Deliverable Report

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Deliverable Title	Optimum hydrodynamics and light conditions during greater amberjack larval rearing.		
WP No:	15	**WP Lead beneficiary:**	P2. FCPCT
WP Title:	Larval husbandry- greater amberjack		
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Task Title:	Effect of environmental parameters during rearing.		
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Objective: The objective of this Deliverable is to define some environmental parameters towards an optimum larval rearing methodology of greater amberjack, to study the influence of tank hydrodynamics and tank type and to define the effect of light (intensity and duration) on the growth, survival, biochemical composition, and skeletal deformities.

Description:

Different trials were conducted to determine the effect of environmental parameters during rearing.

Table of contents

Summary
Greater amberjack (*Seriola dumerili*) is a fast growing fish and a very interesting species for the diversification of aquaculture. However, scarce information exists on its rearing in captivity. For this reason, it is necessary to obtain new information on its rearing protocols. The main objective of this study was to define some environmental parameters towards an optimum larval rearing methodology of greater amberjack: these include tank type-shape (40,000 l and 2,000 l) and stocking density effects on the growth, survival, biochemical composition, and skeletal deformities of the larvae. Greater amberjack eggs came from natural spawning and were stocked at two 40,000 l tanks (10 eggs l^{-1}), two 2,000 l tanks (10 eggs l^{-1}) and two 2,000 l tanks (20 eggs l^{-1}). The higher currents occurred in the 2,000 l tanks followed by the 40,000 l tanks. Severe cannibalism and size dispersion were observed from 10-15 days post hatching. In this trial, survival was significantly higher in 2,000 l tanks, independent of density, compared to 40,000 l, while the larvae of 2,000 l tanks of 10 eggs l^{-1} had the highest total length and body weight compared to the other treatments.
The histological study showed regular hepatocyte morphology with few cytoplasmic lipid vacuoles in mesocosm treatment but in 2,000 l tanks a higher degree of vacuolization was observed. The larvae showed different types of anomalies in all treatments.
The study of the photophase (24L:00D vs 18L:6D) did not result in any significant effect on the performance of the larvae in the 2015 trials. However, in the 2016 trials a better performance, although not significant, was observed, both in terms of growth and survival, in the groups reared with 18 hours of light. The photophase duration had limited effect on the mRNA expression levels of the growth axis during the 2015 trials. Analysis of the 2016 samples showed that the photophase affected the mRNA expression levels of IGF-I, IGF-BP1, IGF-BP3 and IGF-BP5 with higher levels for the 18L:06D compared to the 24L:00D period at certain stages of development.
Different background color of the rearing tanks (white, green and black) resulted in no differences in larval growth (total length and body weight) but differences were observed in the survival rates among the groups. Larvae in the white tanks exhibited the highest survival rate compared to the black and the green tanks. These differences were more apparent during the 2016 trials with mean survival rates of 22.2%, 16.5% and 8.2% for the white, green and black tanks respectively. The gene expression analysis revealed significant differences among the treatments. The fish reared in the white background showed increased levels of the genes implicated in the growth axis system compared to the fish reared in the black and green backgrounds. The presented results are of importance as the achieved survival rates indicated a significant technological step in the larval rearing of the greater amberjack.

Introduction

Greater amberjack, *Seriola dumerili* (Risso, 1810) is a leading candidate species for enhancing European aquaculture, showing growth rates ten times higher than the European seabass *Dicentrarchus labrax* (Muraccioli *et al.,*2000). Greater amberjack culture in the Mediterranean region started in the 80s using standard culture conditions, where the feed was first based on fresh fish but quickly progressed to artificial feeds (García-Gómez, 2000; De la Gándara, 2006). In recent years, interest for this species in the aquaculture industry is expanding, due to its high demand and market price, rapid growth (Thompson *et al*., 1999), excellent fillet quality, and its capacity to accept inert food (Nakada, 2000). Today in Malta, the commercial production of amberjack has reached 500 metric tons (FAO, 2016) while other aquaculture companies in countries around the Mediterranean have begun to produce this species (Mylonas and Robles, 2014).
One of the major bottlenecks in the industrial production of greater amberjack is larval rearing and the production of a sufficient number of fry. Although amberjack larvae can readily shift from consuming rotifers to microdiets (Shiozawa *et al*., 2003), there is still very little information on the larval rearing of this species. The semi-intensive method characterized by stocking 0.25 eggs l^{-1} gave a 3.4% survival rate at 40 dph (Papandroulakis *et al*., 2005). In general, low stocking densities such as in the gilthead seabream (*Sparus aurata*) and the red porgy (*Pagrus pagrus*), improve growth by increasing food accessibility and providing vital space (Kentouri *et al*., 1994; Hernández-Cruz *et al*., 1999; Roo *et al*., 2005a, b). In contrast, a high stocking density is associated with low growth due to poor water quality (Yu and Perlmutter, 1970) or

increased food competition (Hagen, 1993). In other fast-growing species, such as meagre (*Argyrosomus regius*), similar results were obtained (Roo *et al*., 2010).
Nevertheless, protocols for the larviculture of amberjack have to be developed to maximize the egg stocking density and light and duration in order to achieve suitable production levels for a successful industry. In order to increase productivity during the larval stages, specific parameters of two different cylindro-conical tank types with volumes of 40,000 l and 2,000 l tanks were tested in duplicates for a period of 30 days. Studies included the effect of tank hydrodynamics, as a function of volume, on larval performance in terms of growth, survival, histology, biochemical composition and skeletal deformities.
Fish larvae are visual predators and their capacity to identify prey is dependent on the light conditions in their environment (Puvanendran, and Brown, 2002). The effect of light on the background color of the fish's environment plays an important role in the physiology, behavior and ecology of exotherms, and several studies have shown that it also affects the growth and survival of fish larvae (Duray *et al.,* 1996; Downing and Litvak, 1999; Tamazouzt *et al*., 2000; Jentoft *et al*., 2006). In addition, the duration of photo phase has a significant role in the feeding efficiency of the larvae as it prolongs the foraging period of the individuals especially at the early stages (Papandroulakis *et al*., 2002; Papandroulakis *et al*., 2010). A prolonged photo phase has been found beneficial for the performance of some species although concerns have been expressed for the possible stress, as well as retinal damage (Migaud *et al* 2007; Vihtelic, *et al*, 2006) that an extended photoperiod can induce (Villamizar *et al*., 2011)
The growth hormone/insulin-like growth factor axis plays a key role in the coordination of vertebrate growth, including fish. Circulating growth hormone (GH) stimulates Insulin-like Growth factor I and II IGF synthesis and IGFs, in turn, act in a wide variety of tissues by binding to specific receptors that trigger physiological processes. In addition, the distribution and bioavailability of these receptors is modulated by a number of binding proteins (Insulin-like Growth factor Binding Proteins, IGFBPs) (Reindl and Sheridan, 2012). In the present study, the effect of three different background colors as well as two photophase regimes on amberjack larval performance and on the expression of genes implicated in the growth axis were characterized for the first time during early ontogeny in amberjack.

1. The effect of tank hydrodynamics and stocking density on larval performance in terms of growth, survival, histology, biochemical composition and skeletal deformities.

Materials and Methods
The effect of tank hydrodynamics (type-shape) was studied. Two different tank types: 40,000 l and 2,000 l and stocking densities were tested in duplicates for a period of 30 days. The current profile was analyzed with a Vectrino (high-resolution acoustic velocimeter) and its hydrodynamic effect on larval performance in terms of growth, survival, histology, biochemical composition and skeletal deformities was determined.

Current measurements

The applied water exchange rates (as % of total water volume) in 2,000 and 40,000 l tanks were 10% and 4% per hour, respectively, while air flow was 350 and 1,400 ml min^{-1}, respectively. The conditions regarding water exchange and air flow were similar to the ones applied during the current protocol for larval rearing. Current measurements were performed using a Vectrino velocimeter. The Vectrino is a high-resolution acoustic velocimeter used to measure 3D water velocity in a wide variety of applications from the laboratory to the ocean. The basis measurement technology is coherent Doppler processing. The water velocity measurements have a range of ± 1 cm sec^{-1} with an accuracy of ±0.5% of the measured value or ±1 mm sec^{-1}. The sampling volume is at a distance of 5 cm from probe with a diameter of 6 mm and a height of 7 mm (**Fig. 1).**
Measurements of current field at specific depths or layers were performed. The 2,000 l tank was divided into 5 layers at 0.1, 0.65, 1.10, 1.25 and 1.5 m depth. In the first 3 layers, 17 measurements were taken, while on the fourth layer 5 measurements were taken and 1 on the last layer, at normally distributed points. For the mesocosm, 40,000 l tanks, 3 layers were examined at 0.3, 0.7 and 1.5 m where 17 measurements were taken at each layer.

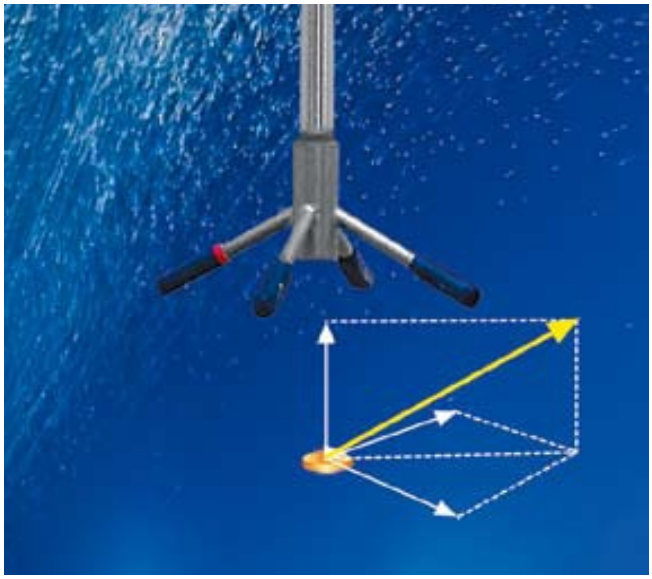


Figure 1. Representation of current sampling volume.

A specially prepared construction on the top of the tanks allowed the accurate positioning of the sensor in the tank in order to perform the measurements (**Fig. 2**)

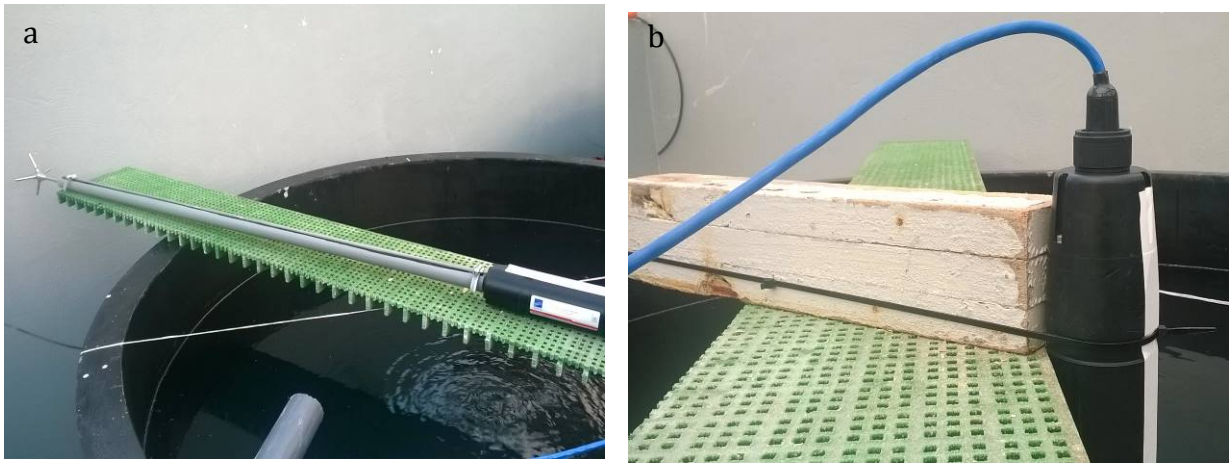


Figure 2. The holding construction of the velocimeter on the top of the tank (a) and detail of the positioning of the sensor (b).

Larval rearing

The present experiment was conducted for the first time in 2015, when there was mass larval mortality between 5-10 dph, and was repeated more successfully in 2016. The eggs in both trials were obtained from natural spawning from a greater amberjack broodstock of the Grupo de Investigación en Acuicultura (GIA) and were maintained in the facilities of "Fundación Canaria Parque Científico Tecnológico" (FCPCT) de la Universidad de Las Palmas de Gran Canaria, Spain. The greater amberjack eggs were stocked at two different densities in the four 2000 l tanks (10 and 20 eggs l^{-1}) while the two 40,000 l tanks were stocked with 10 eggs l^{-1} each (**Fig. 3**).

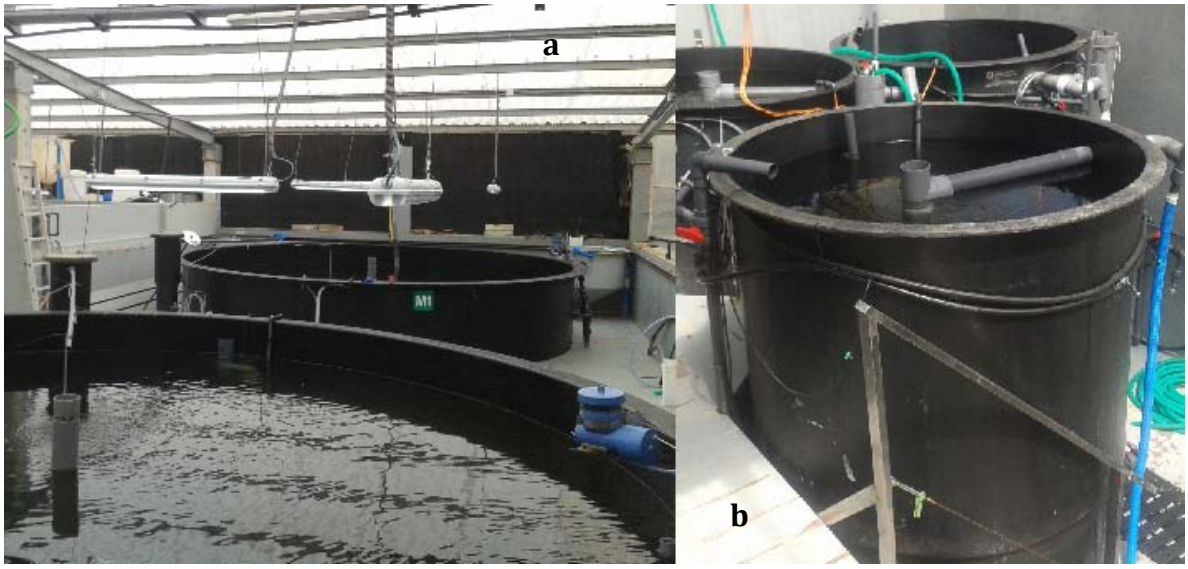


Figure 3: Cylindrical tanks of 40,000 l **(a)** and 2,000 l cylindro-conical tanks **(b),** where greater amberjack eggs were stocked at different densities: 10 and 20 eggs l^{-1}.

Table 1. Greater amberjack larval rearing protocol.

Dph	Photop.	Renewal	Phyrop.	Rotifer		Artemia			Artificial diet
				8:00	15:00	8:00	11:00	15:00	
Stocking	Natural	25%/day	Clear water	10 rot/ml	10 rot/ml	0,5 art/ml	0,5 art/ml	0,5 art/ml	75-150-300 µm. The size of microdiet will be evaluated depending size of the larvae
Hatching									
1									
2		15%/h	40 l						
3									
4			20 l						
5									
6		25%/h							
7									
8									
9									
10		50%/h							
11									
12		75%/h							
13			30 l						
14									
15									
16		100%/h							
17									
18		150%/h							
19									
20		200%/h							
21									
22			15 l						
23									
24									
25			Clear water						
26									
27									
28									
29									
30									

Larval rearing was performed according to the protocol of the GIA (**Table 1**), which requires a natural photoperiod (14:10 h, light: dark), as well as recommended salinity (37 psu) and temperature conditions (24.98-27ºC). Water renewal flow was increased progressively from 25% day^{-1} to 200% $hour^{-1}$, as described in **Table 1**. Water entered the tank from the bottom and exited from the top where water quality parameters were measured daily. Water was continuously aerated (125 ml min^{-1}), attaining 5–8 g l^{-1} of dissolved O_2

(saturation ranging between 60% and 80%). All tanks were equipped with a surface skimmer for removing surface organic material. The green water technique was used which meant adding live phytoplankton (*Nannochloropsis sp*.) to maintain a concentration of 250,000 cells ml^{-1} in the rearing tanks during feeding with rotifers (1-30 dph) while *Artemia* (12-30 dph) were enriched with Ori-Green (Skretting TM, France). From 13-30 dph, the fish were fed progressively larger microdiets of 75, 150 and 300 µm (Gemma (Skretting, France).

The larval sampling schedule is shown in **Table 2.** During the sampling, larvae were sacrificed by immersion in water and ice according to the current regulations (Spanish Royal Decree 1201/2005) which were accepted by the Spanish Ethic Welfare Committee (Comité Ético del Bienestar) of the University of Las Palmas de Gran Canaria (ULPGC) in 2011.

Larval growth was assessed by measuring the total length of 30 larvae per tank, every 5 days. Total length was measured using a profile projector (Mitutoyo PJ-3000A, Kanagawa, Japan) (Faustino and Power, 1998).

Survival of larvae was calculated based on the number of surviving fish that were individually counted at the end of the experiment.

All samples were flushed with N_2 and kept frozen at -80 °C until analysis was carried out. Total lipids were extracted (Folch *et al*., 1957) where the fatty acids were then transmethylated to their fatty acid methyl esters (FAME) (Christie, 1982). Separation and identification of the fatty acids was carried out by gas chromatography (GC) (GC TERMO FINNIGAN FUCUS GC, Milan, Italia) according to Izquierdo *et al.* (1992). Dry matter, ash and protein content were calculated using the methods of analysis of the Association of Official Analytical Chemists (A.O.A.C, 2012).

Twenty larvae $tank^{-1}$ were sampled for histological analysis of the liver and gut at the end of the trial. Tissues were stored in 10% buffered formaldehyde in a sample: formaldehyde ratio of 1:10 for several weeks prior to processing. Samples were further segmented to allow a better penetration of the alcohol and introduced in histology cassettes. Dehydration of the samples was carried out using a Histokinette 2000 (Leica, Nussloch, Germany) with gradually increasing alcohol grades beginning with 70% and ending with 100%, where the last two steps were fixing in xylene and paraffin. This process substitutes water with fat in the tissues, allowing the staining of the sample. Once the paraffin block was obtained it was sectioned (3µm) using a Leica RM 2135 microtome (Leica, Nussloch, Germany) and then placed on a microscopic slide. Samples were then stained with haematoxylin–eosin (Martoja and Martoja-Pearson, 1970) for microscopic evaluation.

To evaluate skeletal anomalies, 100 larvae (35 dph) were collected from each tank and stained with alizarin red and photographed (Vandewalle *et al*., 1998). Different regions of the axial column were identified and divided according to Boglione *et al.* (2001). Observations were performed on the right side of the stained samples under a stereomicroscope. The numerical data set obtained was processed to calculate incidences for each descriptor (anomaly typology) and treatment.

Table 2. Table of samplings at specific ages (dph) and numbers of fish sampled $tank^{-1}$

Age (dph)	Length and Weight (larvae $tank^{-1}$)	Osteology	Biochemistry	Histology
Eggs			5g	
0	30			
5	30			
10	30			
15	30			
20	30			
25	30			
30	30	100	50	20

All the data were expressed as mean ± standard deviation (S.D.) and were tested using one-way ANOVA according to SPSS Statistical Software System 15.0 (SPSS, www.spss.com) where the significance level was set at 0.05. All percentage values were arcsine transformed before statistical analysis. All values were checked for normality and homogeneity of variance, using the Kolmogorov–Smirnoff and the Levene tests,

respectively. When variances were not homogenous, a non-parametric Kruskal–Wallis test was carried out, notched multiple Box and Whisker plot. To evaluate the differences in skeletal frequency of deformities log linear statistical analyses were performed (Sokal and Rolf, 1995).

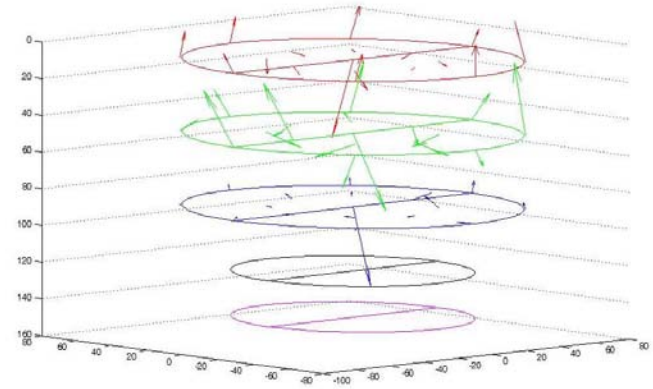
Results

The results are shown in **Table 3**. There were differences in current profiles between the 2,000 l and 40,000 l tanks. Water currents showed that they were generally higher in the 2,000 l tanks.

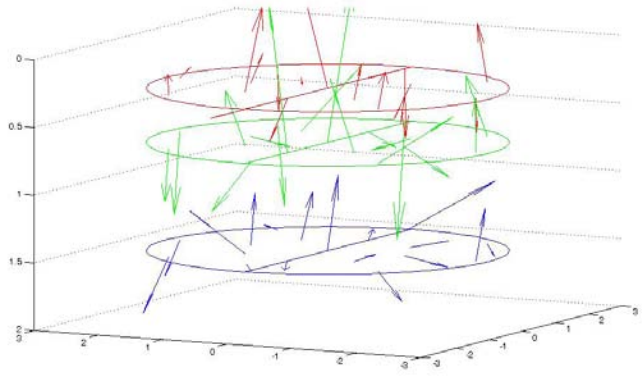
A graphical representation of the current profile is presented in **Figure 4 a and b.** Arrows are in 3-d representation and the observed size does not represent the actual velocity value.

Table 3 Mean velocity at the different layers of the experimental tanks (mean ± standard deviation, n=1,500)

Tank volume (l)	Layer	Mean velocity value (cm s^{-1})
2,000	surface	$1.51x10^{-2} \pm 1.55x10^{-2}$
	medium1	$0.92x10^{-2} \pm 1.38x10^{-2}$
	medium2	$0.84x10^{-2} \pm 3.32x10^{-3}$
	medium3	$0.23x10^{-2} \pm 2.07x10^{-2}$
	bottom	$1.30x10^{-2} \pm 3.65x10^{-2}$
40,000	surface	$9.10x10^{-3} \pm 3.22x10^{-2}$
	medium	$8.70x10^{-3} \pm 3.05x10^{-2}$
	bottom	$7.50x10^{-3} \pm 8.69x10^{-2}$



(a) 2,000 l



(b) 40,000 l

Figure 4. Current profiles in the different layers of 2000 l tanks (a) and 40,000 l tanks (b). Arrows are in 3-d representation and the observed size does not represent the actual velocity value.

Larval performance

The water temperature and oxygen concentration of the second experiment (2016) are shown in **Table 4.**

Table 4. Water temperature and oxygen concentration during the trial.

	40,000l, 10 eggs l^{-1}	2,000l, 10 eggs l^{-1}	2,000l, 20 eggs l^{-1}
Temperature ºC	24.5±1.9	25.9±1.5	25.8±1.6
Oxygen concentration	7.1±0.6	6.1±0.7	7.2±1.3

Significant (P<0.05) differences, in total length and body weight, were observed (**Figs. 5** and **6**, respectively) between all treatments starting from 10 to 30 dph. The larvae of 2,000 l tanks stocked at 10 eggs l^{-1} grew significantly (P<0.05) better than larvae from the same volume tanks, that were stocked at 20 eggs l^{-1} as well as larvae from the 40,000 l tanks stocked with 10 eggs l-^{1} (**Figs. 5** and **6,** respectively**).**
Severe cannibalism and size dispersion were observed in both trials. In the first trial (2015) larvae died between 5-10 dph while during the second trial in 2016 survival was significantly (P<0.05) higher in 2,000 l tanks compared to mesocosm tanks. There was no significant (P>0.05) difference in survival between the 10 and 20 eggs l^{-1} treatments in the 2,000 l tanks. (**Fig. 7)**

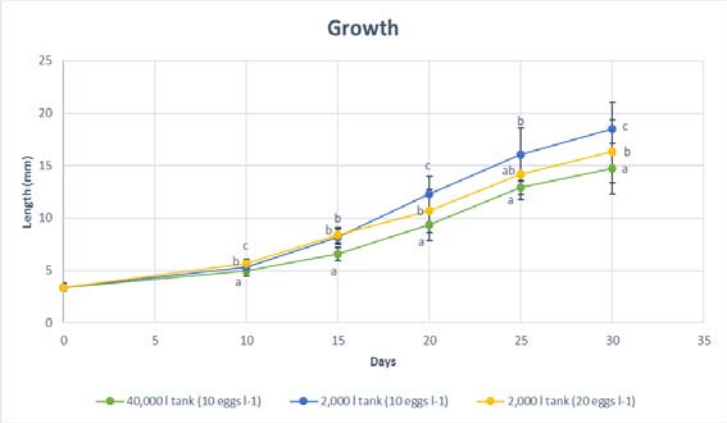


Figure 5. Total length of greater amberjack larvae at different tanks and densities. Values of same age larvae having different letters were significantly (P<0.05) different.

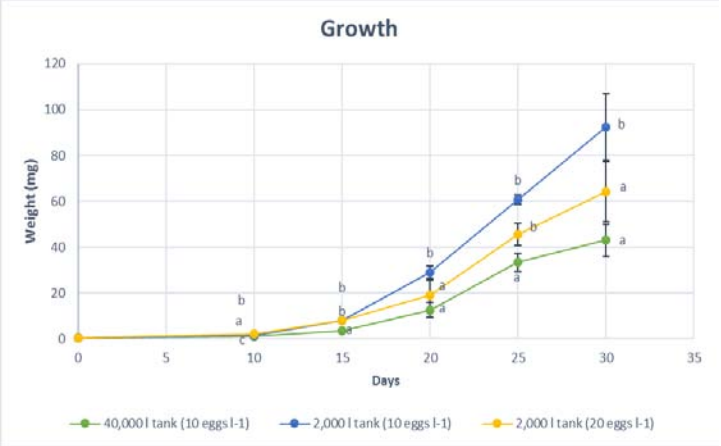


Figure 6: Weight of greater amberjack larvae at different tanks and densities. Values of same age larvae having different letters were significantly (P<0.05) different.

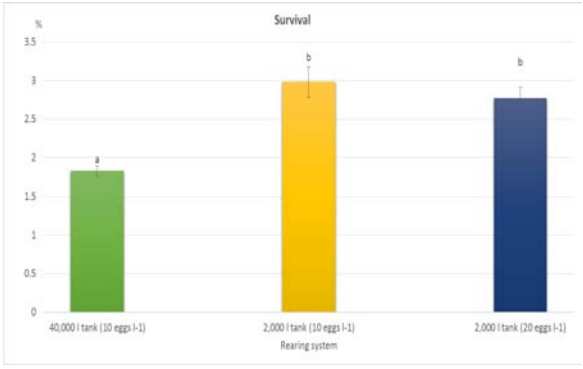


Figure 7. Survival rate of greater amberjack larvae in different tanks and densities. Survival values (following arcsine transformation) having different letters were significantly (P<0.05) different.

The proximate analysis of the samples showed that there was no significant difference in lipid, protein and ash contents between the larvae of all treatments (**Table 4**).

Table 4. The proximal analysis (% DW) of rotifers, *Artemia* and greater amberjack eggs and larvae (P<0.05)

	Rotifers	*Artemia*	Eggs	Larvae 0	Larvae 2,000 l (10 eggs l^{-1})	Larvae 2,000 l (20 eggs l^{-1})	Larvae 40,000 l
Lipids	20.0	21.1	20.9	21.0	10.2	10.3	9.5
Ash	7.80	8.91	5.1	6.0	14.3	14.6	13.2
Protein	71.2	70.0	74.3	72.0	75.0	75.0	76.1

The fatty acids contents of the rotifers and *Artemia* are shown in **Table 5** and no significant difference was ovbserved between them (P<0.05)**.** The fatty acids analysis of the larvae (**Table 6**) showed that the larvae of 2,000 l tanks stocked with 20 eggs l^{-1} had the highest (P<0.05) docosahexaenoic acid (DHA, 22:6n-3) content, while the larvae of 2,000 l tank 10 eggs l^{-1} showed higher arachidonic acid (ARA, 20:4n-6) content. In addition, the larvae of mesocosm 10 eggs l^{-1} showed higher 18:3n-3 (**Table 6**).

Table 5. The fatty acids (% area) of rotifers and *Artemia*. (P<0.05)

Fatty acids	Rotifers	*Artemia*
14:0	2.10	0.63
14:1n-5	2.82	0.75
15:0	0.77	0.18
16:0ISO	0.39	0.42
16:0	17.51	13.54
16:1n-7	12.68	1.88
16:1n-5	0.66	0.59
16:2n-4	0.13	0.03
16:3n-4	0.59	0.53
16:3n-3	0.06	0.06
16:3n-1	1.28	0.14
18:0	8.63	8.52
18:1n-9	20.04	21.38
18:1n-7	7.01	6.44
18:1n-5	0.75	0.09
18:2n-9	1.95	0.63
18:2n-6	8.69	7.99
18:2n-4	0.29	0.07
18:3n-6	0.17	0.43
18:3n-4	0.09	0.06
18:3n-3	1.04	21.50
18:4n-3	0.12	2.87
18:4n-1	0.05	0.01
20:0	0.43	0.30
20:1n-9	0.61	0.14
20:1n-7	2.36	1.11
20:1n-5	0.60	0.15
20:2n-9	0.56	0.06
20:2n-6	0.27	0.36
20:3n-6	0.28	0.13
20:4n-6	0.96	0.81
20:3n-3	0.45	1.16
20:4n-3	0.20	0.65
20:5n-3	1.03	2.17
22:1n-11	0.54	0.24
22:1n-9	0.79	0.15
22:4n-6	0.20	0.05
22:5n-6	0.01	0.01
22:5n-3	0.53	0.22
22:6n-3	2.05	3.32

Table 6: The fatty acids (% area) of greater amberjack larvae. Fatty acid values among treatments having different letters were significantly different (P<0.05).

Fatty acids	2,000 l (10 eggs l-^{1})	2,000 l (20 eggs l-^{1})	40,0000 l (10 eggs l-^{1})
14:0	0.54	0.53	0.42
14:1n-5	0.33	0.37	0.34
15:0	0.20	0.18	0.16
16:0ISO	0.20	0.21	0.24
16:0	16.50	15.40	13.91
16:1n-7	1.80	1.92	1.34
16:1n-5	0.58	0.58	0.57
16:2n-6	0.07	0.11	0.01
17:0	0.09	0.11	0.08
16:3n-4	0.40	0.42	0.49
16:3n-3	0.27	0.30	0.05
16:3n-1	1.53	1.27	1.17
16:4n-3	0.34	0.34	0.28
16:4n-1	0.21	0.19	0.16
18:0	12.86	11.99	11.43
18:1n-9	13.56	14.54	17.29
18:1n-7	5.45 [a]	5.96 [b]	6.44 [c]
18:1n-5	0.10	0.10	0.10
18:2n-9	0.40	0.46	0.57
18:2n-6	7.04 [a]	7.06 [a]	7.44 [a]
18:2n-4	0.08	0.08	0.08
18:3n-6	0.31	0.36	0.38
18:3n-4	0.07	0.07	0.05
18:3n-3	8.34 [a]	11.34 [b]	14.49 [c]
18:3n-1	0.02	0.03	0.01
18:4n-3	0.88	1.19	1.51
20:0	0.36	0.36	0.34
20:1n-9	0.04	0.04	0.04
20:1n-7	0.59	0.60	0.63
20:1n-5	0.21	0.19	0.16
20:2n-9	0.07	0.07	0.08
20:2n-6	0.38	0.39	0.41
20:3n-9	0.03	0.03	0.02
20:3n-6	0.45	0.44	0.40
20:4n-6	3.14 [a]	2.89 [b]	2.44 [c]
20:3n-3	1.04	1.22	1.32
20:4n-3	1.02 [a]	1.17 [b]	1.37 [c]
20:5n-3	5.37	5.32	4.87
22:1n-11	0.08	0.07	0.04
22:1n-9	0.18	0.15	0.17
22:4n-6	0.18	0.15	0.12
22:5n-6	0.12	0.12	0.09
22:5n-3	1.35 [a]	1.19 [b]	0.94 [c]
22:6n-3	13.04 [a]	10.30 [b]	7.40 [c]

The liver of larvae cultured in 40,000 l tanks and 2,000 l tank (10 eggs l^{-1}) showed regular hepatocyte morphology with few cytoplasmic lipid vacuoles that did not alter hepatocyte size or shape. Having said that, a higher degree of vacuolization was observed in the liver of larvae grown in the 2,000 l tank stocked with 20 eggs l^{-1} which also demonstrated a higher vacuolization score (**Table 7** and **Fig. 8 a and b**), although no significant difference was found (P>0.05).

Table 7. Mean scores for larvae liver lipid vacuolization. Cytoplasmic lipid vacuolization score: 0, not observed; 1, few; 2, medium; 3, severe (mean±sd). Values among treatments having different letters were significantly (P<0.05) different.

Tanks	Cytoplasmic lipid vacuolization
40,0000 l (10 eggs l^{-1})	1.30±0.14 a
2,000 l (10 eggs l^{-1})	1.35±0.21 a
2,000 l (20 eggs l^{-1})	1.80±0.14 a

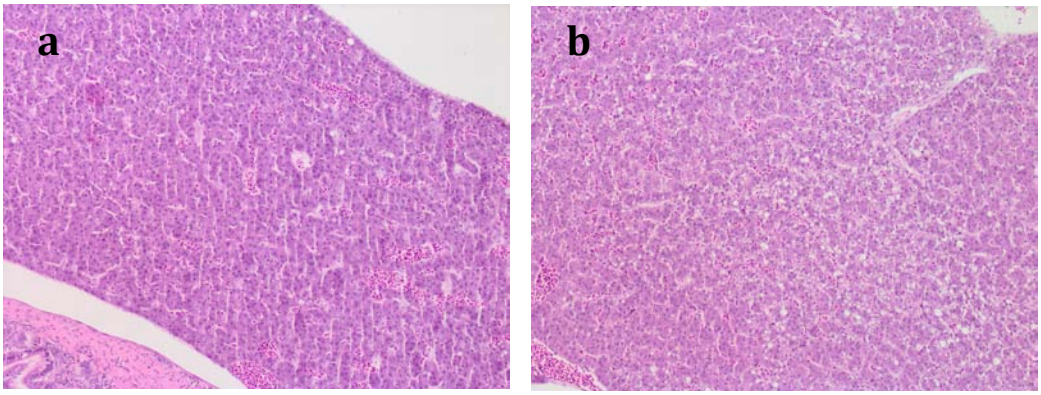


Figure 8. (a) Liver of larvae reared in 40,000 l tanks **(b)** Liver of larvae reared in 2,000 l tanks (20 eggs l^{-1}).

The results of the anomalies evaluation showed that different anomalies were found in all treatments were shown but they were independent of treatment effect (P<0.05, **Fig. 9)**.
Whole mount staining for the description of the anomalies in the skeleton of greater amberjack larvae showed different types of anomalies that are listed in **Table 8** and shown in **Fig.10.**

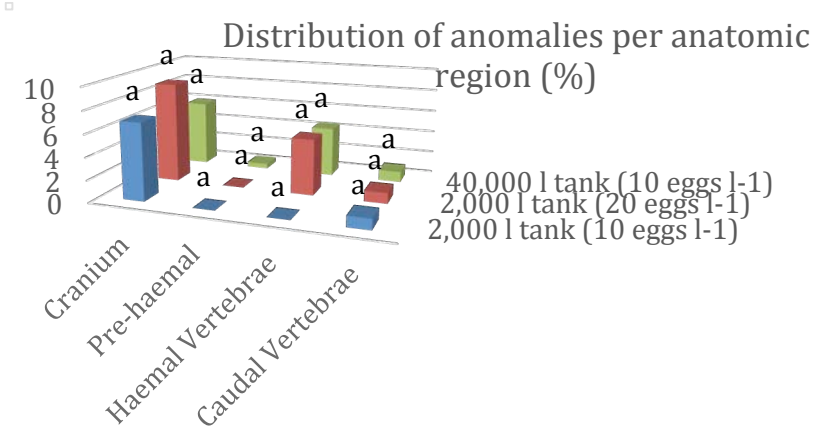
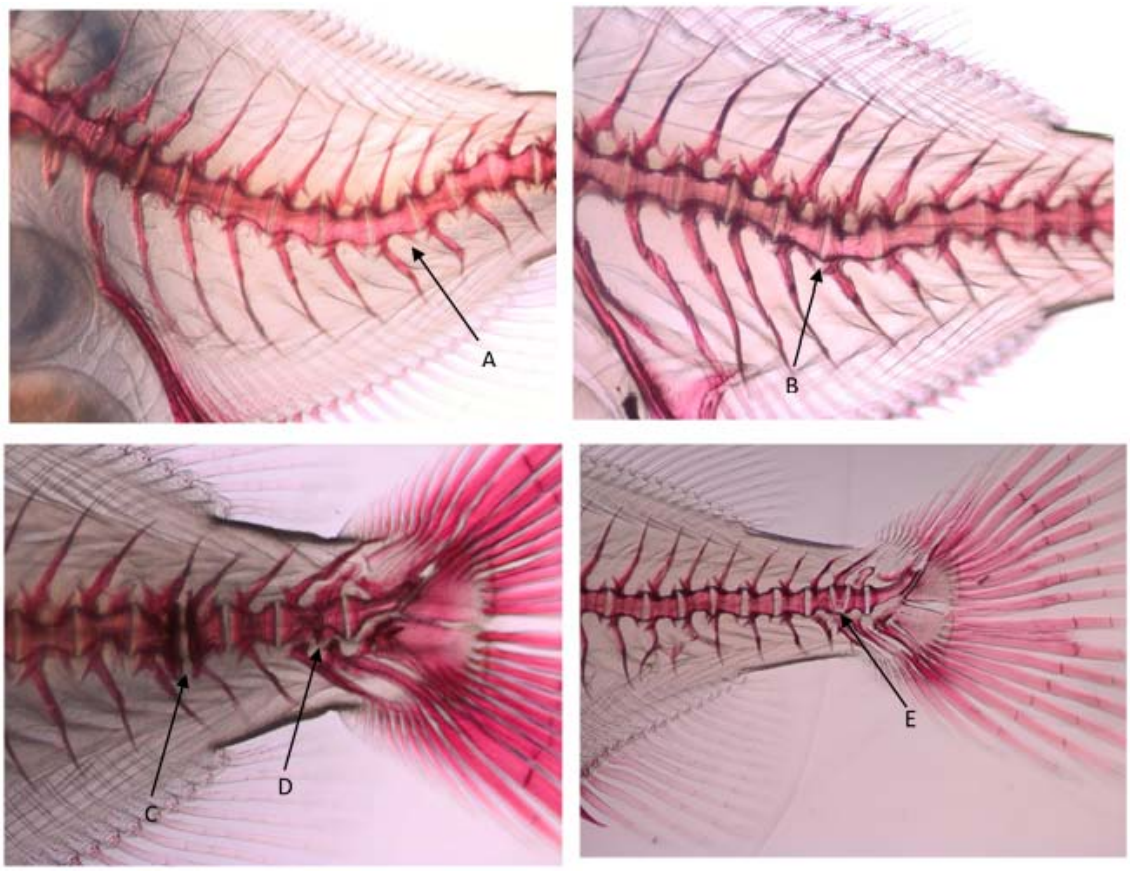
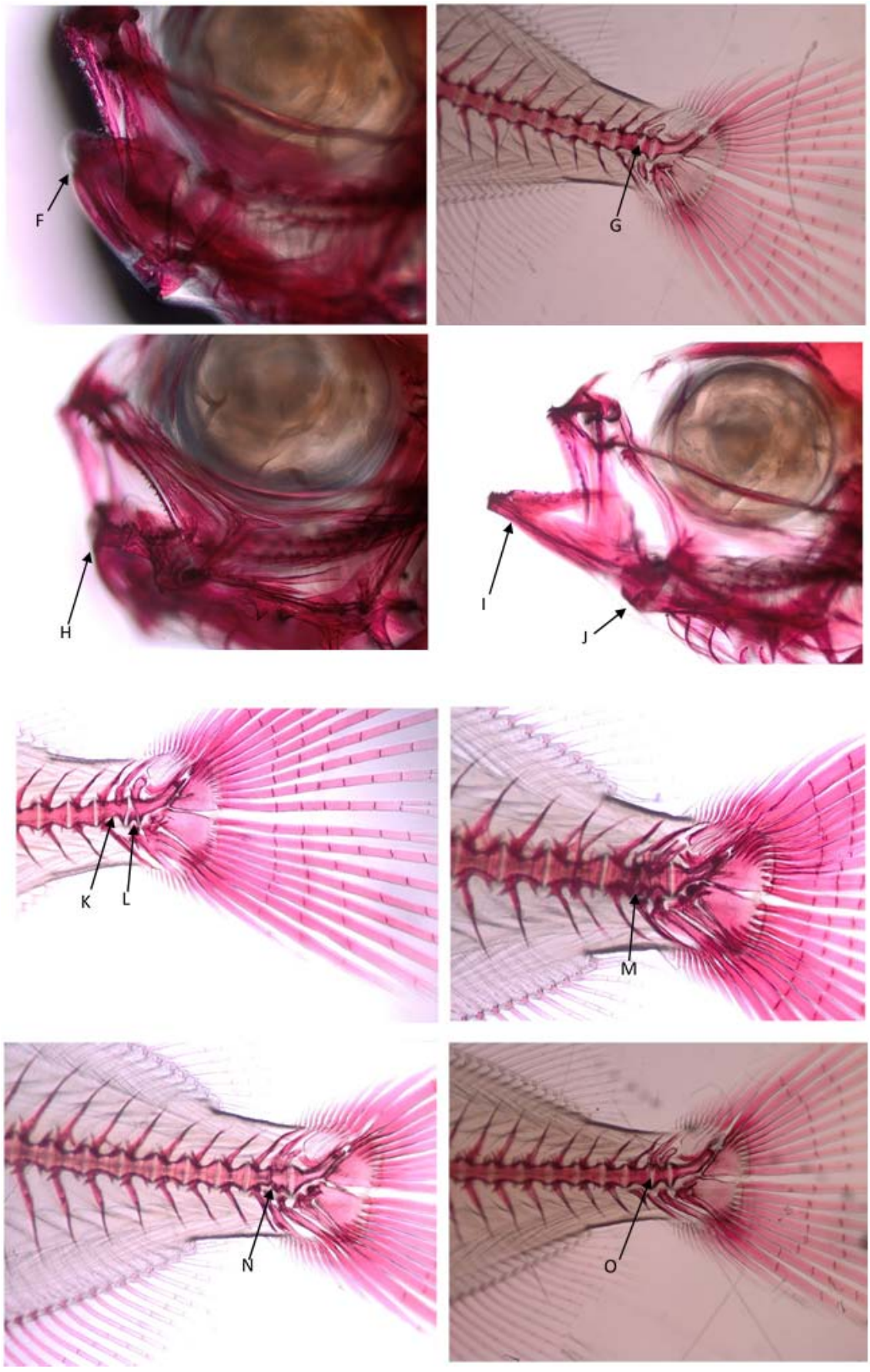


Figure 9. Distribution of anomalies (%) per anatomic region of greater amberjack larvae in different tanks and densities. Values among treatments having different letters were significantly (P<0.05) different.

Table 8. Different types of anomalies observed in greater amberjack larvae at different tanks and densities

Some anomalies observed
A – Lordosis in haemal vertebrae
B – Lordosis in haemal vertebrae
C – Anomalous vertebrae
D - Total vertebral body fusion in caudal vertebrae
E - Partial vertebral fusion in caudal vertebrae
F - Anomalous dentary
G - Partial vertebral fusion in caudal vertebrae
H - Anomalous dentary
I - Anomalous dentary
J - Cephalic anomalies (glossohyal)
K - Total vertebral body fusion in caudal vertebrae
L - Partial vertebral fusion in caudal vertebrae
M - Total vertebral body fusion in caudal vertebrae
N - Total vertebral body fusion in caudal vertebrae
O - Partial vertebral fusion in caudal vertebrae
P - Total vertebral body fusion in caudal vertebrae
Q - Partial vertebral fusion in caudal vertebrae




F
G
H
I
J
K
L
M
N
O

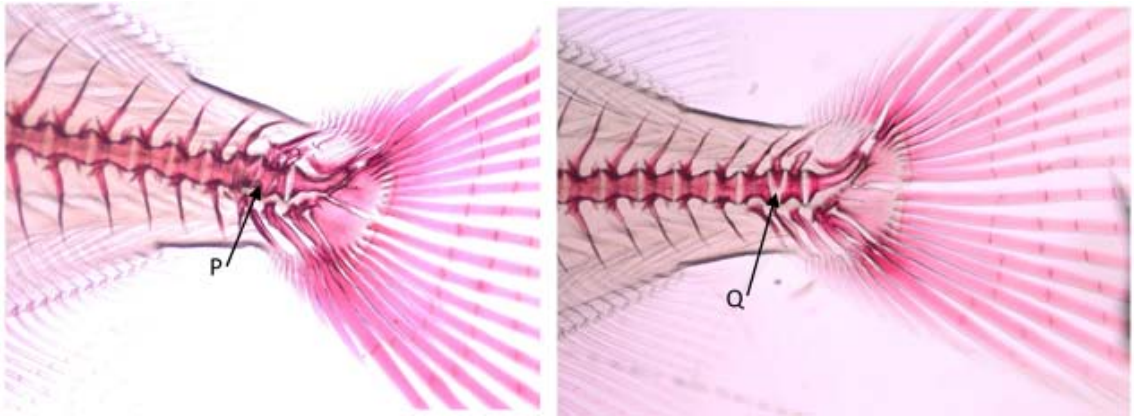


Figure 10. Different types of anomalies observed in greater amberjack larvae at different tanks and densities.

2. The effect of light (intensity and duration) on larval rearing in terms of growth, survival and size distribution.

Materials and Methods

During the 2015 spawning season, a first experimental trial was attempted but showed poor results and in 2016 the studies were repeated. In both years the eggs came from induced spawns from broodstock kept in the Argosaronikos SA cage farm. After collection, eggs were transported to the hatchery facilities of the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC) of the Hellenic Center for Marine Research (HCMR) in polystyrene boxes in ca. 12 hours, and then were incubated.

Larval rearing

The methodology applied was the intensive rearing method, which is characterized by controlled conditions of water quality, light intensity, photophase and feeding. A variation of this methodology is the so-called "pseudo-green" water approach that is based on the frequent addition of phytoplankton and zooplankton in the larval rearing tanks. The tanks were either 2,000 l, used in the 2015 trials, or 500 l, used in the 2016 trials. The 2,000 l cylindro-conical tanks were organized in triplets in a closed water system with a biological filter. Similarly, the 500 l tanks were organized in duplicates in a closed water system with a biological filter (**Fig. 11**).

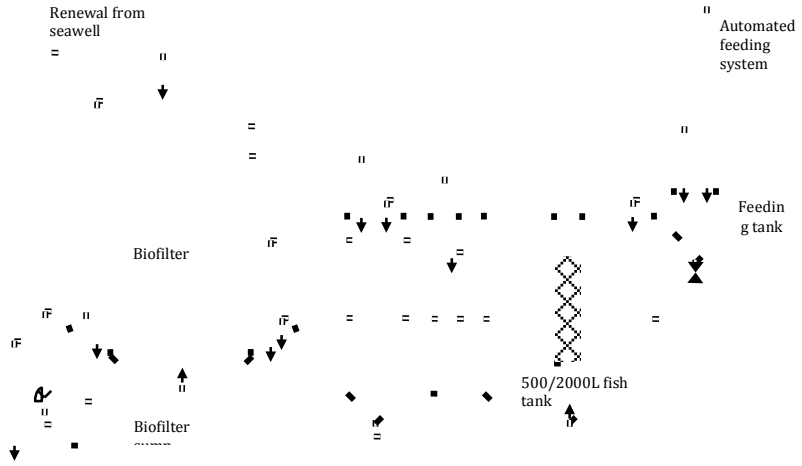


Figure 11. Larval rearing tanks in a closed water system at IMBBC-HCMR.

In both cases, mechanically filtered borehole water of 35 psu was used to supply the tanks. Temperature was kept at 22±0.5 °C during the autotrophic stage and was gradually increased to 24±0.5 °C after mouth opening. The pH fluctuated from 8.3 to 8.5 and the dissolved oxygen from 5.6 to 7.4 mg l^{-1} in the 2015 trials, while in 2016 the temperature was kept at 23.9±0.8 °C, the pH fluctuated from 7.81 to 8.18 and the dissolved oxygen from 4.92 to 7.42 mg l^{-1}. Water circulation was achieved in two ways according to the stage of rearing. During embryogenesis, egg hatching and the autotrophic larval stage, water circulated in the tanks through a biological filter. Aeration was also provided in the tanks (150–250 ml min^{-1}). After first feeding, water circulation was autonomous for each tank by means of an airlift pump. The daily water renewal in the biological filter in the larval rearing tanks was 3% until 15 dph when it gradually increased to 50% at 25 dph. A skimmer was installed during the appropriate period (5 to 15 dph) to keep the surface free from lipids. Light intensity varied between 200 - 800 lux during the day, and was about 200 lux during the night.
Feeding was based on daily administration of enriched rotifers (from 3-21 dph), Instar II *Artemia* nauplii (from 12 dph onwards), and artificial diet (from 21 dph). The concentration of rotifers in the tank was maintained at 3.0 individuals ml^{-1}, while Artemia was added at 0.1 individuals ml^{-1} after measurements of the concentration twice daily. Phytoplankton was added daily from 3 to 22 dph at 300 ± 100 x 10^3 cells ml^{-1}.
The administration of the zooplankton was implemented with the use of an automated feeding system allowing continuous administration of food.

Primer design and qPCR experiments

Primers for GH were based on the available sequences of greater amberjack in genomic databases (NCBI Gene Bank accession no. L43628) and primers for the gene of Growth Hormone Releasing Hormone (GHRH) on the available sequence of gilthead seabream (no. DQ659328). IGFs I and II (IGF-I & IGF-II) were designed based on available sequences of the Japanese amberjack *Seriola quinqueradiata* (no. AB439208 and AB823704, respectively). The products of each primer pair were further checked with sequencing in order to confirm that they amplify the desired genes. Primers for IGFBPs 1, 2, 3 and 5 (IGF-BP1, IGF-BP2, IGF-BP3, IGF-BP5) were as described by Pedroso *et al*. 2009. Primers for b-actin were obtained from previous work of our group (Pavlidis et al., 2011), whereas for ribosomal 18S RNA (18S) they were obtained by the work of Tom *et al.* (2004).

RNA purification and cDNA synthesis

Samples of pre-larvae and larvae were let to thaw on ice, disrupted and homogenized using the Tissue Ruptor (Qiagen, Hilden, Germany) for 20 sec in 600 µl RLT plus buffer (RNeasy Plus Mini Kit Qiagen, Valencia, USA). Total RNA was isolated with the RNeasy Plus Mini Kit (Qiagen, Valencia, USA). RNA yield and purity was determined by measuring the absorbance at 260 and 280 nm using the Nanodrop® ND-1000 UV–Vis spectrophotometer (Peqlab, Erlangen, Germany), and its integrity was tested by electrophoresis in 1% agarose gels. Reverse transcription (RT) was carried out using 1 µg RNA with QuantiTect Reverse transcription kit (Qiagen).

Quantitative real-time PCR (qPCR)

The mRNA expression of genes encoding for GH, GHRH, IGF I and II, IGFBPs 1, 2, 3 & 5 (IGF-BP1, IGF-BP2, IGF-BP3, IGF-BP5) was determined with quantitative polymerase chain reaction (qPCR) assays using the KAPA SYBR® FAST qPCR Kit (Kapa Biosystems). Reactions were cycled and the resulting fluorescence was detected with MJ Mini Thermal Cycler (Bio-Rad) under the following cycling parameters: 95 °C for 3 min (HotStarTaq DNA Polymerase activation step), 94 °C for 15 s (denaturation step), 60 °C for 30 s (annealing step), 72 °C for 20 s (extension step), 40 cycles (step 2–step 4). Levels of GH, GHRH, IGF-I & IGF-II, and IGFBPs 1, 2, 3 and 5 (IGF-BP1, IGF-BP2, IGF-BP3, IGF-BP5)] mRNA were normalized based on the reference genes 18S and b-actin. A relative standard curve was constructed for each gene, using 4 serial dilutions (1:5) of a pool of all cDNA samples. We also performed geNORM analysis (Vandesompele *et al*., 2002) in order to validate which are the most suitable reference genes to serve as an internal control and these were eEF1a and 18S.

All statistical analyses were performed with SigmaPlot 11.0 (Jandel Scientific). All data are presented as means ± standard deviation (SD). Data were initially screened for normality and homogeneity. Statistical

comparisons of temporal patterns of gene expression between the different developmental stages and the various rearing conditions were made using two-way ANOVA. Holm-Sidak's honestly significant difference test for multiple comparisons was used to determine significant differences among groups. The significant level used was $P<0.05$.

Sample collection

The growth of the individuals was estimated with regular measurements of total length and wet weight from a representative sample of larvae per tank. At the end of the rearing period (~25 dph) populations were counted and transferred for pre-growing.

During the 2015 rearing, 5 pooled samples both from the mesocosm and intensive reared fish were taken at 0, 2, 5, 10, 15, 20, 25 and 30 days post hatch (dph). They were used for expression analysis of GH, GHRH, IGF-I, IGF-II, and IGFBPs 1, 2, 3 and 5 (IGF-BP1, IGF-BP2, IGF-BP3, IGF-BP5). During the 2016 trial, samples were limited to 4 pooled samples taken at 3, 5, 17, 25 and 30 dph and used for repeating the gene expression analysis done in 2015.

A. Photoperiod trial

The duration of the photoperiod was tested in 2015 during an experimental rearing in 2 triplicated systems of 2,000 l tanks. Two light:dark conditions that were tested were 24:00 and 18:06 hours d^{-1}. Tanks were covered with non-transparent plastic covers (**Fig. 12**) in order to control the intensity and duration of lighting. In 2016 the experiment was repeated using 500 l tanks in duplicates applying the same photo phases.

Figure 12. Tank configuration for controlling the photoperiod during the 2015 and 2016 trials

B. Light intensity trial

The trial was performed following the intensive methodology described earlier both in 2015 and 2016. Tanks with three different colors (black, green and white) were used for larval rearing, in duplicates. For this, white or green fabric that completely covered the inner walls was used, while black tanks served as controls. Furthermore, underwater lights were used to improve light intensity in the water column without significantly changing the intensity on the tank's surface. Underwater lighting was applied from 8:00 to 20:00 imitating the increased brightness during summer months. In **Fig. 13** the tanks with different backgrounds are shown.

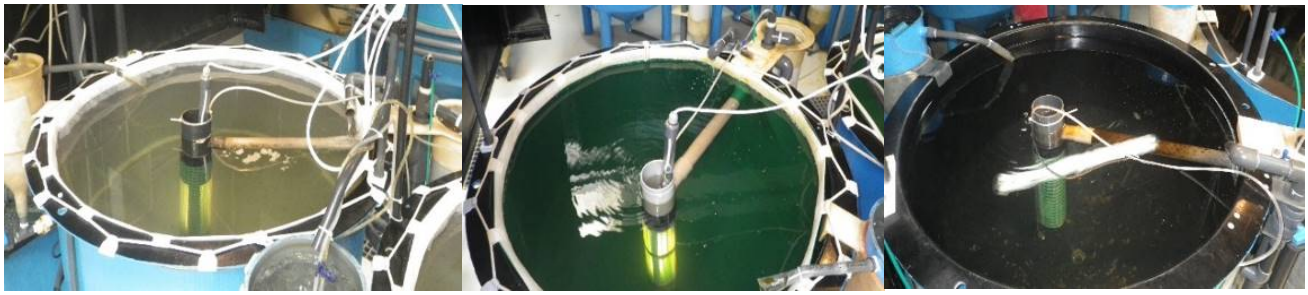


Figure 13. Tanks with different color backgrounds.

Results

A. Photoperiod trial

Rearing was performed as planned and all required samples were collected. Although no pathologies were presented, the overall survival in 2015 was low and at the end of the trial only few individuals survived per tank. The results of the growth performance for 2015 trials are shown in **Table 9** and **Fig 14**.

Table 9. Growth rate (exponential) of greater amberjack larvae in terms of total length and wet weight during the rearing.

Total Length	**2015**
18L:06D	0.0244 d^{-1}
24L:00D	0.0364 d^{-1}
Wet weight	
18L:06D	0.16 d^{-1}
24L:00D	0.21 d^{-1}

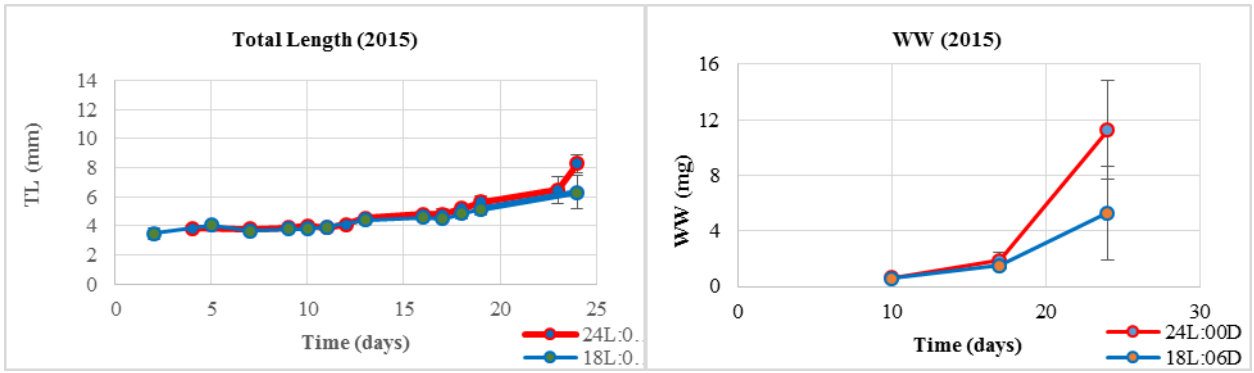


Figure 14. Mean ± SD of total length (TL) and wet weight (WW) of greater amberjack larvae, as a function of age, reared under different photo phases.

In 2016 however, the performance was improved and the survival of the larvae varied between 6% and 13.6%. The mean survival for the 18L:06D photo phase was higher (10.6±4.2%) than the 24L:00D one (8.2±3.1%), although not significantly (P>0.05). In terms of total length (**Fig. 15)**, larvae grew with an exponential rate of 0.310 d^{-1} independent of photophase. Larval growth (total length) for both photophases in 2016 is presented in **Fig. 15.**

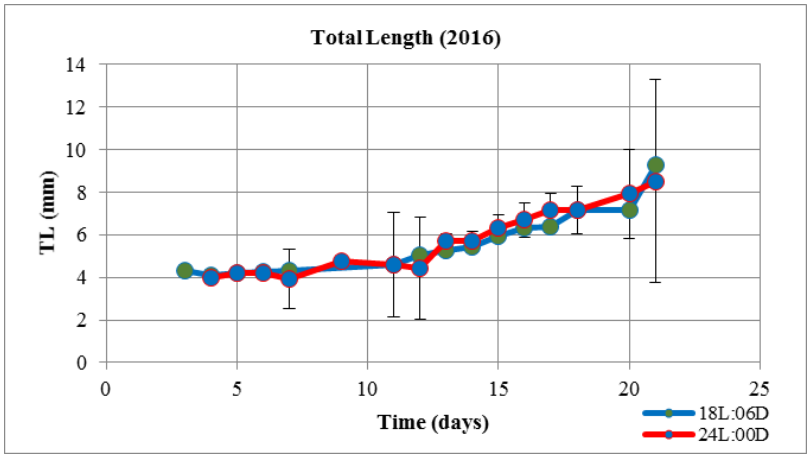


Figure 15. Greater amberjack larvae total length, as a function of age, reared in different photophases during the 2016 trial (Mean ± standard deviation).

The photoperiod (24L:00D vs 18L:6D), based on the 2015 trial, did not appear to affect the mRNA expression levels of IGF-I. However, there was a gradual increase in IGF-I mRNA levels as development proceeded that was significantly ($P<0.05$) higher than levels at hatching at 20, 25 and 30 dph (**Fig. 16**). The mRNA expression levels of IGF-II were not altered based on the photoperiod and appeared high at 0 dph, decreased at 2 dph, and then peaked at 5 dph ($P<0.05$) and remained stable thereafter **(Fig. 17**). The expression of GH was not affected by the rearing method as mRNA levels appeared to be generally stable throughout development apart from 0 and 2 dph, when lower levels were observed (**Fig. 18**). GHRH expression levels were not affected by the rearing method used and remained generally at lower levels until 20 dph, when a significant increase was observed to reach peak values at 30 dph ($P<0.05$; **Fig. 19)**.

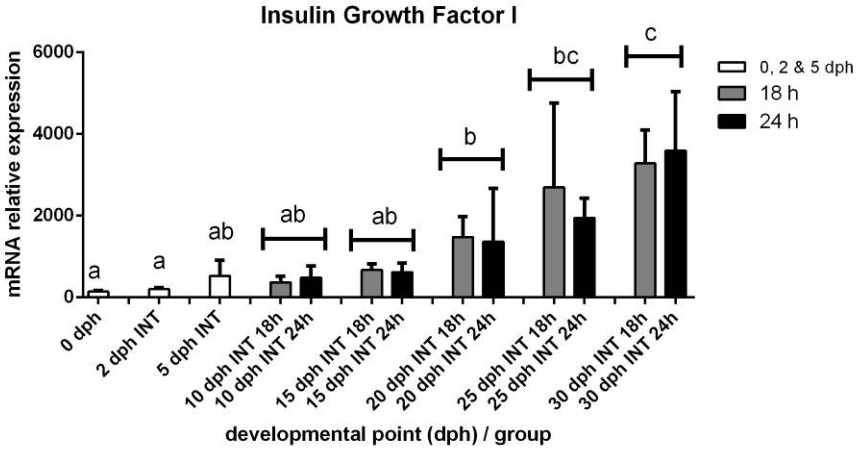


Figure 16. mRNA relative expression levels of IGF-I between the different photoperiod regimes during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 5). Means with different letters differ significantly ($P<0.05$).

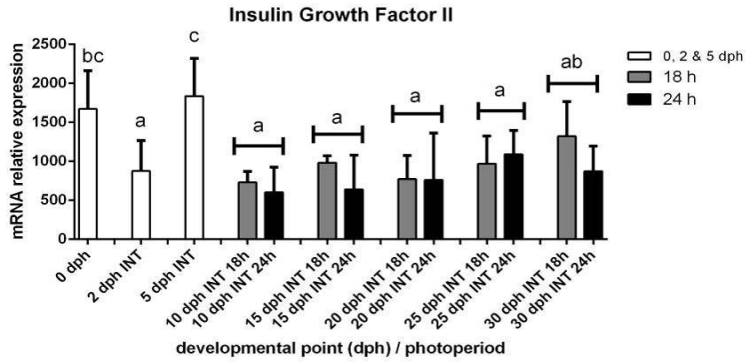


Figure 17. mRNA relative expression levels of IGF-II between the different photoperiod regimes during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 5). Means with different letters differ significantly (P<0.05).

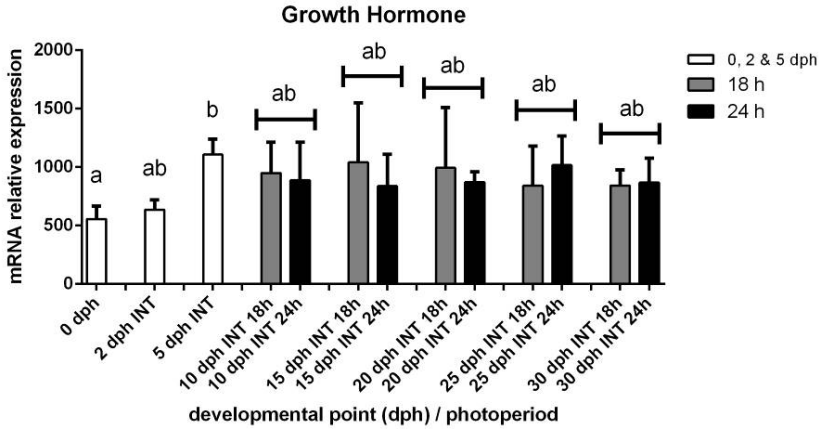


Figure 18. mRNA relative expression levels of GH between the different photoperiod regimes during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 5). Means with different letter superscripts differ significantly (P<0.05).

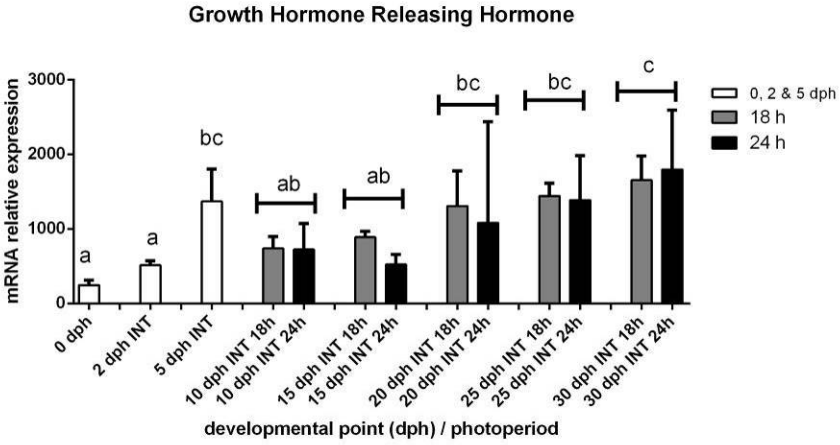


Figure 19. mRNA relative expression levels of GHRH between the different photoperiod regimes during early ontogeny of greater ambberjack. Values are means ± standard deviation (n = 5). Means with different letters differ significantly (P<0.05).

The photoperiod scheme applied did not affect the mRNA expression of any of the IGF binding proteins studied apart from the expression of IGF-BP1 where higher levels (P<0.05) were observed in fish reared under the condition of 24L:00D compared to the fish reared under the condition of 18D:6D at 25 and 30 dph (**Fig. 20a**). Additionally, throughout development the mRNA expression of IGF-BP1 was not consistent between the different groups but at 0 and 2 dph the minimum levels were observed; these levels reached a peak at 5 dph (P<0.05; **Fig. 20a**) and remained stable thereafter with the exception of 30 dph where differences in fish from the two different photoperiod conditions (mentioned above) were observed. The mRNA expression levels of IGF-BP2 appeared low at the beginning of development until 20 dph, when there was a statistically significant upregulation (P<0.05), which continued to gradually increase until 30 dph (**Fig. 20b**). No differences were observed in the case of IGF-BP3 (**Fig. 20c).** However, in the case of IGF-BP5 the mRNA expression levels showed a gradual increase from 0 dph until 5 dph (P<0.05) but its levels appeared stable thereafter (**Fig. 20d**).

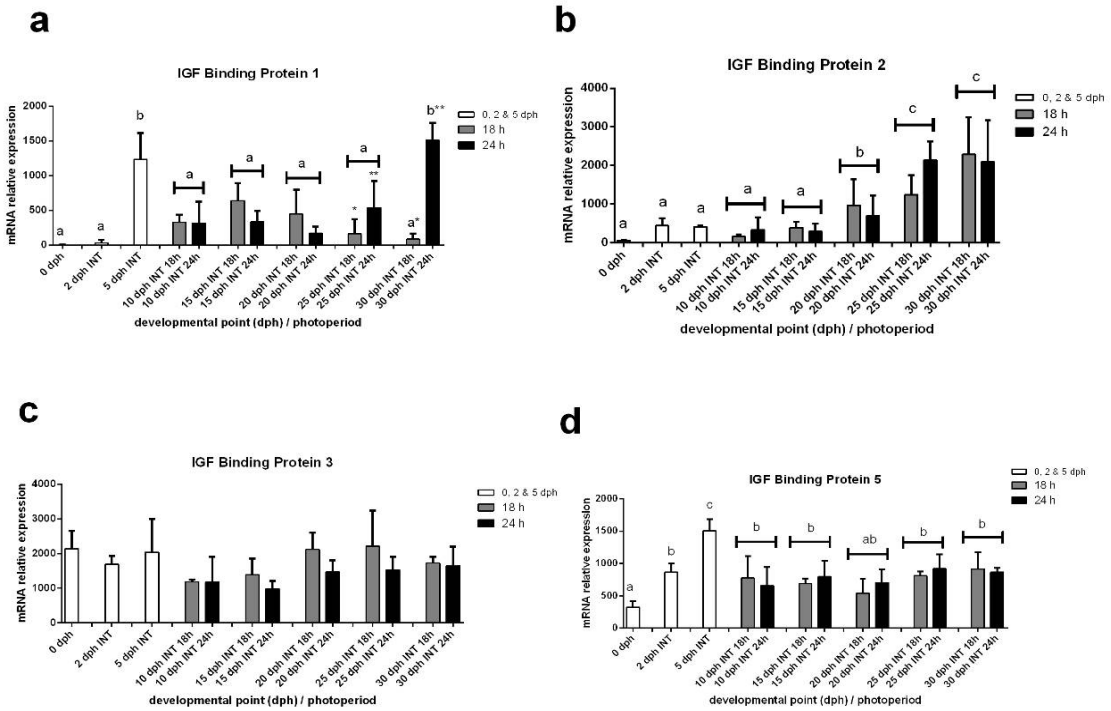


Figure 20. mRNA relative expression levels of IGF-I binding proteins between the different photoperiod regimes during early ontogeny of greater amberjack: (a) IGF-BP1; (b) IGF-BP2; (c) IGF-BP3; (d) IGF-BP5. Values are means ± standard deviation (n = 5). Different letters indicate statistically significant differences between the sampling points during ontogeny whereas asterisks indicate differences between the different photoperiod regimes (P<0.05).

The 2016 trials revealed that the photoperiod (24L:00D vs 18L:06D) affected the mRNA expression levels of IGF-I with higher levels for the 18L:06D group at 17 dph and 25 dph compared to the 24L:00D group. Additionally, there was a statistically significant gradual increase (P<0.05) in mRNA levels as development proceeded, which was observed only in the 18L:06D group with peak values at 25 dph **(Fig. 21).**

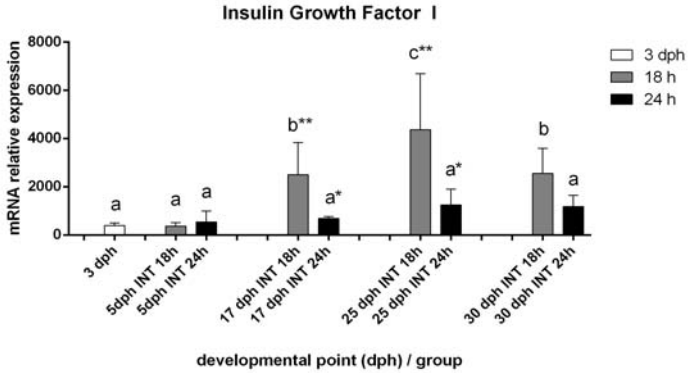


Figure 21. mRNA relative expression levels of IGF-I between the different photoperiod regimes during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 4). Means with different letters indicate differences between the different developmental points whereas asterisks indicate differences between the photoperiod schemes (P<0.05).

The mRNA expression levels of IGF-II were not altered based on the photoperiod and showed a statistically significant decrease at 30 dph compared to all the other developmental points **(Fig.22)**.

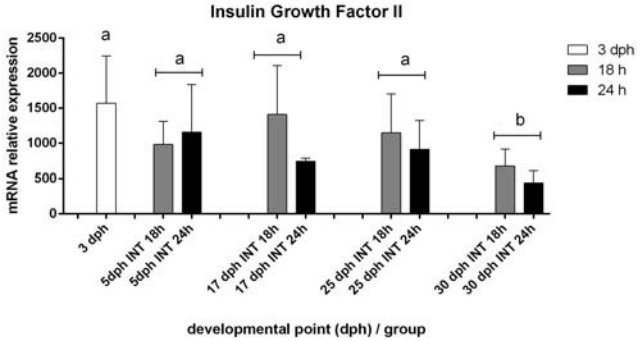


Figure 22. mRNA relative expression levels of IGF-II between the different photoperiod regimes during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 4). Means with different letters indicate differences between the different developmental points (P<0.05).

The expression of GH and GHRH were not affected neither by the photoperiod regime nor by development progression (**Fig.23** and **Fig. 24**).

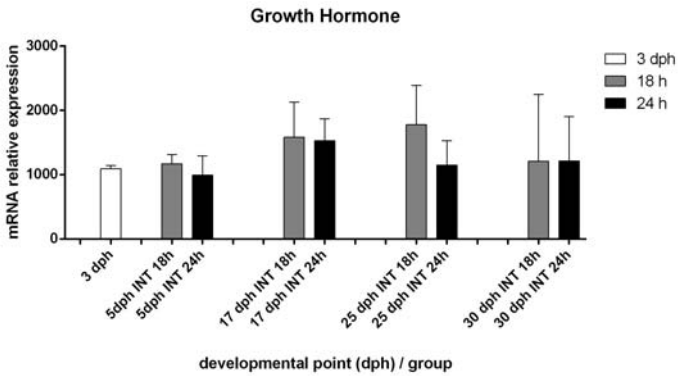


Figure 23. mRNA relative expression levels of GH between the different photoperiod regimes during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 4).

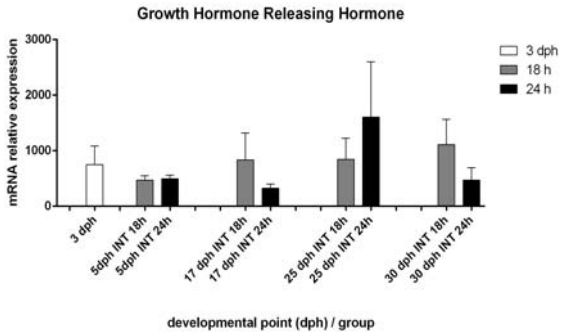


Figure 24. mRNA relative expression levels of GHRH between the different photoperiod regimes during early ontogeny of greater amberjack. Values are means ± standard deviation (n=4).

The mRNA levels of IGF-BP1 appeared to be generally stable apart from 5 dph where a statistically significant upregulation was observed, compared to the other points of development. In addition, the effect of the photoperiod regime increased expression levels in the 18L:06D group compared to the 24L:00D group (P<0.05; **Fig. 25**).

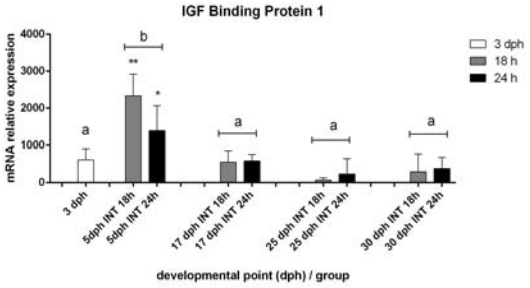


Figure 25. mRNA relative expression levels of IGF-BP1 between the different photoperiod regimes during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 4). Means with different letters indicate differences between the different developmental points whereas asterisks indicate differences between the photoperiod schemes (P<0.05).

The IGF-BP2 expression showed a gradual increase throughout development with statistically higher levels at 25 dph and 30 dph (P<0.05). Additionally, at 30 dph an effect of the photoperiod regime was observed with higher expression levels in the 18L:06D group compared to the 24L:00D group (P<0.05; **Fig. 26**).

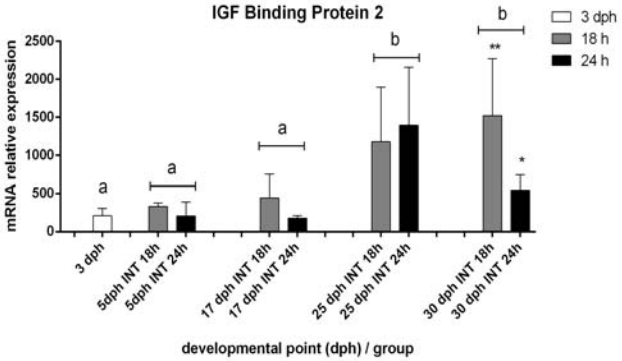


Figure 26. mRNA relative expression levels of IGF-BP2 between the different photoperiod regimes during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 4). Means with different

letters indicate differences between the different developmental points whereas asterisks indicate differences between the photoperiod schemes (P<0.05).

IGF-BP3 expression levels were not affected by the photoperiod regime and remained stable throughout development (**Fig. 27**). However, IGF-BP5 expression at 17 dph and 25 dph not only appeared significantly (P<0.05) higher compared to the other points of development but during this period an effect of the photoperiod regime was also observed with higher expression levels in the 18L:06D group compared to the 24L:00D group (P<0.05 ; **Fig. 28**).

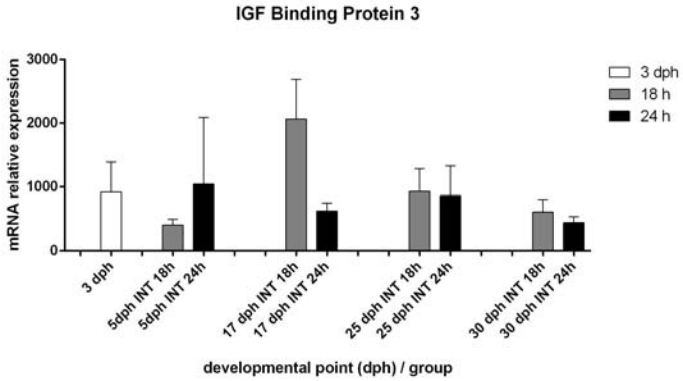


Figure 27. mRNA relative expression levels of IGF-BP3 between the different photoperiod regimes during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 4).

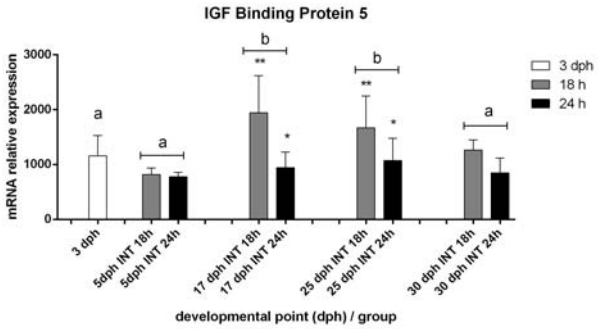


Figure 28. mRNA relative expression levels of IGF-BP5 between the different photoperiod schemes during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 4). Means with different letters indicate differences between the different developmental points whereas asterisks indicate differences between the photoperiod schemes (P<0.05).

Light intensity

No statistically significant (P>0.05) differences were observed in the growth of the larvae in terms of total length and body weight between the different tank colors neither in the 2015 nor in the 2016 trials **(Fig. 29).** Fish growth was exponential in terms of total length (2015: Black: 0.0568 d^{-1}, White: 0.0549 d^{-1}, Green: 0.0578 d^{-1}; 2016: Black: 0.0481 d^{-1}, White: 0.0393 d^{-1}, Green: 0.0355 d^{-1}) and wet weight (2015: Black: 0.2482 d^{-1}, White: 0.2165 d^{-1}, Green: 0.2695 d^{-1}; 2016: Black: 0.1260 d^{-1}, White: 0.1970 d^{-1}, Green: 0.171 d^{-1}).

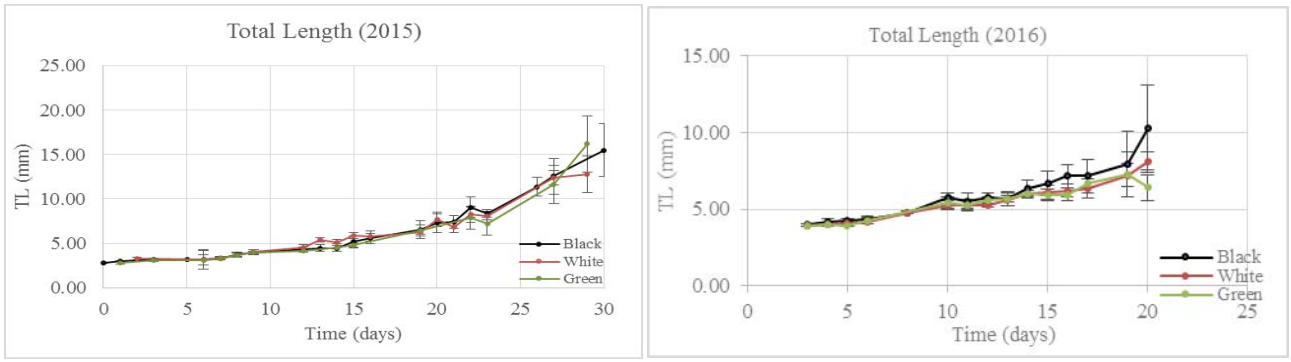


Figure 29: Progression of the total length of greater amberjack larvae reared in tanks with different background color (Mean ± standard deviation).

However, significant differences were observed at the end of the 2015 experimental period in the survival rates among the different groups with the larvae in the white tanks exhibiting the highest rate (1.3%) compared to the black (0.5%) and the green tanks (0.02%). The differences in survival were more profound during the 2016 trial. The white background resulted in a significantly higher survival rate (22.2±0.7%) compared to green (16.5±0.9%) and to black (8.2±3.1%) backgrounds.

Gene expression analysis, based on 2015 trials, revealed significant differences ($P<0.05$) among the groups as well as between the different developmental sampling points. The background color (white, black, green) appeared to affect the mRNA expression levels of IGF-I at 30 dph, with the fish reared in the white background showing higher levels of expression and the fish in the green background the lowest ($P<0.05$; **Fig. 30**). Additionally, during development the expression pattern observed for IGF-I was not consistent between the different groups. From 0 dph until 17 dph the levels remained stable and low in all groups but at 25 dph and 30 dph only fish reared in the white and black background showed a significant increase ($P<0.05$), whereas IGF-I levels in fish reared in the green background remained stable as before (**Fig. 30**). The mRNA expression levels of IGF-II were not altered based on background color but throughout development IGF-II expression was not consistent between the different groups as depicted in **Fig. 31**. The background color appeared to have an effect at the mRNA expression levels of GH at 17 dph with fish reared in the white background exhibiting the highest levels of expression and fish reared in the green background the lowest ($P<0.05$). However, throughout development GH expression was not consistent between the different groups as depicted in **Fig. 32**. GHRH expression levels showed no statistically significant differences either depending on the background color or as development progressed (**Fig. 33).**

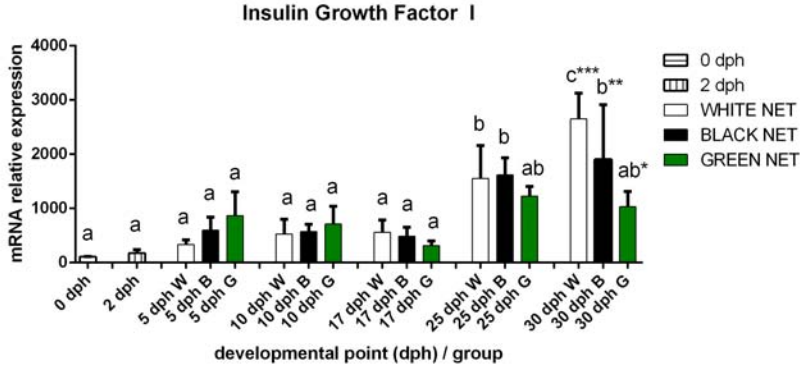


Figure 30. mRNA relative expression levels of IGF-I between the different background colors during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 5). Different letters indicate statistically significant differences between the sampling points during ontogeny whereas asterisks indicate differences between the different background colors ($P<0.05$).

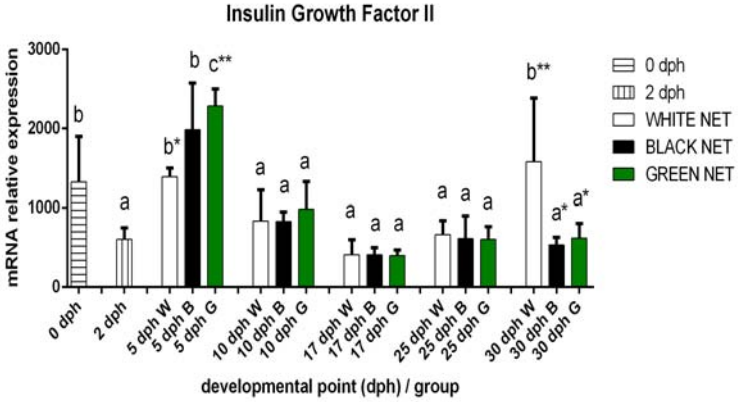


Figure 31. mRNA relative expression levels of IGF-II between the different background colors during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 5). Means with different letters differ significantly (P<0.05).

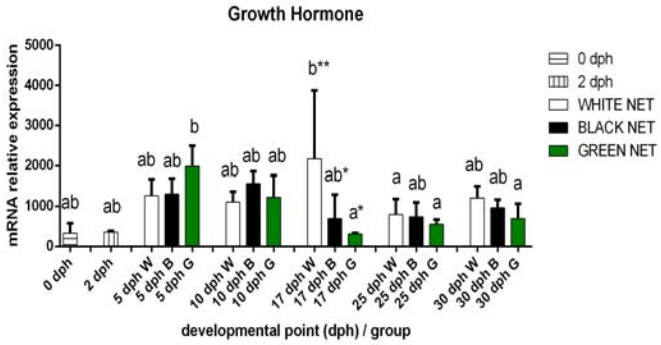


Figure 32. mRNA relative expression levels of GH between the different background colors during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 5). Different letters indicate statistically significant differences between the sampling points during ontogeny whereas asterisks indicate differences between the different background colors (P<0.05).

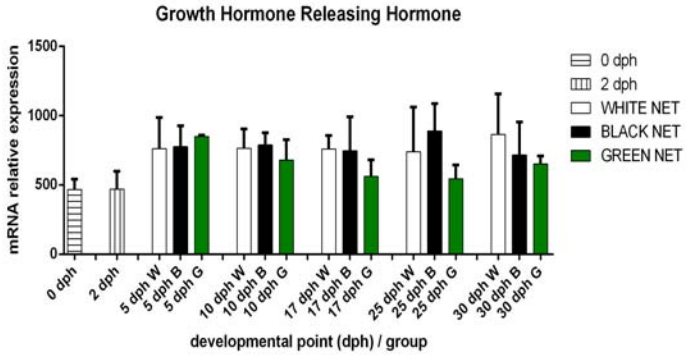


Figure 33. mRNA relative expression levels of GHRH between the different background colors during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 5). No significant (P>0.05) differences were observed.

The different background colors used appeared to have an effect on the mRNA expression of IGF-BP1 in fish at 5 dph. Fish reared in the white background showed the lowest expression while fish reared in the green background demonstrated the highest expression levels (**Fig. 34a**). Additionally, throughout development the mRNA expression of IGF-BP1 appeared very low at 0 dph and 2 dph, while it demonstrated peak values at 5 dph for all groups, and then dropped and remained stable thereafter (P<0.05; **Fig. 34a**). The mRNA expression levels of IGF-BP2 appeared stable at the beginning of development until 25 dph when there was a significant upregulation (P<0.05) for fish from all groups that was also observed at 30 dph. Additionally, at 30 dph the mRNA expression of IGF-BP2 appeared to be affected by the background color in all groups (P<0.05). Fish reared in the white background exhibited the highest expression levels, while fish reared in the black background exhibited medium levels and fish in the green background the lowest (**Fig. 34b**). In the case of IGF-BP3, expression levels appeared to gradually increase until 10 dph where peak values were observed (P<0.05) that dropped back to the initial values as development proceeded (**Fig. 34c**). At 30 dph an effect of the background color on the expression levels of IGF-BP3 was also observed, with an upregulation (P<0.05) observed in fish reared in the white background compared to fish reared in the green background (**Fig. 34c**). Finally, in the case of IGF-BP5 the mRNA expression levels remained generally stable from 0 dph until 25 dph, when a significant (P<0.05) upregulation was observed that continued until 30 dph. The background color appeared to also have an effect on the expression of IGF-BP5 but only at 30 dph, when fish reared in the white background exhibited higher mRNA expression levels (P<0.05) compared to the other two groups (**Fig. 34d**).

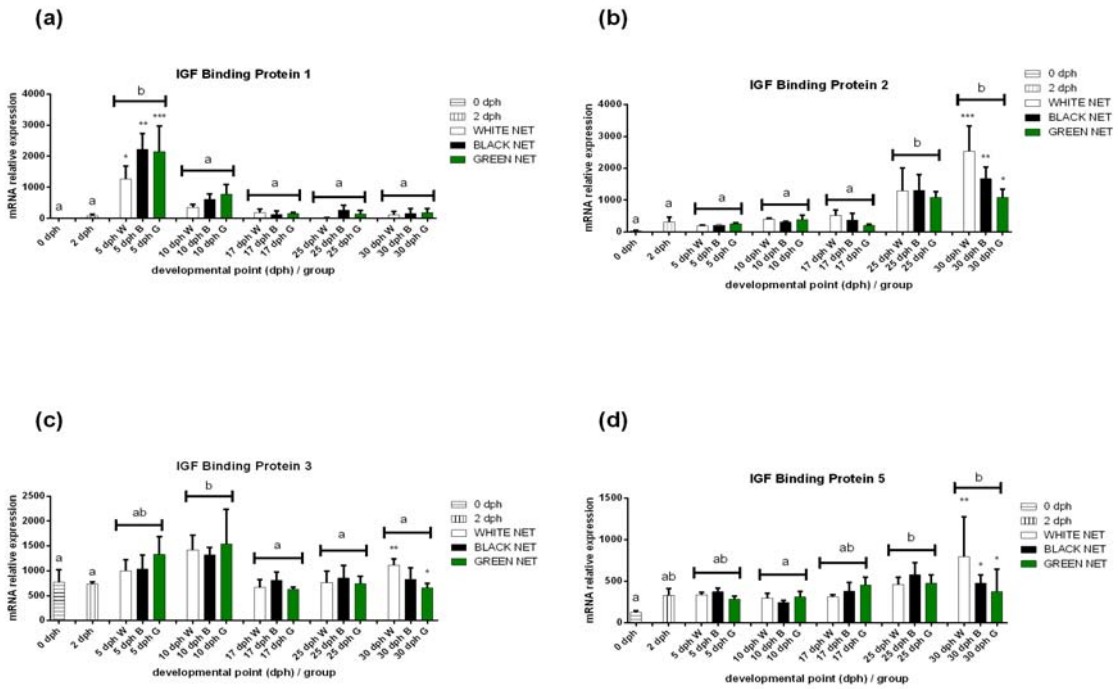


Figure 34. mRNA relative expression levels of IGFBPs between the different background colors during early ontogeny of greater amberjack: (a) IGF-BP1; (b) IGF-BP2; (c) IGF-BP3; (d) IGF-BP5. Values are means ± standard deviation (n = 5). Different letters indicate statistically significant differences between the sampling points during ontogeny whereas asterisks indicate differences between the different background colors (P<0.05).

The analysis of the 2016 results showed that in general the results were comparable to those of 2015, with the fish reared in the white background showing increased levels of the genes implicated in the growth axis system compared to the fish reared in the black and green backgrounds. In particular, IGF-I showed generally higher levels of expression as development proceeds and also it appeared affected by the

background color as higher levels were observed in fish reared in the white background at 17 dph and 30 dph compared to fish reared in the black and green backgrounds (P<0.05; **Fig. 35**).

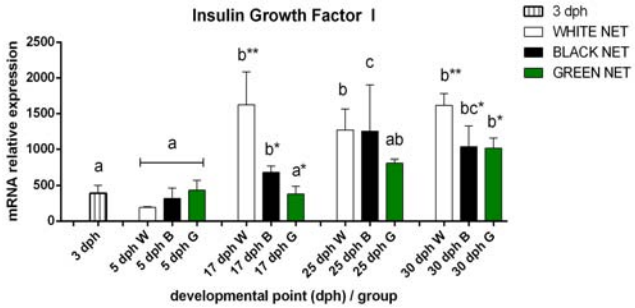


Figure 35. mRNA relative expression levels of IGF-I between the different background colors during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 4). Means with different letters indicate differences between the different developmental points whereas asterisks indicate differences between the background colors (P<0.05).

IGF-II did not show a particular pattern during development. However its expression appeared upregulated in a statistically significant way at 17 dph in fish reared in the white background compared to fish reared in the black and green backgrounds (P<0.05; **Fig. 36**).

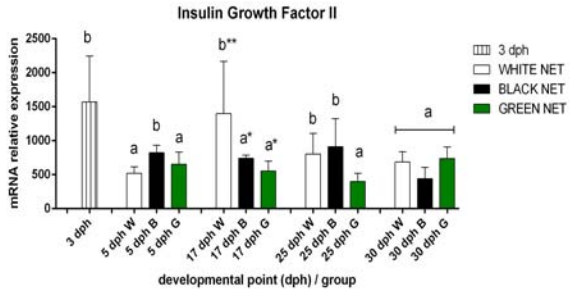


Figure 36. mRNA relative expression levels of IGF-II between the different background colors during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 4). Means with different letters indicate differences between the different developmental points whereas asterisks indicate differences between the background colours (P<0.05).

GH expression levels showed no differences throughout development but they were affected by the color of the tank as at 17 dph fish in the white background showed higher levels compared to the fish reared in the green background (**Fig. 37**).

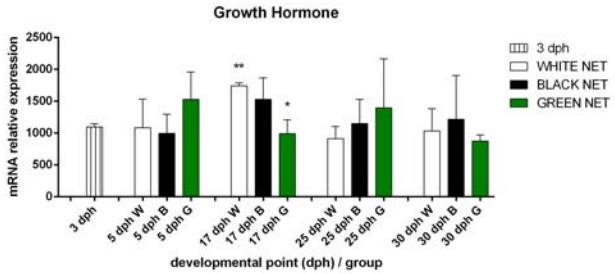


Figure 37. mRNA relative expression levels of GH between the different background colors during early ontogeny of *greater amberjack*. Values are means ± standard deviation (n = 4). Asterisks indicate differences between the background colors (P<0.05).

GHRH expression levels remained low and stable throughout development in fish reared in the green background whereas in fish reared in the white background there was a statistically significant upregulation at 30 dph and for fish reared in the black background at 25 dph (P<0.05; **Fig. 38**). Additionally, background color affected the mRNA levels of GHRH with higher levels for the white background at 17dph, 25 dph and 30 dph (P<0.05; **Fig. 38**).

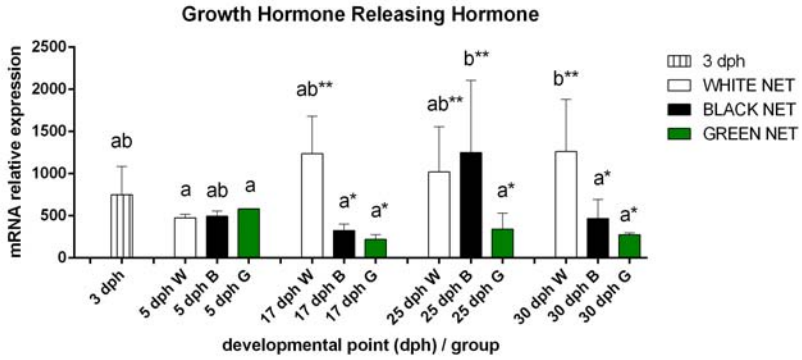


Figure 38. mRNA relative expression levels of GHRH between the different background colors during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 4). Means with different letters indicate differences between the different developmental points whereas asterisks indicate differences between the background colors (P<0.05).

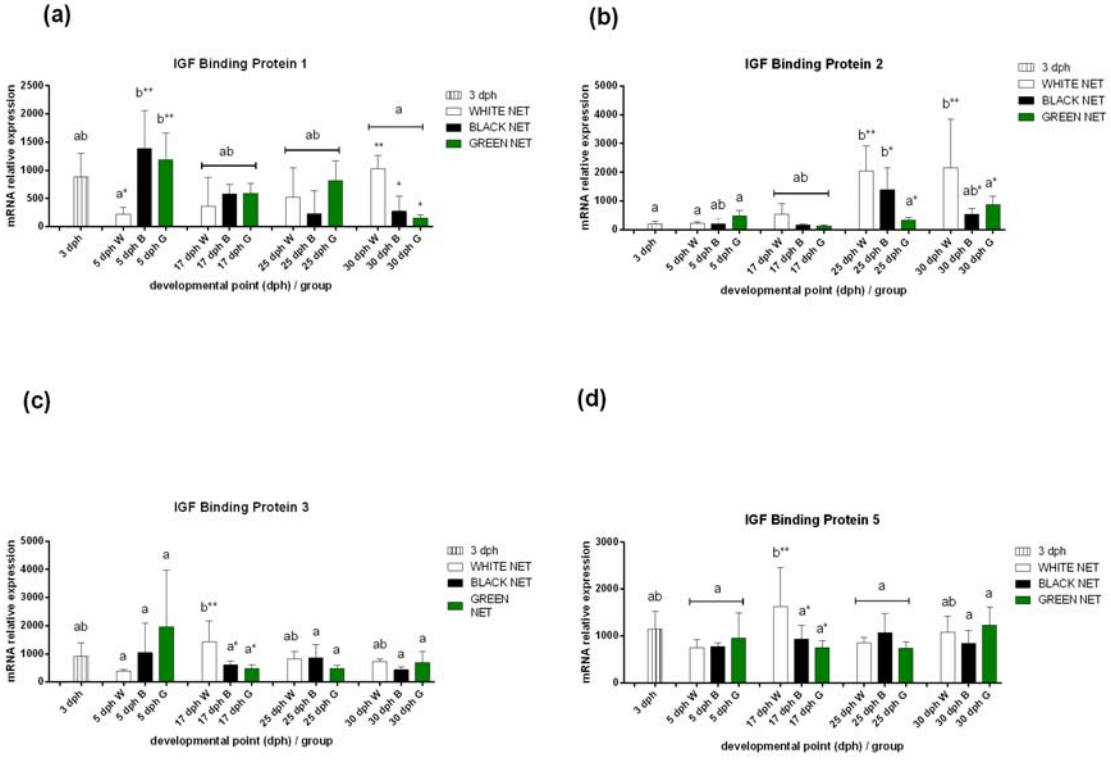


Figure 39. mRNA relative expression levels of IGFBPs 1, 2, 3 & 5 between the different background colors during early ontogeny of greater amberjack. Values are means ± standard deviation (n = 4). Means with different letters indicate differences between the different developmental points whereas asterisks indicate differences between the background colors (P<0.05).

IFG-BP1 expression remained stable throughout development with the exception of 5 dph, when a statistically significant upregulation was observed in fish reared in the green and black backgrounds with these levels being higher than the levels observed in fish reared in the white background **(Fig. 39a**). Expression levels of IGF-BP2 showed no differences throughout development for fish reared in the green background. However, at 25 dph for fish in the other two background colors, there was a statistically significant increase, which remained high until 30 dph only for fish in the white background (**Fig. 39b**). Finally, statistically significant differences in the mRNA expression levels were observed also in the cases of IGF-BP3 and IGF-BP5 where fish in the white background exhibited higher levels at 17 dph compared to the other two backgrounds (**Fig. 39c-d**).

Discussion

In this study on the effect of tank hydrodynamics on larval performance higher survival at the end of the experiment was shown in 2,000 l tanks, independent of egg stocking density, compared to the 40,000 l mesocosm tanks, which was also true for growth, measured in total length and body weight. This was particularly apparent in 2,000 l tanks stocked with 10 eggs l^{-1}. In meagre, which is a rapid growing species, the best growth rate was found at low density (Roo *et al*., 2010). The histological study showed regular hepatocyte morphology with few cytoplasmic lipid vacuoles in the mesocosm system, denoting better digestion and absorption of dietary lipids. On the other hand, the 2,000 l tanks with an egg density of 20 eggs l^{-1} had a higher degree of vacuolization , which denotes poor digestion and absorption of dietary lipids, a fact that could be due to the stress of high density, as has been also observed in seabream and meagre larvae

(Salhi *et al*., 1999; Rámirez-Bolaños, 2016).The results of the deformity evaluation showed a markedly appearance of different types of skeletal anomalies which could lead to lower survival, such as lordosis, vertebral body fusion, and anomalous dentary, in all treatments throughout the larval stages,. In conclusion and according to these results, the adequate rearing density of greater amberjack is 10 eggs l^{-1} in 2,000 l tanks.

This study did not reveal any significant effect of background color on larval growth (total length and body weight). In the 2015 trials when survival was generally low in all tested conditions, there was an indication that other factors (such as initial quality of eggs and nutrition) should be also considered. Fish reared in the tanks with the white background exhibited the highest survival rates in contrast to the groups reared with green background. During the 2016 trials however, all tested conditions presented a better performance, suggesting a higher quality of the gametes used. The difference between the white background and all the other conditions persisted and it was more apparent. The beneficial effect of the brighter environment on the performance of the greater amberjack may reflect requirements related to the pelagic nature of the species. Larvae of greater amberjack are found in nature in open seas sometimes associated with floating objects such as medusas etc. (personal observations of N. Papandroulakis and of the Gulf of Mexico Fisheries Management Council, GMFMC, 2004). The light conditions there are characterized by high intensities and the associated transparency of the water, which is difficult to mimic in rearing conditions. The use of white background and the addition of a submerged light changed the rearing environment allowing the better adaptation of the larvae to the rearing conditions.
Additionally, we carried out mRNA expression analysis of GH, GHRH, IGF-I & IGF-II and IGF-BPs during the early ontogeny of greater amberjack. During development, IGF-BP1 and GH expression appeared to be affected by the background color of the tank, with lower expression levels of IGF-BP1 at 5 dph and higher levels of GH at 17 dph (flexion) in the white group compared to the other two (data not shown). Additionally, at 30 dph higher mRNA expression levels of several genes (IGF-I, IGF-II, IGF-BP2, IGF-BP3 and IGF-BP5) were observed in the larvae reared in tanks with white background compared to the other two backgrounds, particularly the green color, which coincides with the differences observed in the survival rates. This preliminary study provides for the first time information on the regulation of the various components of the IGF signaling pathway in greater amberjack and may serve for the better understanding of the complex relationship between background color and fish performance during early ontogeny.
The present results from the trials with the modified "light environment" of the tanks improved survival an order of magnitude from previous trials, reinforcing the validity of the tested hypothesis and indicating a clear technological step forward in the larval rearing of the greater amberjack.

Deviations: There were no deviations from the DOW, except the delay in submitting the deliverable, which was due to technical problems with the experiments**.**

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