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Early weaning of yellowtail amberjack *Seriola lalandi dorsalis* (Gill 1863) larvaeJing Hu^{1,2}, Rui Yang^{1,2}, Xu Chen^{1,2}, Qibin Yang^{1,2}, Zhenhua Ma^{1,2*}, Gang Yu^{1,2*}¹Tropical Aquaculture Research and Development Centre, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Sanya 572018, China²Key Laboratory of South China Sea Fishery Resources Exploitation and Utilization, Ministry of Agriculture, Guangzhou 510300, China

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ABSTRACT

Objective: To understand the effect of weaning time on the rearing performance of yellowtail amberjack *Seriola lalandi dorsalis* in the experimental condition.**Methods:** The same weaning protocol started on four different days of post hatching (DPH), including 12 DPH (W12), 15 DPH (W15), 18 DPH (W18), and 21 DPH (W21), respectively. Growth, survival, and jaw malformation were used as the assessment criteria to evaluate the impact of weaning time on the performance of yellowtail amberjack larvae.**Results:** The highest specific growth rate was observed in W21 treatment, and the lowest specific growth rate was found in W12 treatment. The highest survival was achieved in W21 treatment, and the lowest survival was recorded in W12 treatment. With postponing the weaning started time, jaw malformation rate significantly reduced. At the end of this study, the highest malformation rate was observed in W12 treatment, and the malformation rate was not significantly different in W15, W18, and W21 treatment.**Conclusions:** Base on the results obtained in this study, we suggest that weaning of yellowtail amberjack larvae should be started from 15 DPH.

1. Introduction

In larval fish rearing, weaning is a process to gradually replace live feeds with artificial diets. In practice, weaning of most temperate marine fish species such as *Trachinotus ovatus* and *Seriola lalandi* are usually commenced after metamorphosis[1,2]. Previous studies have demonstrated that early introduction of micro-particles diets to marine fish larvae can have positive effects on fish adapting to the micro-particles diets[3,4]. But premature introduction of the micro-particles diets can also inhibit fish growth and survival as fish

cannot digest the artificial diets when their digestive system is not properly developed[2,5,6]. To overcome the barriers of poor digestive ability associating to the artificial diets in early development of fish larvae, the co-feeding protocol for fish larvae with live feeds and artificial diets has been developed in marine fish hatchery. Co-feeding protocol can nutritionally pre-condition fish larvae to adapt the artificial diet when live feeds are gradually withdrawn in the weaning period[2,7,8]. By applying co-feeding protocol in weaning of species such as red drum *Plectropomus leopardus*[7], and golden pompano *Trachinotus ovatus*[2], significant improvement of growth and survival has been reported.

The yellowtail amberjack [*Seriola lalandi dorsalis* (*S. lalandi dorsalis*)] belongs to the Carangidae family and is widely distributed throughout warm-temperate waters of the Northern Hemisphere. It has been introduced as a new species for aquaculture due to its fast growth, high flesh quality and suitability for cage culture for 20 years. However, low survival and unreliable fingerling quality have greatly hindered the fingerling production of yellowtail amberjack in China. Up to present, several key issues regarding to the larval rearing of this species have not been solved. High mortality in the weaning period has severely hindered the production efficiency of yellowtail amberjack. The objective of this study was to determine the weaning time by using a co-feeding protocol in yellowtail

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The handle of fish was carried out in strict accordance with the recommendation in the Animal Welfare of Chinese Academy of Fishery Sciences Animal Welfare Committee. The protocol, species and number of animals used in this study were approved by the South China Sea Fisheries Research Institute Animal Welfare Committee (Approved Number: A201601A01).

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amberjack larvae to improve fish growth and survival. Results from the present study will provide practical guide for rearing of yellowtail amberjack in hatchery.

2. Materials and methods

2.1. Ethical approval

In this study, the handle of fish was carried out in strict accordance with the recommendation in the Animal Welfare of Chinese Academy of Fishery Sciences Animal Welfare Committee. The protocol, species and number of animals used in this study were approved by the South China Sea Fisheries Research Institute Animal Welfare Committee (Approved Number: A201601A01).

2.2. Experimental design and larval fish rearing

Fertilized eggs were obtained from a local hatchery in Lingshui Town, Hainan Province, China and transported to the Tropical Aquaculture Research and Development Centre, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. Upon arrival, all eggs hatched in 500 L fiberglass incubators at 23.5 °C. After hatching, the larvae were stocked into the 1000 L fiberglass rearing tanks at a density of 60 larvae/L per tank. All rearing tanks were supplied with filtered seawater with a 6-µm filter in a flow-through system at a daily water exchange rate of 300% tank volume. Three air stones were used in each tank to maintain dissolved oxygen at saturation and to homogenize the distribution of microalgae, rotifers and *Artemia nauplii* (*A. nauplii*). Light intensity at 2400 lx and a photoperiod of 14 h light and 10 h dark was used. Salinity was maintained at 36‰ throughout the experiment.

Rotifers (*Brachionus plicatilis*) were fed to the 3-days post hatching (DPH) larvae until 13 DPH at 10 rotifers/mL. The rotifers fed with microalgae (*Nannochloropsis* sp.) were enriched with DHA Selco (INVE Aquaculture) for 12 h before adding into fish rearing tanks. Instant microalgae (*Nannochloropsis* sp.) was also added into the larval rearing tanks as food for rotifers, and to create a green background for fish larvae. Starting from 8 DPH, *A. nauplii* were enriched with DHA Selco (INVE Aquaculture) before they were introduced into the larval rearing tanks at 5 nauplii/mL. On 8 DPH, fish larvae were harvested and restocked to 12 500 L fiberglass tanks at a density of 40 larvae/L.

2.3. Experimental design and operating protocols

In this study four co-feeding and weaning treatments were tested (Figure 1), including co-feeding of micro-particulate diets starting from 12 DPH (W12), 15 DPH (W15), 18 DPH (W18), and 21 DPH (W21). In each treatment, feeding protocol was designed as a 5-days

co-feeding period, in which fish larvae were fed with live feeds and micro-particulate diets, the amount of live feeds in the co-feeding period was gradually reduced to zero while the amount of micro-particulate diets was increased. Live feeds were added 3-times per day at 08.00, 12.00, and 16.00 h to maintain the targeted live feeds densities. Before adding to the larval fish rearing tanks, rotifers and *A. nauplii* were enriched with commercial enrichments following the same protocols described in the above section. Three sizes of the micro-particulate diets were used starting from small to larvae, including Otohimi A1 (~250 µm, Marubeni Nisshin Feed Co., Ltd., Tokyo, Japan), Huacheng No. 3 (360–600 µm), and Huacheng No. 5 (850–1,100 µm, Huacheng Aquaculture, P.R. China). The micro-particulate diets were fed to fish by hand at 1-h interval from 07.00 h to 17.00 h daily, and the amounts of feed were adjusted to reach the level of apparent feeding satiation. During the experimental period, each tank bottom was cleaned daily by siphoning the bottom of the tank to remove dead fish, uneaten food and feces.

2.4. Growth and survival measurement

On each of the sampling days, 10 larvae were randomly collected from each rearing tank to measure fish growth. Fish were anaesthetized with Aqui-S (AQUI-S New Zealand Ltd., Lower Hutt, New Zealand) and measured under a stereo microscope at 10× magnification. Growth was determined by the specific growth rate (SGR) as %/day using the following equation:

$$SGR = 100 \times (\ln SL_f - \ln SL_i) / \Delta t$$

where SL_f and SL_i were the final and initial fish standard length (mm), respectively, and Δt was the time between sampling intervals. Mortality were recorded daily by counting dead fish on the bottom of each tank.

2.5. Jaw malformation assessment

At the end of this study, 50 larvae from each rearing tank were randomly collected and anaesthetized with Aqui-S and analyzed directly under a stereo microscope using the criteria described by Cobcroft and Battaglene[9]. Jaw morphology of fish larvae was categorized as normal jaw and malformed jaw based on the observations of misalignment maxilla and premaxilla, and elongation or reduction in the lower and upper jaws.

2.6. Statistical analysis

The data in this study were expressed as mean ± SD and tested by One-way ANOVA (SPSS 20.0). When a significant treatment effect was found, Tukey’s test was performed for multiple range comparisons with the level of significant difference set at $P < 0.05$. All the data were tested for normality, homogeneity and independence to satisfy the assumptions of ANOVA.

DPH	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
W12	Artemia			Co-feeding					Weaning					Post-weaning												
W15	Artemia				Co-feeding					Weaning					Post-weaning											
W18	Artemia							Co-feeding					Weaning					Post-weaning								
W21	Artemia										Co-feeding					Weaning										

Figure 1. Weaning design used in this study.

W12: Co-feeding and weaning from 12 DPH; W15: Co-feeding and weaning from 15 DPH; W18: Co-feeding and weaning from 18 DPH; W21: Co-feeding and weaning from 21 DPH.

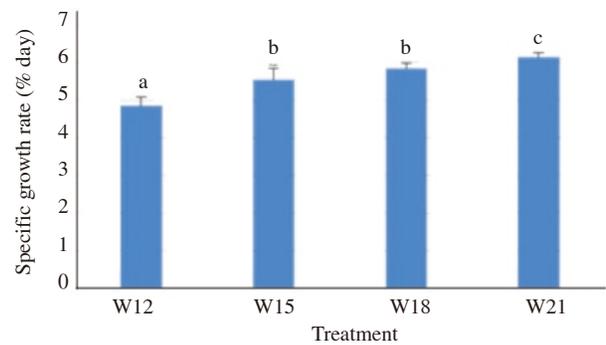
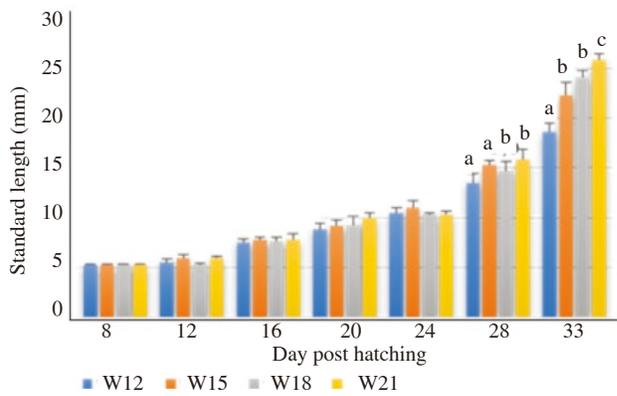


Figure 2. Standard length and specific growth rate of yellowtail amberjack larvae at different weaning time.

W12: Co-feeding and weaning from 12 DPH; W15: Co-feeding and weaning from 15 DPH; W18: Co-feeding and weaning from 18 DPH; W21: Co-feeding and weaning from 21 DPH. Different letters represent significant difference ($P < 0.05$).

3. Results

On 8 DPH, the mean standard length of fish larvae was (5.26 ± 0.14) mm. Upon weaning trial started on 12 DPH, the mean standard length of fish larvae reached to (5.58 ± 0.33) mm (Figure 2). At the end of the experiment, the highest standard length was obtained in W21 treatment, and lowest standard length was observed in W12 treatment ($P < 0.05$). The specific growth rates of fish larvae in W15 and W18 were (5.54 ± 0.29)/day and (5.85 ± 0.13)/day, respectively, which were significantly lower than those in the treatment of W21 ($P < 0.05$, Figure 2). At the end of this experiment, the lowest SGR was observed in the treatment of W12 [(4.85 ± 0.22) /day, $P < 0.05$]. The highest final survival was achieved in the treatment of W21 [(10.08 ± 1.17) %, Figure 3]. The survival of fish larvae in the treatment of W15 and W18 was not significantly different ($P > 0.05$). In this study, the lowest survival was observed in the treatment of W12, only 5.32 ± 0.43 fish survived at the end of this experiment ($P < 0.05$, Figure 3).

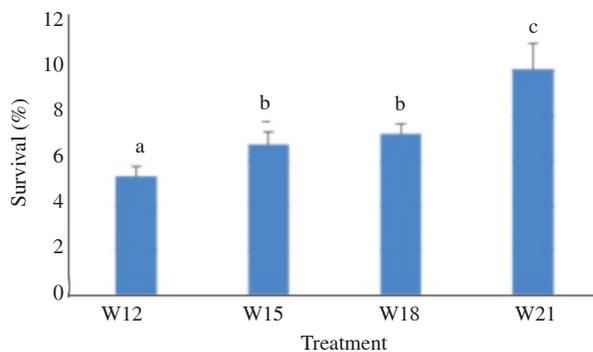


Figure 3. Survival of yellowtail amberjack larvae at different weaning times.

W12: Co-feeding and weaning from 12 DPH; W15: Co-feeding and weaning from 15 DPH; W18: Co-feeding and weaning from 18 DPH; W21: Co-feeding and weaning from 21 DPH. Different letters represent significant difference ($P < 0.05$).

Weaning time significantly affect the jaw malformation of yellowtail amberjack larvae ($P < 0.05$, Figure 4). The lowest survival was observed in the treatment of W21 [(10.99 ± 1.54) %, and the highest jaw malformation was recorded in the treatment of W12 [(21.35 ± 3.75) %, $P < 0.05$]. Jaw malformation in the treatment of W15 and W18 was not significantly different ($P > 0.05$, Figure 4).

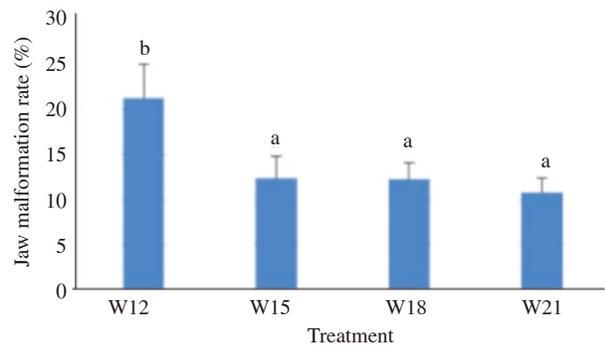


Figure 4. Jaw malformation of yellowtail amberjack larvae at different weaning times.

W12: Co-feeding and weaning from 12 DPH; W15: Co-feeding and weaning from 15 DPH; W18: Co-feeding and weaning from 18 DPH; W21: Co-feeding and weaning from 21 DPH. Different letters represent significant difference ($P < 0.05$).

4. Discussion

In hatchery, weaning marine fish larvae from live feeds to artificial diets can fulfill the increasing demand of fish larvae for food intake and nutrition requirement. In practice, high mortality can occur during the weaning period when unsuitable weaning protocol applied leading to serve economic losses for finfish hatchery. Evidence has shown that early introduction of artificial diets to fish larval can make fish easily adapt to it, but too early to introduce the artificial diets have negative effect on fish growth and survival[6,10,11]. Early introduction of artificial diets can also reduce the amount of usage of *A. nauplii*, which can significantly reduce the production costs. This is important for yellowtail amberjack larvae rearing as the live feeds consumption is higher when fish reaching to the metamorphosis.

Weaning of marine fish larvae can affect the growth of fish larvae[2,12,13]. Unsuitable weaning time and protocol can cause starvation of fish larvae[1,6]. Within the weaning period, fish may use their body energy to maintain basic metabolism and allocate less energy to growth when fish cannot obtain enough nutrition. Once this scenario occurred, the growth rate of fish larvae during weaning period will be reduced significantly. This phenomenon has been observed in species such as Senegalese sole[12] and *Paralabrax maculatofasciatus*[14]. In the present study, weaning time significantly affected the growth of yellowtail amberjack

larvae. The highest specific growth rate was observed in W21 treatment, and lowest SGR was observed in W12 treatment. This result was similar to our previous study in yellowtail kingfish *Seriola lalandi*[1]. Compared to W12, higher SGR in W15, W18, and W21 treatment may correspond to the functional development of the digestive system in this species[1,15].

As the most efficient weaning protocol developed in finfish hatchery, co-feeding of live feeds with artificial diets has been proved to be the most suitable method to improve fish survival during weaning period[7]. By employing co-feeding strategy in weaning of species such as haddock *Melanogrammus aeglefinus* larvae and alligator *Atractosteus spatula* larvae, survivals are significantly improved[16,17]. In this study, co-feeding and weaning of yellowtail amberjack larvae were successfully achieved. The overall survival was above 5%, and the highest survival rate was 10.08% which was significantly higher than the results reported in yellowtail kingfish larvae[1].

In many fish species, early weaning of fish larvae may reduce the quality of fish, and skeletal malformations have been frequently reported in this process[3,5]. The cause of skeletal malformations in early weaning larvae may be due to the malnutrition supply to fish larvae and early cannibalisms (mechanical damage) during the weaning process[2,18,19]. In the present study, jaw malformation of fish larvae in the W12 treatment was significantly higher than other treatments. Such high malformation rate in W12 treatment may cause by the early weaning associated malnutrition supply, and suggest that the weaning time of 12 DPH may be too early for yellowtail amberjack larvae. Since we didn't examine the nutrition requirement of fish larvae during the weaning process, future study should be carried out toward quantify the nutrient requirement of yellowtail amberjack during weaning period to improve the fingerlings quality.

In conclusion, this study explored the impact of weaning time on the rearing performance of yellowtail amberjack larvae. Growth, survival, and jaw malformation evidence suggest that weaning of yellowtail amberjack larvae can be commenced on 15 DPH, and a better weaning performance can be achieved when weaning started on 21 DPH.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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