



# Feeding habits of hatchery-reared larvae of greater amberjack *Seriola dumerili*

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## ABSTRACT

To obtain basic knowledge on the feeding habits of greater amberjack *Seriola dumerili* larvae during the early phase of seed production, larvae were reared until 15–20 days post hatching. Larvae were fed on rotifers *Brachionus plicatilis* sp. complex with different body sizes, i.e., super small type, small type, and large type rotifers, and the survival rate, standard body length (SBL) and gut contents of the larvae were examined. There were no significant effects of rotifer size on survival and growth of greater amberjack larvae, except for the smallest SBL of larvae fed with super small type rotifers. The body sizes (lorica length and lorica width) of rotifers ingested by larvae did not change in accordance with the growth in larval body size and mouth size from the onset of feeding to ~7.6 mm SBL, despite the larvae having a chance to prey on larger body size rotifers in the tanks. Counting the number of rotifer eggs in the guts revealed that larvae  $\geq 4.5$  mm SBL appeared to selectively prey on egg-bearing rotifers, whose body sizes are relatively large among the rotifer populations in the tanks, indicating an ontogenic change in the feeding habit of greater amberjack larvae.

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## 1. Introduction

Greater amberjack *Seriola dumerili* (Risso, 1810) is a marine pelagic fish species with a circumglobal distribution throughout warm and tropical waters (Ochiai and Tanaka, 1986; Thompson et al., 1999). This species has been targeted for aquaculture in Japan and the Mediterranean region because of its high growth rate, excellent flesh quality and high commercial value (Masuma et al., 1990; Tachihara et al., 1993; Mazzola et al., 2000; Nakada, 2002; Mylonas et al., 2004; Papandroulakis et al., 2005; Jerez et al., 2006; Mushiaki, 2006; Takakuwa et al., 2006). Aquaculture of this species began in the 1960s in Japan and was reliant on wild juveniles caught around southern Japan for seed (Takaoka, 2005). In 1986, wild juveniles of greater amberjack caught in the South China Sea were first imported to Japan for aquaculture seed; since then, these imported seed have supported the development of an aquaculture industry for this species (Nakada, 2002; Takaoka, 2005; Mushiaki, 2006). However, there is a great need to develop hatchery technology for mass seed production of greater amberjack. This is because in the spring of 2005, imported juveniles for aquaculture were found to be infected with larval anisakid nematodes, the adults of which can be hazardous to humans

(Mushiaki, 2006; Yoshinaga et al., 2006). In addition, larval survival rates remain low, and mass culture techniques for larval greater amberjack have not been fully developed (Seoka et al., 2000; Shiozawa et al., 2003; Mushiaki, 2006).

Knowledge of the optimal feeding regime for rearing larvae is essential for the successful seed production of fish. Feeding success of fish larvae can be affected by species, density and size of prey organisms (Puvanendran and Brown, 1999; Dou et al., 2000; Olsen et al., 2000; Shaw et al., 2003; Georgalas et al., 2007), as well as by environmental factors such as light intensity, photoperiod and water turbidity (Cobcroft et al., 2001; Puvanendran and Brown, 2002; Carton, 2005; Teruya et al., 2008). Further, it has been reported that the maximal and optimal size of prey ingested by larvae is correlated to varying degrees, depending on the species, with larval body size and mouth size (Shirota, 1970; Oozeki et al., 1992; Cunha and Planas, 1999; Krebs and Turingan, 2003; Shaw et al., 2003; Akazawa et al., 2008). Live feed organisms used for larviculture of greater amberjack are small strain rotifers and/or large strain rotifers of *Brachionus plicatilis* sp. complex (see Hagiwara et al., 2007 for terms of rotifers morphotypes), and brine shrimp *Artemia* spp. (Seoka et al., 2000; Shiozawa et al., 2003). Live feed protocols should be based on understanding the feeding habits of larvae; however, these are not well known for greater amberjack. Empirically, prey with different body sizes are sequentially fed in accordance with larval growth in the

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seed production of greater amberjack (Seoka et al., 2000; Shiozawa et al., 2003).

The aim of the present study was to obtain basic knowledge on the feeding habits of greater amberjack larvae during the early phase of seed production. We elucidated the ontogenetic changes in prey preference by examining the relative growth of mouth size and prey size in the gut of greater amberjack larvae fed with rotifers of different body sizes.

## 2. Materials and methods

### 2.1. Broodstock and egg incubation

The wild caught 0+ aged greater amberjack were reared to reach sexual maturity in a rectangular net cage (5–10 m wide, 5–10 m long and 5–10 m deep) at the Komame Branch, Stock Enhancement Technology Development Center, National Research Institute of Aquaculture, Kochi Prefecture, Japan. The fish were fed to satiation 3 days a week with defrosted raw mackerel, jack mackerel and squid. The 4+ age mature fish, four males and four females, with an average weight of 12.3 kg in 2006 and 12.8 kg in 2007, were transferred to an indoor octagonal tank (60 m<sup>3</sup>) for spawning during the breeding season from April to July in 2006 and 2007. Sand-filtered seawater was supplied to the tank at the rate of eight turnovers per day. Water temperature was regulated at around 22 °C using a heating system and the photoperiod was allowed to fluctuate naturally during the rearing period.

The fish were allowed to spawn naturally after being treated with human chorionic gonadotropin (HCG) (ASKA Pharmaceutical Co. Ltd., Tokyo, Japan) injected at a dosage of 600 IU/kg of body weight. The fish were unfed after HCG treatment. A total of 165,000 (cohort 1) and 281,600 (cohort 2) buoyant eggs, spawned on April 26, 2006 and May 30, 2007, respectively, were transferred to the Stock Enhancement Technology Development Center, National Research Institute of Aquaculture, Oita Prefecture, Japan. They were then incubated in 1000 L cylindrical tanks until 1–2 days post hatching (DPH) under a controlled temperature of ~23 °C with a flow-through system of sand-filtered seawater disinfected by ozone (12 L/min) and aeration (1.5 L/min). The fertilization rate to buoyant eggs and hatching rate to fertilized eggs of incubated eggs were 57.6% and 52.3% for cohort 1, and 98.8% and 97.2% for cohort 2, respectively. Larvae from cohorts 1 and 2 were used for larval rearing in experiments 1 and 2, respectively.

**Table 1**  
Water temperature, DO and pH of larval rearing water during the course of experiments

Expt. no.	Tank	Water temperature (°C)		DO (mg O <sub>2</sub> /L)		pH	
		Mean	SD	Mean	SD	Mean	SD
1	SS1	25.0	0.5	6.17	0.07	8.45	0.11
	SS2	25.3	0.3	6.01	0.05	8.53	0.28
	S1	24.9	0.2	5.96	0.15	8.42	0.13
	S2	24.8	0.4	6.14	0.12	8.44	0.13
	L1	25.3	0.4	5.93	0.14	8.40	0.14
	L2	25.1	0.3	6.15	0.03	8.41	0.13
	M1	24.7	0.4	5.92	0.14	8.47	0.03
	M2	25.1	0.3	5.97	0.08	8.42	0.13
2	S1	24.3	0.9	6.19	0.34	8.36	0.20
	S2	24.1	1.4	6.37	0.38	8.48	0.10
	SL1	24.6	1.0	6.27	0.28	8.38	0.17
	SL2	24.5	0.8	6.16	0.43	8.39	0.16
	L1	24.6	1.0	6.14	0.45	8.35	0.19
	L2	24.9	1.0	6.36	0.28	8.40	0.14

Larvae were fed with SS-type (tanks SS1 and SS2), S-type (tanks S1 and S2), L-type (tanks L1 and L2), and a mixture of three types of rotifers (tanks M1 and M2) in experiment 1, and fed with S-type (tanks S1 and S2) and L-type (tanks L1 and L2) rotifers and S-type rotifers until 7 days post hatching and then L-type rotifers (tanks SL1 and SL2) in experiment 2. Larvae were reared until 15 DPH except for tank SS1 and SS2 (14 DPH) in experiment 1 and until 20 DPH except for tank S2 (10 DPH).

**Table 2**

Survival rate of larvae of greater amberjack reared under different feeding conditions

Expt. no.	Days post hatching	Survival rate (%)					
		Tank-SS2	Tank-S2	Tank-L2	Tank-M2		
1	3	78.0	47.1	71.8	70.2		
	5	31.3	17.1	38.5	69.4		
	10	4.0	7.8	9.5	12.2		
	14	0.1	–	–	–		
	15	–	2.0	4.0	8.4		
		Tank-S1	Tank-S2	Tank-SL1	Tank-SL2	Tank-L1	Tank-L2
2	5	72.7	93.8	100.0	93.8	87.5	84.8
	10	38.7	0.0	40.0	15.6	40.0	100.0
	15	1.4	0.0	2.0	7.1	20.0	6.7
	20	0.2	0.0	1.1	3.4	7.8	2.2

### 2.2. Larval rearing

In experiment 1, 5700 larvae at 2 DPH were stocked in each of eight 500 L black polyethylene tanks with a flow-through water system (0.3 L/min), and reared until 15 DPH under four feeding conditions. Larvae were fed with super small strain (SS-type) rotifers of *Brachionus plicatilis* sp. complex (Thai strain obtained from the Yaeyama Station, National Center for Stock Enhancement (NCSE), Fisheries Research Agency (FRA), Okinawa, Japan; mean lorica length, 0.135 mm; tanks SS1 and SS2), small strain (S-type) rotifers (unknown strain obtained from the Oita Prefecture Public Fisheries Corporation, Oita, Japan; mean lorica length, 0.174 mm; tanks S1 and S2), large strain (L-type) rotifers (Obama strain obtained from the Notojima Station, NCSE, FRA, Ishikawa, Japan; mean lorica length, 0.211 mm; tanks L1 and L2), and a mixture of SS-, S-, and L-type rotifers (tanks M1 and M2). In experiment 2, 5000 1 DPH larvae were stocked in each of six 500 L black polyethylene tanks with still water, and reared until 20 DPH under three feeding conditions, i.e., fed with S-type rotifers (Chikugo strain purchased from the Chlorella Industry Co. Ltd., Tokyo, Japan; mean lorica length, 0.142 mm; tanks S1 and S2), L-type rotifers (Kinki University strain obtained from the Notojima Station, NCSE, FRA; mean lorica length, 0.199 mm; tanks L1 and L2), and S-type rotifers until 7 DPH and then L-type rotifers (tanks SL1 and SL2). The body size of the S-type rotifers used in experiment 1 was significantly larger than that of the S-type rotifers used in experiment 2 (Welch's *t*-test,  $P < 0.0001$ ). Seawater was filtered with sand and disinfected by ozone before being supplied to the rearing tanks. Aeration was provided to each tank at 1 L/min through an air-stone. Photoperiod was 12 h light (6:00–18:00): 12 h dark, and the light intensity on the surface of the rearing seawater was regulated at ~2400 lx and ~1000 lx using fluorescent lights in experiments 1 and 2, respectively. Water temperature was maintained at around 25 °C using a heater. The mean values of the daily water temperature, DO, and pH during the rearing period were 24.7–25.3 °C, 5.92–6.17 mg O<sub>2</sub>/L, 8.40–8.53 in experiment 1 and 24.1–24.9 °C, 6.14–6.37 mg O<sub>2</sub>/L, 8.36–8.48 in experiment 2, respectively (Table 1). There were no differences between running and still water rearing systems.

Rotifers were fed from 4 DPH (mouth opening of larvae). All types of rotifers were cultured with commercial, condensed freshwater *Chlorella* (*Chlorella* V12, Chlorella Industry Co. Ltd., Tokyo, Japan) at around 30 °C, 30 °C and 23 °C for SS-, S-, and L-type rotifers, respectively, and enriched with commercial, condensed freshwater *Chlorella* containing n-3 highly unsaturated fatty acids (Super *Chlorella* V12, Chlorella Industry Co., Ltd., Tokyo, Japan) for 12 h prior to feeding. Rotifer densities in the larval rearing tanks were checked by sampling 5 mL of seawater twice a day (9:00 in experiment 1, 6:00 in experiment 2 and 13:00 in both experiments) and the number of eggs attached to the rotifers was also counted in experiment 2. The number of eggs attached to an individual rotifer was one or two, and was mainly one. Eggs isolated from rotifers were not observed in the seawater samples. Newly enriched rotifers were added to the tanks at around 10:00 and 14:00 to maintain a density

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