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The effects of light, darkness and intermittent feeding on the growth and survival of reared Atlantic bonito and Atlantic bluefin tuna larvae

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ABSTRACT

In larval culture, long light photoperiod regimes are used to maximize ingestion rates by increasing the accessibility to prey and therefore enhancing larval growth. Intermittent feeding could provide a viable alternative to the commonly used continuous feeding regimes that aim to improve larval growth and survival. In this study, we investigate the effect of alternating light/darkness regimes with intermittent feeding on the growth and survival of piscivorous larvae of two Scombrid species: Atlantic bonito, *Sarda sarda* (Bloch, 1793) and Atlantic bluefin tuna *Thunnus thynnus* (Linnaeus, 1758). First we tested if the manipulation of a light regime generated intermittent feeding by analyzing the larval stomach content. Then, we conducted two laboratory experiments to identify the best alternating light regime that maximized larval growth and survival by comparing the results to those obtained using continuous light regimes. The manipulation of light was optimized to provide intermittent feeding opportunities for the larvae, since we discovered a clear interruption of feeding in darkness. An increase in specific ingestion throughout the day was observed in all experiments, reaching a maximum peak late in the day. Bluefin tuna larval growth rates were similar despite different alternating conditions whereas the bonito larvae grew best when provided with light at three hour intervals. In both species, growth under alternating light conditions was similar to the 15 hours continuous light treatment. No differences between the alternating and the continuous light treatments were observed in terms of their survival. Our results suggest that alternating light/feeding periods may have a beneficial effect on ingestion rates; possibly because feeding is less satiation-limited, metabolic costs are lower; or food digestion is more efficient under these conditions. Changes in the light regime, that result in pulse feeding, can thus be an optimal strategy to increase growth at no apparent survival cost in bonito or bluefin tuna larval cultures.

1. Introduction

In larviculture, the effect of different light regimes has been studied with the main objective of improving the growth and survival of fish larvae to enhance mass production (*e.g.* Duray and Kohno, 1988; Puvanendran and Brown, 2002; Stuart and Drawbridge, 2012). Most fish larvae are visual feeders: dependent on light to increase their feeding incidence (Hunter, 1980). For this reason, it is common to extend the duration of day light in intensive culture to maximize ingestion rates, along with providing constant high concentrations of prey during these light periods. However, providing food may represent one of the highest economic costs of farming fish and one of the principal factors deciding the profitability of intensive fish farming. Therefore, an appropriate feeding and photoperiod schedule is important to guarantee the most efficient production.

When the results of different photoperiods have been compared in terms of growth in length and weight of larvae, the continuous 24 h light or the extended 18 h light and 6 h darkness have been the most beneficial (Hart et al., 1996; Puvanendran and Brown, 2002; Shi et al., 2010). However, even when survival has positively attributed to growth rate (Duray and Kohno, 1988; Partridge et al., 2011; Shi et al., 2010), the effect of long light regimes on larval survival is uncertain, and there are situations in which no differences were found in growth or survival when comparing long and short light regimes (Cañavate et al., 2006; Fielder et al., 2002; Hart et al., 1996). In some cases, negative effects have even been discovered due to the damage of the metamorphic

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process generating abnormal development (Cañavate et al., 2006) or manifesting in increasingly aggressive and cannibalistic behaviour (Vallés and Estévez, 2013).

In order to improve larval growth and survival, intermittent feeding could be an alternative to the commonly used continuous feeding regimes. As witnessed in previous laboratory studies, intermittent feeding could enhance the growth or survival of fish larvae compared to constantly fed larvae under long light regimes, probably due to the increase of assimilation efficiencies (Brown et al., 1997; Rabe and Brown, 2000). In the sea, fish larvae may not necessarily feed constantly during daylight hours if the food is spatially distributed in patches (Owen, 1989) and may need to overcome periods during the day without food.

Atlantic bluefin tuna (*Thunnus thynnus*) is a large-sized pelagic predator that reproduces in the Mediterranean Sea and the Gulf of Mexico. It is considered a new candidate for aquaculture due to the increase of its global demand that has caused an overexploitation of the wild population (Ottolenghi, 2008). Atlantic bonito (*Sarda sarda*) is a medium-sized pelagic predator that only reproduces in the Mediterranean Sea. The success of the completion of its life cycle in captivity (Ortega et al., 2013), its rapid growth which can reach 1 kg in just a few months (Santamaria et al., 2005) and its capacity to reproduce in the first year of life (Rey et al., 1984), makes bonito an accessible and good model species to improve culture techniques.

The complexity in scombrid larval rearing makes it difficult to mass produce both species (Masuma et al., 2011; Sawada et al., 2005). Our knowledge of the processes that improve larval survival under controlled conditions is very limited and protocols have not yet been described in detail. The larvae of both species are characterized by turning piscivorous after an initial phase of planktivory. Their high growth potential generates a precocious development of the jaw and the digestive system earlier than larvae of other fish species, which allows for this early piscivory behaviour (Kaji et al., 1996, 2002). The development of the digestive system, in general, occurs after the flexion phase of the larvae (Kaji et al., 1996, 1999), and in bonito, this stage occurs earlier than in bluefin tuna (Ortega and De la Gándara, 2009). The transition from their planktivorous to piscivorous diet is critical for the growth and survival of the larvae of both species, as shown in laboratory experiments (Reglero et al., 2014). Besides, since bluefin tuna larvae inhabit oligotrophic environments, their switch to piscivory behaviour may be a key step to sustain the feeding requirements in wild populations (Reglero et al., 2011).

The aim of this work is to investigate the effect alternative feeding regimes have on the growth and survival of bonito and bluefin tuna larvae during the piscivorous feeding phase, one of the most critical and least studied larval stages. We propose that intermittent feeding can be manipulated by alternating the light and darkness regimes due to the visual feeding behaviour of the larvae. With that aim in mind, we conducted laboratory experiments to test if the manipulation of light regimes induced intermittent feeding behaviour by looking into larval stomach contents. Furthermore, we studied the effect of continuous and various alternating light/feeding regimes on larval growth and survival. The results improved our understanding of the feeding dynamics of the two scombrids with the potential of applying it to the mass production of juveniles.

2. Materials and methods

Fertilized eggs of two cohorts of bonito (*Sarda sarda*) and three cohorts of bluefin tuna (*Thunnus thynnus*) were collected from naturally-spawning captive adults (De la Gándara et al., 2011; Ortega et al., 2013). Three experiments conducted in 150 L cylindrical tanks were initiated when the larvae were in the post-flexion stage, being able to develop piscivory (7–8 mm SL and 8 days post hatch (dph) for bonito and 9–10 mm and 21 dph for bluefin tuna). Previously, bonito larvae were reared in 5000 L and bluefin tuna larva in 1500 and 5000 L tanks, both with a photoperiod set at 15L: 9D, light intensity of 500 lx, and a planktivorous *ad libitum* diet of enriched rotifers (*Brachionus plicatilis species complex*) (from 2 dph for bonito and 3 dph for bluefin tuna) and enriched *Artemia* nauplia (*Artemia salina)* (from 6 dph for bonito and 14 dph for bluefin tuna) (see Reglero et al., 2014 for details). Twice a day the number of prey in the tanks was counted taking three water subsamples to ensure the prey remained in the tank at any time. New prey was added at 11:00 and 17:00 h to maintain concentrations of 10 rotifers ml^{-1} and 0.5 *Artemia* ml^{-1} constant throughout rearing and the experiments. Bonito and bluefin tuna larvae were also fed *ad libitum* with sea bream (*Sparus aurata*) yolk-sac larvae of 1–2 dph (3.4 ± 0.04 mm), providing up to 300 prey per individual twice daily from 1 to 2 days prior to the onset and throughout the experiments. All the larvae were moved to the 150 L tanks one day prior to the onset of the experiments for acclimatization. The light regime was manipulated by covering the 150 L tanks with opaque lids that were periodically slowly removed to match light regimes. The larval behaviour was visually checked for several minutes after every light regime change to ensure no mortality due to collisions of the larvae with the walls while removing the lids. Periodical observations of the larvae in the tanks during the course of the day, ensured the lack of cannibalistic behaviour during the experiment. Larvae from different cohorts were used in each experiment in order to work with similarly-sized and aged larvae (Fig. 1).

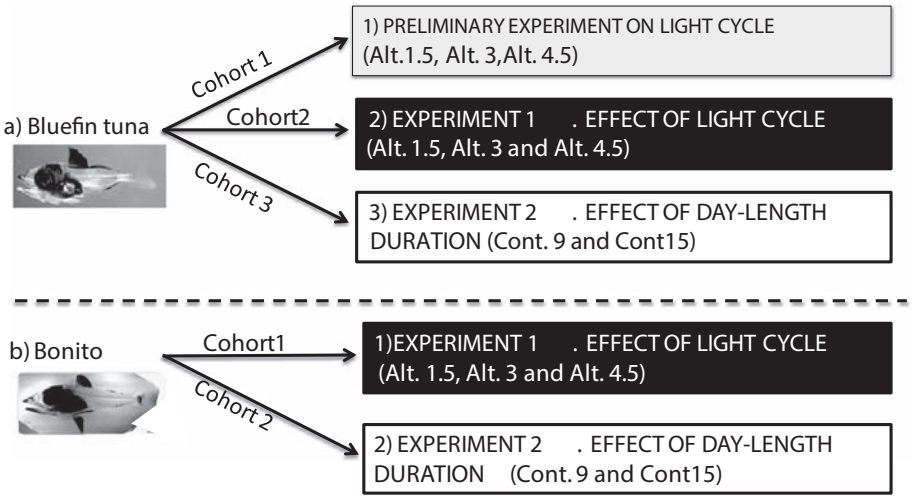

Fig. 1. Conceptual "time-line" of performed experiments for each species. Three cohorts of bluefin tuna and two cohorts of bonito were used. Cohort 1 of bluefin tuna was used for the preliminary experiment in light cycle was performed. Exp. 1 and Exp. 2 were conducted using the second and third cohort of bluefin tuna larvae. In bonito, Cohort 1 was first used for Exp. 1 and later, Cohort 2 was used for Exp. 2. The larval size range used in each experiment was similar.

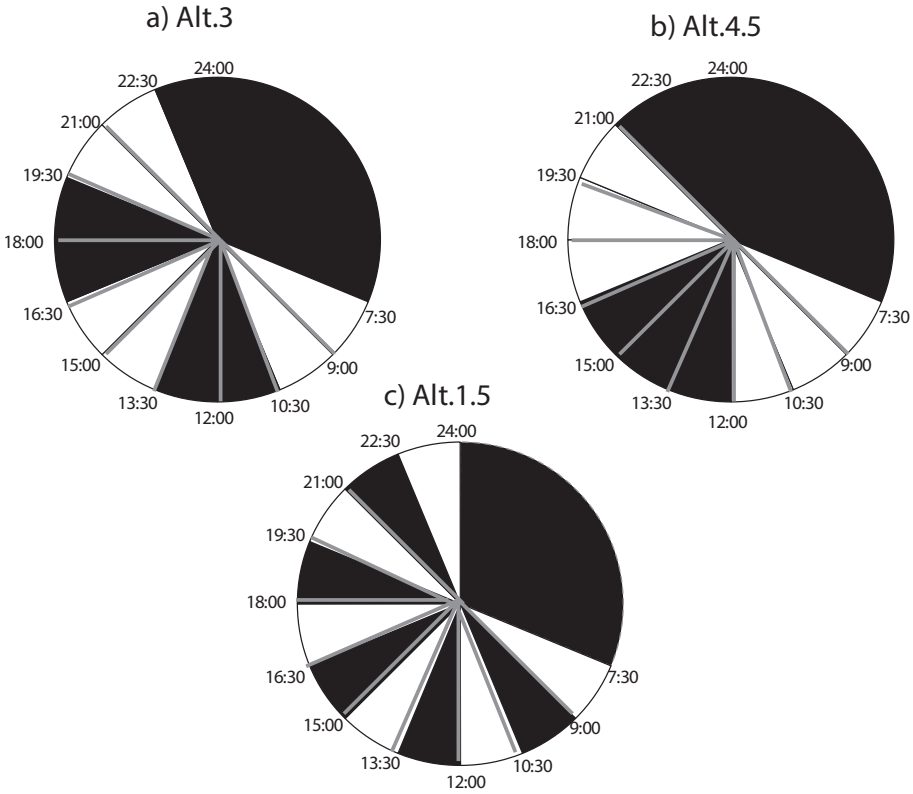

Fig. 2. Bluefin tuna samplings times (grey discontinuous lines) for the stomach content analyses are shown over the 24 h cycle for the three different alternating light treatment, a) Alt. 3 b) Alt. 4.5 and c) Alt. 1.5. White color indicates the light period and black color the darkness period. Every 1.5 h 3 larvae were sampled for stomach content analysis.

2.1. Preliminary experiment on light cycle

We conducted three experiments, each of one day duration, to test if the manipulation of the photoperiod resulted in pulse feeding. Each consecutive day (from 23 to 25 dph) around 30 bluefin tuna larvae were transferred from a 5000 L tank to one experimental 150 L tank at 25 °C (no replication). One day after the first transfer, the photoperiod was manipulated every 3 h (alternating 3 h of light and 3 h of darkness to complete a total of 9 h of light and 15 h of darkness (Alt. 3) (Fig.2a)). The day after the second transfer, the photoperiod was manipulated every 4 and a half hours (alternating 4.5 h of light and 4.5 h of darkness to complete a total of 9 h of light and 15 h of darkness (Alt. 4.5) (Fig. 2b)). The day after the third transfer, the photoperiod was manipulated every hour and a half, (alternating 1.5 h of light and 1.5 h of darkness to complete a total of 9 h of light and 15 h of darkness (Alt. 1.5) (Fig.2c)). Prior to the onset of the experiment, all test groups were maintained in darkness from around 24:00 until 7:30 when light was introduced. In the three alternating experiments, photoperiod manipulation started at 7:30 in the morning when 3 larvae were sampled, photographed and stored in formaldehyde (3%) every 1.5 h until 21:00 (Fig. 2). Afterwards, each larva was wet weighed, the stomach excised and the content counted and weighed for further stomach content ratio analysis. The initial average size of the larvae at the beginning of the experiments was statistically similar among tanks. We used larvae sizes between 8 and 15 mm lengths, representative of the larvae used in the next experiments.

2.2. The effect of light cycle (Exp. 1)

In Exp. 1, the effect of three alternating light treatments on the growth and survival of bonito and bluefin tuna larvae was tested (Table 1). These alternating light treatments were the same as those explained in section 2.1: Alt. 1.5, Alt. 3 and Alt. 4.5. Each treatment had three tank replicates. In both species, 63 larvae were added into each of the nine 150 L tanks and the experiment terminated 6 days after. The experiment was conducted at an average temperature of 24.7 ± 0.4 °C for bonito and 25.3 ± 0.7 °C for bluefin tuna (see Table 1 for further details).

2.3. The effect of the day-length duration (Exp. 2)

The aim in Exp. 2 was to test the effect of day light duration on the

Table 1
Bonito and bluefin tuna average survival in % (± SD) for different treatments (Alt 1.5, Alt 3, Alt 4.5, Cont. 9 and Cont. 15) estimated at the end of Exp. 1 and Exp. 2. Number of replicates per treatment is three. All treatments had 9 h of daylight per 24 h except Cont. 15 which had 15 light hours. Temperature, initial number of larvae and larval age at the end of the experiments, is indicated for each species.

Species	Final survival (%) in experimental groups					Temp	Larvae	Age
	Exp. 1			Exp. 2				
	Alt. 1.5	Alt. 3	Alt. 4.5	Cont. 9	Cont. 15	(°C)	(n)	(DPH)
Bonito	65.6 (± 0.7)	57.4 (± 3.2)	63.9 (± 8.2)	60.4 (± 12.4)	47.5 (± 3.2)	25	63	15
Bluefin tuna	25.0 (± 7.7)	32.8 (± 0.7)	32.9 (± 6.9)	32.8 (± 10.0)	21.7 (± 9.2)	25	63	28

growth and survival of piscivorous bonito and bluefin tuna larvae. We considered two light treatments: i) continuous 9 h of light followed by continuous 15 h of darkness (Cont. 9), and ii) a continuous 15 h of light followed by a continuous 9 h of darkness (Cont. 15) (see Table 1 for details). Each treatment was conducted in three tank replicates. 63 larvae of bonito were added into each six 150 L tanks and kept at an average temperature of 24.7 ± 0.4 °C. The bluefin tuna experiment was conducted at one temperature: 25.3 ± 0.7 °C when 63 larvae were added into each of the 6 tanks (see Table 1 for further details). Both experiments in bluefin tuna and bonito ended 6 days later.

2.4. Larval sampling in Exp. 1 and Exp. 2

Early in the morning, in darkness, 3–4 larvae per tank were sampled as the starting point for the first day of experimentation. All survivors were sampled the last day. Immediately following sampling, the larvae were anesthetized using clove oil, individually photographed using an image analysis system connected to a microscope (Leica Microsystems, Inc., Bannockburn, IL), and individually frozen in vials at − 20 °C for later dry weight estimations. Once in the laboratory, the larval pictures were measured in standard length (SL) using Image-Pro Plus 6.2 software (Media Cybernetics, Bethesda, MD). The frozen larvae were rinsed with distilled water and dried in a 60 °C oven temperature for 48 h to later weigh to the nearest 0.0001 g (Seljeset et al., 2010).

2.5. Statistics

All the statistical analysis was carried out using R statistical software package (Development Core Team, 2011). Dry weight data was transformed (natural log) and survival percentage data was root-squared and arcsin transformed before the statistical analysis to normalize the distributions. Differences among replicates within each treatment were tested using one way ANOVA, and Bonferroni correction was applied to avoid type I error. Statistical differences among the various treatments for each species were first analyzed using one-way ANOVA following which the means were compared using the Tukey HSD post-hoc test. A significance level of $\alpha = 0.01$ was considered in all test. Stomach content ratio (SCR) was only calculated in the preliminary experiment for all the larvae, individually, every 1.5 h, using the following formula:

$$\text{SCR} = \text{Stomach content wet weight}/(\text{full larval wet weight} - \text{stomach content wet weight})(\mu g - \mu g) \quad (1)$$

Daily length increment data was obtained for Exp. 1 and Exp. 2 using the larvae sampled at the beginning and end of the experiment from different percentiles, obtained from the cumulative size distribution CSD, in which the sizes-at-age between repeated samplings of the same cohort can be compared in a single graph with minimal overlap and crossing of lines (Folkvord et al., 2009). We assume a static ranking of fish sizes within a cohort:

$$\text{DLI} = (SL_2 - SL_1)/(t_2 - t_1)(\text{mm/day}) \quad (2)$$

Where, *DLI* is the daily length increment of a given percentile of the population (5%, 50% and 95%) on day t_1 and t_2. Specific growth rates (SGR) were obtained in a similar manner for dry weight data in the Exp. 1 and Exp. 2 using the following formula:

$$\text{SGR} = 100 * [\text{Log}(DW_2) - \text{Log}(DW_1)/(t_2 - t_1)](\%/\text{day}) \quad (3)$$

Where, Log *DW* is the natural logarithm of the dry weight increment of a given percentile of the population (5%, 50% and 95%) on day t_1 and t_2 respectively.

3. Results

In general, there were no significant differences in the length and weight at the end of the experiments among replicates for each treatment (ANOVA, p-adj. > 0.01). Therefore replicates were combined by treatments.

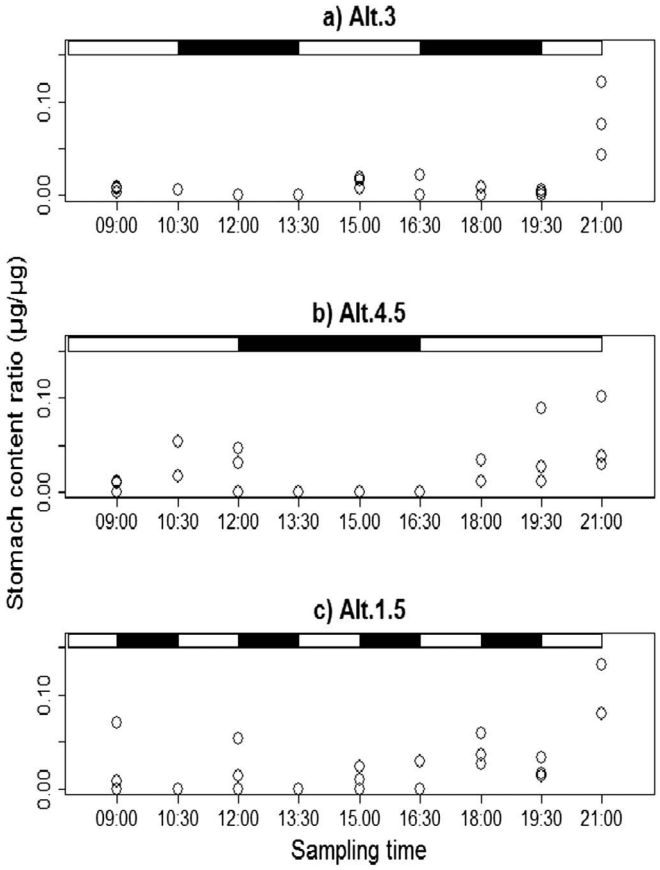

Fig. 3. Stomach content ratio (white dots) per larva at each sampling time (daytime hour of sampling in the X axis) for the three bluefin tuna larvae sampled in the a) Alt. 3, b) Alt. 4.5 and c) Alt. 1.5 treatments. On the top of the graphs, white color rectangles represent the light period, and black color represent the darkness period.

3.1. Preliminary experiment on effect of light-cycle on stomach content

There was a clear interruption of feeding in darkness as observed in the experiments of one-day duration under the three alternating light regimes (Fig. 3). A clear decrease in the stomach contents was observed 1.5 h after the start of the darkness period when most larvae had completely or almost completely emptied their stomachs. Therefore, the manipulation of the photoperiod resulted in pulse feeding. Stomach contents varied from below 2% of the total body weight in the Alt. 3 to around 2–6% in the Alt. 4.5 and 2–1 0% in the Alt. 1.5 treatment. In all three experiments an important increase in food consumption throughout the day was observed. Stomach content ratios were maintained below 0.5 before 16:30, and later increased to 0.10 at 21:00 (Fig. 3a–c). Once the effect of light manipulation on feeding was studied we conducted two laboratory experiments to identify 1) the alternating light regime (Exp. 1) and 2) the continuous light regime that resulted in the best larval growth and survival (Exp. 2).

3.2. Effect of different light-cycles on growth and survival (Exp. 1)

The average survival rate at the end of the experiments were not significantly different for the three alternating light treatments in bonito and bluefin tuna (Table 1, ANOVA p-adj. > 0.01). Bonito attained the largest body sizes under the Alt. 3 treatment (Average DW 21.23 mg), followed by Alt. 1.5 (Average DW 16.75 mg) and Alt. 4.5 (Average DW 15.91 mg) respectively. In bluefin tuna, similar final

Table 2
Daily length increments in standard length (SL, mm/day) and specific growth rates in dry weight (DW, %/day) estimated after 6 experimental days for small (5 percentile), medium (50 percentile) and large (95 percentile) bonito and bluefin tuna larvae. Table a) includes growth rates for the alternating treatments in Exp. 1 and Table b) results of the continuous treatments in Exp. 2.

a) Exp.1			Alt. 1.5			Alt. 3			Alt. 4.5		
	Growth	Species	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
	SL	Bonito	1.41	1.97	2.50	1.64	2.28	2.84	1.23	1.83	2.79
	SL	Bluefin tuna	0.68	0.96	1.98	0.70	1.06	1.69	0.46	1.10	1.55
	DW	Bonito	51.9	55.0	57.3	54.5	59.1	62.1	48.1	53.2	60.8
	DW	Bluefin tuna	31.3	32.7	40.9	29.9	34.7	36.7	22.7	33.7	35.0

b) Exp. 2			Cont. 9			Cont. 15		
	Growth	Species	Small	Medium	Large	Small	Medium	Large
	SL	Bonito	0.75	1.34	2.03	2.11	2.52	3.12
	SL	Bluefin tuna	0.34	0.62	1.41	0.93	1.31	2.01
	DW	Bonito	32.7	43.6	51.7	62.7	62.7	65.3
	DW	Bluefin tuna	17.2	25.0	32.9	36.3	39.0	40.6

weights were obtained across alternating treatments (Average DW 12.72 mg). Daily length increments (in mm day^{-1}) in bluefin tuna were almost half those observed in bonito (Table 2a). Similar results were obtained in specific growth rates (% day^{-1}) where bonito larva grew around 50–60% day^{-1}, and bluefin tuna larvae 25–35% day^{-1}, half than those of bonito (Table 2a). In all cases, DLI and SGR increase with size-at-age (Table 2a).

3.3. Effect of different day-length duration on growth and survival (Exp. 2)

No survival differences were found between the Cont. 9 and Cont. 15 treatments for the two species (Table 1, ANOVA, p-adj. > 0.01). Bonito and bluefin tuna larvae attained the largest weight at the end of the experiment in the Cont. 15 treatment (Average DW 26.25 mg for bonito and 16.16 mg for bluefin tuna) compared to the Cont. 9 treatment (Average DW 8.80 mg and average DW 7.51 mg respectively).

The specific growth rate (% day^{-1}) and daily length increment (mm day^{-1}) were twice as high in bonito compared to bluefin tuna. Also, between Cont. 15 and Cont. 9 treatments, growth rates were twice as high in Cont. 15 compared to Cont. 9 (Table 2b). The largest larvae grew the most in length and weight followed by the medium and small-sized larvae, independent of the light treatment in both species (Table 2b).

3.4. Comparisons Exp. 1 vs. Exp. 2

In bluefin tuna, the body weight and length in the Cont. 15 treatment was similar to any of the other alternating treatments (Fig. 4c–d, Table 3c–d, Tukey p-adj. > 0.01). In bonito, only the Alt. 3 treatment had similar growth to the Cont. 15 treatment (Tukey p-adj. > 0.01). Somatic growth rates under the Cont. 9 treatment were always the lowest, regardless of the group size, as observed in the cumulative-size distribution graphs (Fig. 4a–d) of length (mm) and weight (mg) (Table 3a–d). In both species, larvae under any alternating regime grew faster than those under the Cont. 9 treatment despite having the same total number of light hours (Fig. 4).

4. Discussion

Our laboratory experiments demonstrate that in piscivorous larva, alternating feeding regimes can enhance larval growth and maintain or improve survival rates, compared to continuous feeding. The analyses of stomach content revealed that the larvae only fed during the light hours and stopped feeding during darkness. Therefore, it was appropriate to use alternating light regimes to provide intermittent feeding for the larvae.

4.1. Feeding-ingestion

Piscivorous bluefin tuna larvae feed continuously in light, as observed from the co-occurrence of newly-ingested and digested larval prey in their stomachs. Stomach analyses from field-captured planktivorous tuna larvae show active feeding during day light hours in most specimens (Catalán et al., 2011; Morote et al., 2008; Uotani et al., 1990; Young and Davis, 1990). However, apart from the Scomberomorus species, for which piscivory is observed already at first-feeding (Shoji et al., 2001), continuous piscivory had never previously been tested.

During the dark phase on the one-day duration experiments, bluefin tuna larvae had completely or almost completely emptied their stomachs after 1.5 h of darkness, independent of the light regime. Such evacuation time (~1.5–2 h) is shorter than the 3–4 h reported from field observations on similar planktivores (Llopiz et al., 2010; Young and Davis, 1990). Our results may be caused by the high digestion capacity, when the stomach is completely formed (Rønnestad et al., 2013), an event that in tuna matches the start of piscivory (Yúfera et al., 2014). The larvae may also show more rapid energy absorption in the stomach and gastric evacuation when feeding on yolk-sac fish larvae than when feeding on other planktonic prey. More than that, the digestibility of sea bream yolk-sac larvae should be higher than the digestibility of *Artemia*, as it was suggested by Seoka et al. (2007).

The continuous feeding behaviour and the increase of ingested prey throughout the day may have been caused by the lack of satiation-regulating hormones in the larval stage, and an endocrine system that is not completely functional (Rønnestad et al., 2013). In scombrid larvae fed *ad libitum*, the limit for maximum food ingestion may be determined by the maximum expansion of the stomach cavity and the sum of the cost of prey capture, ingestion, digestion and assimilation, rather than satiation.

The increase of prey in the stomach cavity during light time, from the morning to late in the day, may be explained by an improvement in the skills of this species in capturing prey within these hours, regardless of the photoperiod, or may be caused by a circadian rhythm that prevails from the previous rearing period, signaling a starvation period at night. The same trend has been reported in the stomach content of laboratory reared Japanese Spanish mackerel (*Scomberomorus niphonius*) (Shoji et al., 2001). Stomach content ratios were lower in the Alt.

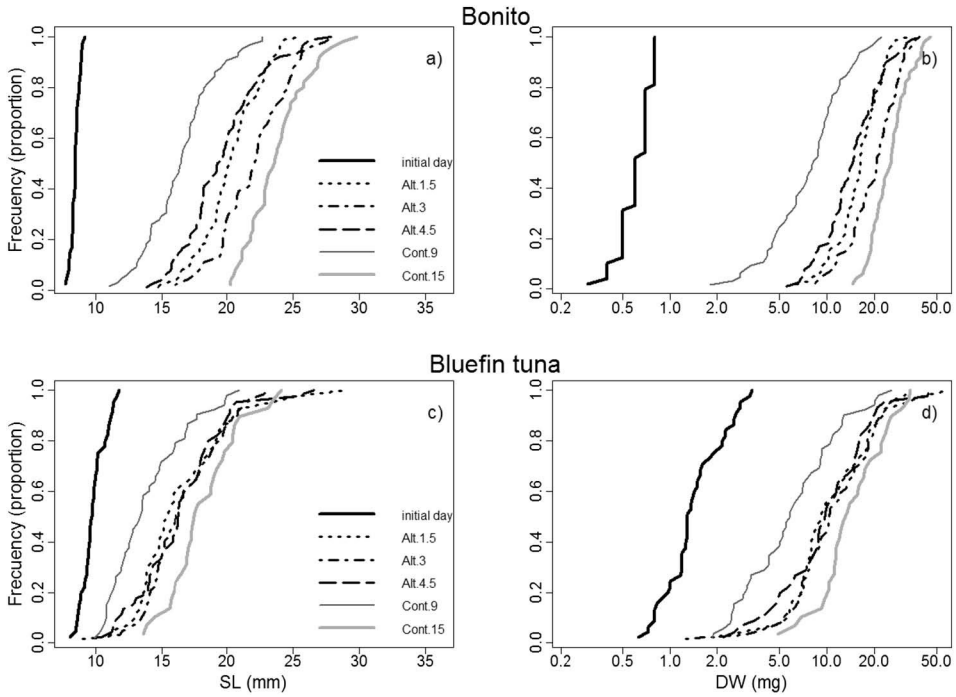

Fig. 4. Cumulative size distributions on standard length (SL, mm) and dry weight (DW, mg) from Exp. 1. and Exp. 2 for a) bonito SL, b) bonito DW, c) bluefin tuna SL and d) bluefin tuna DW. Cumulative size distributions at the end of the experiment are shown for the different treatments: Alt. 1.5 (…), Alt. 3 (- - -), Alt. 4.5 (.-.-.) Cont. 9 (–) and Cont. 15 (-). Note: the x-axis is log transformed for DW in panels b) and d).

Table 3
Tukey HSD post-hoc results comparing larval weight and length (DW, SL) at the end of the experiments among all treatments for bonito (top) and bluefin tuna (bottom). Treatments within each species and size measure with non-overlapping letters are significantly different (Tukey HSD < 0.01), with "a" assigned to the treatment with largest mean.

		DW (mg) Tukey HSD	SL (mm) Tukey HSD
Bonito	Alt. 1.5	bc	b
	Alt. 3	ab	a
	Alt. 4.5	c	b
	Cont. 9	d	c
	Cont. 15	a	a
Bluefin tuna	Alt. 1.5	a	a
	Alt. 3	a	a
	Alt. 4.5	a	a
	Cont. 9	b	b
	Cont. 15	a	a

3 experiment where the larvae were the youngest, 23 dph, compared to larvae in the Alt. 4.5 and the Alt. 1.5 experiments that were 24 and 25 dph respectively. Increasing feeding rates with age was also evident in the amount of sea bream yolk-sac prey that was necessary to add daily to the tanks in order to ensure food *ad-libitum*.

4.2. Growth-survival

The positive effect of increasing the number of continuous light hours on growth has been extensively recorded in numerous fish larval species and stages (*e.g.* Fielder et al., 2002; Hart et al., 1996; Puvanendran and Brown, 2002) including two scombrids, yellowfin tuna (*Thunnus albacares*) (Papandroulakis et al., 2010; Partridge et al., 2011) and bluefin tuna (*Thunnus thynnus*) (Ortega et al., 2014). Growth enhancement has been related to longer foraging times and subsequently greater food intake (Ortega et al., 2014; Partridge et al., 2011). In this study, best growths rates have been obtained in bonito and bluefin tuna larvae under the Cont. 15 treatment closely followed by larvae in the alternating treatments.

Resting periods during darkness may enhance growth in the alternating light regimes. In darkness, fish larvae mostly use their energy source to grow, digest and absorb food, instead of swimming, as when they are in a light environment. Bonito and bluefin tuna larvae grew larger in size, from the alternating light treatments (Alt. 1.5, Alt. 3 and Alt. 4.5) than those from the Cont. 9 treatment despite the fact that all were exposed to 9 h of daily light feeding hours. In both species, growth under alternating treatments was always similar to the Cont. 15. However, larval behaviour in darkness needs to be recorded to document resting behaviour. The increase of the expression of the growth hormone during darkness (Adachi et al., 2008), may stimulate the appetite (Johnsson and Björnsson, 1994) and promote lipid mobilization and protein deposition that might be reflected in an increase in somatic growth (Björnsson et al., 2002). Further biochemical analyses are needed to verify if digestion efficiencies and energy utilization are the main reasons for these differences.

Our survival data show no significant differences across the five different alternating/continuous regimes for each species. This result may be caused by the high fluctuations in survival rates between tanks. Therefore, even though they are not significant (ANOVA p-adj. $>$ 0.01), survival seems to be affected by the light regimes, as it was lower under Cont. 15 than under Cont. 9 treatment, both for bonito and bluefin tuna,. These differences might decrease if looking into survival results at a specific size instead of specific age. The high survival results obtained for bonito are in accordance with previous results (Reglero et al., 2014) but in bluefin tuna, we obtained slightly lower survival rates than in previous piscivorous studies (Reglero et al., 2014; Seoka et al., 2007). Larval stress may be diminished by slowly removing/covering the lids of the tanks to generate gradual light attenuation. The sudden on/off of the lights produce a short excitement in the larvae which could increase collision to the tank walls, though this was not considered the case in our experiments. Our survival data have not been affected by cannibalism. We were able to overcome cannibalism by using homogenous size batches (or by diminishing variability in size), planning experiments of short duration (1 and 6 days) and by feeding with enough amount of sea bream yolk-sac larvae (Masuma et al., 2011; Sawada et al., 2005).

Alternating light regimes may be a good alternative in intensive cultures; reducing rearing cost due to the reduction of the timing of feeding and lighting, especially when rearing piscivorous species whose diet in that critical developmental stage is not easy to obtain due a dependency on available fish larvae (Sawada et al., 2005). However, specific optimum light regimes may be species-stage dependent. Manipulation of the photoperiod can affect feed-conversion ratios or efficiencies, demonstrating the importance of investigating the effect of photoperiod on feeding efficiency (Rabe and Brown, 2000). The effect of endocrine factors on the appetite and growth of fish larvae should also be studied. Our optimal growth results support the idea that larvae do not need to feed constantly to maximize growth rates. The option to feed at certain time intervals may prevent them from becoming satiated and then increase their total ingestion, per unit time of active foraging. This leads to more efficient use of the available food. In addition, our results suggest that larvae may survive short periods of starvation in the field if food is distributed in patches. Our experimental food-deprived larvae could reduce their swimming in darkness, which also could improve the growth rate. However, in the field they may continue swimming due to the daylight present. These differences in energy expenditure should be considered in further research before applying the results to the field.

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