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Behavioral responses of European sea bass (*Dicentrarchus labrax*) larvae and *Artemia* sp. exposed to constant light or darkness vs. light/dark cycles of white, red or blue wavelengths

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ABSTRACT

The performance and survival of fish larvae are strongly influenced by their surrounding photic environment. The aim of this study was to investigate the effect of light characteristics (spectrum and photoperiod) on the feeding and locomotor behaviors of European sea bass larvae and its prey (*Artemia* sp.). To this end, constant light (LL), constant darkness (DD) and 12:12 h LD cycles of red, blue or white LED lights were applied from 1 to 30 days post-hatching. The Modal Action Patterns (swimming duration, orientation, capture, miss and pass frequencies) of larvae and *Artemia* distribution in the tank were video recorded and analyzed using newly developed tracking software. The results showed that under LD_B the phototactic response of sea bass larvae led to a significatively homogeneous distribution in the tanks and aquaria, while under LD_W and LL the highest larvae density (52%) was seen on the tank walls. LD_B and LD_W resulted in longer swimming duration and earlier weaning. Larvae exposed to darkness and red light showed the lowest swimming and feeding activity, and a higher aggregation tendency of both fish larvae and the live prey. White light exposure resulted in a strong phototactic response from fish larvae and *Artemia*, which consisted of a tendency to congregate at the corners or close to the walls of the tank/aquaria. *Artemia* hatching rate under blue light was highest ($56.5 \pm 2.9\%$) in contrast with red light ($26.3 \pm 1.4\%$) and total darkness (27.9 ± 3.9). These results showed that the relationship between the behavioral responses of sea bass larvae and *Artemia* is strongly affected by lighting conditions, which has both basic and practical implications for understanding their behavioral ecology and for improving culture protocols.

1. Introduction

Although organism responses to light have often been ascribed to one basic mechanism (i.e. visual processing), the regulation of circadian rhythms by light led to the discovery of non-visual photoreceptors that are involved in several physiological processes related to the task of extracting seasonal and time-of-day information (Foster et al., 2007). In fish, the photosensory systems and extraretinal photoreceptors are complex and far from fully understood, as the response to light is often species-specific, depending on phylogenetic and ecological factors (Marchesan et al., 2005). Scarce information about the features of the environmental light and the photoresponse behavior of fish, particularly at the larval stage, makes the interpretation and correlation of existing data a difficult task.

In nature, the spectral characteristics of underwater light are determined by a combination of the ambient skylight and the optical properties of the water. Light becomes increasingly monochromatic with depth, because the spectral profile is selectively attenuated as light passes into deep water (Jerlov, 1968). Thus, clear ocean waters transmit maximally at blue wavelengths (470 nm), while coastal waters transmit better at blue-green wavelengths (500 nm) and estuarine waters at green wavelengths (580 nm) (Cohen and Forward, 2002). Furthermore, fish have adapted their photopigment sensitivity according to their surrounding environment (Kusmic and Gualtieri, 2000).

European sea bass (*Dicentrarchus labrax*) is one of the most widely cultured fish species of the Mediterranean Sea and large numbers of larvae are produced at industrial level. However, the impact of environmental factors such as lighting conditions is poorly understood, although recent work reviewed the response to certain light spectra, intensities and photoperiod, which were seen to have a great influence in terms of larval growth, development and survival (Villamizar et al., 2011). Nevertheless, the behavioral responses of larvae and their prey under different lighting conditions remain unexplored.

The aim of this research was to investigate the Modal Action Patterns (swimming duration, orientation, capture, miss and pass frequencies) and distribution of sea bass larvae from 1 day post hatching (DPH) to 30 DPH; exposed to different light spectra and photoperiod. In addition, live prey (*Artemia*) hatching and distribution

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were observed in order to investigate daily patterns and predator–prey relationships.

2. Materials and methods

2.1. Eggs and larval rearing

The experiments were conducted at the Laboratory of Aquaculture at the Naval Base of Algameca (ENA, Murcia, Spain) and at the Laboratory of Fish Chronobiology at the University of Murcia (Spain). Fertilized eggs of European sea bass were obtained from the Spanish Oceanographic Institute (IEO) at Mazarrón (Murcia) from spontaneous spawning by captive broodstock. Newly hatched larvae (0 DPH) were transported in total darkness and distributed into (exp. 1) fifteen glass-reinforced polyester (GRP) tanks of 150-L capacity with an open seawater circulation system and (exp. 2) fifteen 80 L glass aquaria filled with artificial seawater (Ocean Reef, Prodac, Italy) in recirculation system. The density of fish was established at 30 larvae L^{-1}. Aeration was provided by means of continuous slow seawater flow. Rotifers *Brachionus picatilis* were cultured and enriched with commercially available freeze-dried green algae *Nannochloropsis sp.* (Phytobloom Prof®. Necton, Portugal), and added to the tanks/aquaria daily (09:00 h) as an early live food at a density of 20 individuals mL^{-1} from 8 to 20 DPH. *Artemia* nauplii at a density of 2–3 nauplii day^{-1} mL^{-1} were introduced twice a day (09:00 and 13:00) from 18 to 30 DPH. One day before being fed to the larvae, *Artemia* were enriched with a microencapsulate mixture of phytoproteins, HUFAs and phospholipids (ORI-PRO, ORI-GO, Skretting AS., Spain). From 26 DPH to 30 DPH, 2 g L^{-1} of dry food (Gemma wean 0.2, Skretting AS, Spain) was fed to the larvae.

2.2. Experimental design

Two experiments were performed, subjecting animals to continuous (exp. 1) or acute (exp. 2) exposure to different lighting conditions.

For experiment 1, sea bass larvae were reared from 1 to 30 DPH under five lighting regimes with three replicates: 12L:12D cycle with red (LD_R, peak at 685 nm), blue (LD_B, peak at 463 nm) or white (LD_W), 24L:0D white (LL), and 0L:24D (DD). The white light used had a broad-spectrum, with 95% irradiance within the range of 367–700 nm. The spectral analysis was performed using a spectroradiometer (FieldSpec®, ASD, Colorado, USA). To avoid the effects of any background light on the experiments, the experimental tanks were covered with a light-tight black screen. For the different spectral trials, lamps were constructed using 17 light emitting diodes (LED, Kopa Electronica; Barcelona, Spain) mounted on a fiberglass plate (160×232 mm) and encased in a waterproof container. The circuit was powered by a 3 V DC supply connected to a variable resistor (0–2 KΩ) that allowed the light intensity to be adjusted to 0.42 Wm^{-2} s^{-1}, which is low but well above the light threshold (0.06 Wm^{-2} s^{-1}) required to modify the melatonin content in both the eye and plasma of European sea bass (Bayarri et al., 2002).

The effect of the light spectrum and photoperiod on vertical and horizontal distributions was observed from 1 DPH to 18 DPH. Sampling was performed every other day in all tanks, 1 h after live prey addition to allow larvae to return to 'normal' behavior following the disturbance of feeding. Horizontal distribution was described by counting the larvae over a white secchi disk placed in three positions within the GRP tanks (wall, intermediate and center) at 12 cm depth. Vertical distribution and the shift from live prey to dry food (weaning) were observed in 6 L experimental glass aquaria (25×17×14 cm) filled with the same water of the rearing tanks and illuminated using the spectral LED lamps. For vertical distribution, 1 h after feeding, larvae were randomly sampled and introduced to the experimental aquaria at the same density as in the rearing tanks. Observations were made after a 30 min acclimatization period when larvae had recovered from the stress associated with the transfer. The larvae were video recorded for 1 h (Sony Handicam SR55), after which, those present in the three levels (upper, intermediate and bottom) of the glass aquaria were counted. This sampling method was repeated three times in all the replicates and treatments.

Weaning was observed from 26 DPH, when *Artemia* nauplii and dry food were added to the experimental aquaria. The focal animal technique (Altman, 1974) was applied to collect information from one randomly chosen larva during 1 min, 20 larvae per sampling point (60 per treatment). Prey item captures and dry food ingestion were counted by means of 20 min video recordings.

For experiment 2, fish larvae were first reared under a 12L:12D photoperiod of white light (LED lamp) from 1 DPH to 30 DPH and then exposed for 1 h to total darkness and blue and red LED lights in order to observe their behavioral responses after such an acute light treatment. For all video recordings, IR lights (λ = 880 nm) were used as the principal illumination source.

Sea bass larvae and *Artemia* behavioral observations were performed on alternated days 2 h after lights on, in three 6 L glass aquaria (25×17×14 cm for fish larvae and 25×17×7 cm for *Artemia*) with 16 cm water depth and illuminated using the LED lamps (red, blue or white). *Artemia* was incubated at 25 °C, 32 ppt. of artificial seawater at a density of 10 g/L of cysts.

The occurrence of five Modal Action Patterns (MAP) in sea bass larvae including swimming duration, orientation, capture, miss and pass frequencies (Table 1; Puvanendran and Brown, 1998) was video recorded after 30 min of light exposure, during a 30 minute period (Sony Handicam SR55) and quantified using the focal animal technique, either for frequency (events/minute) or duration (s). The resulting data for each MAP, larva and replicate were averaged to obtain a single value of a given MAP in each treatment. *Artemia* were incubated separately under the LED lamps (red, blue or white) in LL or under total darkness for 24 h to observe hatching time differences among treatments as well as distribution patterns.

The distribution of newly hatched *Artemia* nauplii was observed from one hour video recordings (1 frame per second; Sony Handicam SR55) performed three times per treatment and 30 min after being introduced into the aquaria. Image analysis was performed by means of specialized software designed by the Computer Vision Research Group of the University of Murcia, which offers an in-house alternative to other methods. The program transforms each image (I) of the recordings into an 8 bit gray scale image, where a pixel with a value of intensity I(x,y) = 0 is completely black (total absence of *Artemia* in the location (x,y)) and a pixel with a value of I(x,y) = 255 is completely white (representing maximum occupation by *Artemia*). To process the video recordings, the software divided the image of each aquarium into nine equal sections prior to the analysis.

A parameter of *Artemia* occupation (Occ) was defined as:

$$\mathrm{Occ} = 100/255 \cdot \overline{I(\cdot,\cdot)};\quad \overline{I(\cdot,\cdot)} = 1/N \cdot \sum_{\forall \text{ pixel } (x,y)} I(x,y)$$

where $\overline{I(\cdot,\cdot)}$ represents the average intensity value of the N pixels in the chosen region of the aquarium; to better interpret the results, Occ was scaled by 100/255, so it ranges from 0 to 100. The image analysis

Table 1
Modal action patterns (MAP) for European sea bass larvae adapted from Puvanendran and Brown (1998).

MAP	Description
Swim	Forward movement of larvae accomplished by caudal fin action.
Orient	Larvae are stationary and align themselves towards a prey item.
Capture	Prey item is ingested by larvae.
Miss	An attempt is made but larvae fail to capture the prey item.
Pass	Larvae orient towards prey item but instead of making a capture attempt, they swim in a different direction.