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Size-selective mortality of laboratory-reared Atlantic bluefin tuna larvae: evidence from microstructure analysis of otoliths during the piscivorous phase

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ABSTRACT

Atlantic bluefin tuna (*Thunnus thynnus*) larvae show strong piscivorous feeding behavior at the very early larval stage and this enables them to grow at high rates. We conducted a laboratory experiment in which Atlantic bluefin tuna larvae were offered larval prey for the first time at different ages to simulate the early onset of piscivory at three treatments: yolk-sac larvae (YSL), delayed onset of piscivory (DYSL) and a solely planktivorous diet (Rotifers). The otolith microstructure was then used to compare the larval size distribution at the onset of the experiment with the estimated previous size-at-age of the survivors at the end of the experiment by back-calculation. Within a cohort, our results show size-selective mortality of the largest larvae independent of the differences in the timing of onset of piscivory and differences in growth patterns. The results also corroborate the rapid response of Atlantic bluefin tuna to piscivory in terms of growth reflected in the otolith increment widths. Being bigger did not infer a survival advantage and mortality rates did not decline with increasing larval size. Smaller size at a given age could under certain conditions and stages of development confer a survival advantage of individual members of a larval cohort when suitable small-sized prey is available.

1. Introduction

The highest mortality during the life of a fish occurs during the larval stage (Houde, 2008). The transition period from endogenous feeding, when energy is available in the yolk-sac, to exogenous feeding, when the larva is typically dependent on available planktonic food resources, is considered a "critical period" (Hjort, 1914). However, in large pelagic species such as bluefin tuna, as in other Scombridae species, piscivory is also observed during the larval stage (Kaji et al., 2002; Sawada et al., 2005). This second dietary transition, from planktivory to piscivory feeding, might represent another major bottleneck in larval survival (Reglero et al., 2014a). Mortality during the larval stage has been suggested to be strongly size-dependent (McGurk, 1986) and large size and fast-growing larvae are typically considered to have higher survival rates (*e.g.*Anderson, 1988; Takasuka et al., 2003, 2004) although the opposite is also true (Meekan and Fortier, 1996). Piscivorous and cannibalistic behaviors can increase size differences dramatically among individuals of the same cohort (Hecht and Pienaar, 1993). Our knowledge is scarce regarding how mortality affects the size spectrum of the larvae that survive through piscivory and become part of the juvenile population.

Atlantic bluefin tuna (*Thunnus thynnus*) is a scombrid with fast-growing warm water larvae that exhibit most of the juvenile anatomical characteristics at sizes around 17 mm when reared under *ad libitum* feeding conditions (21 days post hatch at 23–24 °C, Yúfera et al., 2014). Evidence for the onset of piscivorous behavior in Atlantic and Pacific bluefin tuna (*Thunnus orientalis*) has been observed in the laboratory at larval lengths between 7 and 9 mm (Reglero et al., 2014a; Tanaka et al., 2010, 2014b, 2015). In contrast, there is little evidence of piscivory in the field only reported in some individuals captured in the Gulf of Mexico and recently in the Mediterranean Sea (Llopiz et al., 2015; Uriarte et al., 2017), since larvae longer than 7 mm are rarely captured during icthyoplankton surveys probably due to net avoidance (Tilley et al., 2016). Growth has been well studied in Pacific bluefin tuna and developmental variability among individuals is apparent during the planktivorous stage (Takebe et al., 2012; Tanaka et al., 2006), and

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particularly after the transition to piscivory feeding behavior (Tanaka et al., 2010, 2014a, 2014b). Since mortality during the larval stage of fish can be size dependent, empirical evidence of the survival size spectrum is particularly important in order to determine the survival strategy of the species and the contribution to the life stages that come afterwards. In Pacific bluefin tuna, large and fast-growing larvae have increased probability of survival to the post-flexion stage, according to results from repeated field-sampled otoliths of planktivorous larvae (Satoh et al., 2013), and by comparing growth trajectories of planktivorous larvae and juveniles captured in the field (Tanaka et al., 2006; Watai et al., 2017). No potential piscivorous larvae (post-flexion developmental stage) were sampled in the previously cited field studies and therefore size and growth-selective mortality during the transition to piscivorous feeding of Pacific bluefin tuna larvae remains unknown. For Atlantic bluefin tuna (ABT) larvae, no analyses on size- and growth-dependent mortality have ever been undertaken in the field.

Larval rearing in controlled conditions can provide a set-up to study how growth and survival relate to other factors in the field that can have an effect, such as predation, prey density and temperature variability. These factors can be isolated and studied where piscivorous larvae can be easily obtained. To date, only Tanaka et al. (2014a, 2017) have studied the effects of size and growth selective mortality during the piscivorous phase of Pacific bluefin tuna larvae. Their studies on hatchery-reared Pacific bluefin tuna larvae suggested that larger and faster-growing larvae could have advantages both prior to and after the onset of piscivory. However, no size and growth selective mortality patterns in ABT larvae have yet been described during either the planktivorous or piscivorous phases. The knowledge of how selective mortality can affect the size distribution of the surviving larvae during the piscivorous phase may influence processes that will affect juvenile populations of ABT due to cumulative mortality during the larval stage. In this study, we examine how manipulating the timing of onset of piscivory might affect the size-dependent mortality of a population of laboratory-reared ABT larvae based on otolith microstructure back-calculation analysis. Otolith size and increment width are representative of size and growth rates during the larval stage and can therefore serve to trace the growth history of individuals (Campana and Jones, 1992). The use of repeated samplings within the same population at an initial point and at later points in time allowed the estimation of which individuals were most likely to survive (size-selective mortality) by comparing the population characteristics at different times (Mosegaard et al., 2002).

2. Material and methods

2.1. Larval rearing

Fertilized eggs of ABT were provided by the private company Caladeros del Mediterráneo S.L. from naturally spawning captive adults maintained in sea cages. The eggs were placed directly in three 1500 L larval rearing cylindrical tanks at a density of 10 eggs per liter, where they hatched one day after transfer. During the larval rearing period, the photoperiod regime was 16 h of light and 8 h of darkness (16L:8D) and water temperature ranged between 22.4 °C and 25.7 °C. Feeding during larval rearing consisted of enriched rotifers (*Brachionus plicatilis*) supplied twice per day to keep a concentration of 10 rotifers mL^{-1} inside the tanks. This guaranteed *ad libitum* feeding conditions during daylight hours. Pseudo green water culture was obtained by adding cultivated microalgae (*Nannochoropsis gaditana*) twice per day during the first rearing days, and later by changing to a paste of concentrated *Chlorella* (Super fresh Chlorella SV-12, Chlorella industry Co., Ltd., Japan) which was added four times a day. ABT larvae were maintained in the rearing tanks for four days before the experiment began or 15 days post hatch (dph). For further details on rearing procedures, see Reglero et al. (2014a).

2.2. Experiment setup

At 15 dph or Experimental day (Eday) -4, four days before the onset of the experiment, a total of 1800 larvae were transferred to 15 experimental tanks of 150 L (120 larvae per tank) for acclimation purposes with a light regime of 15L:9D hereafter. Three times a day, enriched rotifers were added to maintain a concentration of 10 rotifers mL^{-1} for *ad libitum* feeding conditions as explained above. At 19 dph, when the larvae size was between 7 and 9 mm in standard length (SL), and anatomical characteristics enabled the larvae to start with piscivory, the experiment started (Eday 0).

In order to see the effect that different timings of onset of piscivory have on ABT size distribution, we conducted an experiment with three different feeding treatments, 5 tank replicates each. In the first treatment, referred to as Rotifers from now onwards, only enriched rotifers were provided from Eday 0 until the end of the experiment 8 days later (Eday 8). In the second treatment, referred to as DYSL (delayed yolk-sac larvae) from now onwards, after 4 days with only rotifers provided (Eday 4, 23 dph), sea bream (*Sparus aurata*) yolk-sac larvae were added *ad libitum* in the tanks along with the enriched rotifers until Eday 8. In the last treatment, referred to as YSL from now onwards, sea bream yolk-sac larvae were added *ad libitum* from Eday 0 along with enriched rotifers to Eday 8. Up to at least 300 sea bream yolk-sac larvae of 0–2 dph (3.4 ± 0.04 mm) per individual ABT were provided to ensure to remain in the tanks at any time in the corresponding treatments which is known to represent *ad libitum* conditions in this species (Ortega, 2015; Reglero et al., 2014a). The number of larval preys incorporated into the tank to ensure *ad libitum* conditions was estimated based on hourly observations to ensure there was always prey available in the tank. The experiment lasted for 8 days (from 19 dph to 27 dph). The average water temperature was 24.8 ± 0.8 °C.

On Eday 0, early in the morning and in darkness, 3 larvae per tank were sub-sampled for body size measurements at the start of the experiment. Shortly after, with light, all the remaining larvae in each tank were counted for survival estimates. On average, 25 larvae remained in each tank after acclimation on Eday 0. On Eday 8 all the surviving

Table 1
Description of the quantities, larval sizes and otoliths analyzed for the 19 days post hatch (dph) and the 27 dph sampling groups for Rotifers, delayed yolk-sac larva (DYSL) and yolk-sac larva (YSL) treatments are provided. One sagittal otolith was used for microstructure analysis however, a subset ($n = 76$) of larvae are represented by two sagittal otoliths. Final mean survival and instantaneous mortality during the acclimation time for the Experiment day (Eday) -4 to 0 and during the Eday 0 to 8 are shown. Different letters (a,b) indicate significantly different treatment means (ANOVA, Tukey HSD, $p < .05$).

Sampling	Treatment	Sampled larvae	Otoliths	Standard length (mm)	Otolith radius (μm)	Mean survival (%)	Instantaneous mortality (% day^{-1})
19 dph, Eday 0	Rotifers	15 (3 per tank)	13	8.2 ± 0.6^{a}	47.9 ± 11.0^{a}	17.5 ± 4.4^{a}	44.2 ± 0.1^{a}
	DYSL	15 (3 per tank)	15	7.9 ± 1.0^{a}	48.8 ± 15.7^{a}	19.7 ± 7.1^{a}	41.8 ± 0.1^{a}
	YSL	15 (3 per tank)	14	8.2 ± 0.8^{a}	49.9 ± 11.7^{a}	18.2 ± 6.5^{a}	43.9 ± 0.1^{a}
27 dph, Eday 8	Rotifers	19 (all survivors)	18	8.7 ± 0.6^{b}	75.5 ± 13.3^{b}	14.4 ± 11.5^{b}	23.6 ± 0.1^{a}
	DYSL	25 (all survivors)	24	10.4 ± 1.5^{b}	93.2 ± 24.0^{b}	$21.7 \pm 15.3^{a,b}$	$21.6 \pm 0.1^{a,b}$
	YSL	42 (all survivors)	40	13.0 ± 2.8^{a}	130.8 ± 40.8^{a}	39.7 ± 16.2^{a}	12.5 ± 0.0^{b}
	TOTAL	131	124				

larvae were counted and sampled for accurate survival estimates (Table 1). Daily survival estimates were not possible to obtain by collecting dead larvae from the bottom of the tanks due to their rapid degradation. The larvae were terminally anesthetized using clove oil (Guinama© Spain), individually photographed for morphometric measures (standard length) to the nearest 0.1 mm and individually frozen in cryotubes at −80 °C (see Reglero et al. (2014a) for more details). The frozen larvae were then rinsed with distilled water and dried in a 60 °C oven for 24 h, then weighed to the nearest 0.1 mg. At the end of the experiment, a total of 131 larvae had been sampled, 45 larvae from Eday 0, and 86 from Eday 8.

2.3. Otolith microstructure analysis

Both sagittal otoliths from each sampled larva were removed to assess size-selective mortality (Table 1). The otoliths were extracted using fine needles under a stereo microscope and cleaned. Immediately after, otoliths were individually placed on a glass slide and mounted in a small drop of thermoplastic cement (Buehler®) with the distal side of the sagitta facing upwards. Sagittal otoliths were chosen due to their larger size among the other otolith pairs (Itoh et al., 2000).

Otoliths were analyzed using a microscope with transmitted light at magnification of 100× and 63×. The microscope was connected to a Leica DFC450 video camera and each otolith was photographed at a resolution of 2560 × 1920 pixels. Later, several measurements were made on the otolith image using Image J® software (National Institute of Health, Bethesda, MD). Otolith radii (OR) were measured along the longest possible post-rostrum axis from the core to the outer edge, perpendicular to the widest part of the otolith (Fig. 1), following the procedure described in Folkvord et al. (2010). Increment widths were obtained as the difference between the distance from the core to two adjacent increments.

Back-calculation estimates to Eday 0 were obtained from the Eday 8 otoliths by back-calculating 8 increments from the edge along the OR. These back-calculated OR at Eday 0 were then compared to the initially sampled OR on Eday 0 to obtain estimates of size-selective mortality within each treatment group. Both sagittae were read twice, with one month between reads. In general, no significant age estimate differences have been reported between the left and right sagitta (Campana, 1990). Therefore, the otolith which was easiest to measure was selected for further microstructure analysis.

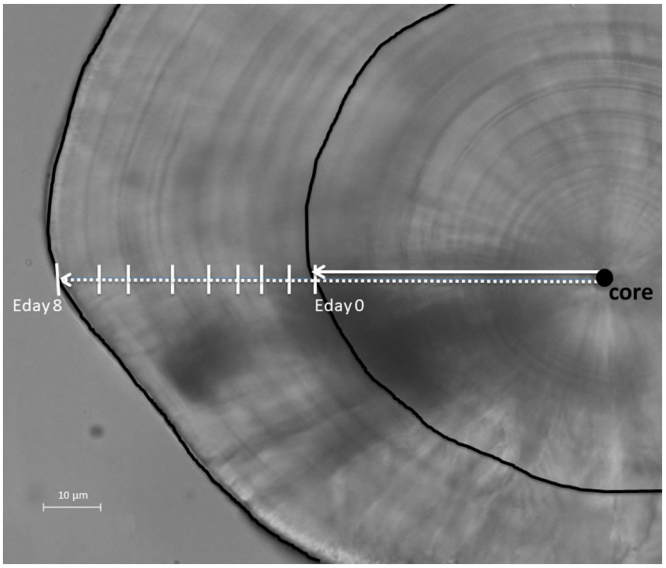

Fig. 1. Atlantic bluefin tuna sagittal otolith showing the increment measurement methodology utilized. The white dashed line represents the otolith radius-at-age for Experimental day (Eday) 8 and the white solid line represents the otolith radius at Eday 0. Black lines highlight the otolith edge on Eday 8 and the increment that represents back-calculated Eday 0. Vertical white lines along the dashed white line indicate daily increments on completed side of the darker layer. Arrows are offset for clarity (OR = 89.4 µm). The otolith core is shown and named. The image was taken using transmitted light.

2.4. Statistical analysis

All statistical analyses were carried out using the R statistical software (Development Core Team, 2011). Survival percentages were root-squared and arcsine transformed for normality. Survival during acclimation and during the experiment were compared among treatments using values from replicate tanks in a factorial one-way ANOVA. Instantaneous mortality, M, during the acclimation and the experiments was calculated using the equation:

$$M = 100 \cdot [\mathrm{Log}\,(N_1) - \mathrm{Log}\,(N_2)/(t_2 - t_1)]\ (\%\ \mathrm{day}^{-1}) \quad (1)$$

where Log N_2 is the natural logarithm of the number of surviving larvae (N) on Eday 8, and Log N_1 is the natural logarithm of the initial number of larvae on Eday 0 at time t_2 and t_1 respectively. To study the correlation between final otolith size *vs.* previous back-calculated otolith size within fish in each treatment and overall, Spearman's rank correlation coefficient (SRC) was calculated. Otolith size distributions were first analyzed for normality and homoscedasticity (Shapiro-Wilk and Levene). Statistical differences in mean larval size and otolith size-at-age between treatments was analyzed by a two-way nested ANOVA with treatment as independent fixed factor and tanks as a random factor nested in treatments. For the size-selective mortality estimation based on otolith size-at-age, a three-way nested ANOVA was used with age of sampling and treatment as independent fixed factors and tanks as a random factor nested. Differences in increments width between days were also analyzed using a three-way nested ANOVA. Significant ANOVAs were followed by a Tukey's honestly significant difference (HSD) multiple comparisons test to determine differences among treatments. All test results were considered significant at a level of 0.05.

Cumulative size distributions and specific growth rate in otolith growth were estimated as described in Folkvord et al. (2009). Assuming static ranking of fish sizes within a cohort is unlikely to change much in the short term, cumulative size distributions were used for visualizing variability of sizes within cohorts over time in a single graph. Size-at-age analysis was estimated from cross-sectional data methods by using the mean OR from two different sampling days whereas back-calculation data analysis was done by longitudinal data method with repeated measurements on the same individual (Chambers and Miller, 1995). OR of sampled larvae from Eday 0 (19 dph) of the three treatments were pooled together since the different treatments had not yet started.

Specific growth rates (SGR) in otolith growth were estimated using the following equation:

$$SGR = 100 \cdot [\mathrm{Log}\,(OR_2) - \mathrm{Log}\,(OR_1)/(t_2 - t_1)]\ (\%\ \mathrm{day}^{-1}) \quad (2)$$

where Log OR_2 is the natural logarithm of the final otolith radius (µm) on Eday 8, and Log OR_1 is the natural logarithm of the OR on Eday 0, of back-calculated Eday 0 or of actual OR observed from Eday 0. We used *OR* data corresponding to a given percentile of the population (5%, 50% and 95%) at time t_1 and t_2 respectively, obtained from the cumulative size distributions (CSD). The growth of all size classes was estimated by following the size of a given percentile of the population from one sampling to the next (Folkvord et al., 1994).

3. Results

Survival during the acclimation time, 4 days prior to the experiment, was similar among the three treatments (one-way ANOVA, $p > .05$). During the experiment, survival was significantly higher in the YSL treatment than in the Rotifers treatment (Table 1, one-way ANOVA, Tukey HSD, $p < .05$) and similar to the DYSL treatment

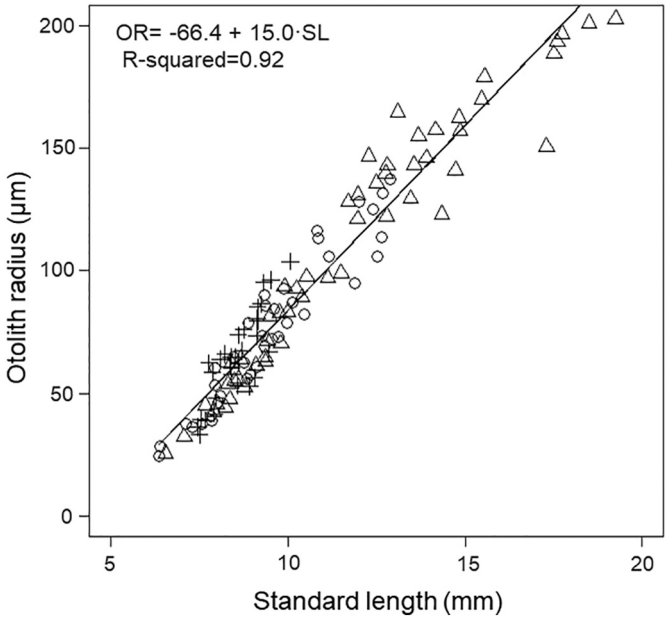

Fig. 2. The relationship between larval standard length (mm) and otolith radius (μm) for the three treatments combined: Rotifers (+), delayed yolk-sac larvae (O, DYSL) and yolk-sac larvae (Δ, YSL).

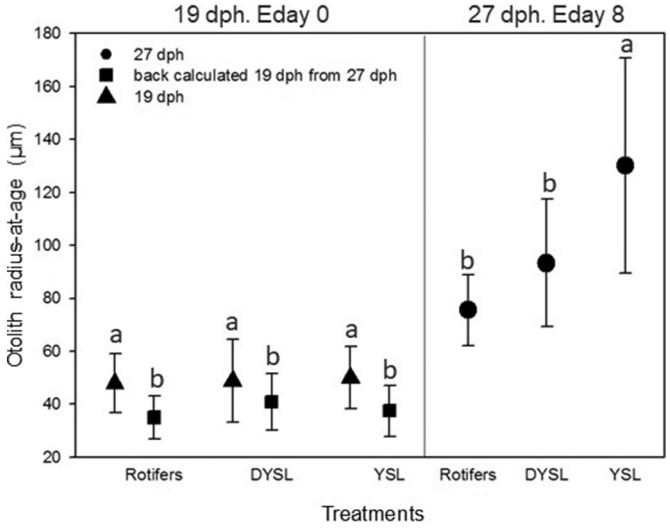

Fig. 3. Mean otolith radius from treatment groups Rotifers, delayed yolk-sac larvae (DYSL) and yolk-sac larvae (YSL) sampled at different times and showing the data corresponding to Experimental day (Eday) 0 and Eday 8. The circle and triangle symbols represent otolith total radii at 27 dph and 19 dph respectively. Rectangle symbols represent the otolith back-calculated radii to 19 dph from 27 dph sampled otoliths. On Eday 0, different letters at respective groups indicate significantly different otolith sizes-at-age of larvae sampled at different dates (three-way nested ANOVA, Tukey HSD, $p < .05$). The two different letters (a,b) indicate differences in otolith sizes among treatments within each day of the experiment (two-way nested ANOVA, Tukey HSD, $p < .05$). Whiskers indicate standard deviation.

(Table 1, one-way ANOVA, Tukey HSD, $p > .05$). Instantaneous mortality during the acclimation period was on average 43% day^{-1} and during the experiment it was on average 19% day^{-1} (Table 1). In those individuals with both sagittae available there was high correlation among OR measurements at 19 dph ($R^2 = 0.99$, $p < .001$, $n = 22$) and 27 dph larvae ($R^2 = 0.97$, $p < .001$, $n = 54$). There was a high correlation between the first and the second otolith total radius readings using all the otoliths extracted, at 19 dph ($R^2 = 0.99$, $p < .001$, $n = 64$) and 27 dph ($R^2 = 0.99$, $p < .001$, $n = 137$). The standard length of the larvae was linearly correlated to the OR (Fig. 2, $R^2 = 0.90$, $p < .001$, $n = 124$), and thus the otolith increment widths were considered proportional to fish growth. Final OR at 27 dph was positively correlated with the previous back-calculated radius within each treatment (Spearman rank correlation, Rotifers: SRC = 0.64, $p = .005$, $n = 18$, DYSL: SRC = 0.83, $p < .001$, $n = 24$, YSL: SRC = 0.80, $p < .001$, $n = 40$), as it was when treatments were pooled together (Spearman rank correlation, SRC = 0.63, $p < .005$, $n = 82$).

The average OR at the onset of the experiment at 19 dph was 48.8 ± 12.8 μm and did not differ among the three experimental treatments (Fig. 3, triangle symbols, two-way nested ANOVA, $p > .05$). The back-calculated OR at 19 dph of the surviving larvae sampled at 27 dph did not differ significantly between treatments and overall averaged 35.0 ± 8.1 μm in the Rotifers treatment, 40.9 ± 10.6 μm in the DYSL treatment and 37.5 ± 9.7 μm in the YSL treatment. They were significantly smaller than the OR measured at 19 dph at the beginning of the experiment (Fig. 3, rectangle and triangle symbols respectively, three-way nested ANOVA, Tukey HSD, $p < .05$). At 27 dph the OR of the surviving larvae were significantly larger in the YSL treatment (average 130.2 ± 40.6 μm) than those in the DYSL and Rotifers treatments (average 93.3 ± 24.0 μm and 75.6 ± 13.3 μm respectively) (Fig. 3, circle symbols, two-way nested ANOVA, Tukey HSD, $p < .05$), the latter two not being significantly different (two-way nested ANOVA, Tukey HSD, $p > .05$).

Due to the similar otolith sizes among treatments of the initial sampling population (19 dph), the otolith sizes were pooled together for the cumulative analysis. The cumulative size distributions between subsequent sampling days (19 dph and 27 dph) were almost parallel in Rotifers and DYSL while in the YSL treatment cumulative size distribution increased with the biggest individuals (Fig. 4). Similar cumulative distributions of the back-calculated data were obtained in all treatments.

The otolith-specific growth rates during the eight experimental days (Eday 0 to 8) of the surviving population based on the back-calculated data were higher than the specific growth rates obtained using initial and final sampled OR data in all the treatments and in all the population percentiles (Table 2). The growth rates of the median and large larvae obtained in all the treatments was similar, considering both with and without back-calculation. The higher growth rate of the small larvae in the Rotifer and DYSL treatments differs from the lower growth rate in the small larvae in the YSL treatment (Table 2).

The growth rate of the surviving larvae sampled at 19 dph four days prior to the experiment (Eday −4 to 0) by back-calculation of the otoliths was determined and resulted to be similar among the median and large larvae, and smaller in the small larvae (Table 2). The daily increment width increased with age (Fig. 5). Four days prior to the experiment, sampled larvae in all the treatments at 19 dph (Eday 0) had relatively constant and similar daily increment growth (three-way nested ANOVA, $p > .05$). In general, during the experimental period, the narrowest increment widths, on average 3–4 μm, were observed during the first experimental days in all the treatments (Fig. 5). An increase in otolith width was already observed one day after the onset of piscivory both in the DYSL and YSL treatments, whereas for the Rotifers treatment otolith increment widths were very similar throughout the entire experiment (Fig. 5A). Final otolith increments widths from Eday 26–27 averaged 7.2 ± 2.4 μm, 9.4 ± 3.8 μm and 22.2 ± 8.6 μm for the Rotifers, DYSL and YSL treatments respectively.

4. Discussion

The switch from a planktivorous to a piscivorous diet can be considered the second critical window in ABT and an early transition benefits the survival and growth of the larvae (Reglero et al., 2014a). We have studied the effect that the different timing in the onset of piscivory has on the growth of the population and on the survival of the

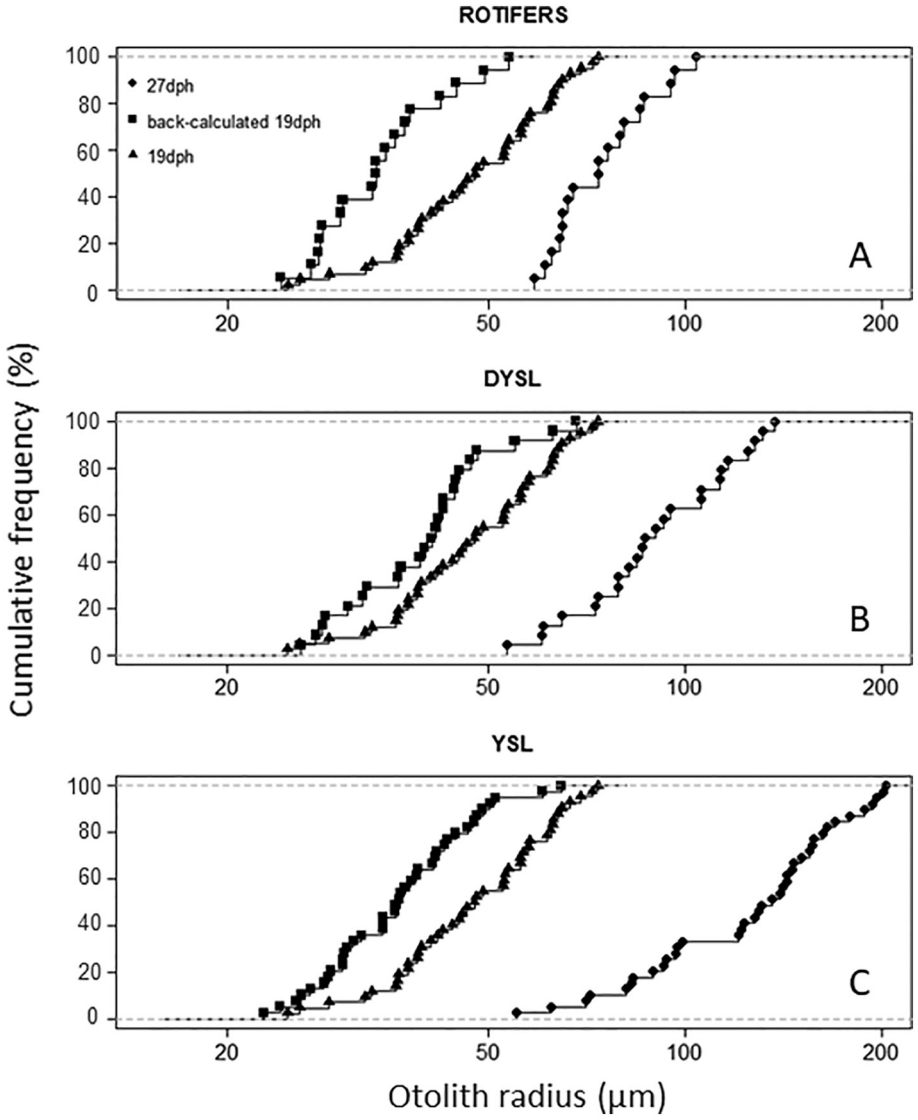

Fig. 4. Combined cumulative size frequency distributions of otoliths radii at 19 dph (▲), at 27 dph (●) and back-calculated 19 dph radii (■) for the three treatments treatment groups Rotifers, delayed yolk-sac larvae (DYSL) and yolk-sac larvae (YSL). Data from the three treatments at 19 dph are pooled together. Note that the X-axes are log (natural log) transformed, values are untransformed.

different size classes of laboratory-reared ABT using otolith microstructure analysis. Our laboratory experiments show size-selective mortality of the largest larvae at 19 dph independently of the timing of onset of piscivory. This study represents the first attempt to infer size-selective mortality in piscivorous ABT larvae.

A proportional relationship between otolith and larval size through time has also been reported in the field for smaller ABT larvae than those considered in our study (Brothers et al., 1983; García et al., 2006). Therefore, we were justified to proceed with larval size back-calculations based on the otolith analyses (Campana, 1990). The first increment formation in the otoliths of laboratory-reared Pacific bluefin tuna correspond with the onset of exogenous feeding and continuous daily periodicity in increment formation has been reported to at least 71 dph (Itoh et al., 2000). In our otoliths all the increments were easy to identify and we concluded that at least one increment was formed per day from 15 dph to 27 dph regardless of diet composition and growth rate.

We found high daily mortalities of around 40% during the acclimation time, probably due to the handling of the larvae during transportation from the rearing tanks to the culture tanks. We think most of the mortality was produced during the first and second acclimation days (pers.obs. 60–65% mortality after handling 5–6 mm larvae). The similar growth rates of the surviving larvae during the experiment after acclimation suggest that the high mortality did not affect size variability within groups.

Our otolith back-calculated results show size-selective mortality of the larger larvae in the three treatments. The survivors at the end of the experiment were the smallest larvae at the beginning of the experiment. This could be linked to the conversion of energy intake into growth, determined by the interaction between the larval rate of feeding, assimilation efficiency and the larval metabolic expenditure (Kiørboe et al., 1987). When large larvae in the Rotifers and DYS treatment fed on rotifers, the net energy gain might be lower than that obtained with optimal diet (yolk-sac larvae) and the larval metabolic expenses will exceed its aerobic capacity leading to energy deficiency, and eventually death (Billerbeck et al., 2001; Lankford Jr et al., 2001). We found high daily mortalities around 20% in both treatments during the experiments that most probably represent these larger larvae. In Pacific bluefin tuna, larvae have very low tolerance to starvation with 100% of the larvae dying after 3–4 days at 25 °C and 2–3 days at 28 °C (Tanaka et al., 2008).

The increase in metabolic expenses related to sub-optimal diet

Table 2
Growth rates in the experiment. The specific otolith growth rate (SGR, μm % day^{-1}) of small (5 percentile), median (50 percentile) and large (95 percentile) larvae are shown for Rotifers, delayed yolk-sac larva (DYSL) and yolk-sac larva (YSL) treatments. The growth rate during the "Experimental time" is shown using otolith back-calculation data from 27 dph sampled larvae (longitudinal data) and without back-calculation analysis using otoliths size-at-age of larvae sampled at 19 dph and 27 dph (cross-sectional data). Also, the growth rate during the "Acclimation time" (four days prior to the start of the experiment) is shown using back-calculated data to 15 dph from larvae sampled at 19 dph (longitudinal data). Data from the different treatments at 19 dph sampled larvae were pooled together, and therefore, the same growth was obtained for the small, median and large larvae.

			Experimental time		Acclimation time
Larval group	Size	Interval (per.)	Back-calculated from 27 dph	Size-at-age 19 and 27 dph	Back-calculated from 19 dph
Rotifers	Small	5%	10.4	9.3	8.4
	Median	50%	9.8	5.4	11.5
	Large	95%	8.4	4.3	11.2
DYSL	Small	5%	9.9	9.3	8.4
	Median	50%	9.5	7.7	11.5
	Large	95%	9.4	8.0	11.2
YSL	Small	5%	12.6	11.1	8.4
	Median	50%	16.4	13.0	11.5
	Large	95%	16.6	13.1	11.2

compositions cannot explain the size-selective mortality of the larger larvae in the early switch to piscivory treatment (YSL). These larvae were expected to be preying on large prey items that could be caught avoiding digestion and metabolic limitations. We could not analyze the otoliths of the dead larvae to have information about their previous growth history. The high temperatures in the tanks degraded the larvae in very few hours. Besides, given the short duration of the experiments, we tried to stress and alter the larvae as less as possible avoiding daily cleaning of the tanks that would have caused them additional stress and most probably higher mortality. We suggest a possible tank effect that affects the large larvae of the YSL treatment. Under laboratory conditions, large larvae might not be able to express their behavior fully and grow at their maximal rates. Besides, some individuals may have died due to collisions with the tank walls (pers.obs.). Also, larger larvae geared towards high growth rates may have high energy demands due to elevated food processing (SDA), and thus be more at risk if food supply is not continuously available (Lankford Jr et al., 2001).

Our results of the lower survival rates of the large and probably faster growing larvae regarding the feeding regime agree with some studies that also show this pattern in the field (*e.g.*Litvak and Leggett, 1992; Meekan and Fortier, 1996; Pepin et al., 1992, 2003), despite the general belief that larger and faster growing larvae have an increased probability of survival (*e.g.*Anderson, 1988; Takasuka et al., 2003, 2004). Under enclosed aquaculture conditions, factors such as predation, transport, environmental variability and prey patch distribution are isolated and larval behavior might be modified compared to the field. Behavior might be especially variable in scombrids with highly variable morphology, physiology and nutritional requirements during their short larval period.

The increased survival of small larvae could be related to the type of food provided to the ABT larvae during the experiment. Prey type can be particularly important in fast-growing species, like scombrids, where growth is strongly linked to feeding success (Pepin et al., 2015). Before piscivory, *Thunnus* larvae primarily feed on copepod nauplii, cladocerans and appendicularians (Llopiz et al., 2010). Most species have a particularly narrow diet during their larval stage (Llopiz, 2013; Robert et al., 2014). It could be possible that a rotifer-based diet played against the largest individuals before they initiated piscivory. Rotifers are not natural prey in the field. In other species, the nutritional composition, growth and survival of larvae fed copepods was higher than those fed rotifers (Folkvord et al., 2018). However, we still do not have the technological development necessary to culture ABT larvae on a copepod-based diet.

Our size-selectivity results evidence the type of mortality acting on ABT during a specific larval phase, when they are potential piscivorous. No studies of this type have been conducted in other life stages of this species. Different patterns of size-selective mortality may be acting from larval emergence until the point where recruitment is set. During the larval stage when dramatic changes in the morphology occur (Kendall et al., 1984), multiple mortality scenarios can directly affect fish larvae against slow-growing individuals or against fast-growing individuals and is the integration of all selective mortality pressures along the early development that will determine the recruitment. In

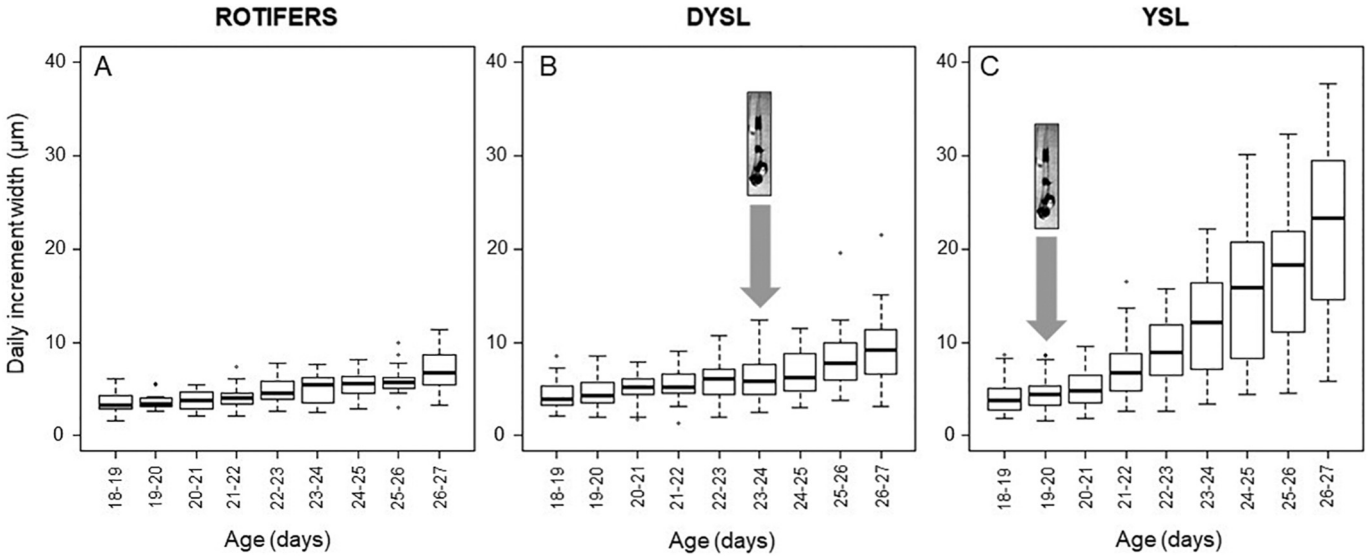

Fig. 5. Boxplots of daily increment widths back-calculated at 18 to 27 dph for the three sample treatments: A) Rotifers, B) delayed yolk-sac larvae, DYSL and C) yolk-sac larvae, YSL. The line within each box indicates the median daily width (μm), and the whiskers are the 25th and 75th percentiles. A sea bream yolk-sac larvae image is inserted along with a gray arrow in panels b and c showing the days where they were added for the first time to each treatment, at 23 dph in DYSL and at 19 in YSL.

another piscivorous scombrid, Atlantic mackerel (*Scomber scombrus*), different mortality scenarios during different years have been found with fast larval growth in surviving juveniles associated with both strong and weak recruitment (Robert et al., 2007). In *Thunnus maccoyii* density-dependent reduction of growth rate has been observed when larvae are competing for food, increasing the general high mortality of the larval stage and also increasing the cumulative mortality of slow-growing larvae (Jenkins et al., 1991). Therefore, in the field, with just a few additional days to larval stage duration may result into a massive difference in cumulative survival (Jenkins et al., 1991).

At the beginning of the experiment, probably not all of the larvae were morphologically ready to start piscivory, with large teeth, well-developed stomachs with gastric glands and differentiated pyloric caeca (Yúfera et al., 2014). Consequently, during the YSL treatment, the growth of the small larvae, less developed to prey on fish larvae, was lower than for the median and large larvae, already developed to prey on fish larvae. In contrast, in the Rotifer treatment, the small larvae may be in their optimal size range for rotifer consumption, which explains their higher growth rates compared with the median and large ones that were ready to start piscivory. In the DYSL treatment the median and large larvae may show growth compensation after the onset of piscivory, resulting in similar growth rates to those observed for the small larvae feeding on an optimal diet consisting of rotifers (Hunt Von Herbing and Turnbough, 2011). Presenting successive cumulative size distributions is quite useful in revealing growth differences. However, a significant mortality event can have important implications for the interpretation of percentile-based growth estimations, since it can affect cohort size variability (Folkvord et al., 2009).

In our study, we found an increase in growth rates across treatments and also an increase in the daily variability. The early piscivory feeding group (YSL) attained the largest sizes and had the fastest growth rate followed by the group in which piscivory was delayed (DYSL) and the planktivorous group (Rotifers). In the maximum growth treatment, YSL, at 26 dph, 6 days after the start of the piscivory, daily increment width of the 75th percentile was twice that of the 25th percentile found. Such individual differences in growth and the strong growth autocorrelation, generally observed in scombrids, suggests that the cumulative size difference would likely lead to cannibalism in the tank (Pepin et al., 2015). However, we avoided cannibalism by stopping the experiment the day before the start of an aggressive behavior. Little is known about potential factors driving variability in growth under piscivorous diet. In Pacific bluefin tuna larvae, prey utilization could be significantly different among individuals of the same age and reared in the same culture tank, because the first days of the piscivory diet overlaps with a planktivorous diet (Tanaka et al., 2014a). Growth variability during the planktivorous phase can induce large growth variability after the onset of piscivory (Takebe et al., 2012). Several different larval developmental stages have been distinguished at a specific age during the planktivorous stage in ABT larvae (Blanco et al., unpublished).

In hatchery-reared piscivorous Pacific bluefin tuna, bigger larvae had advantages in terms of growth-selective survival before and after the start of the piscivory (Tanaka et al., 2014a, 2017). These larvae were reared in big mass-rearing tanks (50000 L) that may favor larval growth and behavior and diminish the chances of tank collision compared to smaller tanks. We chose to use 150 L tanks because they provided the possibility of having 5 replicates per treatment and it was easy to ensure *ad libitum* conditions (homogeneous prey distribution within the tank and easy recognition of available prey). Larger tanks, of 20000–50000 L, are probably better to rear planktivorous and piscivorous larvae, although smaller tanks of 1500 L or 5000 L have also been successfully used for 8–15 mm ABT (De la Gándara et al., 2012). Given the installations available, there was a trade-off between the number of tanks available for replicates and the volume of the tanks. In general, less number of replicates could be conducted when using larger tanks (note one replicate in Tanaka et al. (2014a)), and we selected to have replicates. In the field, several authors have studied size-selective mortality in Pacific bluefin tuna larvae (Satoh et al., 2013; Tanaka et al., 2006; Watai et al., 2017), however, net avoidance by the larger piscivorous larvae, along with the different predation pressure might bias representative sampling (Satoh et al., 2013). Besides, in the field, it is difficult to define a cohort originating from the same environmental conditions.

In summary, our results broaden the previous knowledge in which Reglero et al. (2014a) determined that onset of piscivory is crucial for survival to recruitment in ABT larvae. To our knowledge, this study represents the first study on size-selective mortality in piscivorous ABT larvae under culture conditions. The results derived suggest that being bigger it is not always the best option to survive and mortality rates do not always decline in culture conditions with increasing larval size. When culturing tuna one of the most important issues faced is when to start piscivory feeding. The mortality of the large larvae along with the rapid response of piscivory in terms of growth reflects the fact that there is a cost to fast growth. A delay in the onset of piscivory under certain culture conditions might promote the growth of the smaller larvae, as seen in our results. Further research, along the different developmental stages from larvae to juvenile, will allow to determine the mortality causes acting during early development in order to integrate survival data across phases to obtain a more precise estimation of recruitment. Different patterns in size-selective mortality directed against fast-growing or slow-growing ABT individuals need to be integrated to determine the fraction of the cohort that constitutes the majority of the recruits.

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