in a flow-through system with a daily water exchange rate of 300% tank volume. Two air stones were used in each rearing tank to maintain dissolved oxygen at saturation and to homogenize live food distribution. Photoperiod of 14h light and 10h dark was implemented in this study, and the light intensity was 2,400 lux. Salinity was maintained at 36‰ throughout the experiment. The feeding trial began on 3 DPH, and ended on 12 DPH.

This experiment had three dietary treatments: 1) *Nannochloropsis* sp (cell density: 8 × 10^6 cell/mL); 2) S.presso® (INVE Aquaculture) half dose (175 mg/L); and 3) S.presso® full dose (350 mg/L) full dose with three replicates each. S.presso is a live food enrichment product for *Artemia*, and rotifers produced by a suspension/emulsion technique. The composition of S.presso includes: moisture 58%, crude protein 3%, crude lipids 32%, crude ash 2%, phosphorus 0,5%, vitamin A 110.000 IU/kg, vitamin. D3 10.000 IU/kg, vitamin E 5.400 mg/kg, vitamin C 8.000 mg/kg, Σω3 HUFA 150 mg/g dry weight (dwt), DHA/EPA ratio = 9. During the enrichment, the density of rotifers was maintained at 700 rotifers/mL. After 12h enrichment the rotifers were harvested and fed to fish larvae from 3 DPH to 12 DPH at a rate of 10 rotifers/mL. On 3, 6, and 10 DPH, 4 g (wet weight) rotifers were collected after enrichment for analysis. Instant and live *Nannocholoropsis* sp. (cell density: 1:1) was added into the larval rearing tanks to create a green environment for fish larvae.

Fish growth was assessed as follows:

Specific growth rate (SGR) as %/day: $SGR = 100 \times (Ln(SLf) - Ln(SLi))/Dt$, where SLf and SLi are the final and initial standard length (mm), respectively, and Dt is the time interval (days) between samplings.

At the end of this experiment, fish from each rearing tank were harvested and counted. Fish larvae were collected from each rearing tank for the analysis of fatty acids. The lipids were extracted according to the method described by Folch et al. (1957), and the fatty acids analyzed according to the method described by Ma and Qin (2014). At the end of this experiment, 50 fish larvae from each rearing tank were collected for jaw malformation analysis according to the method described by (Saravanan et al., 2003, Cobcroft, et al., 2004).

In this study, all the percentage data were arcsine-transformed before analysis and are presented as untransformed values in the figures. The data were expressed as mean ± SD, and tested by one-way ANOVA (PASW Statistics 18.0, SPSS Inc.). Tukey's test was used for multiple range comparisons when a significant treatment effect was observed ($P<0.05$). In this study, all the data were tested for normality, homogeneity and independence to fulfill the assumptions of ANOVA.

Results

The growth of yellowtail amberjack larvae was not significantly affected by live food enrichment ($P>0.05$, Fig. 1). The specific growth rate of fish ranged from 1.49 ± 0.46 %/day to 1.62 ± 0.51 %/day. The final survival of fish larvae was significantly affected by enrichment ($P<0.05$, Fig. 2). Highest survival was observed in fish fed with Sp1 enriched rotifers. Survival in *Nannochloropsis* sp. and Sp1/2 treatments was not significantly different ($P>0.05$). Jaw malformation of fish larvae was significantly affected by the enrichment ($P < 0.05$, Fig. 3). Jaw malformation of fish fed with *Nannochloropsis* sp. enriched rotifers was significantly higher than those fed with S.presso enriched rotifers but was not significantly different between the Sp1/2 and Sp1 groups($P>0.05$).

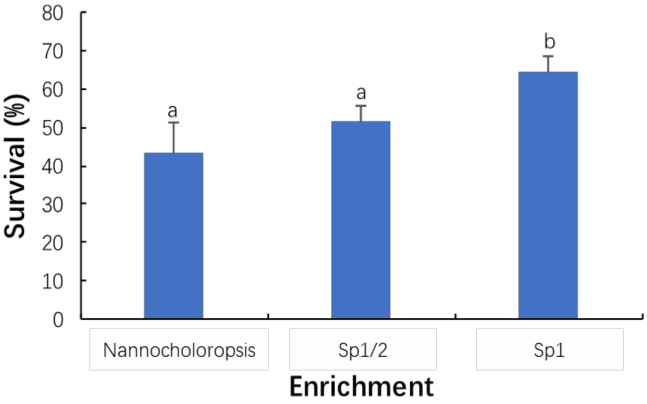

Fig. 1 Survival of yellowtail amberjack larvae fed with rotifers enriched with *Nannochloropsis* sp. and S.presso.
Different letters represent significant differences at $P<0.05$.
Abbreviations:
Sp1/2, S.presso half dose;
Sp1, Spresso full dose.

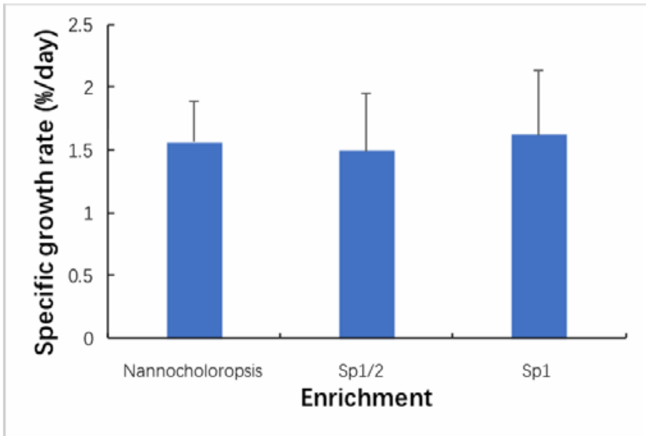

Fig. 2 Specific growth rate of yellowtail amberjack larvae fed with rotifers enriched with *Nannochloropsis* sp. and S.presso. Abbreviations: Sp1/2, S.presso half dose; Sp1, Spresso full dose.

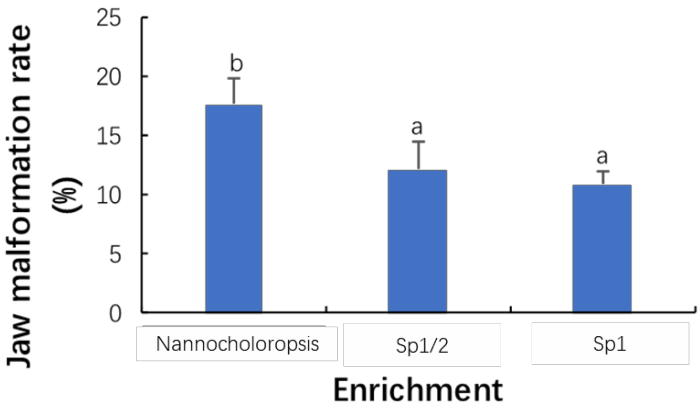

Fig. 3 Jaw malformation of yellowtail amberjack larvae fed with rotifers enriched with *Nannochloropsis* sp. and S.presso.
Abbreviations: Sp1/2, S.presso half dose; Sp1, Spresso full dose.

In this study, the total amount of lipids in the rotifers was not significantly affected by the enrichment (P>0.05, Table 1). The fatty acid compositions in rotifers were significantly affected by the enrichment treatments (P < 0.05, Table 1). The highest ARA (20:4n-6) was observed in rotifers enriched with *Nannochloropsis* sp., and the lowest value was found in rotifers enriched with Sp1/2. The amount of EPA (20:5n-3) in rotifers enriched with *Nannochloropsis* sp. was highest but the EPA content in Sp1/2 and Sp1 was not significantly different (P>0.05). After 12h enrichment, the highest DHA (22:6n-3) level was observed in Sp1 treatment and was almost four times higher than the amount in *Nannochloropsis* sp. treatment. In this study, the amount of total saturated fatty acids in rotifers was not significantly affected by the S.presso enrichment (P>0.05), but the total polyunsaturated fatty acids content in the rotifers was significantly affected by this enrichment (P<0.05). The DHA/EPA ratio in rotifers was significantly affected by the enrichment (P<0.05, Table 1). The highest DHA/EPA ratio was observed in Sp1 treatment, and the lowest ratio in *Nannochloropsis* sp. enriched rotifers. The highest total polyunsaturated fatty acids was in rotifers enriched with the Sp1 treatment.

Table 1. Fatty acid composition (% of total fatty acids) of enriched rotifers

	Nannochloropsis	*Sp1/2*	*Sp1*
20:1n-9	2.85±0.36a	2.99±0.08a	2.74±0.13a
20:4n-6 (ARA)	2.18±0.07c	1.46±0.12a	1.78±0.03b
20:4n-3	2.21±0.05b	0.64±0.05a	0.68±0.04a
20:5n-3 (EPA)	9.05±0.45b	4.12±0.47a	4.02±0.17a
22:0	0.01±0.00a	0.28±0.22b	0.78±0.11c
22:1n-9	1.14±0.13b	0.82±0.13a	0.75±0.06a
22:5n-3 (DPA)	0.08±0.11a	1.29±1.56b	5.39±0.29c
24:0	0.00±0.00a	0.28±0.41b	0.65±0.05b
22:6n-3 (DHA)	4.11±0.09a	13.49±0.38b	18.23±0.68c
Total n-9	7.01±0.46c	6.01±0.18b	5.04±0.21a
Total n-6	9.48±0.73a	11.19±0.25b	12.20±0.18b
Total n-3	22.99±1.43a	22.00±2.03a	31.93±1.08b
Total saturated	24.37±1.12a	25.12±1.87a	25.57±0.93a
Total poly unsaturated	34.79±1.08a	34.64±1.82a	45.36±1.28b
DHA/EPA	0.45±0.21a	3.27±0.16b	4.53±0.32c
DHA/DPA	51.38±2.31c	10.46±1.23b	3.38±0.18a
EPA/ARA	4.15±0.16c	2.82±0.09b	2.26±0.11a
DPA/ARA	0.04±0.01a	0.88±0.06b	3.03±0.19c
Total lipids (mg/g)	8.32±3.21a	13.76±2.48a	14.88±2.97a

Different letters represent significant differences at P < 0.05.
Abbreviations: Sp1/2, S.presso half dose; Sp1, Spresso full dose; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; ARA, Arachidonic acid; DPA, Docosapentaenoic acid.

On 12 DPH, the fatty acid composition in yellowtail amberjack larvae was significantly affected by the nutrients enhancement (P<0.05, Table 2). The highest EPA was found in fish fed with *Nannochloropsis* sp. enriched rotifers, and the EPA in fish fed with Sp1/2 and Sp1 enriched rotifers was not significantly different (P>0.05). The highest DHA was observed in fish fed with Sp1 enriched rotifers, and the lowest DHA was found in fish fed with *Nannochloropsis* sp. enriched rotifers. The highest DHA/EPA ratio in fish was found in Sp1 treatment, and the lowest DHA/EPA ratio was observed in *Nannochloropsis* treatment. In this study, the total polyunsaturated fatty acid in fish from Sp1/2 and Sp1 was similar to that observed in the fertilized eggs of yellowtail amberjack (P>0.05).

Table 2. Fatty acid composition (% of total fatty acids) of fertilized eggs and 12 DPH fish larvae fed with rotifers enriched with *Nannochloropsis* sp. and S.presso.

	Fertilized egg	*Nannochloropsis*	*Sp1/2*	*Sp1*
20:1n-9	1.87 ± 0.03b	1.03 ± 0.21a	0.92 ± 0.52a	0.99 ± 0.26a
20:4n-6 (ARA)	1.93 ± 0.05a	4.68 ± 0.23c	3.49 ± 0.23b	3.75 ± 0.27b
20:4n-3	0.43 ± 0.07a	0.38 ± 0.09a	0.39± 0.14a	0.36 ± 0.05a
20:5n-3 (EPA)	5.28 ± 0.03b	8.01 ± 0.42c	3.19 ± 0.46a	2.61 ± 0.54a
22:0	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
22:1n-9	0.53 ± 0.08b	0.13 ± 0.22a	0.26 ± 0.13a	0.20 ± 0.13a
22:5n-3 (DPA)	1.63 ± 0.09a	2.38 ± 0.03b	1.34 ± 0.25a	1.07 ± 0.28a
24:0	0.58 ± 0.05a	1.25 ± 0.22b	1.00 ± 0.41b	0.85 ± 0.25b
22:6n-3 (DHA)	26.42 ± 0.19b	16.79 ± 0.69a	25.98 ± 0.95b	28.27 ± 0.98c
Total n-9	4.62 ± 0.06a	5.11 ± 0.29b	4.26 ± 0.31a	4.16 ± 0.38a
Total n-6	3.37 ± 0.07a	9.14 ± 0.15b	8.03 ± 0.16b	8.36 ± 0.14c
Total n-3	36.47 ± 0.21c	29.03 ± 0.35a	31.22 ± 0.81b	32.79 ± 0.76b
Total saturated	26.76 ± 0.26a	33.47 ± 0.58b	32.45 ± 0.78b	31.85 ± 0.64b
Total poly unsaturated	40.47 ± 0.29b	38.28 ± 0.87a	39.97 ± 1.02b	41.72 ± 0.74b
DHA/EPA	5.00 ± 0.61b	2.10 ± 0.23a	8.14 ± 0.09c	10.83 ± 0.11d
DHA/DPA	16.21 ± 2.23b	7.05 ± 1.15a	19.39 ± 1.09b	26.42 ± 3.31c
EPA/ARA	2.74 ± 0.07c	1.71 ± 0.23b	0.91 ± 0.17a	0.70 ± 0.25a
DPA/ARA	0.84 ± 0.06d	0.51 ± 0.11c	0.38± 0.07b	0.29± 0.05a
Total lipids (mg/g)	29.23 ± 4.68a	28.54 ± 5.21a	32.44 ± 5.21a	35.58 ± 6.21a

Different letters represent significant differences at $P < 0.05$.
Abbreviations: Sp1/2, S.presso half dose; Sp1, Spresso full dose; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; ARA, Arachidonic acid; DPA, Docosapentaenoic acid.

Discussion

In the present study, enrichment with S.presso did not alter the total lipid composition in rotifers. This result is consistent with previous studies (Diaz, et al., 1997; Fernández-Reiriz, et al., 1993; Ma & Qin, 2014; Palmtag, et al., 2006). However, fatty acid composition in rotifers was significantly affected by live food enrichment. The highest DHA/EPA ratio (4.53 ± 0.32) was found in rotifers enriched with Sp1, while rotifers enriched with *Nannochloropsis* sp. had the lowest DHA/EPA ratio (0.45 ± 0.21). Previous studies have demonstrated that unbalanced dietary fatty acids reduced the survival of fish larvae because unbalanced lipid composition affects fatty acid digestion and absorption (Diaz, et al., 1997; Salhi, et al., 1999; Watanabe, 1993). Although specific growth rate was not significantly different in the present study, higher survival was achieved in fish fed with Sp1 enriched rotifers. Lower survival observed in fish fed with *Nannochloropsis* sp. enriched rotifers is consistent with lower DHA/EPA ratio in the rotifers. Low survival in this treatment may have been cause by unbalanced lipid composition.

Previous studies suggested that the ideal diet for fish larvae should contain similar lipids to those found in the fertilized eggs (Sargent, et al., 1999). The optimal DHA/EPA ratio initially recommended was 2:1 for marine fish larvae (Sargent, et al., 1995). However, increasing evidence indicates that the optimum dietary DHA/EPA ratio for fish larvae varies between species (Copeman, et al., 2002; Harel, et al., 2002; Ma & Qin, 2014). In yellowtail kingfish *Seriola lalandi lalandi*, the optimum dietary DHA/EPA ratio is 4.2:1–4.8:1 (Battaglene & Cobcroft, 2007; Ma & Qin, 2014). In this study, the DHA/EPA ratio in fertilized eggs of yellowtail amberjack was 5.00 ± 0.61 and higher than the DHA/EPA ratio (0.45 ± 0.21) in the rotifers enriched with *Nannochloropsis*. Nevertheless it was not significantly different from the DHA/EPA ratio of rotifers enriched with Sp1 (4.53 ± 0.32). Low survival of fish fed rotifers enriched with *Nannochloropsis* and Sp1/2 suggests that rotifers should be enriched with a formula close to the nutrition composition of fertilized eggs or embryos.

In marine fish, DHA and EPA are essential to fish growth, and the ratio of DHA/EPA can also regulate fish growth (Rezek, et al., 2010; Rodriguez, et al., 1997). For instance, the DHA/EPA ratio of 1.4:1-0.3:1 in rotifers can improve the growth of gilthead sea bream *Sparus aurata* (Rodriguez, et al., 1997). However, in species such as yellowtail flounder *Limanda ferruginea* fed higher DHA/EPA ratio (8:1) rotifers grow faster than those fed lower DHA/EPA ratio (1.9:1) rotifers (Copeman, et al., 2002). In species such as yellowtail kingfish, Japanese flounder *Paralichthys olivaceus*, and turbot *Scophthalmus maximux*, dietary DHA/EPA ratio the impact on growth appears to be lower (Esteveza, et al., 1999; Furuita, et al., 1999; Ma & Qin, 2014). Similarly, the growth of yellowtail amberjack larvae was not significantly different when fed rotifers with DHA/EPA ratios ranging from 0.45-4.53.

Jaw malformations are common and have frequently been observed in artificially reared finfish (Cobcroft, et al., 2001; Ma, et al., 2016). Jaw malformation not only affects fish growth and survival but also reduces the market value of marine fish (Cobcroft, et al., 2004; Ma & Qin, 2014; Ma, et al., 2016). Polyunsaturated fatty acids are important in bone formation, and several studies have indicated that diet can affect the fatty acid composition in bone and cartilage (Izquierdo, et al., 2010; Liu, et al., 2004; Watkins, et al., 1997). As the source of dietary lipids is primarily from rotifers, enrichment formula on rotifers may possibly affect jaw malformation of fish larvae. In the present study, jaw malformation of fish larvae fed rotifers enriched with *Nannochloropsis* was significantly higher than those fed with S.presso enriched rotifers. This may suggest that the nutrition composition of *Nannochloropsis* enriched rotifers does not meet the nutritional requirement for jaw development of yellowtail amberjack larvae.

In summary, the present study evaluated the effect of live food enrichment on the rearing performance of yellowtail amberjack larvae. Fish growth was not significantly affected by the enrichment enhancements, but jaw malformation reduced when fish were fed S.presso full dose enriched rotifers.

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