Effects of Various Water Cultivation Regimes on Plankton Community in Grouper *Epinephelus coioides* Larviculture Ponds*

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Abstract: As a member of grouper family, *Epinephelus coioides* is one of the most commonly cage-cultured species in southern areas of China. Although groupers production are becoming more popular in worldwide markets, the aquaculture industry of this specie is still poorly developed when compared to other marine fish species aquacultured such as stripped bass, cobia, gilthead seabream. Despite of other factors affecting the expansion of cultured area for groupers, the price and steady provision of larvae are important determinants, which depend on effectiveness of the larval culture. Nowadays, grouper larvae for aquaculture in China are mostly from Hainan province due to its favorable conditions for larval culture such as good water quality, suitable temperature. However, mass production of grouper larvae is still encountering many difficulties, and high mortalities were often reported in grouper larvae culture, which affects its industry development. The low survival rate of grouper larvae cultured is due to the poor first-feeding conditions such as live prey deprivation or environmental stress. The aim of this paper is to compare and discuss effects of various water cultivation regimes on plankton community in grouper *Epinephelus coioides* larviculture ponds (2.5 m × 4.0 m × 1.1 m, W × L × H). Four cultivation regimes were designed. In group 1 (G1) and group 2 (G2), two different levels of prepared effective microorganisms solution (EMS) (40 mL · m^{-3} for G1 and 80 mL · m^{-3} for G2, respectively) and two different levels of shrimp chip (SC) (4 g · m^{-3} for G1 and 8 g · m^{-3} for G2, respectively) were daily added one week prior to hatching. Effective Microorganisms comprised of *Clostridiums*, *Photosynthetic bacterium*, *Lactobacillus*, *Saccharomyces* and *Nitrobacteria* species. In group 3 (G3) and group 4 (G4), only prepared *Platymonus* spp. solution (PS) was daily added one day prior to hatching at a level of 2.5 L · m^{-3} for G3 and a level of 5 L · m^{-3} for G4, respectively. Each group contained three replicates. Results showed that G1 and G2 had more species of phytoplankton and zooplankton than G3 and G4 did. Both phytoplankton and zooplankton populations' density could be significantly improved by EMS and SC additions. G2 had a high density of zooplankton populations ($1.3 \times 10^5 \sim 2.1 \times 10^5$ ind · L^{-1}) during the larval culture period. Zooplankton populations density in G1 and G2 were significantly higher than noted in G3 and G4 during the larval culture period ($P \leqslant 0.05$). In the present studies, high density of zooplankton populations observed in G2 indicates that effective microorganisms do have a positive role in improving zooplankton biomass of grouper experiment ponds. Additionally, a brown water environment came into being in ponds which been added shrimp chip and brown sugar. This may play a role in alleviating possible external stress to grouper larvae. Overall, abundant zooplankton biomass as live prey for grouper larvae growth

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would be constantly attained by additions of EMS and together with SC.

Key words: grouper *Epinephelus coioides*; plankton community; water cultivation regime; experiment pond

不同培水方式对斜带石斑鱼育苗池中浮游生物群落的影响

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摘　要：斜带石斑鱼在中国的养殖主要位于南方，养殖规模仍不及其他海水养殖鱼类，如卵形鲳鲹、海鲈、军曹鱼等。苗种培育成活率低是造成斜带石斑鱼养殖业发展滞后的主要“瓶颈”之一，这与石斑鱼仔鱼开口期缺乏适宜的生物饵料有关，尤其是浮游动物的缺乏。为此，文中设计了 4 种不同培水方式，研究其对斜带石斑鱼育苗池中浮游生物群落的影响。方式 1 中分别添加益生菌、虾片 40 $mL \cdot m^{-3}$ 和 4 $g \cdot m^{-3}$（组 1），方式 2 中分别添加益生菌、虾片 80 $mL \cdot m^{-3}$ 和 8 $g \cdot m^{-3}$（组 2），方式 3 中添加小球藻液 2.5 $L \cdot m^{-3}$（组 3），方式 4 中添加小球藻液 5 $L \cdot m^{-3}$（组 4）。每组设 3 个重复。育苗池规格 2.5 m ×4.0 m×1.1 m（宽×长×高）。结果表明，组 1 和组 2 中浮游植物、浮游动物种类数高于组 3 和组 4。益生菌和虾片的添加可显著提高育苗池中浮游植物及浮游动物密度。整个育苗过程中。组 2 所含浮游动物密度维持在较高水平（$1.3 \times 10^5 \sim 2.1 \times 10^5$ $ind \cdot L^{-1}$），组 1 和组 2 中浮游动物密度要显著高于组 3 和组 4（$P \leq 0.05$）。从实验中可看出石斑鱼育苗池中添加一定量的益生菌和虾片，可获得较丰富的浮游动物群落，为石斑鱼仔稚鱼生长提供活饵料。

关键词：斜带石斑鱼；浮游生物群落；培水；育苗池

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Groupers (Family: Serranidae), a very diverse family of carnivorous fish, are widely distributed throughout the tropical and subtropical seas of the world[1]. As a member of grouper family, *Epinephelus coioides* is one of the most commonly cultured species[2], especially in China. Although groupers production are becoming more popular in worldwide markets[1], the aquaculture industry of this species is still poorly developed when compared to other marine fish species aquacultured such as stripped bass, cobia, gilthead seabream. Despite of other factors affecting the expansion of cultured area for groupers, the price and steady provision of larvae are important determinants.

Over the past few years, the hatchery technology for grouper has gotten improved, which resulted in a rapid increase in grouper aquaculture production in the Asia-Pacific region[1,3-4]. However, mass production of grouper larvae is still encountering many difficulties, and high mortalities were often reported in grouper larvae culture[5-8].

The first-feeding is regarded as a critical point and improvement of the rearing conditions can play an important role in improving food intake, growth and survival of grouper larvae[9]. Taking into account that it has been widely reported to improve fish larval growth, survival and feed ingestion[10-11], ‘green water technique’ has been extensively used in larval culture of many marine fish species[3,12-14]. Though the effect of microalgae on fish larvae performance is not completely understood[15], providing live prey and a colored water environment may be the main functions. Green water environment has been proved to be beneficial to larvae performance for many fish species[15-18]. Apart from direct additions of microalgae, fertilization of ponds before stocking is also able to produce live prey and bring a green water environment[19-21].

Therefore, it is worth finding a new more effective approach to improving survival rates and growth of grouper larvae. Marine plankton communities are often looked as a stabilizer for fish larvae[22]. Hence, plankton composition and populations density play an important role in fish larvae culture[21,23-24]. Effective microorganisms have been reported to play a great role in natural and man-made aquatic ecosystems such as adjusting algal population, speeding up decomposition of organic matter[25-26], but it is unknown whether effective microorganisms will have an effect on plankton growth performance after they are added to grouper experiment ponds. The purpose of this study was to compare and discuss response of plankton composition, populations' density, respiration and primary productivity in grouper *Epinephelus coioides* experiment ponds under various additions of effective microorganisms' solution (EMS), shrimp chip (SC) and *Platymonus spp* solution (PS).

1 Materials and methods

1.1 Preparation of effective microorganisms and *Platymonus* spp. solutions and experiment ponds

Before the experiment ponds' filling, ten litre of commercially available effective microorganisms (Dongfang Ocean Biology Corporation, Ltd, Haikou city, China) were co-cultured with 20 L clean seawater and 2 kg brown sugar in an enclosed polyethylene bucket. Effective microorganisms were comprised of *Clostridiums*, *Photosynthetic bacterium*, *Lactobacillus*, *Saccharomyces* and *Nitrobacteria* species, and total concentration of active bacterium contained was 1.0×10^8 cells · mL^{-1}. The *Platymonus* spp. were produced by standard protocols in an alga lab of Ocean Colledge of Hainan University, and the concentration of *Platymonus* spp. solution used was 8.0×10^6 cells · mL^{-1}. The prepared effective microorganisms'solution (EMS), shrimp chip (SC) (Chengdian Feed and Oil Corporation, Shaoan County of Haikou City, China) and *Platymonus* spp. solution (PS) was used as materials for water cultivation.

All experimental ponds were totally drained, sundried and flooded, and water was flushed out to clean the pond bed. After disinfected by calcium hypochlorite (60% chlorine) at 20 mg · L^{-1}, twelve 11 m^3 concrete grouper experiment ponds (2.5 m × 4.0 m × 1.1 m, W × L × H) were randomly distributed to different treatments, and each treatment had three replicates. Each of the experiment ponds was provided with 12 air stones connected to low-pressure electrical blowers, and dissolved oxygen (DO) levels were maintained at saturation. Water temperature was measured daily and was maintained at (27 ± 1.0)℃. The salinity kept at a 23.5 ~ 24.5 g · L^{-1} level. Light was applied 24 h a day by fluorescent light tubes.

For PS groups, experiment ponds were filled with clean sand-filtered seawater to an 80 cm depth and incubated *Platymonus* spp. solution equivalently twice daily at 08:00 and 15:00 one day before hatching until the end of the experiment. For EMS + SC groups, experiment ponds were added clean seawater to the same depth as PS groups one week before hatching, and subsequently, prepared effective microorganisms' solution (EMS) and shrimp chip (SC) were added equivalently by hand twice daily at 08:00 and 15:00 until the end of the experiment. The details of different water cultivation regimes are presented in Table 1.

Table 1 Details of the daily adding schedule of different water cultivation regimes

Materials	Group 1	Group 2	Group 3	Group 4
Effective Microorganisms1) solution / (mL · m^{-3})	40	80	0	0
Shrimp chip / (g · m^{-3})	4	8	0	0
Platymonus spp. Solution / (L · m^{-3})2)	0	0	2.5	5

1) Effective Microorganisms comprised of *Clostridiums*, *Photosynthetic bacterium*, *Lactobacillus*, *Saccharomyces* and *Nitrobacteria* species, with a total active bacterium concentration of 1.0×10^8 cells · mL^{-1};
2) *Platymonus* spp. solution with a concentration of 8.0×10^6 cells · mL^{-1}

1.2 Larval feed

After experiment ponds'filling, eggs from a grouper-spawning cage in Hongsha Bay of Shanya city of China were hatched at a density of 1.0×10^4 ind · m^{-3}. One day after hatching, rotifers (*Brachionus plicatilis*) were daily added to reach a density of 2×10^4 ind · L^{-1} till the end of the experiment.

1.3 Water sampling and measurements

Water was sampled at two locations in each experiment pond on 2, 7, 12 and 17 DPH, respectively. Soluble reactive phosphate (SRP) was analyzed by colorimetry after reaction with ammonium molybdate and stannous chloride[27]. Total NH_3-N of analyses were conducted according to methods described byAPHA [28].

Water samples for phytoplankton were collected with a 100 mL plastic bottle, and samples were preserved with 1% Lugol's iodine solution. Quantitative analysis of phytoplankton was done using a haemacytometer and a compound microscope. Phytoplankton was identified under a compound light microscope using keys and illustrations by Stafford [29] and Prescott [30] and other phycological taxonomic books.

Zooplankton samples were collected by taking a standard plankton net of 1 m length and mesh size of 63 μm fitted with a flowmeter (Hydrobios, Kiel) at two locations in each experiment pond. Samples were preserved in 240 mL of buffered formalin-sucrose solution prior to enumeration by light microscopy[31]. All organisms in three 1 mL subsamples from each pond were counted by using a Sedgwick-Rafter counting cell as described by Geiger & Turner [31] and identified with the taxonomic keys of Thorp & Covich[32].

1.4 Statistic analysis

All data are presented as means ± S. D. and subjected to one-way analysis of variance (ANOVA) to test the effects of experiment treatments using the software of the SPSS (version 11.5) for windows. Duncan's multiple range test was used to resolve the differences among treatment means[33]. Differences among means were considered significant at $P \leqslant 0.05$.

2 Results

2.1 Response of water quality in grouper experiment ponds

Changes in water quality were presented in Table 2. On 17 DPH, total NH_3-N level (3.41 μmol · L^{-1}) in G2 was significantly higher than that in other groups ($P \leqslant 0.05$). In all groups, total NH_3-N value increased with an increasing of larvae culture time. G4 exhibited significantly higher SRP levels than other groups did during the whole experiment period ($P \leqslant 0.05$). SRP levels were significantly improved by an increment of EMS + SC or PS ($P \leqslant 0.05$).

2.2 Response of plankton composition and populations density in grouper experiment ponds

Phytoplankton and zooplankton compositions observed in all groups were shown in Table 3 and Table 4, respectively. G2 had more abundant and complex plankton composition than other groups did. Thirteen different phytoplankton species and 16 different zooplankton species were observed in G2. On 2 DPH, phytoplankton populations density in G3 (381.85×10^4 cell · L^{-1}) and G4 (563.25×10^4 cell · L^{-1}) were significantly higher than values in G1 (66.10×10^4 cell · L^{-1}) and G2 (141.35×10^4 cell · L^{-1}) ($p \leqslant 0.05$) (Fig. 1). On 7, 12 and 17 DPH, differences in phytoplankton populations density between G2, G3 and G4 were not significant ($p > 0.05$). G2 showed the highest zooplankton populations density (138.74×10^3 ind · L^{-1} on 2 DPH, 155.90×10^3 ind · L^{-1} on 7 DPH, 178.50×10^3 ind · L^{-1} on 12 DPH and 208.62×10^3 ind · L^{-1} on 17 DPH, respectively) among all groups (Fig. 2), and differences were significant ($P \leqslant 0.05$) compared to values in G3 and G4 during the whole experiment period.

Table 2 Changes in water quality in grouper larviculture ponds under different water cultivation regimes1)

	Group 1	Group 2	Group 3	Group 4
Total NH_3-N/ (μmol · L^{-1})				
2 DAH	1.33 ±0.01^a	1.15 ±0.20^a	0.79 ±0.18^b	0.58 ±0.09^b
7 DAH	1.26 ±0.07^a	1.24 ±0.03^a	1.33 ±0.11^a	0.95 ±0.04^b
12 DAH	1.14 ±0.12^c	1.15 ±0.13^c	2.37 ±0.31^a	1.94 ±0.08^b
17 DAH	2.18 ±0.59^b	3.41 ±0.24^a	2.10 ±0.12^b	2.20 ±0.19^b
SRP/ (μmol · L^{-1})				
2 DAH	0.034 ±0.000^d	0.072 ±0.000^c	0.101 ±0.013^b	0.127 ±0.003^a
7 DAH	0.082 ±0.024^b	0.098 ±0.031^b	0.164 ±0.001^a	0.195 ±0.004^a
12 DAH	0.100 ±0.012^d	0.144 ±0.012^c	0.185 ±0.008^b	0.295 ±0.002^a
17 DAH	0.173 ±0.027^c	0.286 ±0.044^b	0.253 ±0.035^{bc}	0.396 ±0.031^a

1) Values are means ± S. D. of three replicates and values within the same row with different letters are significantly different ($P \leqslant 0.05$)

Table 3 Changes in phytoplankton composition in grouper larviculture ponds under different water cultivation regimes1)

Phytoplankton species	Group 1	Group 2	Group 3	Group 4
Chlorella vulgaris	+	+	+	+
Nannochloropsis oculata	+	+	+	+
Navicula sp.	+	+	+	+
Cocconeis scutellum	+	+	−	+
Skeletonenma costatum	+	+	−	−
Nitzschia sp.	+	+	−	−
Cryptophyta sp.	+	+	+	+
Melosira sp.	+	+	+	−
Biddulphia sp.	−	+	+	+
Amphora sp.	−	+	−	−
Diploneis sp.	+	+	−	−
Prorocentrum sp.	+	+	−	−
Diatoma sp.	+	+	+	+
Platymons subcordiforiformis	−	−	+	+
Chaetoceros sp.	−	−	−	+

1) ' + ' means observed, and ' − ' means not observed

Table 4 Changes in zooplankton composition in grouper larviculture ponds under different water cultivation regimes[1)]

Zooplankton species	Group 1	Group 2	Group 3	Group 4
Cyclidium litomesum Stoke	+	+	+	+
*Gymnodinium coeruleum*Doyiel	+	+	+	+
Mesodimium sp.	+	+	+	+
Strobilidium gyrans	+	+	+	+
Euplotes eurystoma	+	+	+	+
Halteria sp.	+	+	+	+
Actinophrys sp.	+	+	−	−
Strombidinopsis sp.	+	+	−	−
Heterotrophic flagellates	+	+	+	+
Globigerina sp.	+	−	−	−
Brachionus sp.	+	+	+	+
Peridinium sp.	+	+	+	+
Calanus sinicus	+	+	+	+
Oxytricha sp.	−	+	−	−
Prorodonvi ridis	−	+	−	−
Lacrymaria sp.	−	+	−	−
Holophrya atra	−	+	−	−
Chilodonella calkinsi	−	−	+	−
Trachelocerca tenuicollis	−	−	−	+

1) ‘ + ’ means observed, and ‘ − ’ means not observed

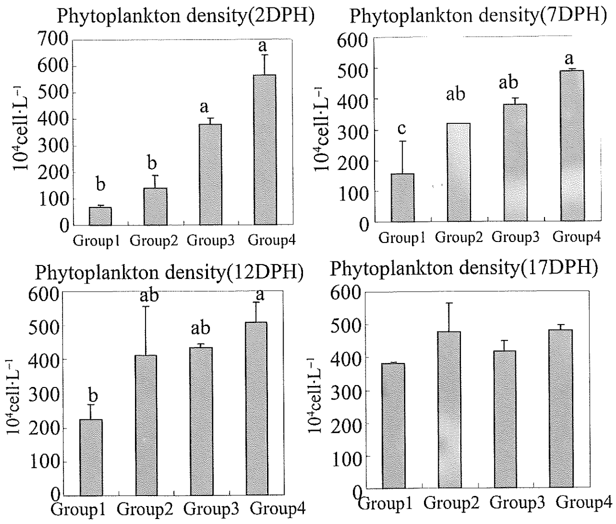

Fig. 1 Changes in phytoplankton density in grouper larviculture ponds under different water cultivation regimes. Values within the same number of DPH with different letters are significantly different ($P \leq 0.05$)

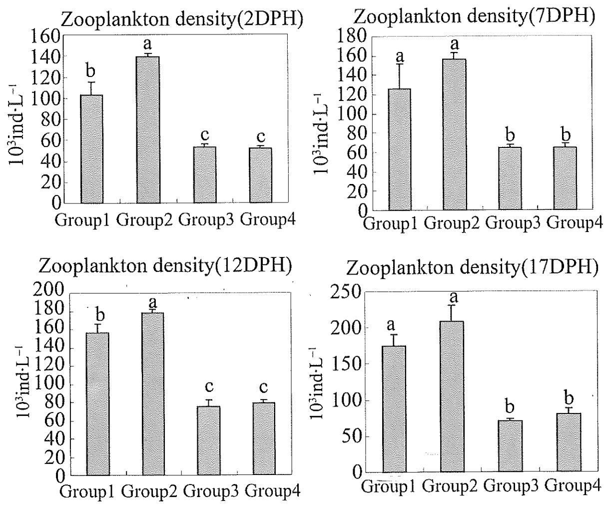

Fig. 2 Changes in zooplankton density in grouper larviculture ponds under different water cultivation regimes. Values within the same number of DPH with different letters are significantly different ($P \leq 0.05$)

3 Discussion

In concrete grouper *Epinephelus coioides* experiment ponds, abundant plankton community, especially zooplankton community, would be produced by additions of EMS and together with SC. Water cultivation to attaining abundant plankton community has been widely regarded as a necessary procedure for fish larvae culture[21]. Effects of effective microorganisms on aquatic animals' immunity and water quality have been studied by some researchers[25-26,34], but in fish experiment ponds, plankton growth performance responsible for effective microorganisms is less concerned. In the present study, high density of zooplankton populations observed in G2 indicates that effective microorganisms do have a positive role in improving zooplankton biomass of grouper experiment ponds.

It has been reported that early survival of aquacultured grouper larvae is very low compared to other finfish[35-37], which may be due to the poor first-feeding conditions such as live prey deprivation or environmental stress[38]. Live zooplankton has been regarded as the main source of larval food for marine fish[24,39]. In this study, G2 had more complex plankton composition and significantly higher zooplankton populations' density than other groups did. This indicates that water cultivation regimes of G2 are more beneficial to grouper larvae survival and growth compared to other regimes. Additionally, a brown water environment came into being in ponds which been added shrimp chip and brown sugar. This may play a role in alleviating possible external stress to grouper larvae.

From 7 DPH, the phytoplankton populations' density of G2 reached a high level as G4. This shows that effective microorganisms' solution would also enhance phytoplankton biomass. These phytoplankton may provide enough live feed for zooplankton thriving. During the larval culture period, zooplankton populations' density observed in G3 and G4 were low, which indicates that *Platymonus* spp. solution did not have an obvious effect on zooplankton biomass.

The increased total NH_3-N and soluble reactive phosphate (SRP) concentrations observed in all groups may indicate that there have N and P accumulations in experiment ponds. In this study, there was a positive relationship between water SRP levels and phytoplankton population density. These results show that SRP is one of the limiting factors affecting water phytoplankton biomass, which was in accordance with the report of[31].

In conclusions, for concrete grouper *Epinephelus coioides* experiment ponds, abundance of phytoplankton and zooplankton community could be attained by additions of EMS and together with SC. We suggest filling concrete grouper experiment ponds a week before hatching and adding EMS and SC at daily levels of 80 $mL \cdot m^{-3}$ and 8 $g \cdot m^{-3}$, respectively. It is possible that regimes of water cultivation for grouper *Epinephelus coioides* proposed in the present study are also of practical use for other marine fish larvae culture. Further works should be required to consider the mechanism of foodchain variations in fish-culturing ponds.

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