Light effect on octopus paralarvae (*Octopus vulgaris*).

Irene Moltó Martín

Master thesis Aquaculture

September 2017

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

INDEX

Abstract

The common octopus (*Octopus vulgaris*, Cuvier 1797) is a promising species for aquaculture diversification, but its massive mortality during the first life stage is the main bottleneck for its commercial production. Light is a key environmental factor that synchronizes all life-stages, from embryo development to sexual maturation. The aim of this master thesis is to evaluate the effect of light on behaviour, predation and survival using distinct colours (white, blue, green and red) and intensities at different DPH to assess the performance, development and welfare of *O. vulgaris* paralarvae. Results show that white and blue wavelengths contribute to the best outcomes. Paralarvae exhibit a strong positive phototachtism with a clear preference for white colour. Additionally, polarized vision was evaluated using polarized filters but no remarkable results were obtained. These results highlight the role of lighting conditions during the early development of paralarvae and should be considered for the optimization of rearing protocols in the hatchery phase.

Keywords: *Octopus vulgaris*, light, FluoSpheres, colour, intensity, polarization, survival, predation.

1. Introduction

1.1. *Importance and challenges of Octopus vulgaris culture*

The common octopus (*Octopus vulgaris*, Cuvier 1797) is a species with increasing interest for marine aquaculture diversification, given its high growth rate and easy adaptation to captivity (Iglesias et al., 2007, 2014[a]).

According to several authors (Iglesias and Fuentes, 2013; Iglesias et al., 2007, 2014[a]), the high mortalities could be due to: (i) inadequate and/or unbalanced diets that do not satisfy paralarvae nutritional requirements; (ii) lack of standardized rearing techniques, and (iii) little knowledge about octopus paralarvae physiology and behaviour. In the last years, the interest in octopus culture has increased and many studies have been published arising relevant data on physiology and farming (Garrido, 2016[a]).

Light is a key environmental factor, several studies, have showed that artificial light affects paralarvae foraging, growth and survival (Monk et al., 2006; Yoseda et al., 2008; Villamizar et al., 2009). However, light influence in *O. vulgaris* paralarvae has not been deeply investigated.

1.2. *Underwater photo-environment and biological responses*

The incident light from the sun is quickly modified (absorbance and reflection) depending on the specific properties of the water. Radiant energy is selectively absorbed and scattered by particles present in the water column which thus affect the magnitude (square of the electric field vector), polarisation (direction of oscillation of the electric field vector), wavelength (frequency of oscillation), direction and propagation of the light generating great photic variability in the aquatic environment. The water column acts as

a chromatic filter with wavelengths below violet (λb390 nm) and beyond red (λN600 nm) being quickly absorbed. Blue wavelengths (λ~450 nm) however, penetrate deeper in the underwater environment, reaching depths of up to 150 meters. Importantly, artificial lights differ greatly from the sun's spectrum, particularly underwater, as most light bulbs provide red-rich wavelengths and few blue photons (Villamizar et al., 2010).

Biological rhythms enable organisms to measure time and to synchronize their endogenous behaviour and physiology with the time constraints of their environment. Octopuses are perhaps among the most interesting inhabitants of the littoral zone, they inhabit the photic zone of the sea where sunlight is known to influence the activity of many organisms. Since these animals have both well-developed visual and camouflage systems, it may be expected that *O. vulgaris* activity may be strongly influenced by light. However, light may only play a minor role in synchronizing activity. Other environmental cues may be used by *O. vulgaris* to synchronize their activity and behaviour with the environment.

For most species of octopus, hatching events occur at night to avoid predation. The physiological mechanisms that promote hatching process are unknown. After 15-20 days of residence in the littoral zone, pelagic paralarvae are passively transported by current to the open sea, where they are located to about 100-200 m of depth. Planktonic stage lasts for approximately 2 months. After a brief period in contact with hard surfaces, paralarvae definitively settle to the seafloor, this is known as presettlement period. Young octopuses, considered juveniles from now onwards, live on the benthos and have similar habits to that of adults (Villanueva et al., 2008).

The biological response to light depends on the species-specific ecology: in deep sea fishes, photoreceptors have a maximised visual contrast in the blue band, while coastal fish species have maximum sensitivity in the green band (Villamizar et al., 2010). *O. vulgaris* paralarvae probably change their sensitivity during their life cycle. Newly hatched paralarvae must be able to survive in a high intensity photoenvironment (20-40m depth), later they must adapt to dim light conditions (100-200m depth) and finally adapt to benthonic lifestyle (1-100m depth).

Moreover, planktonic paralarvae have nictemeral migrations in response to zooplankton migrations. The zooplankton experiences vertical migrations with daily frequency, rising from the depths in the early evening to occupy higher levels during the night, and then, before dawn, descend again to the depths where they normally inhabit during the day. This migratory phenomenon represents the largest migration of living beings on the planet, both by the number of individuals and by the amount of biomass, and the distance traveled varies considerably from one species to another. Among the factors that have been cited as causes of vertical migration, the most relevant is light, but there are also gravity, pressure and others that remain unclear. The truth is that, in any case, vertical migration must have an ecological sense, feeding events, is perhaps the most logical, since a large part of the migratory population is mainly found in the 100 meters surface, precisely where primary production is located by phytoplankton. It has also been argued

that nictemeral migrations constitute a defense mechanism: during the day, zooplanktonic organisms remain in the depths, where they would be safe from their predators, ascending at night, when they are more difficult to detect by these.

The visual abilities of nocturnal and deep-sea animals are remarkable, ranging from high sensitivity to dim light by a variety of means such as large eyes, pupils and photoreceptors, tapeta to aid photon capture, neural summation, large visual receptive field, specialized foveas, etc. Even though many animals sacrifice colour vision for increased sensitivity to low light, colour vision has been found in nocturnal hawkmoths and geckoes, as well as in some deep-sea fish and cephalopods, which opens the possibility that colour vision under low-light conditions might not be as unusual as previously thought (Allen et al., 2010). However, care should be taken in making broad conclusions about any particular species photic sensitivity as during their life cycle, marine species can undergo radical morphological changes or significant geographical migrations which generally involve adaptations to their new photic environment (Villamizar et al., 2010). After residence in the plankton, paralarvae undergo an intense morphological and ecological transition from free-swimming pelagic animal to a dominant benthic life style which characterises the juvenile and adult stages. This transition implies a series of adaptations over a relative brief time (Villanueva et al., 2008). It is thought that *Octopus vulgaris* varies its visual system from a high visual acuity to a high visual sensitivity during transitions from pelagic (paralarvae) to benthic habitats (juvenile). Therefore, when designing an artificial lighting system for octopus culture, its ecology and developmental stage should be considered.

Because visual predation occurs day and night, many predators must have good night vision. Prey therefore exhibit antipredator behaviours in very dim light. Cuttlefish use their excellent night vision to perform adaptive camouflage in dim light. It is likely that nocturnal camouflage behaviour is an anti-predator tactic and/or increases their hunting success (Allen et al., 2010). Cephalopods vary their spectral reflectance by active control over their chromatophores in response to natural backgrounds rather than simply varying their luminance. Across a diversity of taxa, all cephalopod studies to date have found rhodopsin transcripts in the skin identical to those in the eye, and the skin's spectral response to light is nearly identical to that of the retina (Stubbs et al., 2016).

1.3. Polarization vision

Under water, the wavelength spectrum reflected from an object varies with depth, while the reflected e-vector orientation remains relatively constant, and the percentage polarization of the background scattered light is high even at depths exceeding 50 m. Like other cephalopods, octopuses are sensitive to the orientation of the e-vector of linearly polarized light and thus possess polarization sensitivity. The function of polarization sensitivity in navigation, body orientation and the location of large bodies of water is well established (Shashar et al., 1996). Cephalopods, squid and octopus are known to be sensitive to the orientation of polarization of incoming light. This sensitivity arises from the orthogonal orientation of neighbouring photoreceptors in the retina. Irregularities in

the retina or movements of the eye enable cephalopods to sense any polarization orientation. Cuttlefish use polarization vision to hunt in the natural environment, they use it for breaking camouflage of potential prey. Cuttlefish also display intraspecific recognition and communication through polarization (Shashar et al., 1996, 2000, 2002). Cephalopods possess a single photoreceptor for spectral discrimination. Octopuses use a combination of off-axis pupil shape and chromatic aberration to yield spectral information (Stubbs et al., 2016). *O. vulgaris* are colour blinded and polarized vision may provide information similar to that available from colour vision and thus serve to enhance the detection and recognition of objects.

1.4. Objectives

I. *Behaviour*: Evaluate the influence of light over paralarvae behaviour. Detect preferences and maximal sensitivities between distinct colours and intensities. Ascertain if these preferences change with age.
II. *Predation*: Assess the influence of light on predation under different colours. Test if polarized filters enhance prey capture and observe if paralarvae perform best under simulated underwater conditions with the use of neutral density filters. Assessment of feeding performance at different ages.
III. *Survival*: Study how light influences paralarvae survival using different colours and evaluate survival rates under simulated natural environment intensities with neutral density filters. Evaluate if tolerance to light stress changes with age.

2. Material and Methods

All the experiments were performed according to the Spanish Law 6/2013 based on the Directive 2010/63/EU regarding the protection and humane use of animals for scientific purposes. Experiments were carried out from July- September 2016 in the IEO Tenerife installations.

2.1. *Brodstock*

Broodstock rearing was carried out under common standard conditions as described by Reis et al. (2015). The adult specimens were kept in 1000 L tanks (with a maximum density of 10 kg per tank) with water renovation (5L·min-1), under oxygen saturation conditions and low light intensity. Broodstocks female weight average and physicochemical parameters of water are presented in **Table 1**. The availability of food and centre logistic conditioned the broodstock diet. Considering the study carried out by Quintana et al. (2015), crabs or cephalopods (e.g. squid *Loligo gahi*) were included to ensure an optimal spawning quality. Adults of *O. vulgaris* were captured from local fisheries using octopus traps. As a result, paralarvae geographical origin was Tenerife-Central Atlantic area (28°30'N, 16°12'W).

Table 1. Female weight and physochemical parameters for broodstock.

Female weight (kg)	4.5
Temperature (°C)	19-21
Salinity (PSU)	36.8

Oxygen (mg/L)	6.8-7.4
NH3/NH4 +(mg/L)	0
NO2 -(mg/L)	<0.3

2.2 . Paralarvae rearing conditions

Newly hatched paralarvae were cultured at density of 5 paralarvae·L^{-1}, in 1000 L black fiberglass cylinder-conical tanks. Temperature and oxygen were measured daily, and nitrite, ammonium and salinity once a week. Dissolved oxygen levels were kept close to saturation and nitrite and ammonia were <0.3 mg·L-1 and 0 mg·L-1, respectively. A renovation flow of 1L·min-1 (corresponding to more than 1.5 renovations per day) was applied from 18:00 to 8:00. A flow-through seawater system equipped with 20, 5 and 1 μm filter cartridges and UV lamps were used.

2.2.1 Paralarvae feeding

In all experiments, *Artemia* nauplii were obtained from cysts that hatched in fiberglass cylinder-conical tanks for 24h at 28°C, with 37 PSU, vigorous aeration and 2000 lx. After the on-growing period, *Artemia* enrichments were carried out with phytoplankton (*Isochrysis galbana* (Iso) *and Nannochloropsis* sp.). The phytoplankton enrichments were performed according to Iglesias and Fuentes (2014). Two prey sizes were used along the experimental period: nauplii (1day old) from day 0 to 15, and metanauplii (4 days old) from day 16 to 29. Paralarvae were fed 3 times per day, at a density of 0.3 nauplii·mL^{-1} from day 0 to 15 and at 0.15 metanauplii·mL^{-1} from day 16 to 29. The enriched *Artemia* was kept in the dark at 4°C with soft aeration until paralarval feeding. The *Artemia* cysts were obtained from INVE Aquaculture (Dendermonde, Belgium), freeze dried *Isochrysis galbana* and *Nannochloropsis* sp. were provided by Fitoplancton marino S.L (Cádiz, Spain).

2.3 Effect of light on larval behaviour

A wide range of light conditions (natural light, incandescent bulbs, fluorescent tubes) has been used in paralarvae culture. In this experiment, we are going to test the behaviour of *O. vulgaris* paralarvae under new artificial lighting technologies such as light emitting diodes (LED). To test the effect of light on larval behaviour, we designed a light preference experiment. This experiment was carried out in a dark room using a horizontal tube divided in length in 16 sections and with LEDS at its ends. The tube structure consisted of a pipe tube divided by half held by the sides by other pipes positioned vertically. At the bottom of each of the vertical pipes a faucet was placed to allow filling and emptying of water. The horizontal pipe was filled with a salt water hose and after each filling the pipe was calibrated with a bubble gauge to maintain the horizontal plane. Inside the vertical pipes at each end of the horizontal pipe, a LED was placed hanging from the roof at 7 cm from the water surface, this distance was modified when necessary to adjust intensity between colours.

Tests were performed with newly hatched paralarvae (age 0 days) using 4 distinct colours: white (W), blue (B), green (G), and red (R). A total of 30 paralarvae per trial were dropped

in the middle of the tube and after 60 minutes the number of paralarvae at each point of the tube was counted to evaluate its light preference. Each experiment consisted of 3 replicates (R%) and 3 controls (C%). Control tests were performed by exchanging the position of the lights to discard a possible tube effect. Intensities are provided in **Table 2**.

Similar test was performed using two types of filter: chromatically neutral density filter "neutral filter" and linearly polarizing dichroic sheet filters (Polaroid, HN38S) "polarized filter". Each neutral density filter sheet reduces the PAR (Photosynthetically Active Radiation) 15%, an attenuation found between 5-20 meters deep, these filters can be used to mimic natural light conditions. White colour with neutral filter (6 layers of neutral density filter) was tested against white without filter. These experiments were performed for age 0 and 15 DPH (days post hatching) with the aim of observing possible changes with the increasing age of the paralarvae. Further tests were performed using white colour comparing high intensity (Wmax) *vs.* low intensity (Wmin) with paralarvae age 0, 12 and 19 DPH.

Intensity was firstly measured using a digital lux meter model LX-101. Most lighting engineers measure lighting levels in lumens per square meter (lux). A series of calculations were made to equal light intensities between the two LEDS. As white and blue had very similar intensity values, distance remained the same for both (7cm). However, to equal intensities between white and green, white was raised from the water surface 11cm and to equal white with red, white led was raised 12cm. Importantly, when studying light, measurements should consider the full visible spectrum and not only what the human eye can detect. Therefore, the use of measurement unit's specific to the spectral sensitivity of the human eye (Lumens, lux etc.) is not appropriate and should be replaced by unbiased irradiance measurements like watts/ m2 or photons/s/m2. Therefore, intensity was measured with Avantes Spectrograph Model AvaSpec-ULS2048x16 at 57 cm. When distance is reduced to half, intensity multiplies by four for this reason, if distance is reduced to 7 cm all values should be multiplied by 64. Intensity data is provided in **Table 2.**

Table 2. Light intensity measurements from the light emitting diodes (LEDS) at 57 cm (with pane glass) using Avantes Spectrograph Model AvaSpec-ULS2048x16 for the different colours: White (W); Blue (B); Green (G); Red (R); W2(6 neutral filters); W3 (4 neutral +1polarized). Calculation of intensity data when distance is reduced to 7,1cm. Digital lux meter measurements at distance 7cm. Data conversion from lux to W/m^2.

Colour	Avantes Spectrograph (57 cm)	Avantes Spectrograph (7 ,1cm)	Digital lux meter (7 cm)	W/m^2
WHITE	2,07 E+06 counts	132,48 E+06 counts	6,48 lux.	26,05
BLUE	1,958E+06 counts	124,8 E+06 counts	6,10 lux.	24,53
GREEN	1,195E+06 counts	76,48 E+06 counts	3,51 lux	14,11
RED	1,323E+06 counts	84,67 E+06 counts	4,13lux	16,60

W2 (6 neutral filters)	1,24E+06 counts	79,36 E+06 counts	3,88 lux.	15,60
W3 (4 neutral +1pol)	1,11E+06 counts	71,04 E+06 counts	3,47 lux.	13,95

2.4 Effect of light on predation

Predation experiments were developed in a dark room isolated from light on a table which was divided physically in 4 individual spaces, with no possible influence between areas. A LED bulb was placed on the roof in the middle of each area and 4 colours: white (W), blue (B), green (G) and red (R) , where established for each area. 1L glasses painted black were placed below the focus; 3 replicates without filter and 3 replicates with neutral/ polarized filter per colour. In this experiment polarized filter will be used to test if paralarvae, in effect, use polarization to enhance predation. Neutral filter will be evaluated to test if paralarvae perform best in dim light conditions like those found in their natural environment. We were also interested in applying polarized filters as an effort to verify if a decrease in the light conditions shows a less active behaviour from the live prey (*Artemia* sp.) and therefore are easier to catch, for this reason a cover with 4 layers of neutral density filter and 1 layer of polarized filter was fabricated. Intensities for the assorted colours are provided in **Table 3.**

Table 3. Light intensity measurements from the light emitting diodes (LEDS) at 57 cm (with pane glass) using Avantes Spectrograph Model AvaSpec-ULS2048x16 for the assorted colours: White (W); Blue (B); Green (G); Red (R), with no filter, 6 neutral filters and 4 neutral + 1 polarized.

Avantes Spectrograph Model AvaSpec-ULS2048x16			
Colour	**No filter**	**6 neutral filters**	**4 neutral + 1 polarized**
BLUE	1,958E+06	6,683E+05	6,485E+05
RED	1,323E+06	5,831E+05	5,671E+05
GREEN	1,195E+06	5,710E+05	8,002E+05
WHITE	2,07E+06	1,24E+06	1,11E+06

Because white and blue had similar intensity values, distance from the surface of the glass to the LED was not modified and remained at a distance of 57cm, meanwhile green was set at 46 cm and red at 45 cm.

Predation was evaluated with live prey *Artemia* sp. labelled with FluoSpheres (10 microns). 10 paralarvae of different ages (0, 5 and 10 DPH) were placed in 1L dark glasses and acclimatize for 15 minutes, later they were fed with a concentration 0’3 artemia/mL (containing FluoSpheres) for 30 minutes and then they were anesthetized and stored in ethanol (0’1%) for observation under the fluorescence magnifier. A total of 1440 paralarvae were used to complete this experiment.

2.4.1 FluoSpheres (Fluorescent Microspheres) labelling

An experiment was performed to optimize the method of labelling *Artemia* sp. for the purpose of quantifying feed intake. Fluorescent Microspheres were supplied by Life Technologies. Blue fluorescent FluoSpheres beads with excitation/emission maxima of 350/440 nm and contain a blue fluorescent dye that provides brightness. The smallest microspheres are currently about 0.02 µm in diameter, because of their small size, micro spheres are transparent to light in aqueous suspensions and behave very much like true solutions. All FluoSpheres products should be stored at 2–6°C, protected from light but not freeze. Before sampling, FluoSpheres should be mixed well. The micro spheres are stable for at least one year, provided recommended storage conditions are strictly followed. The microspheres were used as an aliquot ($2*10^6$ microspheres/500µl). The concentration of interest in the mixture of *Artemia* was set to 5000 microspheres/mL, (dilution factor 400). The *Artemia* were washed with a 60µm sieve and reuptake in 400 mL saltwater before adding the aliquot of microspheres. Incubation time was 30 minutes, by then it is estimated that the *Artemia* incorporated a representative amount of microspheres by passive filtration of the surrounding water. *Artemia* was re-washed with new seawater to discharge the remained microspheres of the water column and ready to feed paralarvae.

2.5 Effect of light on survival

During this experiment, the influence of 4 distinct colours was tested (white, blue, green and red) on paralarvae of different ages (0, 6 and 12 DPH) with and without neutral filter. Paralarvae (10 PLV/L) were introduced inside 1L black glasses isolated from peripheral light and the only light available was the one provided from de LED. Intensities with and without neutral filter are provided in **Table 3**. The neutral density filter pretends to simulate the light conditions in the natural environment at 200 meters of depth where the wild paralarvae are found. For each age (0, 6, 12 DPH) and colour (W, B, R, G) 3 replicates were made without filter and 3 replicates with neutral filter (NeuF). 24 black glasses and 240 paralarvae were used per trial. In total, for the survival experiment 720 paralarvae were employed. Survival was measured after 3 days with continuous light (LL). Water from the glasses was not renewed and no aeration was provided. Paralarvae were fed once a day with a maintenance dose of 0'3 artemia/mL

2.6 Statistical analysis

Results are presented as means ± standard deviation (SD). ANOVA was performed using 9999 permutations (Anderson and Ter Braak, 2003) based on the Euclidean distances (Anderson, 2004; Anderson and Millar, 2004) for dataset of "behaviour". A multifactorial analysis was used with the fixed factor "filter" (2 levels: neutral filter and no filter), fixed factor "age" (2 levels: 0 and 15 DPH) and factor "position" (2 levels: R and C). Two-way comparisons were made, likewise by permutations (Anderson, 2004), of factor levels that were significant. Additional ANOVA was performed using 9999 permutations for dataset of "behaviour". A multifactorial design was used with factor "intensity" (2 levels: Wmax. and Wmin.), factor "age" (3 levels: 0, 12 and 19 DPH) and factor "position" (2 levels: R and C).

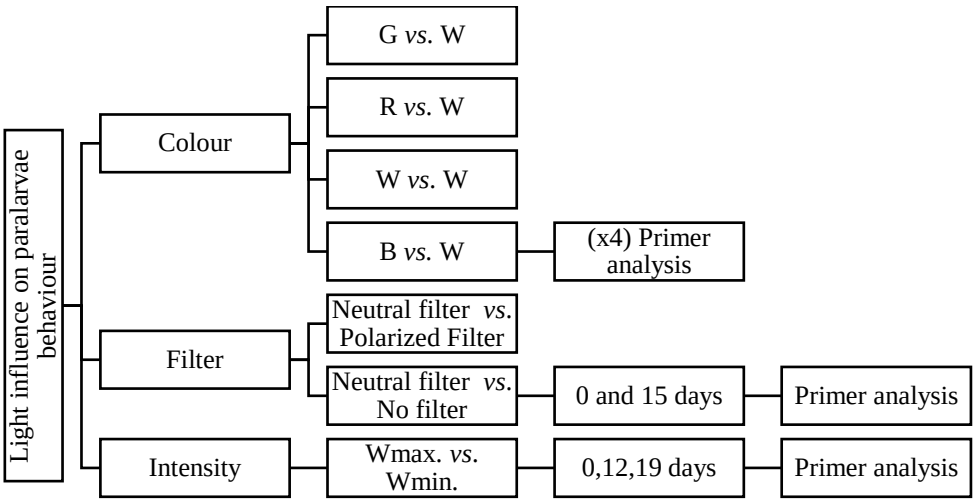
A ANOVA was performed using 9999 permutations based on the Euclidean distances for dataset of "survival". A multifactorial analysis was carried out to assess the effect of fixed factor "colour" (4 levels: white, blue, green and red), fixed factor "age" (3 levels: 0, 6, 12 DPH) and fixed factor "filter" (2 levels: neutral filter and no filter). Subsequent comparisons were made two to two, equally by permutations (Anderson, 2004) of the levels of factors that were significant.

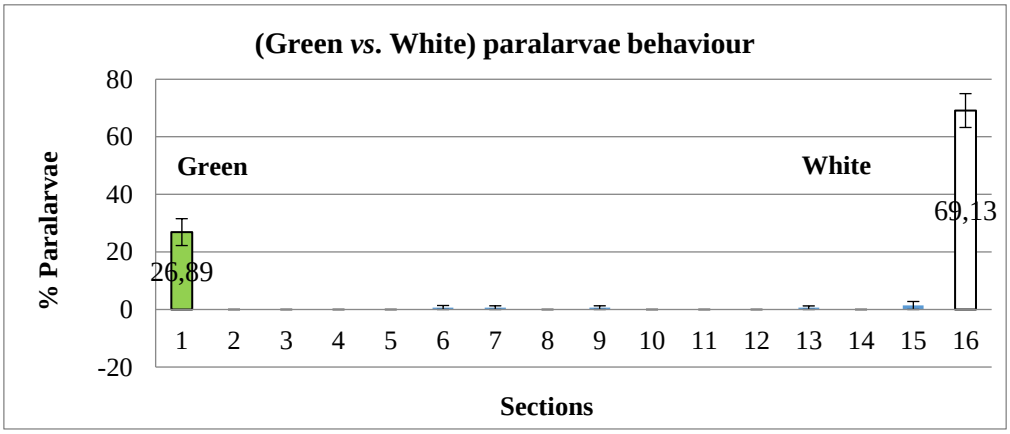
A ANOVA was performed using 9999 permutations based on the Euclidean distances for dataset of "predation". A multifactorial analysis was carried out to assess the effect of fixed factor "colour" (4 levels: white, blue, green and red), fixed factor "age" (3 levels: 0, 5, 10 DPH) and fixed factor "filter" (3 levels: neutral filter, polarized filter and no filter). Subsequent comparisons were made two to two, equally by permutations of the levels of factors that were significant.

All these analyzes were performed with the statistical package PRIMER 6 & PERMANOVA + (www.primer-e.com).

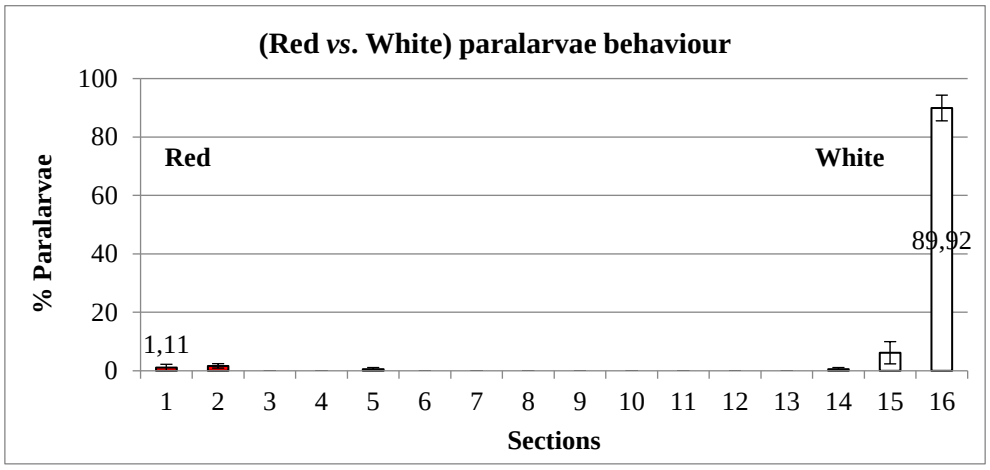
3. Results

3.1 Light influence on paralarvae behaviour

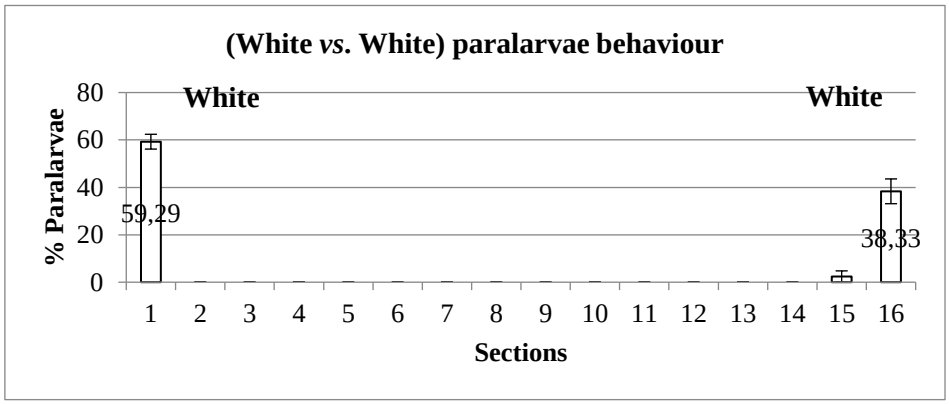




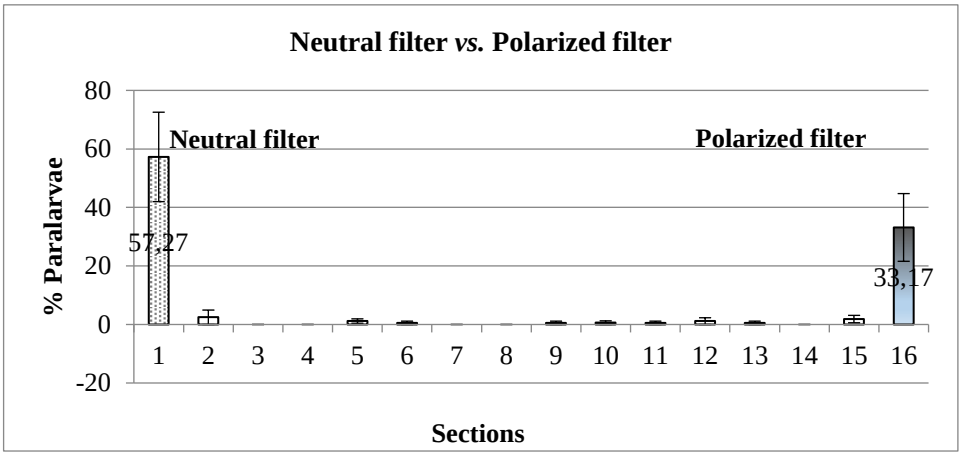
Graph 1. % Paralarvae present in each section of the experimental tube after 60 minutes, when comparing green *vs.* white. Paralarvae age 0 days. Data is expressed as mean ±S.D.



Graph 2. % Paralarvae present in each section of the experimental tube after 60 minutes, when comparing red *vs*. white. Paralarvae age 0 days. Data is expressed as mean ±S.D.



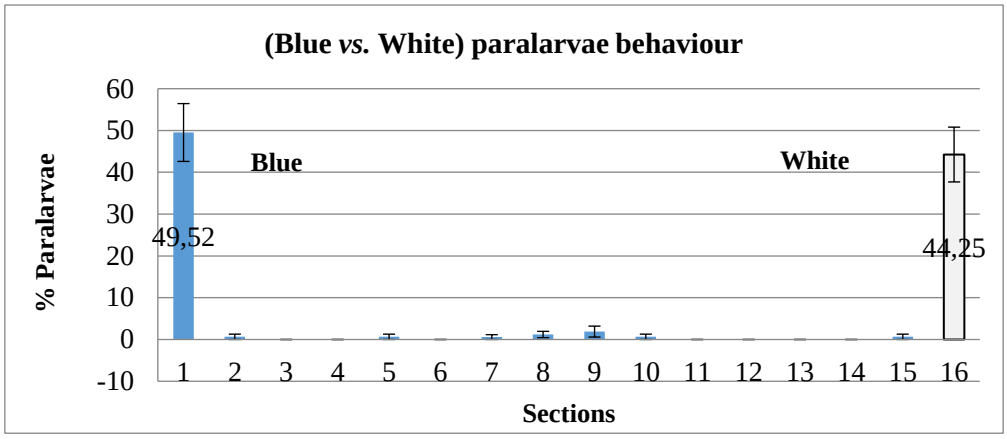
Graph 3. % Paralarvae present in each section of the experimental tube after 60 minutes, when comparing white *vs.* white. Paralarvae age 0 days. Data is expressed as mean ±S.D.



Graph 4 % Paralarvae present in each section of the experimental tube after 60 minutes, when comparing White neutral filter *vs.* White polarized filter. Paralarvae age 0 and 15 DPH. Data is expressed as mean ±S.D. white

Results show a clear dominance of the white, when compared with green and red despite having equalized their intensities by modifying the distance (**Graphs 1** and **2**). Pelagic paralarvae display a strong positive phototactic behavior. However, this dominance is unclear when compared with blue (**Graph 5**). White colour was tested against itself (white *vs.* white) and a possible tube effect was detected, paralarvae tended to flow more occasionally to the left side of the experimental tube rather than the right side (**Graph 3**).

When comparing neutral filter (57,27%) *vs.* polarized filter (33,17%), neutral filter seems to perform the best however some of the paralarvae remained in sections closer to the polarized light as you can see in **graph 4**.

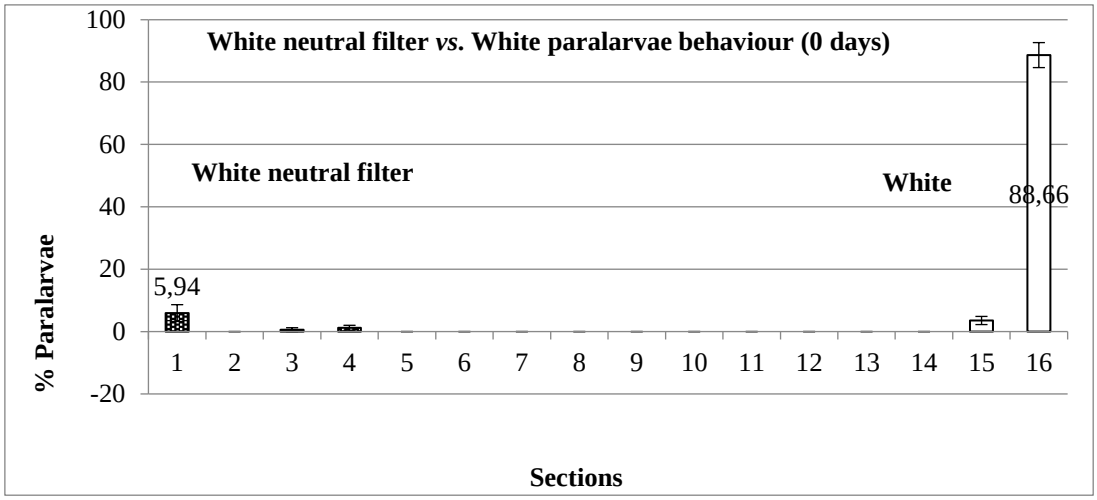


Graph 5. % Paralarvae present in each section of the experimental tube after 60 minutes, when comparing Blue *vs.* White light. Paralarvae age 0 days. Data is expressed as mean ±S.D.

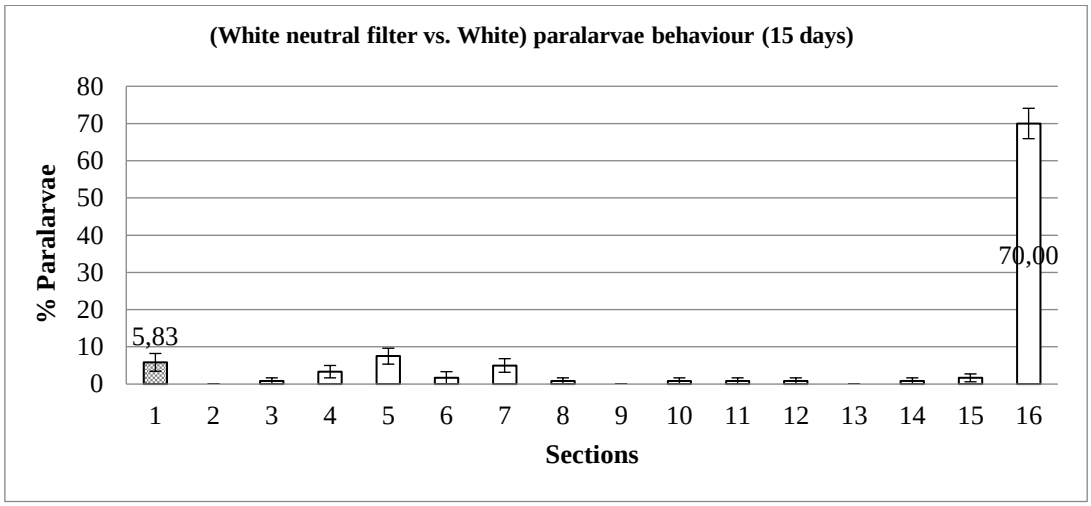
Table 4. Results of the multifactorial (ANOVAs), based on the Euclidian distances for dataset of behaviour comparing colour (Blue *vs.* White), position (R/C), and their possible interaction.

Behaviour					
Blue *vs.* White					
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**
Position	1	7514,1	7514,1	18,353	0,0003
Colour	1	2580,9	2580,9	6,3038	0,0172
Poxco	1	53,071	53,071	0,12962	0,7202
Res	44	18015	409,43		
Total	47	28163			

To determine the dominance between white *vs.* blue the experiment was repeated up to 4 times. The multifactorial ANOVAs made from the data of behaviour were statistically significant for factor "position" ($F= 18,353$; $p< 0,01$) and factor "colour" ($F= 0,0172$; $p< 0,05$) (**Table 4**). Besides having similar intensities blue and white, light display significant differences. Position was also significant this means that there is a possible tube effect or probably the inclination of the tube was accidentally modified during filling or emptying. It is remarkable to mention that factor interaction "position x colour" was not significant.



Graph 6. % Paralarvae present in each section of the experimental tube after 60 minutes, when comparing white neutral filter *vs.* white light. Paralarvae age 0 and 15 DPH. Data is expressed as mean ±S.D.



Graph 7. % Paralarvae present in each section of the experimental tube after 60 minutes, when comparing white neutral filter *vs.* white light. Paralarvae age 0 and 15 DPH. Data is expressed as mean ±S.D.

Table 5. Results of the multifactorial (ANOVAs), based on the Euclidian distances for dataset of behaviour comparing white colour with neutral filter and without filter, position, age, and their possible interaction.

Behaviour					
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**
Age	1	930,88	930,88	18,946	0,0001
Position	1	6,0295E-2	6,0295E-2	1,2272E-3	0,9735
Filter	1	50544	50544	1028,7	0,0001
Agxpo	1	53,533	53,533	1,0896	0,3076
Agxfi	1	1491,1	1491,1	30,348	0,0002
Poxfi	1	15,777	15,777	0,32111	0,5766
Agxpoxfi	1	9,6041	9,6041	0,19547	0,6682
Res	28	1375,7	49,133		
Total	35	67437			

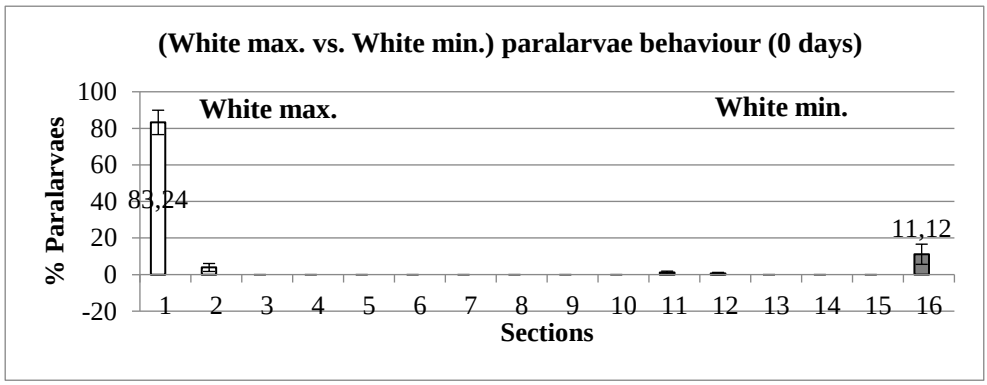
The multifactorial ANOVAs made from the data of behaviour were statistically significant for factor "age" ($F=18,946$; $p< 0,01$), factor "filter" ($F=1028,7$; $p< 0,01$), as well as the interaction between factors "age x filter" ($F= 30,348$; $p< 0,01$). All the same no

differences were found for factor "position" and the interaction between "age x position"; "position x filter"; and "age x position x filter" (**Table 5**). Position was not significant this means that exchanging light positions did not affect the results and therefore tube effect was not detected.

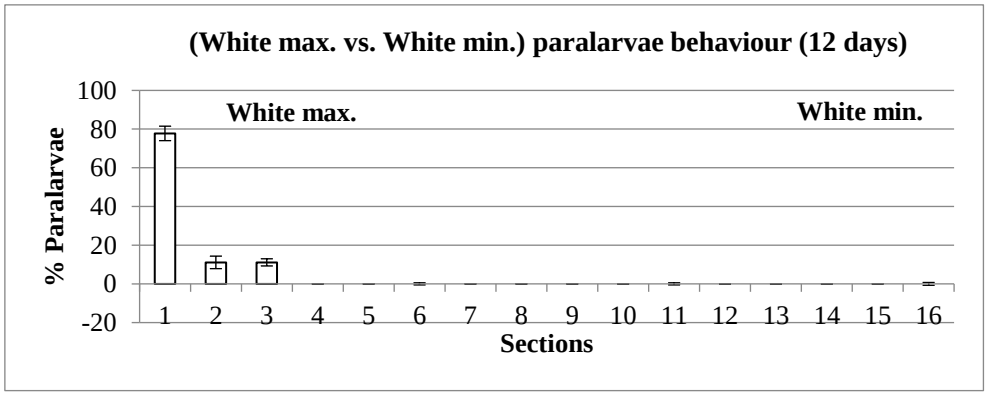
Table 6. Pair-wise comparisons for the levels of the significant factor "age x filter" obtained in the ANOVA analysis of the behaviour data. The values of the statistic (t-Student) and the level of significance P (perm) for the comparison between neutral filter and no filter at ages 0 and 15 days are included.

Behaviour		
"Age x Filter"		
Neutral filter	**t**	**P(perm)**
0 *vs.* 15 days	1,0229	0,313
No filter	**t**	**P(perm)**
0 *vs.* 15 days	5,9766	0,0003

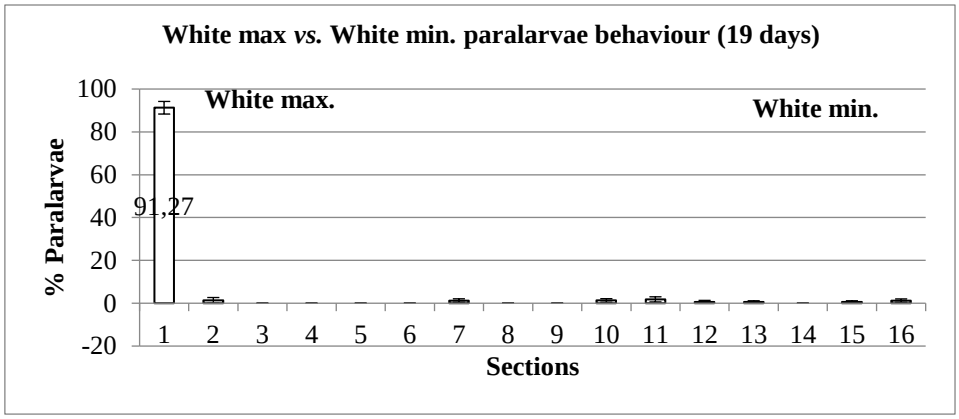
Further analysis of factor interaction "age x filter" showed significant differences between 0 and 15 DPH ($p < 0,01$) (**Table 6**).



Graph 8. %Paralarvae present in each section of the experimental tube after 60 minutes, when comparing White high intensity (max.) *vs.* White low intensity (min). Paralarvae age 0, 12 and 19 DPH. Data is expressed as mean ±S.D.



Graph 9. %Paralarvae present in each section of the experimental tube after 60 minutes, when comparing White high intensity (max.) *vs.* White low intensity (min). Paralarvae age 0, 12 and 19 DPH. Data is expressed as mean ±S.D



Graph 10. %Paralarvae present in each section of the experimental tube after 60 minutes, when comparing White high intensity (max.) *vs.* White low intensity (min). Paralarvae age 0, 12 and 19 DPH. Data is expressed as mean ±S.D.

Table 7. Results of the multifactorial (ANOVAs), based on the Euclidian distances for dataset of behaviour comparing white intensity (maximum/minimum), position (R/C), age (0,12 and 15 DPH), and their possible interaction.

Behaviour					
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**
Age	2	23,306	11,653	0,14829	0,8665
Position	1	99,624	99,624	1,2678	0,2814
Intensity	1	68415	68415	870,61	0,0001
Agxpo	2	480,89	240,44	3,0597	0,0589
Agxin	2	585,56	292,78	3,7258	0,0305
Poxin	1	10,157	10,157	0,12925	0,7285
Agxpoxin	2	17,897	8,9485	0,11387	0,9022
Res	24	1886	78,583		
Total	35	71519			

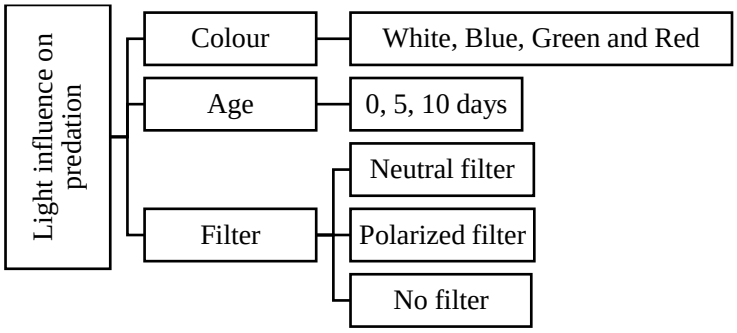
The multifactorial ANOVAs made from the data of behaviour were statistically significant for fixed factor "intensity" ($F=870,61$; $p< 0,01$) and factor interaction "age x intensity" ($F= 30,348$; $p< 0,05$). All the same no differences were found for factors "age"; "position" and the interaction between "position x intensity"; and "age x position x intensity" (**Table 7**).

Table 8. Pair-wise comparisons for the levels of the significant factor "age x intensity " obtained in the ANOVA analysis of the behaviour data. The values of the statistic (t-Student) and the level of significance P (perm) and P (Montecarlo) for the comparison between white intensity (maximum/minimum), age (0,12 and 15 DPH), are provided.

Behaviour			
"Age x Intensity"			
White max.	**t**	**P(perm)**	**P(MC)**
0 *vs.* 12 days	1,347	0,2224	0,2112
0 *vs.* 19 days	0,80538	0,4695	0,4439
12 *vs.* 19 days	1,0442	0,3194	0,3204
White min.	**t**	**P(perm)**	**P(MC)**
0 *vs.* 12 days	1,8717	0,0833	0,099
0 *vs.* 19 days	1,7312	0,1163	0,1199
12 *vs.* 19 days	0,55677	0,6047	0,5945

Moreover, no significant differences were found when evaluating factor interaction "age x intensity" (**Table 8**).

3.2 Light influence on predation

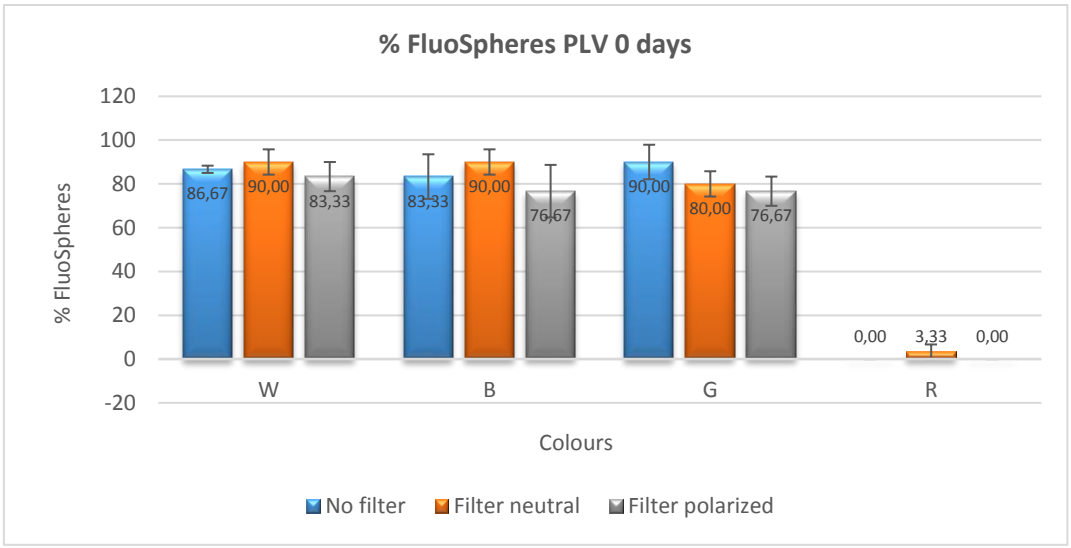


One of the objective of this experiment is to evaluate how predation is affected when using different light colours (white, blue, green and red). Within this experiment we intend to prove that *Octopus vulgaris* paralarvae use polarization to hunt in an analogous way to cuttlefish.

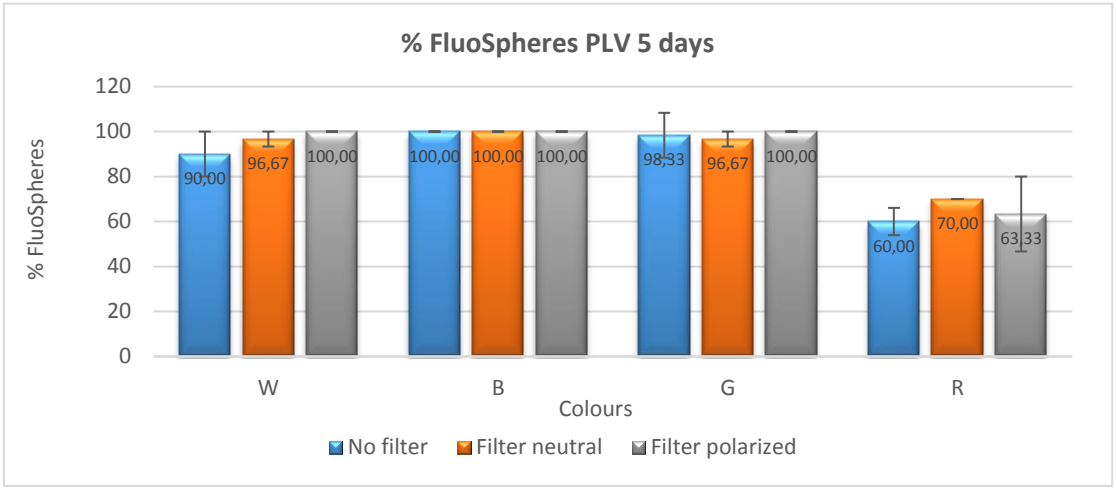
Table 9. Comparison between % FluoSpheres and % Stomach content found in paralarvae of age 0, 5, and 10 days, in 4 different colours: white (W), blue (B), green (G) and red (R), with no filter, neutral filter and polarized filter. Data is expressed as mean ±S.D.

Age	**% FluoSpheres**			**% Stomach Content**		
0 days	**No filter**	**Filter neu**	**Filter pol**	**No filter**	**Filter neu**	**Filter pol**
W	86,66 ± 1,67	90 ± 5,77	83,33 ± 6,67	78,33 ± 9,55	80 ± 11,55	76,66 ± 8,82
B	83,33 ± 10,18	90 ± 5,77	76,66 ± 12,02	78,33 ± 10,60	86,66 ± 6,67	73,33 ± 8,82
G	90 ± 7,89	80 ± 5,77	76,66 ± 6,67	83,33 ± 14,34	80 ± 5,77	60 ± 5,77
R	0 ± 0,00	3,33 ± 3,33	0 ± 0,00	3,33 ± 3,33	3,33 ± 3,33	0 ± 0,00
5 days	**No filter**	**Filter neu**	**Filter pol**	**No filter**	**Filter neu**	**Filter pol**
W	90 ± 10,00	96,66 ± 3,33	100 ± 0,00	93,33 ± 3,33	96,66 ± 3,33	100 ± 0,00
B	100 ± 0,00	100 ± 0,00	100 ± 0,00	100 ± 0,00	100 ± 0,00	100 ± 0,00
G	98,33 ± 10,00	96,66 ± 3,33	100 ± 0,00	96,66 ± 1,67	100 ± 0,00	100 ± 0,00
Red	60 ± 6,08	70 ± 0,00	63,33 16,67	80 ± 10,77	73,33 ± 3.33	50 ± 11,55
10 days	**No filter**	**Filter neu**	**Filter pol**	**No filter**	**Filter neu**	**Filter pol**
W	93,33 ± 4,55	100 ± 0.00	90 ± 5,77	93,33 ± 4,55	100 ± 0,00	93,33 ± 6,67
B	83,33 ± 4,41	90 ± 5,77	76,66 ± 13,33	88,33 ± 7,26	93,33 ± 3,33	76,66 ± 13,33
G	80 ± 4,55	100 ± 0,00	86,66 ± 3,33	83,33 ± 6,08	100 ± 0,00	86,66 ± 3,33
R	20 ± 7, 68	20 ± 10,00	13,33 ± 6,67	20 ± 7,68	20 ± 10	16,66 ± 8,82

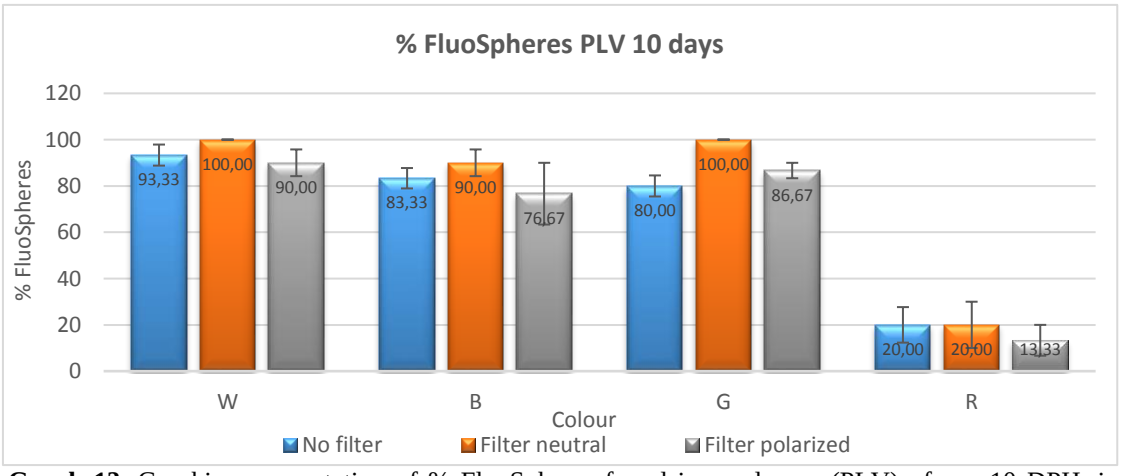
When paralarvae were observed under the fluorescence magnifier and no FluoSpheres were detected each of the paralarvae was checked under normal light to evaluate stomach content. As we can see in **table 9**, stomach content in some occasions shows higher values of predation, however, we decided to take FluoSpheres as a more reliable data in order to avoid errors of interpretation, since evaluating the stomach content is very subjective and it depends on the previous experience of the observer as well as the state of preservation of the paralarvae. FluoSpheres are very visual and facilitate considerably the correct interpretation of the results.



Graph 11. Graphic representation of % FluoSpheres found in paralarvae (PLV) of age 0 days, in 4 different colours: white (W), blue (B), green (G) and red (R), with no filter, neutral filter and polarized filter. Data is expressed as mean ±S.D.



Graph 12. Graphic representation of % FluoSpheres found in paralarvae (PLV) of age 5 DPH, in 4 different colours: white (W), blue (B), green (G) and red (R), with no filter, neutral filter and polarized filter. Data is expressed as mean ±S.D.



Graph 13. Graphic representation of % FluoSpheres found in paralarvae (PLV) of age 10 DPH, in 4 different colours: white (W), blue (B), green (G) and red (R), with no filter, neutral filter and polarized filter. Data is expressed as mean ±S.D.

In general, predation is higher when white colour is applied, followed very closely by green and blue, whereas, red displays the lowest predation rates in all the experiments (**Graphs 11,12** and **13**). Paralarvae age 5 DPH showed greater predation rates, followed by 0 days and 10 DPH.

Table 10. Results of the multifactorial (ANOVAs), based on the Euclidian distances of the data of predation comparing colour, filter, age and their possible interaction.

Predation						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**P(MC)**
Age	2	15707	7853,6	36,429	0,0001	0,0001
Colour	3	95208	31736	147,21	0,0001	0,0001
Filter	2	681,94	340,97	1,5816	0,2087	0,2056
Agxco	6	11495	1915,8	8,8866	0,0001	0,0001
Agxfi	4	634,72	158,68	0,73604	0,567	0,564
Coxfi	6	118,06	19,676	9,1267E-2	0,997	0,9974
Agxcoxfi	12	1115,3	92,94	0,4311	0,9484	0,9476
Res	108	23283	215,59			
Total	143	1,6076E5				

The multifactorial ANOVA made from the data of predation were statistically significant for factor "colour" ($F=147,21$; $p< 0,01$) and factor "age" ($F=36,429$; $p< 0,01$), as well as the interaction between factors "age x colour" ($F= 8,8866$; $p< 0,01$). Nevertheless, no differences were found for factor "filters" and the interaction between "age x filter"; "colour x filter" and "age x colour x filter" (**Table 10**).

Table 11. Pair-wise comparisons for the levels of the significant factor "Age" obtained in the ANOVA analysis of the survival data. The values of the statistic (t-Student) and the level of significance P (perm) and P (Montecarlo) for the comparison between ages are included.

Predation			
Age	**t**	**P(perm)**	**P(MC)**
0 *vs.* 5 days	8,7438	0,0001	0,0001
0 *vs.* 10 days	2,5385	0,0124	0,0142
5 *vs.* 10 days	5,4373	0,0001	0,0001

Further analysis with significant factor "age" showed significant differences between age 0 *vs.* 5 days ($p< 0,01$). There are also significant differences between age 5 and age 10 ($p< 0,01$) (**Table 11)**. Surprisingly, no significant differences were found between age 0 and age 10DPH.

Table 12. Pair-wise comparisons for the levels of the significant factor "colour" obtained in the ANOVA analysis of the survival data. The values of the statistic (t-Student) and the level of significance P (perm) and P (Montecarlo) for the comparison between colours are included.

Predation			
Colour	**t**	**P(perm)**	**P(MC)**
White *vs.* Blue	0,94002	0,3644	0,3557
White *vs.* Green	0,7471	0,46	0,4588
White *vs.* Red	16,344	0,0001	0,0001
Blue *vs.* Green	0,2783	0,7792	0,7812
Blue *vs.* Red	15,168	0,0001	0,0001
Green *vs.* Red	16,556	0,0001	0,0001

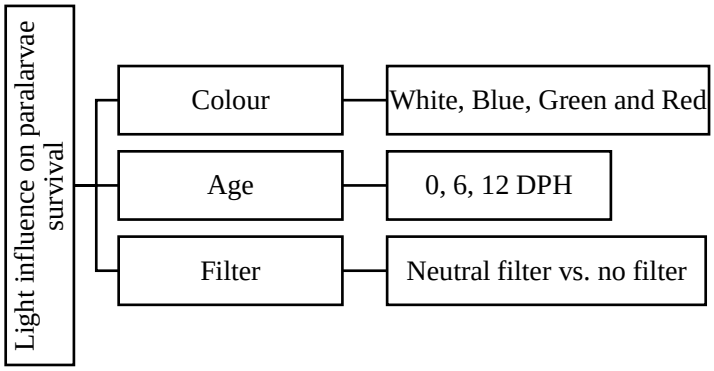
Posteriori analyses of factor "colour" showed significant differences between White *vs.* Red ($p< 0,01$) but not with blue and green. Blue colour displayed significant differences with Red ($p< 0,01$). As well as green *vs*. red which showed significant differences ($p< 0,01$). (**Table 12)**.

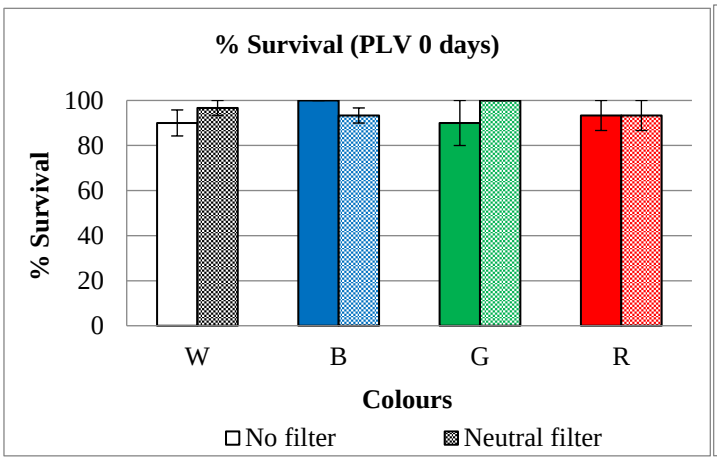
Table 13. Pair-wise comparisons for the levels of the significant factor "age x colour" obtained in the ANOVA analysis of the predation data. The values of the statistic (t-Student), level of significance P(perm) and P (Montecarlo) for the comparison amongst ages and colours, are provided.

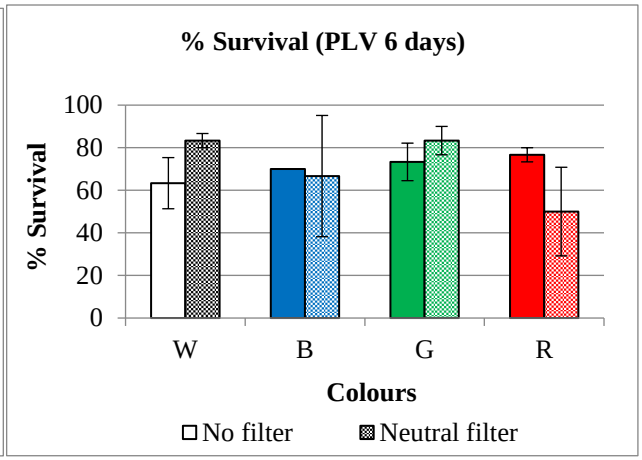
Predation			
"Age x Colour"			
0 days	t	P(perm)	P(MC)
White *vs.* Blue	0,51962	0,6085	0,6158
White *vs.* Green	0,81839	0,4264	0,4277
White *vs.* Red	20,661	0,0001	0,0001
Blue *vs.* Green	0,18091	0,8584	0,8541
Blue *vs.* Red	16,322	0,0001	0,0001
Green *vs.* Red	21,9	0,0001	0,0001
5 days	**t**	**P(perm)**	**P(MC)**
White *vs.* Blue	0,79126	0,5692	0,4372
White *vs.* Green	0,48287	0,7551	0,6299
White *vs.* Red	3,3204	0,0043	0,0038
Blue *vs.* Green	1,3416	0,2269	0,1932
Blue *vs.* Red	4,7411	0,0004	0,0002
Green *vs.* Red	4,4581	0,0008	0,0005
10 days	**t**	**P(perm)**	**P(MC)**
White *vs.* Blue	1,7467	0,1002	0,0971
White *vs.* Green	1	0,3592	0,3317
White *vs.* Red	12,963	0,0001	0,0001
Blue *vs.* Green	0,7151	0,4734	0,4805
Blue *vs.* Red	8,1644	0,0001	0,0001
Green *vs.* Red	9,6	0,0001	0,0001

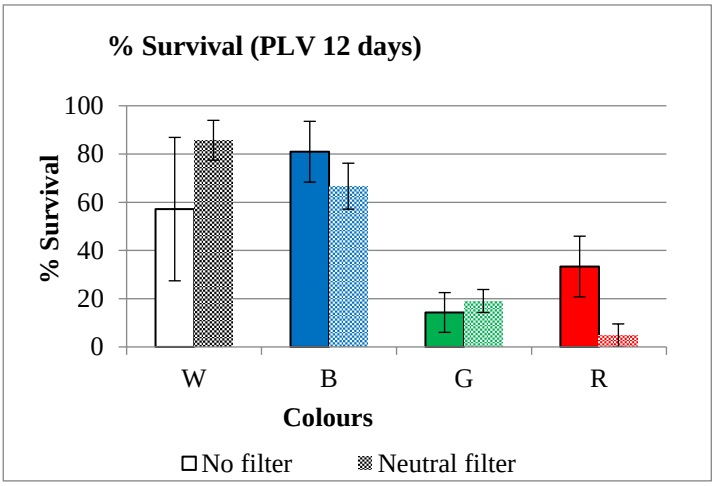
In the case of factor interaction "age x colour", within ages 0, 5 and 10 DPH, White *vs.* red ($p< 0,01$).; blue *vs.* red ($p< 0,01$). and green *vs.* red ($p< 0,01$) displayed significant differences (**Table 13)**. Predation in red light is always lower and displays significant differences with white, blue and green were predation is higher, this trend is also perceived in older paralarvae. Apparently paralarvae do not have good vision in red wavelength and therefore prey capture is low and predation rate decreases even more with age.

3.3 Light influence on paralarvae survival



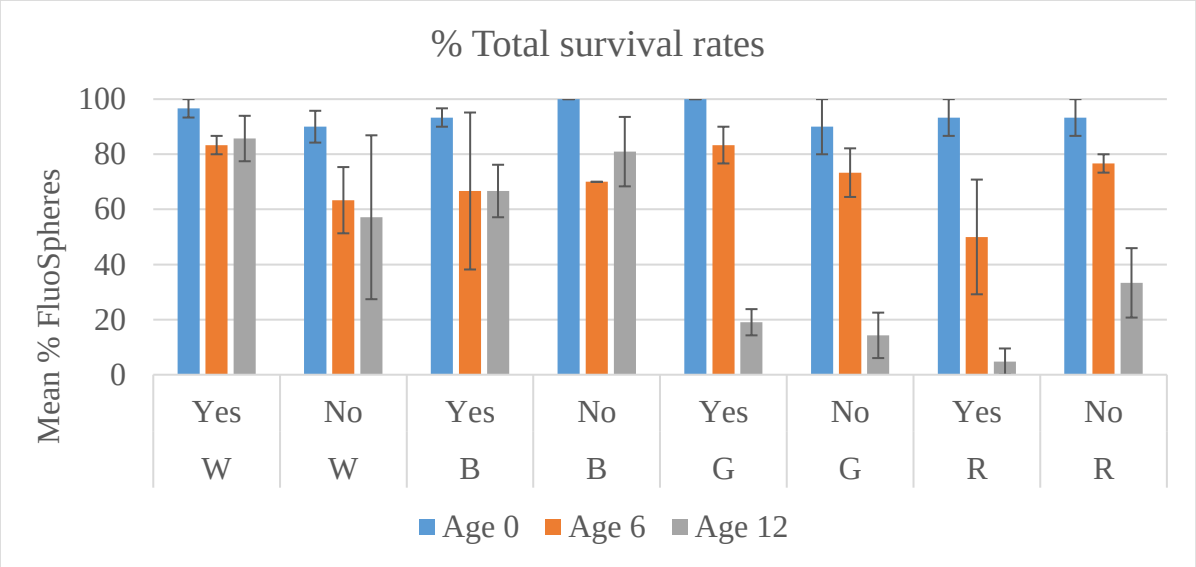






Graphs 14, 15, 16. % Survival observed for ages (0, 6 and 12 DPH) with neutral filter and with no filter in different colours (white (W), blue (B), green (G) and red (R)). Data is expressed as mean ±S.D.

In general, survival is maximal at age 0 days and decreases from age 6 to 12 DPH. Apparently, the use of neutral filter increases slightly survival. Survival rates are maximum when applying neutral filter and white light, followed closely by blue, green and red despite de age **(Graphs 17).**



Graph 17. Graphic representation of the % survival rates observed for age (0, 6 and 12 DPH) with neutral filter and with no filter in different colours (white (W), blue (B), green (G) and red (R)). Data is expressed as mean ±S.D.

Table 14. Results of the multifactorial (ANOVAs), based on the Euclidian distances of the data of survival comparing colour, filter, age and their possible interaction.

Survival					
Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**
Colour	3	0,63863	0,21288	5,2373	0,0028
Filter	1	2,8345E-6	2,8345E-6	6,9735E-5	0,9927
Age	2	2,9233	1,4617	35,96	0,0001
CoxFi	3	0,36527	0,12176	2,9955	0,0412
CoxAg	6	1,2437	0,20728	5,0996	0,0004
FixAg	2	7,1485E-3	3,5743E-3	8,7936E-2	0,9141
CoxFixAg	6	0,11816	1,9693E-2	0,4845	0,8137
Res	48	1,951	4,0646E-2		
Total	71	7,2472			

The multifactorial ANOVAs made from the dataset of survival were statistically significant for factor "colour" (F=5,2373; p< 0,05) and factor "age" (F=35,96; p< 0,01), as well as the interaction between factors "colour x filter" (F=2,9955; p< 0,05) and "colour x age" (F=5,0996; p< 0,01). Nevertheless, no differences were found for factors "filters" and the interaction between "filter x age" and "filter x age x colour" (**Table 14)**.

Table 15. Pair-wise comparisons for the levels of the significant factor "colour" obtained in the ANOVA analysis of the survival data. The values of the statistic (t-Student) and the level of significance P (perm) for the comparison between colours are included.

Survival		
Colour	**t**	**P(perm)**
White vs Blue	3,0292E-2	0,974
White *vs.* Green	2,5129	0,0163
White *vs.* Red	2,8873	0,0087
Blue *vs.* Green	2,6235	0,0158
Blue *vs.* Red,	2,9856	0,007
Green *vs.* Red	0,89126	0,3846

Posteriori analyses of factor "colour" showed significant differences between white *vs.* green and red (p< 0,05) but not with blue. Blue colour displayed significant differences with green and red (p< 0,05). whereas, green and red did not show significant differences (**Table 15)**.

Table 16. Pair-wise comparisons for the levels of the significant factor "Age" obtained in the ANOVA analysis of the survival data. The values of the statistic (t-Student) and the level of significance P(perm) for the comparison between ages are included.

Survival		
Age	**t**	**P(perm)**
0 *vs. 6* days	4,5062	0,0002
0 *vs.* 12 days	9,5231	0,0001
6 *vs.* 12 days	3,7339	0,0008

Further analysis with significant factor "age" showed significant differences between age zero with age 6 (p< 0,01) and age 12 (p< 0,01) There are also significant differences between age 6 and age 12 (p< 0,01) (**Table 16)**.

Table 17. Pair-wise comparisons for the levels of the significant factor "colour x age" obtained in the ANOVA analysis of the survival data. The values of the statistic (t-Student), level of significance P(perm) and P (Montecarlo) for the comparison amongst ages and colours, are provided.

Survival			
'Colour x Age'			
White	**t**	**P(perm)**	**P(MC)**
0 *vs.* 6 days	2,8284	0,0177	0,0224
0 vs. 12 days	1,3876	0,204	0,1933
6 *vs.* 12 days	0,11445	0,9229	0,9099
Blue	**t**	**P(perm)**	**P(MC)**
0 *vs.* 6 days	1,9762	0,0522	0,0868
0 vs. 12 days	2,8321	0,0333	0,0227
6 *vs.* 12 days	0,33631	0,7848	0,7491
Green	**t**	**P(perm)**	**P(MC)**
0 *vs.* 6 days	2,2361	0,0677	0,0607
0 vs. 12 days	11,345	0,0033	0,0001
6 *vs.* 12 days	8,4521	0,0026	0,0001
Red	**t**	**P(perm)**	**P(MC)**
0 *vs.* 6 days	2,5981	0,0329	0,0289
0 vs. 12 days	9,0368	0,0047	0,0001
6 *vs.* 12 days	3,5404	0,0059	0,0089

Factor interaction "colour x age" displayed significant differences for colour white between ages 0 and 6 DPH ($p< 0,01$). For blue significant differences were found between 0 and 12 DPH ($p< 0,05$). Meanwhile, green colour presented significant differences between ages 0 and 12 ($p< 0,05$) and between ages 6 and 12 DPH ($p< 0,05$). Whereas red colour presented significant differences for the three ages ($p< 0,05$) (**Table 17)**.

Table 18. Pair-wise comparisons for the levels of the significant factor "colour x filter" obtained in the ANOVA analysis of the survival data. The values of the statistic (t-Student), level of significance P(perm) and P (Montecarlo) for the comparison between colours with and without filter, are provided.

Survival			
'Colour x Filter'			
No filter	**t**	**P(perm)**	**P(MC)**
White *vs.* Blue	1,2328	0,2559	0,2401
White *vs.* Green	5,0486	0,0005	0,0003
White *vs.* Red	4,8394	0,0009	0,0002
Blue *vs.* Green	0,77576	0,4791	0,4481
Blue *vs.* Red	2,0899	0,0561	0,0585
Green *vs.* Red	2,2786	0,0417	0,0428
Neutral filter	**t**	**P(perm)**	**P(MC)**
White *vs.* Blue	1,1584	0,2803	0,273
White *vs.* Green	0,90852	0,3932	0,3833
White *vs.* Red	0,19993	0,8542	0,8471
Blue *vs.* Green	3,6461	0,0054	0,0035
Blue *vs.* Red	2,4656	0,0277	0,0306
Green *vs.* Red	1,1988	0,2563	0,2565

Factors interaction "colour x filter" showed significant differences when no filter was used between colours white vs. green, red ($p< 0,01$); green vs. red ($p< 0,05$). On the other hand, when neutral filter was used significant differences were found between blue vs. green, red ($p< 0,05$) (**Table 18**).

4. Discussion

Light is a key environmental factor, several studies, have showed that artificial light affects paralarvae foraging, growth and survival (Monk et al., 2006; Yoseda et al., 2008, Villamizar et al. 2009). In general, light influences all aspects of *O. vulgaris* behaviour.

Newly hatched paralarvae inhabit the littoral coasts (20-40m depth) characterized by high luminous intensities so it is not surprising that the paralarvae show a strong positive phototactism as they are adapted to live in very bright environments. Results observed in behaviour experiment, show a clear preference for white colour followed closely by blue. Importantly, artificial lights differ greatly from the sun's spectrum, particularly underwater, as most light bulbs provide red-rich wavelengths and few blue photons. (Villamizar et al., 2010). It is possible that significant differences found between blue and white, are caused because LEDS are unable to provide sufficient blue photons, even though both colours have similar intensities.

After their residence period near the littoral coast (15-20 DPH) paralarvae migrate to open sea and become inhabitants of the photic zone (up to 100-200meter depth). In the behaviour experiment, 15 DPH paralarvae exhibited a change in light preference, where paralarvae started to migrate from white bright side to attenuated zones (simulated natural environmental conditions with neutral density filters). This fact correlates with migration from coastal areas (high intensity) to open sea at depth 100-200m where paralarvae must adapt to dim light conditions. However, this trend was not detected when assessing white high intensity *vs.* low intensity, DPH were found to be no-significant.

Previous experiments with *O. vulgaris* hatchlings showed that light enhanced consumption rates 3-folds in comparison with dark conditions suggesting the importance of light (and vision) in predatory behaviour (Márquez et al. 2007). Because visual predation occurs day and night, many predators must have good night vision. Prey therefore exhibit antipredator behaviours in very dim light (Allen et al., 2010). This behaviour has been evidenced in *Artemia* nauplii which displayed higher aggreation pattern under white light, followed by darkness, red and blue light (Villamizar et al., 2010). Light may not be essential for prey capture as a positive correlation was found between prey density and consumption rates in dark conditions. Paralarvae is affected by the presence of prey, individual paralarvae tend to increase their turning rate and reduce swimming speed in presence of prey to increase residence time in zooplankton patch, increasing the probability of prey encounters (Villanueva et al., 2008). In the predation experiment, prey capture was significantly lower under red and higher under white, blue and green in ages 0, 5 and 10 DPH which may be explained by both the scattering of prey around the glasses and their better visualisation and detection by paralarvae (Monk et al., 2008).

In deep sea fishes, photoreceptors have a maximised visual contrast in the blue band, while coastal fish species have maximum sensitivity in the green band (Villamizar, et al. 2010). It was expected that newly hatched paralarvae, during their residence time in the coastal areas, before migration to open seas might have maximised visual contrast in green and develop maximum sensitivity to blue band when migrating to 100-200m depth. Our predation result confirms that white> blue> and green visual contrast is well developed for newly hatched paralarvae whereas vision in red wavelengths is poor. It is

likely that at later ages, catches in blue, exceed those of white, and that after the presettlement period, the predation rate increases for the green wavelengths in juveniles.

The role of polarization vision in octopus paralarvae is unknown although it is likely to play a significant role in predation behaviour. Adult octopuses can recognize polarization contrast within small objects, suggesting that polarization vision is used in contrast enhancement and target recognition. Up till now, the use of polarization (light or filters) to enhance predation was never reported (Iglesias et al. 2014). Besides, factor filter was not significant, neither of the two filters polarized/neutral enhanced consumptions rates. Though, 10 DPH is a relative short age and polarization vision probably improves more onwards when paralarvae inhabit water column at 100-200m depth. In the complex underwater polarized light environment, polarization sensitivity may be used not only for navigation but also for target recognition, breaking camouflage, increasing detection range, enhancing contrast and detecting transparent objects (Shashar et al., 1996[b]). Paralarvae may target, luminescing prey, particularly in deeper waters and at night during nocturnal feeding (Villanueva et al., 2008).

In the same way, as paralarvae increase in age importance of simulating dim light conditions with neutral density filters increases. It is thought, *O. vulgaris* varies its visual system from a high visual acuity to a high visual sensitivity during transitions from pelagic (paralarvae) to benthic habitats (juvenile). For this reason, when designing an artificial lighting system for octopus in culture, its ecology and developmental stage which will affect its sensitivity should be considered. It is known, that culture conditions alter the normal behaviour of the paralarvae. In the wild, the paralarvae learn to hunt and not to be hunted, they train their visual system to increase the success in their captures and avoid being predated. In laboratory conditions, this learning is limited since on the one hand the variety and number of prey available is not the same and of course they do not have predators. It is necessary to answer questions like: are paralarvae able to use polarized vision in the early stages? does polarized vision need to be trained in order to recognize polarization patterns?

Artemia is frequently used as live food in paralarvae cultures because of its easy availability, fine acceptability and good handling/production logistics, making it a profitable live prey. However, *Artemia* in contrast to natural marine zooplankton, has an inadequate lipid composition, with low levels of polar lipids (PL) and highly unsaturated fatty acids (HUFA), especially docosahexaenoic acid (DHA) (Navarro et al., 1993), which is particularly relevant for octopus paralarvae development (Navarro and Villanueva, 2000, 2003). Recent studies (Monroig et al., 2013; Reis et al., 2014) point out that paralarvae have scarce or no capacity to synthesize HUFA such as arachidonic acid (ARA), eicosapentaenoic acid (EPA) and DHA, from n-6 and n-3 precursors, confirming the essentiality of these fatty acids. The analysis of wild paralarvae have shown DHA levels up to twice high as in culture specimens at the same age (Garrido et al., 2016[b]), estimated by using daily growth increments in the beak (Garrido et al., 2016, Perales Raya et al., 2017). Best results have been achieved when paralarvae were fed with *Zoeae* (8-9% of growth per day) whereas *Artemia* results are poor in comparison (only 3%). This

means that a 10DPH paralarvae fed with *Zoeae* would be equivalent to a 20DPH fed with *Artemia*. Paralarvae fed with *Zoeae* have more developed organs including eyes for vision. Therefore, if our experiments had been performed with *Zoeae*, we might have been able to detect more remarkable differences between the different paralarvae ages assesed. However, this is not possible as *Zoeae* culture is difficult and problematic. Besides, DHA is also a major structural lipid of retinal photoreceptor membranes and it is essential for the proper functioning of photoreceptors, being a key nutrient for eye health. *Artemia* enrichment performed during these experiments, are insufficient because of the poor transfer rate of DHA from *Artemia* to paralarvae (Reis et al., 2016). Results might be affected in a negative way by poor diets in DHA supplied to paralarvae.

When survival is considered among the factors influenced by light, the direct cause of mortality (or higher survival rates) remains unknown or in some cases, contrasting results are found and/or no significant differences are reported (Villamizar et al., 2010). Results confirm that under (LL) white and blue wavelengths contribute to the best outcomes, whereas survival rates for red and green were poor. Sea bass larvae reared under blue and white light performed better in terms of growth and development (Villamizar et al., 2009), this coincides with the results obtained in our study. Comparable results have also been observed in other fish larvae such as Atlantic cod, which performed better under short wavelengths (blue and green). Paralarvae probably starve under red wavelengths because they are unable to catch preys, because their vision is not good. Another possible cause, is that maintenance diet 0,3 Art/mL/day is insufficient and prey density is too low, besides the fact that *Artemia* aggregates more under red light and therefore, prey encounters are lower.

Photoperiodicity should match habitat and lifestyle at given geographical locations (Sykes et al., 2006). Further experiments with colours white and blue applying LD cycles should be performed. Continuous light (LL) generates stress on the paralarvae and thus mortality. The is a need for finding stress biomarkers that give us accurate and quantifiable information on the state of a paralarvae and allow us to improve the assessment of different treatments. Among these markers, we can examine digestive enzymes (trypsin, chymotrypsin, etc.), the antioxidant defense system, thermal stress proteins or RNA / DNA index. Additionally, massive data analysis (proteomics) is being performed to detect new types of these markers. The effect of culture conditions and environmental stress have also been analysed by biochemical indicators of stress such as hormones (corticosterone and catecholamines) (Tur et al., 2017[b]).

The disparate results found in laboratory rearing conditions should not be surprising because, according to Boletky and Villanueva, (2014), they are not always completely reproducible. The existence of such dissimilar results may draw attention to the problems associated with individual variations, epigenetic effects, and phenotypic plasticity that individuals with such complex sensory organs display. Standardization of the culture technology, will allow comparison among different investigation centres and will disclose an important variability among populations and/or specimens (Garrido et al., 2017).

5. Conclusions

I. *Behaviour*: Paralarvae exhibit a strong positive phototachtism with a clear preference for white colour. White dominance is not so strong when assessed with blue. Elder paralarvae exhibit a change in preference from bright to dim light conditions (neutral filter). However this trend is not observed when evaluating white intensities, paralarvae feel attracted by higher intensities despite the age.

II. *Predation*: Predation rates were high in all colours assessed except red. DPH influences predation results.

III. *Survival:* Survival was high except in green and red. No differences were found between white and blue wavelengths. DPH affected survival, as elder paralarvae performed worst.

These results highlight the role of lighting conditions during the early development of paralarvae and should be considered for the optimization of rearing protocols in the hatchery phase as juvenile supply is one of the main production bottlenecks.

6. Acknowledgement

This research was funded by the Spanish Oceanographic Institute of Tenerife. I appreciate the advice provided by my tutor Dr. Eduardo Almansa Berro as well as the support received from my family and friends.

7. Bibliography

Aksnes,D.L., and Giske,J. (1993). A theoretical model of aquatic visual feeding. *Ecological Modelling.* **67:** 233-250.

Allen,J.J., Mäthger,L.M., Buresch,K.C., Fetchko1,T., Meg Gardner,M., and Hanlon,R.T. (2010). Night vision by cuttlefish enables changeable camouflage. *The Journal of Experimental Biology*. **213:** 3953-3960.

Almansa,E., and Cerezo,J. (2013). Hacia el cultivo integral del pulpo de roca (*Octopus vulgaris*). *AquaTIC*. **39:** pp. 23-26.

Anderson,M.J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*. **26:** 32-46.

Anderson,M.J. (2004). PERMANOVA_2factor: a FORTRAN computer program for permutational multivariate analysis of variance (for any two-factor ANOVA design) using permutation tests. Department of Statistics, University of Auckland. New Zealand.

Anderson,M.J., & Millar,R.B. (2004). Spatial variation and effects of habitat on temperate reef assemblages in north eastern New Zealand. *Journal of Experimental Marine Biology and Ecology*. **305:** 191-221.

Anderson,M.J., & Ter, Braak. C.J.F. 2003. Permutation test for multi-factorial analysis of variance. *Journal of Statistical Computation and Simulation*. **73:** 85-113.

Bradshaw,W.E., and Holzapfel,C.M. (2007).Evolution of Animal Photoperiodism *Annu. Rev. Ecol. Evol. Syst.* 2007. **38:**1‑25.

Boletzky,S., and Villanueva,R. (2014). Cephalopod Biology. In *Cephalopod Culture* (pp. 3–16).

Carrasco,J., Arronte,J., and Rodríguez,C. (2006). Paralarval rearing of the common octopus, *Octopus vulgaris* (Cuvier). *Aquaculture Research*. **37** (15)**:** pp.1601-1605

Cobb,C.S., Williamson,R., Pope,S.K. (1995). The responses of the epistellar photoreceptors to light and their effect on circadian rhythms in the lesser octopus, *Eledone cirrhosa*. *Marine and Freshwater Behaviour and Physiology.* **26:** 59 – 69

Davies-Colley, R.J., Vant,W.N., Latimer,G.J. (1984). Optical characterisation of natural waters by PAR measurement under changeable light conditions. *New Zealand Journal of Marine and Freshwater Research.* **18:** 455-460.

DeWolf,T., Lenzi,S., Lenzi,F. (2011) Paralarval rearing of *Octopus vulgaris* (Cuvier) in Tuscany, Italy. *Aquac* Res. **42:**1406–1414.

Fernández-López,A., Roo,J., Socorro,J., Hernández-Cruz,M.C., Fernández-Palacios,H., & Izquierdo, M.S. (2005). Crecimiento y supervivencia de paralarvas de Octopus vulgaris cultivadas bajo diferentes intensidades de luz. In *X Congreso Nacional de Acuicultura*. (pp. 1–3).

Franco-Santos,R.M., Perales-Raya,C., Almansa,E., DeTroch,M., & Garrido,D. (2016). Beak microstructure analysis as a tool to identify potential rearing stress for Octopus vulgaris paralarvae. *Aquaculture Research*. **47** (9)**:** 3001–3015. http://doi.org/10.1111/are.12753

Garrido,D., Martín,V.M., Rodríguez,C., Almansa,E. (2016). Multidisciplinary approach to the culture of common octopus (*Octopus vulgaris*) paralarvae. Feeding, nutrition and stress biomarkers. Tesis Doctoral. Universidad de La Laguna.

Garrido,D., Martín,V.M., Rodríguez,C., Iglesias,J., Navarro,J.C., Estévez,A., & Almansa,E. (2016). Meta-analysis approach to the effects of live prey on the growth of *Octopus vulgaris* paralarvae under culture conditions. *Reviews in Aquaculture*. http://doi.org/10.1111/raq.12142

Garrido,D., Varó,I., Morales,A.E., Hidalgo,M.C., Navarro,J.C., Hontoria,F., and Cardenete,G. (2017). Assessment of stress and nutritional biomarkers in cultured Octopus vulgaris paralarvae: Effects of geographical origin and dietary regime. *Aquaculture*. **468:** 558–568.

Gómez-González,E. (2006) Guía básica de conceptos de radiometría y fotometría. ESI- Universidad de Sevilla.

Iglesias,J., Sánchez,F.J., Bersano,J.G.F., Carrasco,J.F., Dhont,J., Fuentes,L., Linares,F., Muñoz,J.L., Okamura,S., Roo,J., van der Meeren,T., Vidal,E.A.G., Villanueva,R. (2007). Rearing of Octopus vulgaris paralarvae: Present status, bottlenecks and trends. *Aquaculture*. **266:** 1–15.

Iglesias,J., Otero,J.J., Moxica,C., Fuentes,L., and Sanchez,F.J. (2004). The completed life cycle of the octopus (*Octopus vulgaris*, Cuvier) under culture conditions: paralarval rearing using Artemia and zoeae, and first data on juvenile growth up to 8 months of age.*Aquaculture International* **12:** 481–487.

Iglesias,J., Fuentes,L., and Villanueva,R. (2014). Cephalop culture. 1st ed. SPRINGER

Johnsen,S., Widder,E.A., and Mobley,D.C. (2004). Propagation and Perception of Bioluminescence: Factors Affecting Counterillumination as a Cryptic Strategy. *Biol. Bull.* **207:** 1–16.

Mäthger,L.M., Shashar,N., and RogerT., Hanlon,R.T. (2009). Do cephalopods communicate using polarized light reflections from their skin?.*The Journal of Experimen talBiology.***212:** 2133-2140

Meisel,D.V., Byrne1,R.A. ,Kuba1,M., Griebel,U., & Mather,J.A. (2003). Circadian rhythms in *Octopus Vulgaris. Berliner Paläobiol. Abh.* **03***: 171-177*

Monk,J., Puvanendran,V., Brown,J.A. 2008. Does different tank bottom colour affect the growth, survival and foraging behaviour of Atlantic cod (*Gadus morhua*) larvae? *Aquaculture.* **277:** 197-202.

McLean,B.W., Bray,M.R., Boraston,A.B., Gilkes,N.R., Haynes,C.A., and Kilburn,D.G. (2000)Analysis of binding of the family 2a carbohydrate-binding module from Cellulomonas fimi xylanase 10A to cellulose: specificity and identification of functionally important amino acid residues.*Protein Eng***. 13**(11)**:**801-9.

Moxica,C., Linares,F., Otero,J., Iglesias,J., & Sánchez,F. (2002). Cultivo intensivo de paralarvas de pulpo, *Octopus vulgaris* (Cuvier, 1797), en tanques de 9 m^3. *Boletín Instituto Español De Oceanografía*. ***18*****:** 31–36. Retrieved from

Monroig,Ó., Tocher,D., and Navarro,J. (2013). Biosynthesis of Polyunsaturated Fatty Acids in Marine Invertebrates: Recent Advances in Molecular Mechanisms. *Marine Drugs*. **11**(10)**:** 3998–4018. https://doi.org/10.3390/md11103998

Navarro,J., Monroig,Ó., and Sykes,A. (2014). Nutrition as a Key Factor for Cephalopod Aquaculture. *Cephalopod Culture*, pp.77-95. Doi: 10.1007/978-94-017-8648-5_5

Patterson,L., Dick,J., and Elwood,R. (2007). Physiological stress responses in the edible crab, Cancer pagurus, to the fishery practice of de-clawing. *Marine Biology*. **152**(2)**:** pp.265-272.

Quintana,D., Márquez,L., Arévalo,J.R., Lorenzo,A., Almansa,E. 2015. Relationships between spawn quality and biochemical composition of eggs and hatchlings of *Octopus vulgaris* under different parental diets. *Aquaculture*. **446:**206-216.

Reis,D.B., García-Herrero,I., Riera,R., Felipe,B.C., Rodríguez,C., Sykes,A.V., Martín,M.V., Andrade, J.P., & Almansa,E. (2015). An insight on *Octopus vulgaris* paralarvae lipid requirements under rearing coditions. *Aquaculture Nutrition*. **21:** 797–806.

Repolho,T., Baptista,M., Pimentel,M.S., Dionisio,G., Trübenbach,K., Lopes,V.M., Lopes,A.R., Calado, R., Diniz,M., Rosa,R. (2014). Developmental and physiological challenges of octopus (Octopus vulgaris) early life stages under ocean warming. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*. ***184***(1)**:** 55–64.

Roura,A., González,A.F., Redd,K., and Guerra,A. (2012). Molecular prey identification in wild *Octopus vulgaris* paralarvae. *Marine Biology*. **159**(6)**:**1335–1345.

Roura,Á., Álvarez-Salgado,X., González,Á., Gregori,M., Rosón,G., Otero,J., and Guerra,Á. (2016). Life strategies of cephalopod paralarvae in a coastal upwelling system (NW Iberian Peninsula): insights from zooplankton community and spatio-temporal analyses. *Fisheries Oceanography*. **25**(3) **:** pp.241-258. DOI: 10.1111/fog.12151

SanGiovanni,J.P., and Chew,E.Y. (2005). The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina.*Prog Retin Eye Res.* **24**(1)**:**87-138.

Seixas,P., Rey-Méndez,M., Valente,LMP., Otero,A. (2010) High DHA content in *Artemia* is ineffective to improve *Octopus vulgaris* paralarvae rearing. *Aquac.* **300:**156–162

Sánchez,F., Fuentes,L., Otero,J., Lago,M., Linares,F., Pazos,G., and Iglesias,J. (2011). Effect of tank volume on the growth and survival of reared *Octopus vulgaris* paralarvae. *Aquaculture Research*. **44**(4)**:** pp.667-670.

Shashar[s],N., Rutledge,P.S., and Cronin.T.W. (1996). Polarization vision in cuttlefish – a concealed communication Channel *The Journal of Experimental Biology*. **199:**2077–2084.

Shashar[b],N., and Cronin.T.W.(1996).Polarization contrast vision in *Octopus. The Journal of Experimental Biology.* **199:**999–1004

Shashar,N., Hagan,R., Boal,JG. et al (2000) Cuttlefish use polarization sensitivity in predation on silvery fish. Vision Res. **40:**71‑75

Shashar,N., Milbury,CA., Hanlon,RT. (2002) Polarization vision in cephalopods: neuroanatomical and behavioral features that illustrate aspects of form and function. *Mar Fresh Behav Physiol.* **35:**57‑68

Sierra-Flores,R., Davie,A., Grant,B., Carboni,S., Atack,T., Migaud,H. (2016). Effects of light spectrum and tank background colour on Atlantic cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) larvae performances.*Aquaculture.* **450:** 6–13.

Sykes,A.V., Quintana,D., and Andrade,J.P. (2012). The effects of light intensity on growth and survival of cuttlefish (*Sepia officinalis*) hatchlings and juveniles. *Aquaculture Research.*

Sykes.AV., Domingues,PM., Correia,M. et al (2006) Cuttlefish culture-state of the art and future trends. *Vie Milieu*. **56:**129‑137

Stubbs,A.L., and Stubbs,C.W. (2016). Spectral discrimination in color blind animals via chromatic aberration and pupil shape.*PNAS Early Edition*.

Tett,P., Arístegui,J., Barton,D., Basterretxea,G., De Armasd,J.D., Escánez,J.E., Hernández,S., Lorenzo, L.M., Montero,N. (2002). Steady-state DCM dynamics in Canaries wáter. *Deep-Sea Research II*.**49:** 3543–3559.

Tur,R., Almansa,E., Lago,M.J., Chivite,M., López,M.A. (2017) Temporal variation of monoamines content in common octopus (*Octopus vulgaris*) paralarvae subjected to anoxia.

Yasumuro,H., and Ikeda,Y.(2015) Environmental Enrichment Accelerates the Ontogeny of Cryptic Behavior in Pharaoh Cuttlefish (*Sepia phar aonis*)

Young,R.E. (1972). Function of extra-ocular photoreceptors in bathypelagic cephalopods. *Deep Sea Research and Oceanographic Abstracts.***19:**651-652.

Villamizar,N., García-Alcazar,A., and Sánchez-Vázquez,F.J. (2009). Effect of light spectrum and photoperiod on the growth, development and survival of European sea bass (*Dicentrarchus labrax*) larvae. *Aquaculture.* **292:** 80-86.

Villamizar,N., García-Mateos,G., Sanchez-Vazquez,F.J. (2010). Behavioural (activity and feeding) responses of sea bass (*Dicentrarchus labrax* l., 1758) larvae and prey reared under different ligthining conditions.

Villamizar,N., Blanco-Vives,N., Migaud,B., Davie,H., Carboni,A., Sánchez-Vázquez,F.J. (2011). Effects of light during early larval development of some aquacultured teleosts: A review. *Aquaculture.* **315:** 86–94.

Villanueva,R., and Bustamante,P. (2006). Composition in essential and non-essential elements of early stages of cephalopods and dietary effects on the elemental profiles of *Octopus vulgaris* paralarvae. *Aquaculture*. **261:**225-240.

Villanueva,R., and Norman,M.D. (2008). Biology of the planktonic stages of benthic octopuses. *An Annual Review.* 46**:**105–202.