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Running title: Light intensity in common sole larvae

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Abstract

The effects of four light intensities (1000 lx, 500 lx, 50 lx, 3 lx) on growth, survival and feeding activity in common sole (*Solea solea* L.) larvae were studied from 4 till 51 days post hatching (dph). During the pelagic larval stage (4-12 dph), larvae reared at 3 lx showed a lower growth. From 19 onwards, the larvae reared under 3 lx displayed a significant ($P \leq 0.05$) higher SGR than the other treatments and a higher final weight compared to 1000 lx and 500 lx. Survival rate was higher under intermediate light intensities (500 and 50 lx). Larvae reared at 3 lx displayed a significant delay in the degree of metamorphosis compared to the other treatments, while at 33 dph metamorphosis was completed under all treatments. Histological examination revealed the importance of vision and light in the first feeding of this species, while after metamorphosis, the full development of other sensory organs indicated that feeding activity is also mediated by chemosensory perception. Results indicate that high light intensity seems to be more suitable during the pelagic larvae, while the opposite would ensure better growth from the onset of metamorphosis to the benthic phase.

Introduction

Abiotic factors, such as water temperature, salinity, oxygen and light, are known to influence the growth, survival, behavior and development of fish (Howell & Baynes 2004). One of the key environmental parameters is light, which is considered as a complex set of characteristics such as intensity, photoperiod and spectrum (Villamizar, Blanco-Vives, Migaud, Davie, Carboni & Sánchez-Vázquez 2011). Light has been shown to influence fish throughout various stages of their life, from embryonic development to the sexual maturation of adults (Boeuf & Le Bail 1999; García-López, Pascual, Sarasquete & Martínez-Rodríguez 2006; Stuart 2013; Villamizar *et al.* 2011). Among light characteristics, light intensity has been defined as the amount of illumination at the water surface, per unit area (Cobcroft, Pankhurst, Hart & Battaglene 2001; Stuart & Drawbridge 2011). Knowledge about the sensitivities to this parameter in fish larvae is still scarce, though it is an extremely important environmental factor in larval rearing and known to significantly affect growth, development and survival (Stuart 2013; Villamizar *et al.* 2011). Light intensity can be also responsible for the larvae initiating feeding and can affect the ability to forage, since many marine fish larvae are visual feeders and require a minimum amount of light to feed (Hubbs & Blaxter 1986; Fielder, Bardsley, Allan & Pankhurst 2002; Monk, Puvanendran & Brown 2006; Puvanendran & Brown 2002). The feeding responses of larvae in different light environments change with the developing visual capabilities of larvae and have been correlated with larval distribution in the wild (Job & Bellwood 2000) and used to determine appropriate light conditions for larval culture (Pankhurst & Hilder 1998).

On the other hand, high light intensity may also negatively modify larval behavior (Stuart 2013) and can be stressful or even lethal for larvae (Boeuf & Le Bail 1999). Light requirements in terms of intensity in which fish can thrive are species-specific and the optimal values for larval development, growth and survival may also differ during larval ontogeny (Boeuf & Le Bail 1999; Fielder *et al.* 2002; Stuart 2013).

Recently, some improvements in the study of common sole larvae (*Solea solea* L.) have been achieved by focusing on larval feeding, nutrition and physiology (Bonaldo, Parma, Badiani, Serratore & Gatta 2011; Ferraresso, Bonaldo, Parma, Cinotti, Massi, Bargelloni & Gatta 2013; Lund, Steenfeldt & Hansen 2007; Lund, Steenfeldt, Banta & Hansen 2008; Parma, Bonaldo, Massi, Yufera, Martínez-Rodríguez & Gatta 2013). However, abiotic factors such as light intensity at the larval stage are poorly investigated in this species and, to our knowledge, the study carried out by Lund, Steenfeldt & Hansen (2010) is the only reference available on this topic.

We thus investigated the effects of light intensity on growth, feeding activity and development in common sole larvae with the aim of providing useful information for hatcheries. To this end, common sole larvae were exposed to four different light intensity regimes, from 3 to 1000 lux, following previous studies on other marine flatfish (Cañavate *et al.* 2007; Henne & Watanabe 2003; Huse 1994). The trial lasted 48 days, covering the entire sole larval cycle, from before to after metamorphosis. To gain further insight into larval development, we also sought to provide a comprehensive description of the major organs, with special regard to the ontogeny of sensory organs, based on histological examination.

Materials and methods

Experimental design and rearing system

Four treatments were tested in triplicate in order to investigate different light intensity regimes (LRs): light intensity of 1000 lx (LR-1000), light intensity of 500 lx (LR-500), light intensity of 50 lx (LR-50) and light intensity of 3 lx (LR-3) at the water surface.

The experimental facility consisted of twelve 280-liter square grey (RAL 7038) flat bottom (0.64 m^2) tanks supplied with natural seawater and connected to a closed recirculating system (overall water volume: 4000 L). The rearing system consisted of a mechanical sand filter (0.4 m^3 of silica sand, 0.4-0.8 mm. PTK 1200, Astral Pool, Servaqua S.A. Barsareny, Spain), ultraviolet lights (PE 25mJ/cm^2: 16m^3 h^{-1}, Blaufish, Barcelona, Spain) and a biofilter (PTK 1200, Astral Pool, Servaqua S.A. Barsareny, Spain). Each tank was provided with artificial white light supplied by a dimmable lamp (Disano Hydro 951, Disano Illuminazione S.p.A, Rozzano, Italy; Philips master TL-D18 W/840, Philips S.p.A, Monza, Italy) with two bulbs, one lamp for each tank placed at 60 ± 2 cm from the water surface.

In order to obtain a low light intensity for treatment LR-3, the tanks were separated from the other tanks by black plastic sheeting, which were fixed from the floor to the ceiling, to eliminate extraneous light.

At the beginning of the trial, light intensity was regulated (Delta Ohm lightmeter HD2302.0; Probe LP 471 PHOT; Delta Ohm, Padua, Italy) at the water surface according to the LRs and values were monitored daily, in the morning before feeding to avoid large variations. Averages recorded were 1052.7 ± 96.6 lx under LR-1000, 512.0

± 21.4 lx under LR-500, 53.0 ± 8.9 lx under LR-50 and 2.8 ± 0.6 lx under LR-3. The irradiance (rad, W/m^2) at the water surface of each tank was also measured at the same time (Delta Ohm lightmeter HD2302.0; Probe LP 471 RAD; Delta Ohm, Padua, Italy). Averages recorded during the trial were 2.705 ± 0.203 W/m^2, 1.323 ± 0.145 W/m^2, 0.146 ± 0.036 W/m^2 and 0.007 ± 0.002 W/m^2 in the LR-1000, LR-500, LR-50 and LR-3 treatments respectively. The trial lasted 48 days, from 4 days post hatching (dph) to 51 dph.

Larvae origin and feeding

Common sole larvae were obtained from a captive broodstock maintained at the Laboratory of Aquaculture, Department of Veterinary Medical Sciences, Cesenatico, Italy. One batch of fertilized floating eggs was incubated in an 80 L cylinder-conical tank until 4 dph, under a light intensity of 160 lx. At this time, 2050 larvae $tank^{-1}$ were randomly distributed to the experimental rearing tanks using volumetric allocation (Bonaldo *et al.* 2011). The feeding protocol for all LRs consisted in the administration of live feed (*Artemia* nauplii from 4 to 10 dph, subsequently enriched metanauplii) until 18 dph, followed by weaning onto a commercial microdiet (MD) (AgloNorse K/S Tromsø Fiskeindustri A/S & Co., Tromsø, Norway) (Parma *et al.* 2013). *Artemia* nauplii and metanauplii were manually administered twice a day (10.00 am and 4.00 pm). Live feed was added so as to reach an overall amount of 10 individuals mL^{-1} day^{-1}, and 3 days before the end of weaning (18 dph) it was gradually decreased to 2 individuals mL^{-1} day^{-1}. The diet was supplied from 10 to 33 dph at a commercial size of 200-300 µm and subsequently at a commercial size of 300-500 µm and 400-600 µm.

Dry feed was supplied by belt feeders for 12 h day^{-1}. Larvae were fed in excess to guarantee a high availability of MD particles in the water and avoid an excess of uneaten feed on the tank bottom. The feeding rate was adjusted after a daily visual inspection of the tank bottoms, which took place in the early morning (8.30 am) before feed was supplied. The daily feed ratio was decreased in the case of excessive leftover feed (Parma *et al.* 2013). Feed administration ranged from 4 to 8 g $tank^{-1}$ day^{-1}. *Artemia* cysts (Great Salt Lakes, Catvis BV, s-Hertogenbosch, the Netherlands) were incubated and hatched in seawater (salinity 25 g l^{-1}) at 28 °C over an 18 h period. *Artemia* metanauplii were harvested and enriched for 24 h using Algamac-3050 (Aquafauna, Bio-Marine Inc., Hawthorne, CA,USA).

Water quality – Environmental parameters

During the trial, the water temperature was kept constant at 18.0 ± 1.0 °C and the photoperiod was maintained at 16 h light: 8 h dark. The oxygen level was kept constant (7.5 ± 1.0 ppm) using a liquid oxygen system connected to a software program (B&G Sinergia snc, Chioggia, Italy); furthermore strong aeration (200 l min^{-1}) was applied in the stock tank to remove CO_2. Overall daily water renewal was approximately 20 % day^{-1} as a consequence of mechanical filter backwashing and tank flushing. At the beginning of the trial, the water exchange rate of the tanks was 1 L min^{-1}, and it was increased to 2 L min^{-1} following larval development. Ammonia (total ammonia nitrogen ≤ 0.1 ppm), nitrite (≤ 0.2 ppm), nitrate (≤ 50 ppm) and salinity (37 g L^{-1}) were monitored daily by spectrophotometry in the afternoon (5.00 pm) (Spectroquant Nova

60, Merck, Lab business, Darmstadt, Germany). Sodium bicarbonate was added on a daily basis to keep the pH constant at 7.8 - 8.0.

Data collection, growth, survival and metamorphosis

At the beginning of the trial, 3 pools of 50 larvae each were randomly collected from the initial stock for wet weight (WW) determination.

At 12, 19, 33 and 51 dph, 50 larvae tank^{-1} were collected to determine WW. WW was measured with a microbalance (Scaltec SBC31, Scaltec Instruments GmbH, Göttingen, Germany) after rinsing the larvae with deionized water on a 400 µm screen and blotting excess moisture away from behind the screen using a lint free paper towel (Bonaldo *et al.* 2011). The growth rate was calculated as the specific growth rate (SGR, % day^{-1}), according to the following equation:

$$SGR = \frac{(\ln \text{final weight}) - (\ln \text{initial weight})}{\text{Number of Days}} \times 100$$

Twenty larvae per tank were examined using a stereomicroscope (Nikon SMZ 800, Nikon Instruments Inc. Melville, NY, USA) to determine the degree of metamorphosis, evaluated by scoring the position of the migrating left eye. Degrees of metamorphosis were divided into 5 phases (S): 0) symmetrical left and right eye position; 1) an asymmetrical position of the left eye and right eye, the left eye starts to migrate; 2) the migrating eye reaches at maximum the midline of the dorsal surface; 3) the migrating eye can be seen from the right ocular side or migrates within the dorsal side; 4) eye translocation is completed and the orbital arch is visible (Bonaldo *et al.* 2011).

At the end of the trial, the remaining larvae were removed from each tank and counted to determine the survival rate (SR) as a percentage of the initial number of larvae.

All experimental procedures were evaluated and approved by the Ethical-Scientific Committee for Animal Experimentation of the University of Bologna, in accordance with European directive 2010/63/EU on the protection of animals used for scientific purposes.

Gut fullness

At 6, 8, 10 and 13 dph, 10 larvae tank^{-1} were collected to determine gut fullness. Larvae were sampled in the morning (1 h after the meal), anesthetized in phenoxyethanol and immediately observed under a microscope (Nikon SMZ 800, Nikon Instruments Inc. Melville, NY, USA). At 6, 8 and 10 dph, gut fullness was determined by counting the number of *Artemia* ingested, according to Downing & Litvak (2001) and Blanco-Vives, Aliaga-Guerrero, Cañavate, García-Mateos, Martín-Robles, Herrera-Pérez, Muñoz-Cueto & Sánchez-Vázquez (2012). Whole prey or partially digested *Artemia* were clearly visible within the larval gut. At 13 dph, due to the impossibility of clearly distinguishing and counting the *Artemia*, fullness of gut was instead visually estimated through the observation of microscope images and classified into four categories (nominally: empty, medium, full and very full) (Mc Lauren & Avendano 1995; Puvanendran & Brown 2002).

Histology

At the beginning of the trial, a pool of 9 larvae, and subsequently 5 larvae per tank per sampling day (8, 14, 18, 25, 34 and 51 dph), were collected. Samples were fixed in 10% buffered formalin and paraffin embedded. Sections were cut at 3 µm and stained with hematoxylin-eosin. The sections were photographed with a camera (Nikon Digital Sight SD-MS, Tokyo, Japan) connected to an optical microscope (Nikon Eclipse 80i, Tokyo, Japan). A microscopic evaluation was performed, focusing on the major developing organs, with special regard to sensory organs (eyes, olfactory sensory cells, otic vesicles, neuromasts, taste buds and barbels). As histological studies for common sole larval development are lacking, we adopted the approach used in the study by Padrós, Villalta, Gisbert & Estévez (2011) on Senegal sole larvae.

Statistical analyses

The results in terms of WW, SGR, SR and gut fullness at 6, 8, 10 dph were analyzed using one-way ANOVA, followed by Tukey's Multiple Comparison Test. Gut fullness at 13 dph and metamorphosis data was analyzed using Pearson's χ^2 test with Yates' continuity correction. All statistical analyses were performed using the statistical package R version 2.15.3 for Windows (Revolution analytics, Palo Alto, CA, USA) and GraphPad Prism 5.0 for Windows (Graph Pad Software, San Diego, CA, USA). The differences between treatments were considered significant at $P \leq 0.05$.

Results

At the end of the trial, the SR was significantly higher in the LR-50 treatment compared to the LR-1000 and LR-3 treatments; the LR-1000 treatment showed a lower SR than both the LR-500 and LR-50 treatments (Table 1). The SGR data are presented in Table 1. The SGR of the larvae of the LR-3 treatments was significantly lower than in all the other treatments until 12 dph. From 12 to 19 dph, the larvae of the LR-3 treatment had a higher SGR than the LR-50 and LR-500 treatment. From 19 to 51 dph, the LR-3 treatment had a significantly higher SGR than under all the other LRs. The SGR of the LR-50, LR-500 and LR-1000 treatments were not significantly different during the trial.

Table 1.

The larvae of the LR-3 treatment showed a significantly lower WW than those of all the other treatments at 12 dph and of those of the LR-1000 treatment at 19 dph, respectively (Fig. 1). At 33 dph, there were no significant differences among treatments, whereas at the end of the trial (51 dph), the larvae of the LR-3 treatment showed a higher WW in comparison with those of the LR-1000 and LR-500 treatments.

Figure 1.

The gut fullness data are presented in Fig. 2. At 6 dph, the LR-3 treatment showed a lower number of ingested prey compared to the LR-500 treatment. At 8 dph, gut fullness of the LR-3 treatment was lower than that of LR-50 and LR-1000 treatments, whereas at 10 dph, the same treatment showed a lower value compared to the LR-500 and LR-1000 treatments (Fig 2, a). At 13 dph, the LR-3 treatment showed a significant lower fullness of gut compared to all the other treatments (Fig 2, b).

Figure 2 a, b.

For most of the specimens observed (S1, 93-100%), metamorphosis started at 12 dph in the LR-1000, LR-500 and LR-50 treatments (Fig. 3). In the LR-3 treatment, the larvae displayed a significant delay in the degree of metamorphosis development at 12 dph compared to the larvae in the other treatments (S1, 68%). At 20 dph, the larvae in the LR-1000 treatment showed a significant advance in the degree of metamorphosis in comparison with the other treatments and the LR-500 treatment showed a higher degree compared to the LR-3 treatment. From 24 dph onward, no significant differences were observed among the treatments and at 33 dph metamorphosis was completed (S4) under all the LRs.

Figure 3.

No histopathological changes were observed in any individual larvae. At 5-8 dph, eyes were fully pigmented and the retina showed, in the outer nuclear layer (ONL), the presence of retinal stem cells, 15 µm in diameter, with a centrally located, hyperchromatic round nucleus at the base of the photoreceptor layer (PRL) (Fig. 4, a). Olfactory bulbs and otic vesicles appeared; olfactory sensory cells were arranged in two small olfactory placodes. Neuromasts were seen in the epidermal layer covering the cephalic region; taste buds appeared in the ventral pharyngeal region and along the alimentary canal. The yolk sac was fully reabsorbed and gut content was visible. Several mucous cells appeared in the esophageal epithelium and the intestine was divided in two portions. The liver and exocrine pancreas were well developed and hepatocytes contained a large amount of lipid/glycogen. The swim bladder was visible above the gut. Large melanophores (larval type) were arranged in groups in the cephalic region and trunk. Several epidermal mucous cells appeared in the integument.

At 14-18 dph, in some specimens both eyes were visible on the right side. A few retinal stem cells were still present within the ONL. Olfactory cavities and nostrils were present. Otoliths were clearly evident within the otic vesicles. Neuromasts (Fig. 4, b) and taste buds extended along the alimentary canal, reaching the anatomic region corresponding to the stomach. Barbels appeared around the mouth. The kidney appeared well differentiated with evident collecting ducts. The heart and spleen were well evident and formed. Numerous thyroid follicles filled with colloid were evident. Small melanophores of an adult type appeared together with large melanophores of a larval type.

At 25 dph, the metamorphosis of the larvae observed had been completed. The olfactory bulbs increased in size, the otic vesicles became larger and otoliths were clearly detectable inside them. Neuromasts were visible also in the ventral aspect of the body. Taste buds were widely distributed in the buccal cavity and esophagus (Fig. 4, c). The brain was fully developed (telencephalon, hypothalamus and myelencephalon, cerebellum). Pigmentation was similar to that of adults.

After 34 dph and up to 51 dph, all subjects were closely similar to adults; the sensory organs were fully developed and further changes were only related to increases in size (Fig. 4, d).

Figure 4.

Discussion

Successful mass production of high-quality juveniles is dependent on successful larval rearing. The determination of optimal environmental conditions for fish larvae,

299 including light, is important to maximize their growth and survival (Stuart 2013). A
300 better understanding of light requirements during larval rearing may aid in standardizing
301 the optimal culture conditions. The literature shows that light requirements at larval
302 stage are species specific with a wide range of optimal values found among species,
303 extending from a few lux for Atlantic cod (*Gadus morhua* L.) to thousands of lux for
304 California yellowtail (*Seriola lalandi*) (Valenciennes) (Table 2).
305 Table 2.
306 In the present study, common sole showed a different response in relation to light
307 intensity and larval stage. During the first period of the trial (from 4 to 12 dph), which
308 corresponds to the pelagic larval stage, a low light intensity (3 lx) negatively affected
309 WW and SGR and the amount of prey ingested during the pelagic stage seemed to be
310 negatively affected by the lowest light intensity. Hence, the lower growth recorded at 12
311 dph for the larvae reared under 3 lx compared to the other treatments may be
312 attributable to their reduced capability of detecting and capturing live feed, which is
313 consistent with general finding in the pelagic stage of marine fish larvae (Monk *et. al*
314 2006). According to the histology observation, sole larvae observed at 5-8 dph showed
315 undeveloped sensory organs with the exception of eyes, which were fully pigmented
316 and therefore functional enough for feeding activity (Roo, Socorro, Izquierdo,
317 Caballero, Hernández-Cruz, Fernández & Fernández-Palacios 1999; Cobcroft &
318 Pankhurst 2003; Bejarano-Escobar, Blasco, DeGrip, Oyola-Velasco, Martín-Partido &
319 Francisco-Morcillo 2010; Ortiz-Delgado, Iglesias, Sánchez, Cal, Lago, Otero &
320 Sarasquete 2012). However, the presence in the ONL of retinal stem cells, which are
321 recognized as the rod precursors (Otteson & Hitchock 2003), suggest that vision at this

developmental stage is mainly mediated by cones, which limit vision at relatively high light intensity conditions in superficial waters during the day (Hubbs & Blaxter 1986). Different studies have shown that the majority of marine fish larvae are mainly diurnal visual feeders (Boeuf & Le Bail 1999; Peña, Dumas, Saldivar-Lucio, García, Trasviña & Hernández-Ceballos 2004; Puvanendran & Brown 2002) and a positive correlation between high light intensity and increased growth (Henne & Watanabe 2003; Huse 1994; Monk *et al.* 2006; Puvanendran & Brown 2002; Vallés & Estévez 2013) has been found. Visual feeding behavior has also been described in Senegal sole larvae, where the capture of live prey during the early pelagic stage depended on light (Cañavate, Zerolo & Fernández-Díaz 2006). On the other hand, our results suggest that an increment of light intensity from 50 to 1000 lx does not bring about any improvement in prey ingestion, WW or SGR at 12 dph. This last finding was also observed in Senegal sole larvae (Cañavate *et al.* 2007), where different light intensities, in the range of 200 to 2000 lx, had no effect on growth from 3 to 20 dph.

From 12 dph onwards, following the onset of metamorphosis, an inverse growth trend began to emerge, which became more evident in the last part of the trial (settlement and benthonic phase). In fact, between 12 and 19 dph, the larvae reared under 3 lx displayed a higher SGR than those under 50 and 500 lx, while between 19 and 51 dph their growth rate was above that under all the other LRs. As a consequence, the growth in terms of WW was similar among treatments at 33 dph, while at 51 dph the larvae reared under 3 lx displayed a higher WW compared to those under 500 and 1000 lx. Metamorphosis showed a different pattern of development. At 12 and 20 dph, the larvae reared at the lowest light intensity displayed a delay in the degree of metamorphosis while at 24 and 33 dph metamorphosis had been completed in all the LR treatments. A

346 dietary energy limitation during the larval stage has been pointed out as a relevant factor
347 that may prevent fish from reaching the energy reserves required for the onset of
348 metamorphosis (Parra & Yufera 2001; Pinto 2013). Some authors have reported that
349 common sole and Senegal sole larvae continue feeding during metamorphosis (Amara,
350 Lagardere & Desaunay 1993; Fernández-Díaz, Yúfera, Cañavate, Moyano & Díaz
351 2001; Geffen, Van der Veer & Nash 2007), suggesting that the matter and energy
352 required for transformation do not originate exclusively from body reserves.
353 Furthermore, our own findings suggest that the degree of metamorphosis was mainly
354 related to the weight of the larvae and the influence of light intensity on eye migration
355 seems negligible.
356 During this period, the most pronounced physiological and behavioral changes of larvae
357 are related to the shift from pelagic to benthic life (Fernández-Díaz *et al.* 2001;
358 Yamashita, Tanaka & Miller 2001). In the wild, these habitats are characterized by
359 different lighting conditions, since light intensity decreases exponentially with depth in
360 the water column (Thistle 2003). At the end of metamorphosis, members of this species
361 spend most of their life on the sea bottom, generally up to 30 and 100 meters of depth
362 for juveniles and adults, respectively (Salen-Picard, Darnaude, Arlhac & Harmelin
363 Vivien 2002; Grati, Scarcella, Polidori, Domenichetti, Bolognini, Gramolini, Vasapollo,
364 Giovanardi, Raicevich, Celić, Vrgoč, Isajlovic, Jenič, Marčeta & Fabi 2013).
365 Furthermore, sole species such as Senegal sole switch from a diurnal behavioral pattern
366 to nocturnalism during larval metamorphosis (Blanco-Vives *et al.* 2012) and their
367 ability to feed in darkness seems to improve with age (Cañavate *et al.* 2006).
368 Histology evidences that after metamorphosis feeding activity is also mediated by
369 chemosensory perception. In fact, observation revealed a wide distribution of

neuromasts and taste buds, besides the appearance of barbels around the mouth with growth. Moreover, the development of a sophisticated olfactory epithelium, very rich in microvillar and ciliated cells, supports the enhanced olfactory capability required during the benthonic phase (Appelbaum, Adron, George, Mackie & Pirie 1983). Neuromasts are sensory receptors that respond to mechanical stimuli and are probably involved, in low light conditions, in the capture of *Artemia*, which produce vibrations (Mukai & Lim 2012). Taste buds and the olfactory epithelium respond to chemical stimuli such as free amino acids released from live prey and MD (Mukai, Tuzan, Lim & Yahaya 2010), while barbels are mainly necessary in benthic species to recognize the sea bottom and search for prey items (Lombarte & Aguirre, 1997).

SR showed a different trend compared to WW and SGR. In fact, at the end of the trial, SR was higher for larvae reared under an intermediate light intensity (500 and 50 lx). Boeuf & Le Bail (1999) reported that in larvae optimal light for growth is often not the same as for survival, because too much light can be stressful or even lethal. Similar results were found in Southern flounder larvae, where survival at 11 and 15 dph was generally higher under a mid-range intensity compared to the extreme values (Henne & Watanabe 2003). The authors suggested that while the minimum illumination threshold theory explains the poor growth and survival of marine fish larvae under low light intensities, there appears to be a maximum illumination level above which larval growth and/or survival are adversely affected in some species.

In summary, common sole larvae require specific light intensities to maximize feeding activity, growth and survival. Based on the results of the present trial, we suggest different light regimes during larval ontogeny: a high light intensity seems to be the most suitable for first-feeding larvae, while a low light intensity would ensure better

394 growth after larval settlement. Thus, the light intensity regime can be fixed between 50
395 and 500 lx during the pelagic stage (4-12 dph) and reduced to 3-50 lx from the onset of
396 metamorphosis to the benthic phase. Further investigations are needed to determine the
397 effects of environmental factors on the early life stages and larval feeding behavior of
398 this species.
399
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Figure captions

Figure 1. Common sole larvae wet weight (mg) recorded during the trial. Data are given as the mean ± SD from triplicate treatments. The inset graph shows larval wet weight during the first 19 days of the trial in detail. Different letters denote significant differences among the treatments ($P \leq 0.05$).

Figure 2. **a)** Gut fullness expressed as the: number of *Artemia* found at 6, 8, 10 days post hatching (dph) in the gut of sole larvae reared under different light intensities ($P \leq 0.05$); **b)** Gut fullness of larvae at 13 dph classified into four categories: empty, medium, full and very full. Data are given as the mean (n = 30) ± SD. Different letters denote significant differences among the treatments ($P \leq 0.05$).

Figure 3. Percentages in the degree of metamorphosis in common sole larvae as a function of light intensity regime. S0) symmetrical left and right eye position; S1) asymmetrical position of the left and right eyes, with the left eye starting to migrate; S2) the migrating eye reaches at maximum the midline of the dorsal surface; S3) the migrating eye can be observed from the right ocular side or migrates within the dorsal side; S4) eye translocation is completed and the orbital arch is visible. N = 60 larvae per sample per treatment. Different letters denote significant differences among the treatments ($P \leq 0.05$).

Figure 4. Histological section of the main sensory organs. A) Retina of 8 dph larva. Large, oval cells, 15 μm in diameter, with a centrally located, hyperchromatic round

nucleus are present at the base of the PRL (asterisks), interpreted as retinal stem cells (H&E, bar=15 μm). B) 14 dph larva (H&E, bar=200 μm). Flask-shaped neuromasts formed by several elongated cells are present in the frontal region of the head (inset, H&E, bar=15 μm). C) 25 dph larva. (H&E, bar=200 μm). Numerous taste buds formed by two-three cells and projecting into the lumen of the alimentary canal are visible (inset, H&E, bar=15 μm). D) 51 dph larva. (H&E, bar=200 μm). Olfactory bulbs and olfactory epithelium are well developed in these metamorphosed specimens (inset, H&E, bar=100 μm).

Table 1

Specific growth rate (SGR) and survival of common sole larvae at the end of the trial.

Protocols	SGR (% day^{-1}) from 4 to 12 dph	SGR (% day^{-1}) from 12 to 19 dph	SGR (% day^{-1}) from 19 to 51 dph	Survival (%)
LR-1000	11.79 ± 0.78^{b}	16.16 ± 0.92^{ab}	8.37 ± 0.30^{a}	27.4 ± 9.1^{a}
LR-500	12.17 ± 0.16^{b}	14.28 ± 0.44^{a}	8.37 ± 0.35^{a}	43.3 ± 5.7^{bc}
LR-50	11.93 ± 0.42^{b}	15.32 ± 0.97^{a}	8.86 ± 0.18^{a}	48.7 ± 1.8^{c}
LR-3	7.38 ± 2.41^{a}	18.17 ± 1.58^{b}	9.82 ± 0.19^{b}	30.4 ± 1.9^{ab}

Each value is expressed as the mean ± SD. Columns with different superscript letters for a given value are significantly different ($P \leq 0.05$).

Table 2. Summary of optimal light intensity thresholds for some cultured marine fish species larvae

Larvae species	Intensity (Lux)	Age (dph)	References
Southern flounder (*Paralichthys lethostigma*) (Evseenko)	457 – 1,362	0 - 30	Denson & Smith (1997)
Haddock (*Melanogrammus aeglefinus* L.)	110	0 - 41	Downing & Litvak (1999)
European sea bass (*Dicentrachus labrax* L.)	5 - 400	0 - 21	Cuvier-Péres *et al.* (2001)
Fat snook (*Centropomus parallelus*) (Poey)	200 – 1,500	3 - 14	Cerqueira & Brügger (2001)
Atlantic cod (*Gadus morhua* L.)	2.4 – 56.6	0 - 35	Van der Meeren *et al.* (2007)
Senegal sole (*Solea senegalensis*) (Kaup)	200 – 2,000	3 - 20	Cañavate *et al.* (2007)
White sea bass (*Atractoscion nobilis*) (Ayres)	300 – 3,000	0-15	Jirsa *et al.* (2009)
California yellowtail (*Seriola lalandi*) (Valenciennes)	14,850	2 - 16	Stuart & Drawbridge (2011)
Meagre (*Argyrosomus regius*) (Asso)	500 – 1,000	1 - 30	Vallés & Estévez (2013)

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655 **Figure 1**

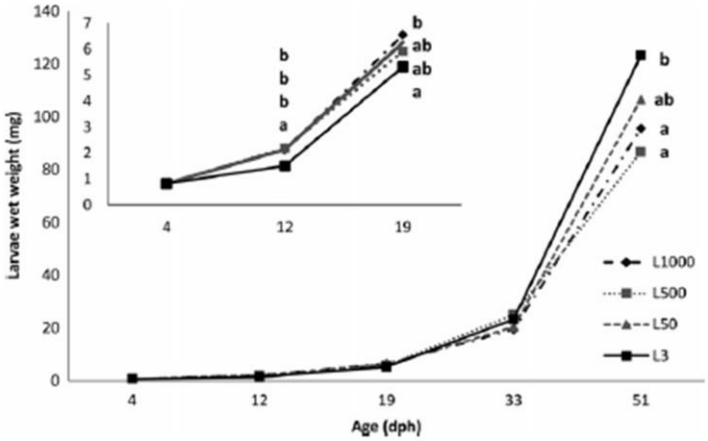

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Figure 2

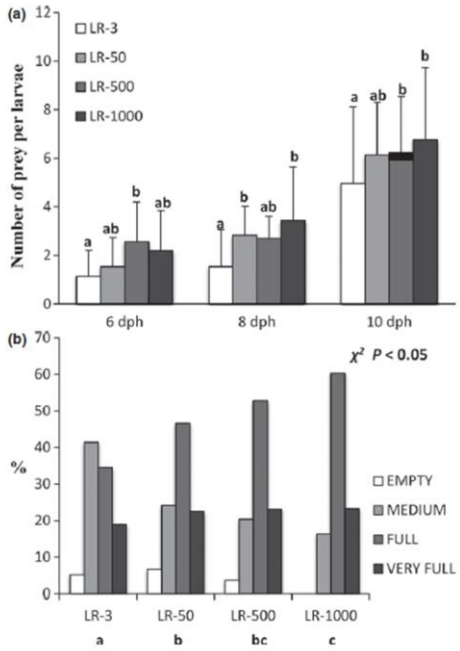
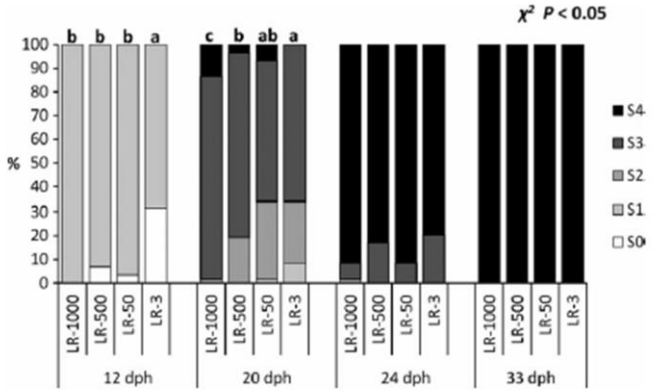
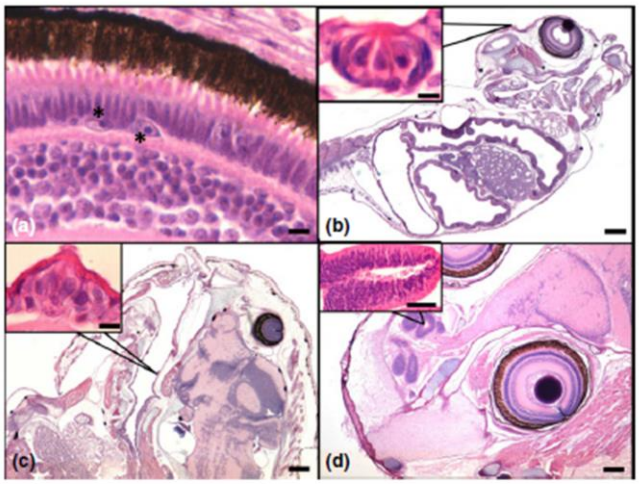

(a)
LR-3
LR-50
LR-500
LR-1000
Number of prey per larvae
6 dph
8 dph
10 dph
(b)
χ^2 $P < 0.05$
%
EMPTY
MEDIUM
FULL
VERY FULL
LR-3
LR-50
LR-500
LR-1000
a
b
bc
c

Figure 3


χ² P < 0.05
b b b a
c b ab a
100
90
80
70
60
% 50
40
30
20
10
0
S4
S3
S2
S1
S0
LR-1000
LR-500
LR-50
LR-3
12 dph
20 dph
24 dph
33 dph

669 **Figure 4**



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