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First results of spawning and larval rearing of longfin yellowtail *Seriola rivoliana* as a fast-growing candidate for European marine finfish aquaculture diversification

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Abstract

The present study describes the adaptation of longfin yellowtail *Seriola rivoliana* as broodstock and first larval rearing trials under intensive and semi-intensive conditions. Fifteen sub-adults were captured in the South coast of Gran Canaria (Canary Islands, Spain) in June 2007. Fish (initial weight 1.76 ± 0.25 kg) reached a weight of 6.0 ± 1.1 kg in July 2010. Once a year, fish were sampled to determine individual growth in weight and size. In addition, the state of sexual maturity was established based on gonadal biopsies. On the basis of repeated hormonal injection (GnRHa, 20 μg/kg), 10 successful spawns were obtained between July and October 2009, with 92.5 ± 5.5% and 72.6 ± 17.2%, fertilization and egg viability respectively. First results of larval rearing under semi-intensive conditions, showed an average survival at 30 DAH of 2.5% as compared with 0.5% under intensive conditions. The low survivals under the two rearing conditions in addition to their failure to pass a stress test could be attributed to deficiencies in essential fatty acids as could be seen in both eggs and feeds. Morphometric parameters showed no significant difference between the two rearing systems in 230 DAH larvae.

Keywords: diversification, seriola, broodstoock, spawning, larval rearing, rearing techniques

Introduction

Longfin yellowtail *Seriola rivoliana* as other seriola species is considered as one of the most important emerging marine finfish species in Japan, Australia and the United States. In contrast, this species has not been under development for mariculture in Europe. This circumtropical carangid species can be found in Eastern Central Atlantic regions from Portugal (Azores and Madeira), Canary Islands to Cape Verde (Fischer, Bianchi & Scott 1981), whereas some individuals were caught sporadically in the Mediterranean sea (Castriota, Greco, Marino & Andaloro 2002). As other seriola species, *S. rivoliana* is well known for its fast growth, reaching a maximum standard length (SL) of 160 cm and a maximum weight of 59 kg (IGFA 2001) and its high market value of 7–10 USD per kg (Nakada 2002). However, the bottleneck to *S. rivoliana* mass production is the unreliable supplies of juveniles resulting from poor spawns and low hatchery survival. Therefore, the industry in Japan, Australia and USA is relying on the collection of fingerlings from the wild, to be ongrown in tank and cages (Nakada 2002; Yamamoto, Teruya, Hara, Hokazono, Hashimoto, Suzuki, Iwashita, Matsunari, Fuguita & Mushiake 2008).

A reproduction protocol for this species in terms of culture conditions, maturation and use of hormonal treatment is still not available. Furthermore, larval rearing studies of this species are scarce and limited to some reports in Ecuador (Benetti 1997;

Blacio, Darquea & Rodríguez 2003) and Hawaii (Laidley, Shields & Ostrowksi 2004). On the other hand, reproduction and larval rearing protocols have been developed for similar species, such as Japanese yellowtail *Seriola quinqueradiata,* greater amberjack *Seriola dumerili* or yellowtail king fish *Seriola lalandi* (Benetti 2000; Poortenaar, Hooker & Sharp 2001; Nakada 2002; Papandroulakis, Mylonas, Maingot & Divanach 2005).

To enhance the development of *S. rivoliana* for aquaculture diversification in Europe, different experimental activities, including broodstock management and larval rearing, are being conducted in the Canary Islands (Spain).

The objective of this study was to test mesocosms or semi-intensive techniques, which have been previously tested and reported successful in the larval rearing of difficult-to-rear fish species, which could be applied later to more intensive, commercial systems to improve biological performance and system productivity (Papandroulakis *et al.* 2005; Jerez, Samper, Santamaría, Villamandos, Cejas & Felipe 2006; Roo, Hernández-Cruz, Socorro, Fernández-Palacios & Izquierdo 2010). The comparison between the intensive and semi-intensive techniques, for larval rearing of *S. rivoliana,* will contribute to better understand of the husbandry needs of the species regarding future application in commercial production.

Material and methods

Broodstock

Fifteen *S. rivoliana* sub-adults (1.76 ± 0.25 kg) were captured at the South coast of Gran Canaria (Canary Islands, Spain), transported to land facilities and adapted to captivity in 10 m^3 squared glass fibber tanks (3 m × 3 m × 1.5 m depth). Fish were kept under natural photoperiod and natural sea water with 37 g L^{-1}salinity and temperature ranging from 18 to 24°C year around. After capture, all fish were weighed, sized, individually tagged with PIT tags of 0.1 g and 152 × 12 mm in length (EID Ibérica SA – TROVAN, Madrid, Spain), and sexed by gonadal biopsy inserting a 1.3 mm internal diameter catheter (Kruuse, Langeskov, Denmark) into the gonadal cavity and applying gentle aspiration. Fish were fed twice a week with commercial pellets (13 mm, Vitalis Repro™; Skretting, Burgos, Spain) corresponding to 1% of the body weight (BW), supplemented once a week with frozen squid (*Illex argentines*) and mussels (*Mytilus galloprovincialis*) at 2% of BW. Once a year (June), the whole population was anaesthetized with clove oil (Guinama S.L, Valencia, Spain; 50 ppm) and standard length (SL), body weight and condition factor (CF) were recorded (Table 1). The maturation stage was assessed using gonadal biopsy, oocytes from females were taken *in vivo* and placed in Serra's solution (6:3:1, 70% ethanol, 40% formaldehyde and 99.5% acetic acid) to be measured using a profile projector (Mitutoyo PJ-3000A, Kanagawa, Japan). Mean diameter of the largest oocytes were determined. The per cent of running males was determined (Table 1). When oocyte diameter (OD) was greater than 500 μm, the whole population (males and females) was injected with gonadotropin releasing hormone analogue (GnRHa, des-Gly^{10},[$_{D}$-Ala^{6}]-; Sigma-Aldrich, St. Louis, MO, USA) at a dose of 20 μg kg^{-1} body weight, based

Table 1 Evolution of body weight, total length and condition factor of *Seriola rivoliana* broodstock

Year	2007	2008	2009	2010
Females (indv)	8	8	6	6
Body weight (kg)	1.83 ± 0.22	2.90 ± 0.42	4.08 ± 2.02	6.22 ± 1.78
Standard length (SL)	45.05 ± 1.41	54.50 ± 3.54	57.19 ± 7.28	65.93 ± 4.58
Condition factor	2.00 ± 0.04	1.79 ± 0.19	2.01 ± 0.40	2.12 ± 0.24
Oocite diameter (μm)	n.a	<500 (8)	>500 (2)	>500 (4)
Males (indv)	7	7	5	5
Body weight (kg)	1.65 ± 0.39	2.32 ± 0.25	3.36 ± 0.97	5.55 ± 1.10
Standard length (SL)	43.25 ± 3.18	51.38 ± 2.95	54.57 ± 5.16	61.75 ± 5.06
Condition factor	2.02 ± 0.06	1.71 ± 0.26	2.02 ± 0.13	2.37 ± 0.38
Maturation	n.a.	Immature (7)	Running (5)	Running (5)

Mean values ± SD; Condition factor = [body weight (g)/[standard length (cm)]3; n.a. no available.
( ), number of individuals.

on the reported dosage for *S. dumerillli* (Mylonas, Papandroulakis, Smboukis, Papadaki & Divanach 2004). These hormonal treatments were applied every 2 weeks from 15 June to 15 October 2009. After the hormone injection, fish were left to spawn naturally in the tank. Eggs collectors, located at the perimeter of the tank were monitored daily until spawns were obtained (32 h after hormonal injection, on average). From the total amount of eggs, different spawning quality parameters, such as number of fertilized eggs, hatched larvae, 3-day-old surviving larvae and spawning indexes, such as fertilization rate and hatching rate, were calculated in accordance to the methodology described by Fernández-Palacios, Izquierdo, Robaina, Valencia, Salhi & Vergara (1995).

Larval rearing

In July 2009, fertilized *S. rivoliana* eggs were obtained for first time and these were used to test semi-intensive (SIS: 4.5 eggs L^{-1} in two 40 m^3 tanks) and intensive (IS: 125 eggs L^{-1} in three 2 m^3 tanks) larval rearing techniques according to Roo *et al.* (2010). Each rearing systems was tested in three consecutive trials, with duration of 1 month each along the spawning season. Experimental tanks were supplied with filtered and UV sterilized seawater. Water exchange progressively increased from 15% of the tank volume per day at 2 DAH for both rearing systems, to 100% from 15 DAH onwards in the IS and from 20 DAH in the SIS one. Water salinity (37 g L^{-1}), dissolved oxygen (6.22 ± 0.21 ppm) and temperature (24.03 ± 0.26°C) were measured daily. In addition, total gas saturation was measured daily using a Weiss saturometer (E-300; Eco Enterprises, Seattle, WA, USA) ($\Delta P = 0$). Pseudo-green water technique was used adding live phytoplankton (*Nannochloropsis sp.*) to maintain a concentration of 250 000 cells mL^{-1} in both rearing systems. Larval rearing was conducted under 12/12 (dark/light) photoperiod, using mixture of artificial fluorescent lights (Mod. TLD 58W/54-765; Philips, Lyon, France) and natural sun light with a light intensity just above the water surface ranging between 1000 and 3500 lux. From 2 to 25 days after hatching (DAH) rotifers, *Brachionus plicatilis* L-strain (205 μm mean lorica length, 125 μm mean lorica width) cultured on baker's yeast, *Saccaromices cerevisiae* and enriched with DHA Protein Selco (Inve Aquaculture, Dendermonde, Belgium) were added twice a day (08:00; 14:00). Rotifers density was adjusted to 4–5 rot mL^{-1} in the semi-intensive and from 7.5 to 10 rot mL^{-1} in the intensive system. *Artemia* Instar II nauplii, enriched with A_1 Easy Selco (INVE Aquaculture) were given equally from 15 DAH onwards to tanks in the SIS and IS treatments. The weaning protocol included hand feeding of an inert diet from 20 DAH (Genma Micro, Skretting, Vervins, France) four times a day for 5 days and automatic feeding every hour from day 25 until 30 DAH.

Growth and survival

Larval meristic parameters were recorded along the larval development (Fig. 1): total length (TL), standard length (SL), pre-anal length (PAL), eye diameter (ED), cephalic height (CH), yolk sac length (YSL), lipid globule diameter (LGD). All parameters were monitored from samples of 25 larvae per tank every 5 days. In addition, at the same time intervals, dry weight (DW) was recorded and specific growth rate (SGR) calculated

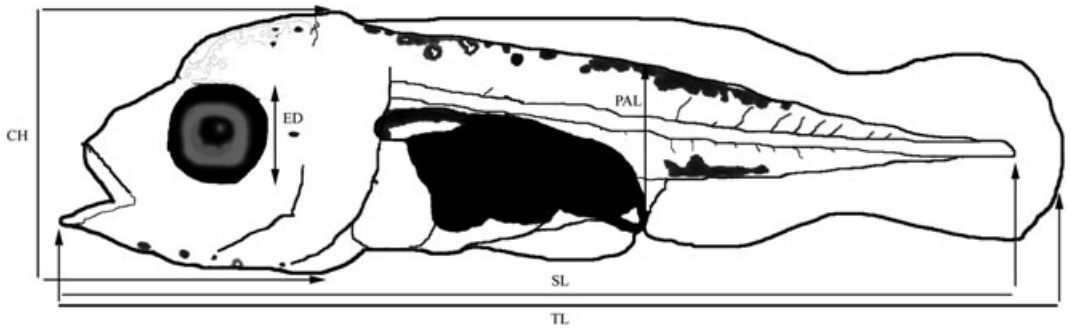

Figure 1 Squematic view of different morphometric determinations.

according with Ricker (1958) using the following equation:

SGR=(e^g − 1) × 100% where g = ([ln(DW_t)-ln(DW_0)]/t) and DW_t is the larval dry weight at the end of time period t, DW_0 is the dry weight at the beginning of time period t and t is the time period in days. On days 2, 5, 8 and 10 after hatching, 25 larvae per tank from each rearing tank and system, were sampled in the morning 9:30 approximately 1:30 hours after rotifer density adjustment. They were looked at *in vivo* under stereomicroscope to evaluate number of larvae with ingested rotifers. Final survival was determined at 30 DAH counting remaining live larvae in the experimental tanks.

Stress test

An air exposure test was performed on 20 and 30 DAH. On day 20, larvae (n = 45) were individually exposed to air for 15, 30 or 60 s in a 500 μm nylon mesh screen (Izquierdo, Watanabe, Takeuchi, Arakawa & Kitajima 1989). After the air exposure, larvae were transferred to an aerated 10-L beaker and survival was recorded 24 h later. At 30 DPH, a new set of stress tests were performed. At this point, two clove oil doses (1 and 2 ppt), as anaesthetic, were evaluated in combination with different air exposure times (15, 30, 60, 75 and 90 s). Larval survival was recorded 24 h after the stress test.

Biochemical analysis

For biochemical analysis, samples of eggs and feeds (rotifers, *Artemia* and microdiets) were collected along the feeding trials for biochemical analysis (Tables 2 and 3): Dry matter, ash and protein content were carried out using the methods of analysis of the Association of Official Analytical Chemists (AOAC 1990). Total lipid content was obtained as described by Folch, Lees and Stanley (1957). Fatty acid methyl esters (FAMEs) were obtained by transesterification with H_2SO_4 (10 mL L^{-1} methanol), (Christie 1982) and purified using adsorption chromatography on NH2 Sep-pack cartridges (Waters, S.A., Milford, MA, USA) as described by Fox (1990), and separated and quantified using gas liquid chromatography (GLC) as described by Izquierdo *et al.* (1989).

Statistical analysis

All the data were statistically treated using SPSS Statistical Software System ver 15.0 (SPSS, Chicago, IL, USA). A t-test for simple mean comparison analysis ($P < 0.05$) (Sokal & Rolf 1995) was applied to compare differences between rearing systems. When data were not normally distributed, arcsin-transformation was applied, and then Kolmogorov–Smirnov non-parametric test was applied to the non-transformed data. Results are presented as mean values ± SD.

Results

Broodstock

After 4 years in captivity, survival of the captured *S. rivoliana* was 73%. Losses in the course of this period were mainly due to repeated parasite outbreaks caused by monogenean ectoparasites (*Neobenedia sp.*). The breeders attained an average weight of 6.0 kg in July 2010 from an initial weight of 1.7 kg in June 2007, reaching a density of ca 10 kg m^3 (Table 1).

The sexual development of the brood stock was followed closely via gonadal biopsies. A year after their capture (2008) the population, independently of the sex, was sexually immature, whereas in 2009, the presence of late developing oocytes (>500 μm diameter) occurred in 33% of the females, in 5.1–6.3 kg BW females and in all males, all of which were running (Table 1). At this

Table 2 Proximate composition of fertilized *Seriola rivoliana* eggs and feeds utilized along the larval rearing (average ± SD, n = 3)

	Moisture	Ash (% DW)	Lipids (% DW)	Protein (% DW)	CHO	Energy (kJ/g)	Ratio P/L
Eggs	90.63 ± 0.18	0.63 ± 0.01	26.92 ± 0.86	68.83 ± 1.42	3.63 ± 2.22	2749.96 ± 27.33	2.56 ± 0.04
Enriched Rotifers	88.69 ± 1.50	2.18 ± 0.91	17.33 ± 3.14	54.95 ± 7.93	25.55 ± 9.32	2420.68 ± 123.97	3.22 ± 0.50
Enriched *Artemia*	91.89 ± 0.88	0.76 ± 0.21	29.29 ± 4.91	54.03 ± 3.75	15.91 ± 5.50	2705.95 ± 105.21	1.90 ± 0.37
Dry feed	5.29 ± 1.44	17.09 ± 0.83	17.70 ± 1.11	57.66 ± 1.09	7.54 ± 1.45	2189.83 ± 19.46	3.27 ± 0.26

Table 3 Fatty acid composition (% total fatty acid) from the total lipid content *Seriola rivoliana* fertilized eggs and feeds utilized along the larval rearing (average ± SD, $n = 3$).

	Eggs	Enriched Rotifers	Enriched *Artemia*	Dry feed
ΣSaturated	23.82 ± 2.18	20.48 ± 1.85	18.31 ± 1.47	27.65 ± 4.21
ΣMonounsaturated	28.55 ± 2.03	41.12 ± 2.67	35.85 ± 0.52	29.09 ± 2.44
Σn-3	29.00 ± 4.27	22.31 ± 2.48	33.78 ± 1.81	14.57 ± 5.20
Σn-6	16.85 ± 2.12	12.23 ± 2.22	9.67 ± 0.06	27.24 ± 1.19
Σn-9	21.18 ± 1.58	23.53 ± 1.47	24.10 ± 0.32	16.19 ± 2.15
Σn-3HUFA	26.91 ± 3.30	19.67 ± 2.17	13.30 ± 0.57	9.97 ± 3.61
14:00	1.03 ± 0.04	1.59 ± 0.11	0.97 ± 0.18	3.53 ± 0.65
16:00	16.19 ± 0.37	14.30 ± 1.58	11.20 ± 0.99	19.36 ± 3.07
16:1 n-7	3.65 ± 0.17	12.40 ± 1.32	3.37 ± 0.10	3.86 ± 0.07
18:00	5.94 ± 1.67	3.38 ± 0.23	5.10 ± 0.25	3.80 ± 0.39
18:1 n-9	20.15 ± 1.34	18.33 ± 0.93	21.82 ± 0.14	10.40 ± 1.64
18:1 n-7	3.57 ± 0.27	3.76 ± 0.42	6.08 ± 0.16	2.15 ± 0.14
18:2 n-6	13.54 ± 1.72	8.67 ± 1.81	6.66 ± 0.06	26.30 ± 1.03
18:3 n-3	0.95 ± 0.02	1.11 ± 1.00	17.71 ± 2.06	3.46 ± 1.19
20:1 n-9	0.49 ± 0.12	2.65 ± 0.22	1.63 ± 0.17	5.48 ± 0.48
ARA	1.48 ± 0.10	1.45 ± 0.05	1.27 ± 0.03	0.38 ± 0.06
EPA	6.72 ± 1.70	5.90 ± 0.24	5.76 ± 0.18	3.54 ± 1.10
DHA	16.50 ± 1.49	10.37 ± 1.97	5.37 ± 0.68	5.71 ± 2.32
DPA (22:5n-6)	0.29 ± 0.02	0.53 ± 0.06	0.29 ± 0.04	0.04 ± 0.02
DHA/22:5 n-6	57.27 ± 73.66	19.42 ± 1.78	18.45 ± 0.29	-
EPA/ARA	4.55 ± 16.24	4.06 ± 0.29	4.54 ± 0.09	9.23 ± 1.37
ARA/EPA	0.22 ± 0.06	0.25 ± 0.02	0.22 ± 0.00	0.11 ± 0.02
DHA/EPA	2.45 ± 0.88	1.76 ± 0.33	0.93 ± 0.14	1.58 ± 0.18
DHA/ARA	11.17 ± 14.22	7.15 ± 1.49	4.24 ± 0.56	14.73 ± 3.72
Oleic/DHA	1.22 ± 0.90	1.83 ± 0.47	4.10 ± 0.51	2.15 ± 1.26
Oleic/n-3HUFA	0.75 ± 0.41	0.94 ± 0.16	1.64 ± 0.06	1.19 ± 0.63
n-3/n-6	1.72 ± 2.02	1.86 ± 0.33	3.49 ± 0.20	0.53 ± 0.17

point, both males and females were hormonally injected with the GnRHa analogue (20 μg/kg BW) 10 times from July to October 2009, resulting in 100% successful spawns 32 h post injection (average 275 000 eggs per spawn). Eggs were transparent, spherical in shape with an average egg diameter of 1.1 mm ($n = 100$), and a single oil droplet of 0.24 ± 0.02 mm in diameter (Plate 1a). Along the different spawning events, average fertilization rate was 92.5 ± 5.5% with 72.6 ± 17.2% of viable eggs.

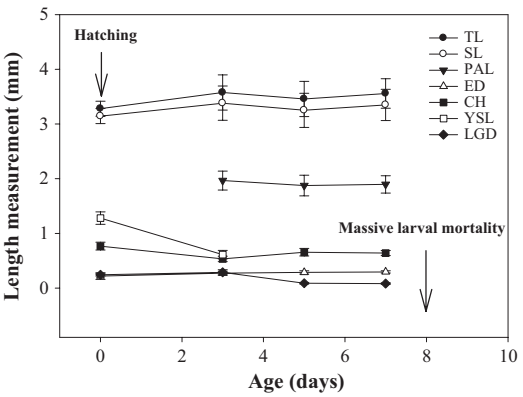

Figure 2 Development of different morphometric indices on starved larvae: total length (TL), standard length (SL), pre-anal length (PAL), eye diameter (ED), cephalic height (CH), yolk sac length (YSL), lipid globule diameter (LGD).

Larval rearing

Hatching time ranged between 36 and 48 h post-fertilization depending on the rearing temperature (22–24°C), with an average hatching rate of 79.0 ± 11.3%. Newly hatched larvae were 3.28 ± 0.13 mm in total length and yolk sac length was 1.27 ± 0.11 mm (Fig. 2; Plate 1b). At 4 DAH, yolk sac reserves were completely absorbed (Fig. 2) and average larval survival was 52.3 ± 19.1%. At this point, survivors showed eye pigmentation and mouth and anus open and ready to start first feeding (Plate 1c). Although yolk sac was quickly depleted, the oil droplet remained in the anterior abdominal cavity until

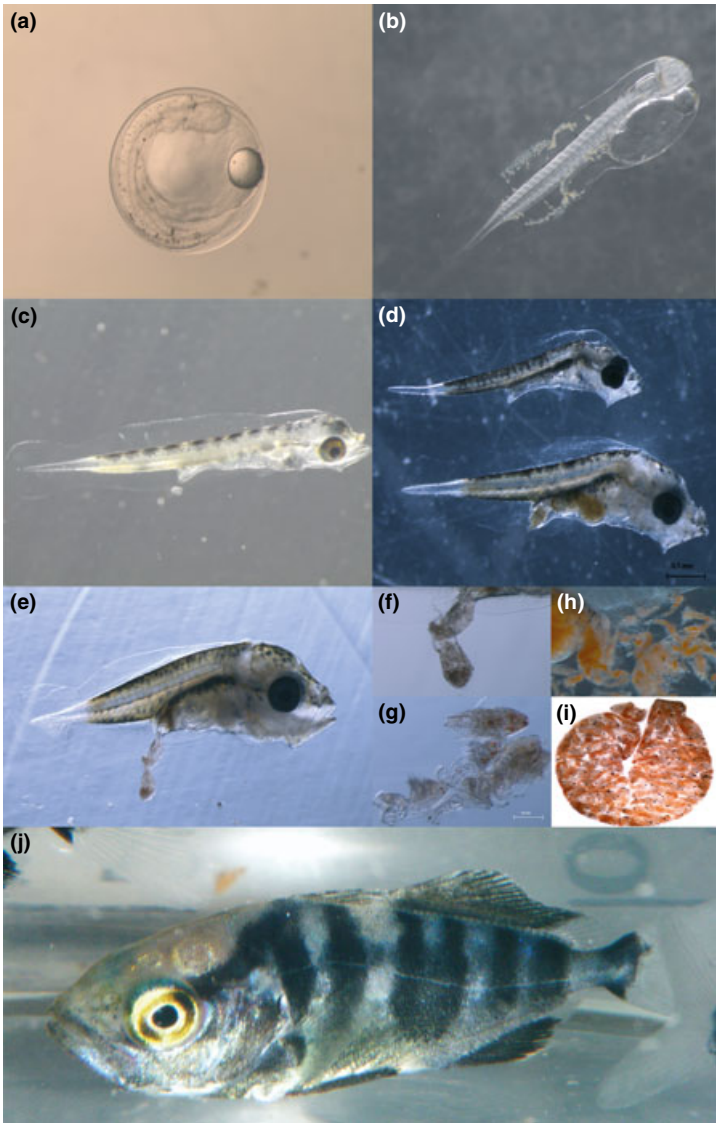

Plate 1 a) *S. rivoliana* egg; b) Newly hatched larvae; c) 3-day old larva; d) 10-day old fed (down) and starved (up) larvae; e) 20-day old larvae; f) excreted copepods in 20 DAH in semi-intensive system reared larvae; g) detail of diggested copepods; h) excreted *Artemia* in 25 DAH larvae; i) detail of faeces packets with undigested *Artemia* in 25 DAH; j) 30 DAH juvenile.

day 8 DAH, and it was not further visible in 10 DAH larvae (Fig. 2; Plate 1d).

Enriched rotifers L-type strain were supplied from 2 DAH onwards, but a low incidence of larvae with rotifers in the gut were recorded from 2 to 5 DAH. Thus, no larvae with ingested rotifers were recorded on day 2, 50% of the larvae were recorded with rotifers inside the gut lumen on day 5, 86% at day 8 and 100% by day 10, regardles of the rearing system utilized.

The transfer to *Artemia* feeding was successful, and it occurred 15–20 DAH. From 20 to 30 DAH, most of the larvae were observed carrying long faeces packets, identified as live undigested *Artemia* (Plate 1h,1i).

At the end of the rearing trials (30 DAH), average larval survival was 2.3 ± 0.7% and 0.5 ± 0.2% in semi-intensive and intensive rearing conditions respectively. The highest larval mortalities occurred from 8 to 12 DAH. In the following days, a continuous larval mortality was recorded until day 20. At this point, 20 and 30 DAH larvae showed a very high sensitivity to the air exposure stress test; even the lowest air exposure (15 s) was associated with an almost immediate total larval mortality. It is interesting to note that the *Seriola* larvae resisted better the stress test if exposed to a 1–2 ppm of an anaesthetic.

Growth performance of 5–30 DAH seriola larvae was independent of the rearing system

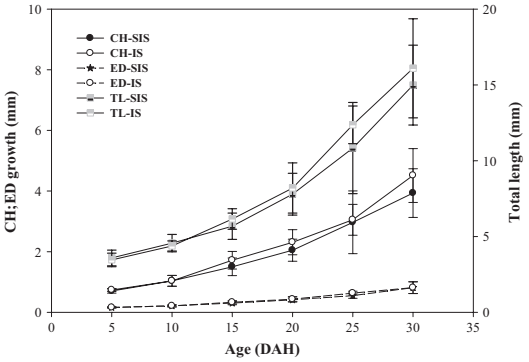

Figure 3 Morphometric development of total length (TL), cephalic height (CH) and eye diameter (ED) of *Seriola rivoliana* larvae cultured under intensive (IS) or semi-intensive (SIS) rearing techniques.

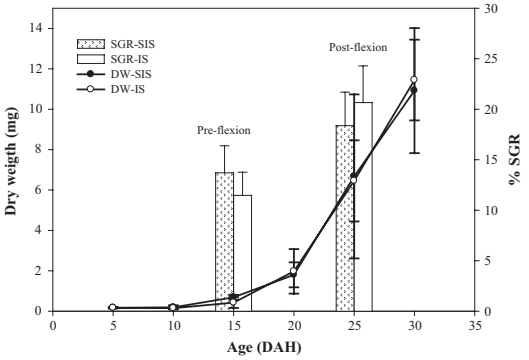

Figure 4 Growth development in dry weight (DW) and standard growth rate (SGR) of *Seriola rivoliana* larvae cultured under intensive (IS) or semi-intensive (SIS) rearing techniques.

($P > 0.05$; Figs 3 and 4). It is interesting to note that we identified two different growing periods; one, of a slow daily growth rate of 12.1–14.7%, in coincidence with pre-flexion (0–15 DAH) and one with an accelerated daily growth rate of 20.1–22.9%, post-flexion of the notochord (15–30 DAH). Specific growth rate was not significantly different in SIS and IS reared larvae (Fig. 4).

Proximate composition

Protein content of the live and dry feeds fed to *S. rivoliana* larvae were similar and ranged from 54% to 57% on a dry matter basis. Ash contents were higher in the dry feed than in live preys. On a dry matter basis, total lipid content was 17%, in enriched rotifers and microdiets in comparison with enriched *Artemia* (29%). Energy content showed a similar trend to lipid content, being higher in enriched *Artemia* than enriched rotifers and microdiets. This was in contrast with the Protein/Lipid (P/L) ratio, which was lowest in enriched *Artemia* (Table 2).

Fatty acid analysis showed that *S. rivoliana* eggs were high in oleic (18:1n-9), docosahexaenoic (22:6n-3) and palmitic acids (16:0) followed by linoleic (18:2n-6) and eicosapentaenoic (20:5n-3) acids. Grouping the fatty acids by families, n-3 fatty acids were the most abundant, followed by the level of monounsaturated and saturated fatty acids. In comparison, fatty acid profiles of rotifers were extremely high in monounsaturated fatty acids, particularly palmitic and oleic acid, which was 4X higher than in *S. rivoliana* eggs. Futhermore, n-3 fatty acids were 20% lower in rotifers than in fish eggs, due to their lower DHA content. *Artemia* fatty acids were particularly high in linolenic acid (18:3n-3), which was 18 times higher than in eggs, and monounsaturated fatty acids and very low in DHA. The dry weaning feed was also very low in DHA, together with ARA, but very high in 18:2n-6 (Table 3).

Discussion

Broodstock

Captured broodstock generally adapted well to culture conditions, but eye anomalies, such as cataracts and exophthalmia, associated with chronic levels of total dissolved gas pressure in water supply (ΔP = 5–10) were observed. Besides, repeated parasite outbreaks by a monogenean (*Neobenedia sp.*) were the main cause of broodstock mortality. Monogenean infections were identified as a major concern for Seriola industry in Australia and Japan, causing loss of fish growth, decreasing market value and causing fish mortality, as it was reported for different seriola species (Chambers & Ernst 2005; Hirayama, Kawano & Hirazawa 2009). Regarding the reproductive biology, even after 3 years in captivity (4.08 ± 2.2 kg; 57.19 ± 7.28 cm SL), most of *S. rivoliana* females were sexually immature with ovaries containing only previtellogenic oocytes in 66% of the fish, whereas 100% males were recorded as running, which could be related to the size at first maturity of this species, which was reported to be around 5 kg in

Hawaii (Laidley *et al.* 2004). In the present study, after 4 years in captivity when the fish reached a weight of 6.22 ± 1.78 kg (65.93 ± 5.06 cm SL), 66% of the females carried late developing oocytes (>500 μm in diameter), demonstrating the capacity of this seriola species to mature in captivity although, no spontaneous spawns were obtained. Lack of oocyte development and maturation in other species in captivity is generally associated with inadequate environmental (photoperiod, temperature) conditions or other stress factors, such as fish density or rearing volume (Micale, Maricchiolo & Genovese 1999; Benetti 2000). Indeed, lower fish densities and bigger tanks used in comparison with the present study, for same species in Hawaii and Ecuador, lead to successful spontaneous spawns (Blacio *et al.* 2003; Laidley *et al.* 2004).

In the present study, the use of hormonal induction of oogenesis and egg maturation with the GnRHa analogue was associated with a high rate of egg fertilization without negative effects on brood fish survival. The dosage applied was based on Mylonas *et al.* (2004) for greater amberjack and Duncan, Estevez and Mylonas (2007) for meagre (*Argyrosomus regius*). The fact that in *S. rivoliana,* in the present study, was induced to spawn at a size of about 5.0 kg would suggest that this seriola species has a practical advantage over other seriola species, such as *S. dumerilli*, as the latter needs very large tanks as to reach sexual maturity (Mylonas *et al.* 2004; Jerez *et al.* 2006).

Larval rearing

Survival and growth

Survival rates obtained in this study (0.5–2.5%) were similar to those reported for the same species in Ecuador (Blacio *et al.* 2003), Hawaii (Laidley *et al.* 2004) and other seriola species, such as *S. lalandi* (Tachihara, El-Zibdeh, Ishimatsu & Tagawa 1997) and *S. dumerili* (Papandroulakis *et al.* 2005; Hamasaki, Tsuruoka, Teruya, Hashimoto, Hamada, Hotta & Mushiake 2009). In the present study, the highest larval mortalities were observed in the early developmental stages, from 8 to 12 DAH. Different studies attributed the early larval mortalities to an unstable bacterial flora in the rearing water and opportunistic bacteria colonization, the larval digestive tract (Hansen & Olafsen 1999; Makridis, Fjellheim, Skjermo & Vadstein 2000; Verner-Jeffreys, Shields, Bricknell & Birkbeck 2003; Reid, Treasurer, Adam & Birkbeck 2009) causing massive mortalities mainly when intensive larval rearing systems are utilized (Skjermo & Vadstein, 1999). The improved larval survival in the SIS in comparison with IS would suggest that seriola larval rearing should be performed following the SIS regime. This could be associated with the use of larger water volumes, with a low renewal rate and low larval density. This regime might help promote the development of a more stable environment, probably associated with a more mature microbial flora, similar to mature waters obtained in recirculation systems (Roo *et al.* 2010; Attramadal, Salvesen, Xue, Øie, Størseth, Vadstein & Olsen 2012). Indeed, the use of microbial matured water has being reported to improved, larval survival and improved feeding incidence at early stages in different marine species, such as Atlantic halibut *Hippoglossus hippoglossus* (Skjermo, Salvesen, Øie, Olsen & Vadstein 1997) or Atlantic cod *gadus morhua*, among others (Attramadal *et al.* 2012). Furthermore, theorical prey availability in relation with initial larval population was tenfold lower in the IS, with around 160 rotifers/larva/day, whereas more than 1600 rotifers/larva/day could be available in the SIS. Similar data where previously reported by Roo *et al.* (2010) in red porgy larvae. Nevertheless, this fact seems not to affect feeding incidence, measured in 5 DAH (50%) and 8 DAH (80%), in the present study in both system regimes. Furthermore, our observations on seriola larval feeding were lower than those reported in similar species, such as *S. dumerilli* (>70% first feeding at 4 DAH; Hamasaki *et al.* 2009), suggest that initial larval mortalities could be also related to the low success for first feeding in *S. rivoliana* under both culture techniques and suggesting a strong dependence on maternal reserves during the early development. Similar results were reported for *S. lalandi* (Chen, Qin, Kumar, Hutchinson & Clarke 2006) and could be related to nutritional imbalances in broodstock diets, and hence, deficiencies in essential nutrients in the endogenous reserves of the larvae, nutritional imbalance of live prey at first feeding or inadequate culture conditions. In fact, it was suggested that broodstock diets for *Seriola sp.* may need an extra supply of neutral lipids as an important energy source for early larval development (Hilton, Poortenaar & Sewell 2008). In the present study, *B. plicatilis* L-strain seems to be adequate

for first feeding of *S. rivoliana* larvae, which is in agreement with the results reported by Hamasaki *et al.* (2009). These authors reported that larval survival in *S. dumerilli* was independent of rotifer size of different strains, which had a range of lorica length from 135 to 211 μm. In the present study, copepods (Harpacticoida, Tisbidae) produced endogenously in the semi-intensive systems tanks were observed in the larval digestive tract (Plates 1e, 1f, 1g). This was particularly true for the small naupliar stages of marine copepods, suggesting the preferences for this type of prey by *Seriola* larvae. These results are similar with those reported by Van der Meeren (1991) when turbot larvae were offered rotifers and copepods. In general, copepods are a suitable prey-size organisms for first-feeding larvae that are rich in a number of biochemical components, such as lipids, highly unsaturated fatty acids, digestible phospholipids, protein, protein-bound amino acids, free amino acids, pigments or vitamins (Støttrup & Norsker 1997; Van der Meeren, Olsen, Hamre & Fyhn 2008), which are essential nutrients and as such would helped promote seriola larval survival under semi-intensive conditions. In older larval stages (>20 DAH), there was a continuous mortality with weak larvae floating at the water surface. *Seriola* larvae at this age also succumbed after stress test. The presence of larvae with faeces, including undigested and even live *Artemia,* demonstrated the low digestibility of this prey (Plate 1i), which leads to larval malnutrition when *Artemia* is the main source of food (15–30 DAH). Moreover, striking differences were found in the fatty acid profiles of eggs and different feeds utilized in this study, suggesting a potential fatty acid deficiency. This is in agreement with the shock syndrome (sudden mortality) observed after the stress test or even at capture as it was reported by Izquierdo *et al.* (1989). Also, sudden massive mortalities were recorded along the different trials in this rearing period. The relation between larval welfare and essential fatty acids, such as 20:5n-3 (EPA), 22:6n-3 (DHA) and 20:4n-6 (ARA), has been emphasized in early stages of development for different marines species (Izquierdo 1996), with 'shock syndrome' being one of the indication of EFA limitations in the diets (Izquierdo *et al.* 1989). Previous results from Yamamoto *et al.* 2008; suggest that in *Artemia*-feeding stages at least 1.4–2.6% of DHA and 2.3–4.1% of n-3 HUFAs are needed in the diet of the yellowtail *S. quinqueradiata*. Present results showed that *S. rivoliana* eggs contents 16.5% total fatty acids (TFA) (about 4.3% DW) in DHA and n-3 HUFA were as high as 26.9% TFA (7.1% DW) suggesting even higher EFA requirements for this species, than for *S. quinqueradiata.* In agreement with these potential high EFA requirements, high mortalities could be related with the low DHA and n-3 HUFA content in rotifers (10.3–19.6%), *Artemia* nauplii (5.3% and 13.3%) and dry feeds (5.7–9.9%). In addition, the ratios of DHA/EPA and oleic/DHA, used to evaluate the essential fatty acids requirements (Izquierdo 1996), greatly differed for feeds (0.93–1.76 DHA/EPA; 1.8-4.1 oleic/DHA) and eggs (2.45 and 1.22 respectively). It is noteworthy to mention, that not only fatty acid composition, but also lipid classes should be taken into consideration in larval feeds. Thus, commercial live prey enrichment, such as the ones utilized in present experiment provides most of EFA as neutral lipids (NL) particularly as triglycerides (TG) and free fatty acids (FFA) forms (data not shown). On the contrary, natural live preys, such as copepods, are rich in phospholipids (PLs) (Van der Meeren *et al.* 2008). It is well known that in marine fish larvae, PL is structural constituents of bio-membranes and therefore highly demanded in the fast-growing larvae. However, there are several indications that fish larvae are unable to efficiently synthesize PL in a rate fast enough to cover their high demand and therefore PL needs to be included in the diet (Izquierdo & Koven 2011), suggesting that nutritional modification in *S. rivoliana* larval feed could be beneficial for improved larval survival.

Total length of newly hatched larvae averaged 2.54 ± 0.01 mm, which is similar to previous studies in this species (Blacio *et al.* 2003) and *S. dumerilli* (2.88 mm TL) (Papandroulakis *et al.* 2005), but shorter than *S. lalandi* larvae (4.30 mm TL) (Chen *et al.* 2006). Nevertheless, both absolute growth was similar to the reported in these Seriola species (0.45–0.51 mm TL/day) and in the range of values reported for *Thunnus* species (0.44–0.68 mm TL/day) according to Kaji, Tanaka, Oka, Takeuchi, Ohsumi, Teruya and Hirokawa (1999). Notochord flexion started at 15 DAH (5.6–6.1 mm TL), similar to *S. dumerilli* (5.5 ± 0.52 mm TL) (Papandroulakis *et al.* 2005), being completed at 20 DAH (7.7–8.2 mm TL). This change in larval external morphology may be used as an indication of digestive system maturity, as it occurs synchronously with the appearance of

digestive glands, as it was reported by Abreu (2010), suggesting changes in larval behaviour and feeding habits, similar to other species, such as red porgy (*Pagrus pagrus*) (Roo, Socorro, Izquierdo, Caballero, Hernández-Cruz, Fernández & Fernández-Palacios 1999). Thus, the appearance of gastric glands marked the formation of a functional stomach with a higher capacity to utilize proteins (Govoni, Boehler & Watanabe 1986) and the capacity to perform a successful early weaning onto dry diets, as it has been found in *S. lalandi* (Chen *et al.* 2006). By day 30 (15.0–16.0 mm TL), juveniles had a wet weight ranging from 0.15 to 0.3 g in wet weight with the characteristic striped pigmentation (Plate 1j). At this point, the elevated mortality recorded in the stress test trials can be reduced by the use of anaesthesia based on the use of low clove oil doses, which was associated with 100% larval survival even after a 90 s air exposure. This protocol could be applied in routine manipulations, such as grading or juveniles transportation. Finally, at 90 DAH juveniles from semi-intensive and intensive systems reached 26.7 ± 4.7 and 14.2 ± 5.2 g in wet weight, respectively, denoting the rapid growth of this species.

In summary, the results of this first experience of *S. rivoliana* culture in Europe showed that this species adapts well to captivity conditions and even with dry commercial feeds, responds with good quality spawns to GnRHa hormonal injection, obtaining similar larval survival and growth behaviour as other Seriola species. Moreover, larval survival results and biochemical analysis of eggs and feeds suggests that this species might have higher n-3 HUFA and particularly DHA requirements during larval stages as compared with other commercial species. Further studies are being conducted to improve the culture performance and the study of EFA requirements of *S. rivoliana* larvae.

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