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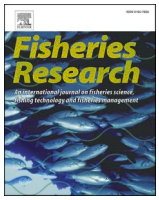

Full length article

Effects of temperature on embryonic development of Atlantic bluefin tuna (*Thunnus thynnus*, L 1758) and Atlantic bonito (*Sarda sarda*, Bloch 1793)

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ABSTRACT

Temperature is the most important abiotic factor controlling the very early life of fish. Data on development, hatching, and survival responses of fertilized fish eggs to varying temperatures are essential to understand fitness of Atlantic bluefin tuna (ABFT) and Atlantic bonito (AB). In this study, we investigated i) the effect of temperature on viable hatching rate and ii) the relationship between temperature and the timing necessary to reach different developmental stages. Our findings show that the thermal range for viable egg hatching is colder in AB than in ABFT. ABFT eggs hatch from 18 to 33 ºC, highest around 26–27 ºC; below 21 ºC or above 30 ºC at least 50 % of the hatched larvae were abnormal, while below 19 ºC and above 32 ºC the larvae had no chance to survive, having highest survival chance at around 26 ºC. In AB, eggs hatched from 16 ºC to 28 ºC, being optimal around 21–22 ºC, the incidence of abnormal larvae was very high above 27 ºC and at 16.5 ºC, and larvae had no chance to survive above 28 ºC and had the highest survival chance at around 21 ºC. ABFT eggs required longer times to hatch than other tuna species. The results obtained in this study are an important tool for aquaculture; here, we describe the best temperature conditions for successful and viable hatching in ABFT and AB, which may help to improve modelling tools and to develop better indices of annual recruitment and the possible impact of future global temperature changes.

1. Introduction

The early life of fish is a period characterized by dramatic changes in morphology and high mortality rates (Peterson and Wroblewski, 1984; Mcgurk, 1986; Leggett and Deblois, 1994). Most marine fish are oviparous and two different stages can be considered until metamorphosis: embryonic development from fertilization to hatching and larval development from hatching to metamorphosis (Balon, 1975). The embryonic stage is characterized primarily by endogenous feeding (acquisition of nutrients from parental sources) inside or outside egg envelopes (Balon, 1986, 1990). During this embryonic stage, temperature is the most important abiotic variable influencing physiological processes, embryonic developmental time, hatching and survival rates, the presence of abnormal larvae, yolk utilization of larvae, and post-hatching health (Kjorsvik et al., 1990; Pepin, 1991; Polo et al., 1991; Blaxter, 1992). The responses of these factors to temperature are highly variable between species (Pauly and Pullin, 1988; Pepin, 1991), stocks, and populations (Geffen et al., 2006).

Atlantic bluefin tuna (*Thunnus thynnus*, ABFT) and Atlantic bonito (*Sarda sarda*, AB) play an important ecological role as top predators and are targets of some of the most important fisheries in the world, which has a fundamental influence on the structure and function of marine communities (Shimose and Wells, 2015). Both species have asynchronous ovarian development; during the spawning season females contain ovaries with oocytes at all developmental stages (Rey et al., 1984; Medina et al., 2002; Corriero et al., 2003, 2005; Macías et al., 2005).

In temperate seas such as the Mediterranean Sea, both species spawn pelagic eggs only a few weeks apart and with a temperature difference of several degrees. ABFT starts spawning at temperatures above 20 ºC in June–July, while AB reproduces at water temperatures above 18 ºC in May–July (Ortega, 2015; Reglero et al., 2018a). The relationship between temperature and embryonic development is necessary to

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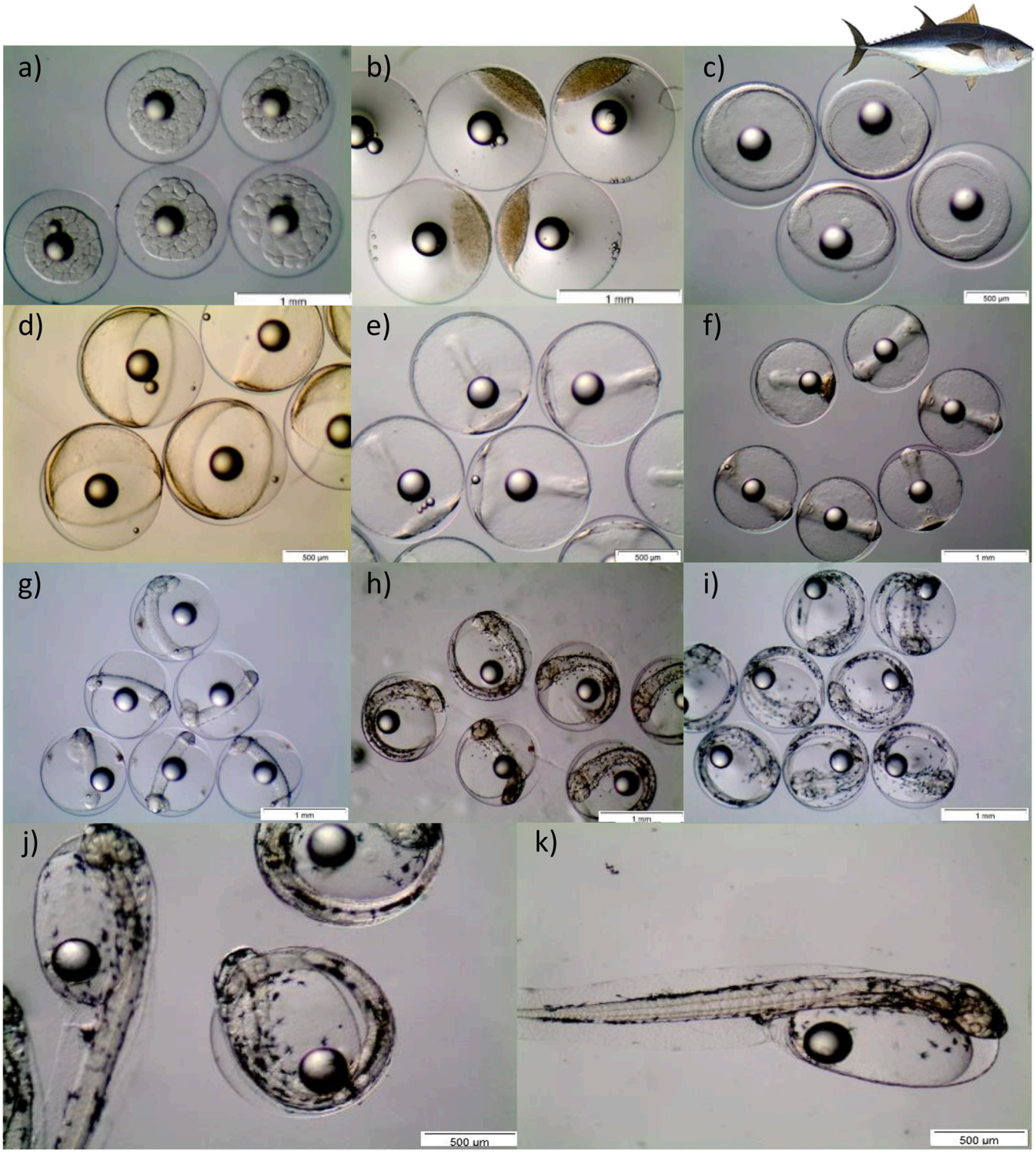

Fig. 1. Ontogeny of ABFT: A, Morula; B, Blastula; C, Gastrula; D, Embryonic shield; E, Appearance of the embryonic body; F, Kupfer's vesicle; G, Onset of pigmentation; H, Heart beating; I, Pre-eclosion; J, Beginning of hatching; K, Larvae that just hatched.

understand the spawning strategies of both species and their thermal tolerance in early life stages.

The growth of the ABFT aquaculture industry in recent years (Mylonas et al., 2010) and the expansion of experimental aquaculture for AB (Reglero et al., 2014a, 2018c; Blanco et al., 2017, 2019) provide us with a unique opportunity to improve the process-based knowledge of the factors influencing survival and growth, during the egg and larval stages of these species.

ABFT and AB are the only two scombrid species of the Mediterranean Sea that have been successfully reared in captivity (Ortega, 2015); therefore, experimental research on these species is still very limited. For example, thermal tolerance for hatching success and egg developmental time have been scarcely studied, and only over a limited temperature range in ABFT (Gordoa and Carreras, 2014; Reglero et al., 2018a). There is no detailed description of the embryonic development over a wide temperature range for any of the two species.

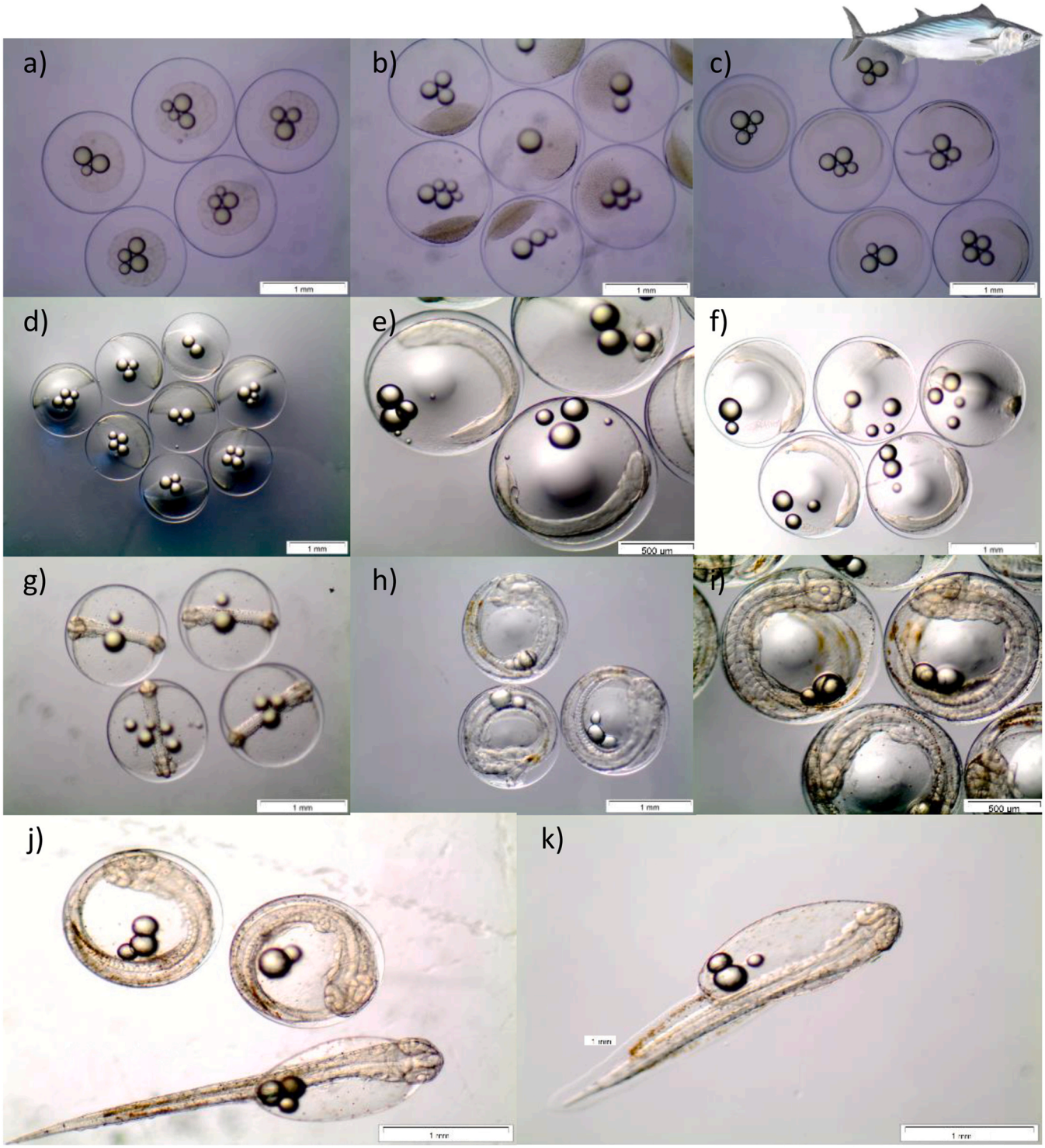

Fig. 2. Ontogeny of AB: A, Morula; B, Blastula; C, Gastrula; D, Embryonic shield; E, Appearance of the embryonic, body; F, Kupfer's vesicle; G, Onset of pigmentation; H, Heart beating; I, Pre-eclosion; J, Beginning of hatching; K, Larvae that just hatched.

The present study aimed to determine (i) the effect of temperature on the viable hatching rate of ABFT and AB, and (ii) the relationship between temperature and the time to reach different developmental stages in ABFT and AB in the laboratory at different temperatures.

Understanding the processes that drive growth and survival during the early life stages of ABFT and AB is essential to better manage fisheries and ensure the sustainable exploitation of these resources (Hjort, 1914; Houde, 2008). The response of fertilized ABFT and AB eggs to changes in temperature is essential not only to understand the processes that determine the recruitment of the species, but also to predict the potential response of both species to climate change (i.e., global heating of oceans). This study will contribute to improving the rearing conditions for aquaculture purposes in the hatchery production of ABFT larvae, because temperature ranges must be narrowed to maximize hatching rate and larval survival.

Table 1
Exponential relationship ($H=a*exp^{bT}$), where H is hours after fertilization and T is temperature (ºC), in ABFT and AB for each egg developmental stage.

	ABFT			AB			
Developmental stages	**Coefficients**		**R^2**	**Coefficients**		**R^2**	**Developmental stage description**
Morula	a b	- -	-	a b	4.1758 -0.011	0.210	Early-stage embryo consisting of 16 cells called blastomeres
Blastula	a b	10.851 -0.027	0.352	a b	17.865 -0.044	0.578	The 128 blastomeres cell are oriented together in one side of the egg
Gastrula	a b	22.954 -0.042	0.804	a b	35.39 -0.055	0.756	The blastula is reorganized into a multilayered structure
Embryonic shield	a b	38.460 -0.052	0.774	a b	66.608 -0.071	0.958	An embryonic shield is formed
Embryo	a b	59.231 -0.059	0.747	a b	152.07 -0.1	0.925	Appearance of an initial embryonic body from one side to another
Kupfer's vesicle	a b	41.900 -0.043	0.663	a b	135.63 -0.091	0.956	A few small vacuoles (Kupffer's vesicles) appear at the underside of the caudal (posterior) end of the body, which is in contact with small blastopore.
Pigmentation	a b	75.324 -0.054	0.683	a b	186.27 -0.093	0.960	Beggining of pigmentation
Heart beat	a b	222.110 -0.091	0.962	a b	229.39 -0.092	0.961	Beggining of the first heart beat
Beggining of hatching	a b	237.800 -0.078	0.894	a b	282.4 -0.081	0.907	Beggining of the larval hatching
End of hatching	a b	235.040 -0.073	0.929	a b	491.41 -0.101	0.939	Moment at which 100 of the viable embryos hatched

2. Material and methods

Coinciding with the spawning season, in June 2013 and 2014, newly-fertilized ABFT and AB eggs were collected. ABFT eggs were collected at early morning hours from spontaneous spawning of captive populations in sea cages. Eggs were transported for around 1 h in soft plastic containers, reaching the experimental facilities of the Spanish Institute of Oceanography when the eggs were in the 4–16 cell phase. Newly-fertilized AB bonito eggs were collected in the afternoon using an egg collector from spontaneous spawning in a captive broodstock kept in a mesocosm at the experimental facilities of the Spanish Institute of Oceanography. Experiments were conducted separately for AB and ABFT in a temperature-controlled room set at 16 ºC and 18ºC respectively using the air conditioning system, resulting in the tank with the lowest temperature. The remaining tanks were warmed up, and the temperature was stabilized using heaters. Seventeen 400 L tanks were prepared each one with a determined temperature from 16 and 18ºC to 28ºC and 33 ºC. To homogenize the water temperature, each 400 L tank was equipped with an aeration system. Temperatures were monitored continuously every 5 min using a temperature logger placed in each tank (HOBO). AB and ABFT eggs were acclimated at the incubation water temperatures by increasing or decreasing the temperature at a rate of 1 ºC/45 min (for a maximum of 4 hours) until the target temperature was reached.

To monitor survival and hatching rates, approximately 50 eggs were placed into 250 mL flasks, at the controlled incubation temperature in triplicates (18–33ºC for ABFT and 16–28ºC for AB). To determine the stages of embryonic development, 250 eggs were placed into a 1000 mL flask for each experimental temperature. Flasks were previously filled with filtered seawater and placed inside 400 L tanks.

Ten eggs were taken from each 1000 mL flask every 2–3 hours until hatching and examined under binocular stereomicroscope to identify the embryonic developmental stage according to (Miyashita et al., 2000). An exponential curve was used to fit the data. Once late developmental stages were identified in the 1000 mL flasks, we examined the 250 mL flasks to ensure the eggs were monitored right after the onset of hatching. Once all eggs had hatched, larvae were counted to calculate the total hatching rate (total number of larvae/total inoculated eggs). Abnormal larvae were identified and counted to estimate the deformity rate (abnormal/total hatched larvae). Viable hatching rate was calculated as the relation between the total hatching rate and deformity rate (total hatching rate × 1 - deformity rate). Viable hatching rates represent the number of larvae that survive and have normal development after hatching.

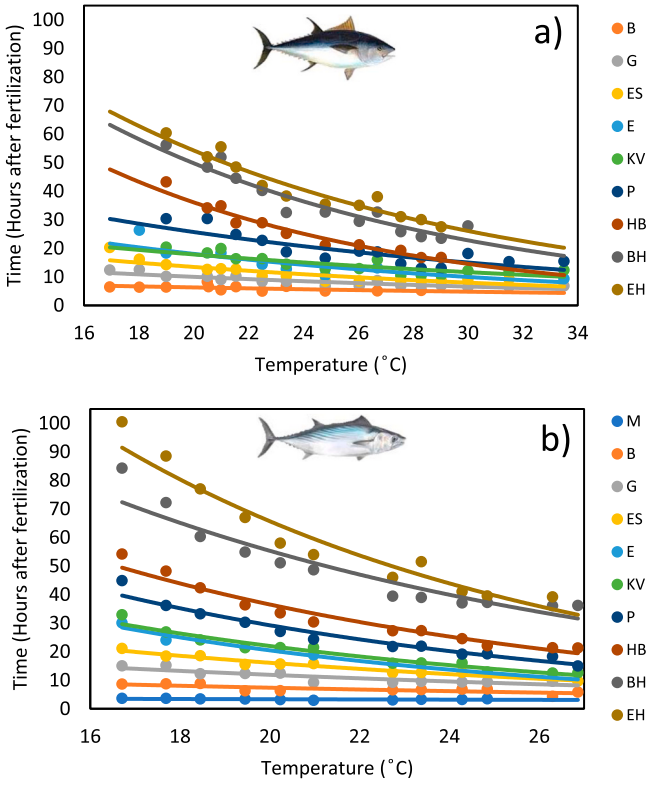

Fig. 3. Time required to reach the different developmental stages in a) ABFT and b) AB. M, Morula; B, Blastula; G, Gastrula; ES, Embryonic shield; E, Appearance of the embryonic body; KV, Kupfer's vesicle; P, Onset of pigmentation; HB, Heart beating; BH, Beginning of hatching; EH, End of hatching.

3. Results

ABFT eggs were spherical, with an average diameter of 1.07 ± 20.8 mm. In general, they had a single oil globule, although some eggs had two or three globules that merged as development progressed. At

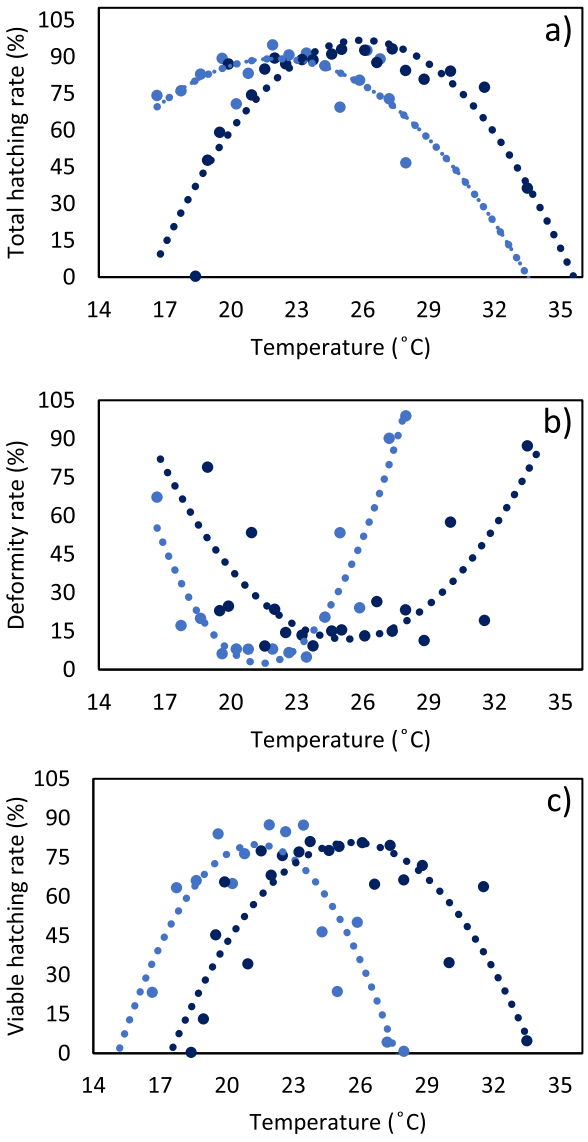

Fig. 4. a) Total hatching rate of eggs incubated at different temperatures. b) Incidence of abnormal larvae reared from eggs incubated at different temperatures. c) Relationship between water temperature and viable hatching rate of ABFT eggs. ABFT in dark blue and AB in light blue.

hatching, larvae had a total length of 3.82 ± 0.25 mm (Fig. 1). AB eggs had an average diameter of 1.29 ± 32.9 mm and the average number of oil globules was 3.4 ± 0.6. At hatching, the larvae had a total length of 4.19 ± 0.19 mm (Fig. 2). The sequence of events from fertilization to hatching followed the general scheme of vertebrate embryology: morula, blastula, gastrula, development of the embryonic shield, appearance of the embryonic body, appearance of the Kupfer's vesicle, onset of pigmentation, start of the heart beat and hatching (Figs. 1 and 2, Table 1).

The developmental time (DT) from morula to the end of hatching (EH) of ABFT and AB eggs was strongly correlated with temperature (Fig. 3a and b, $r^2 > 0.90$). DT decreased significantly with increasing temperatures (Fig. 3a and b, Table 1). At temperatures below 27 ºC, DT for AB was always longer than for ABFT; at 27–28 ºC both species had the same DT (Fig. 3a and b). The embryonic DT or EH decreased exponentially as incubation temperature increased in both species (Fig. 3a and b, Table 1). We could not determine the onset of the morula stage in ABFT, since the eggs were obtained from sea cages, and they reached the morula stage while they were being acclimatized.

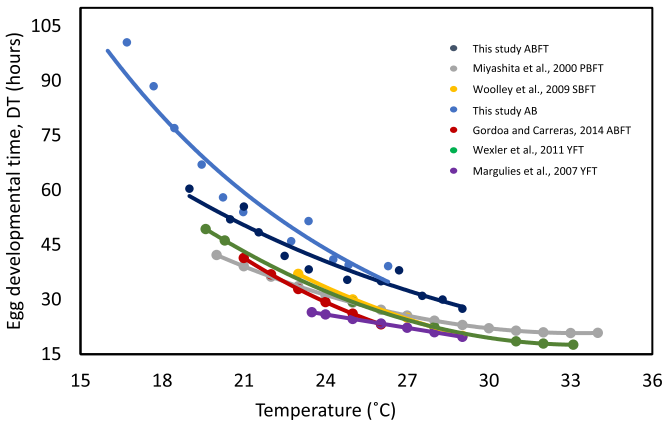

Fig. 5. Relationship between egg developmental time (DT) and temperature for other tunas: ABFT (this study, dark blue), AB (this study, light blue), ABFT (Gordoa and Carreras, 2014, red), Pacific bluefin tuna (Miyashita et al., 2000, grey), Southern bluefin tuna (Woolley et al., 2014, yellow), yellowfin tuna (Wexler et al., 2011, green and Margulies et al., 2007, purple).

ABFT eggs were able to hatch from 18 to 33 ºC (Fig. 4a). Below 21 ºC and above 30 ºC, at least 50 % of the hatched larvae were abnormal (Fig. 4b). Below 19 ºC or above 32 ºC, larvae had no chance of surviving (Fig. 4c). In AB, eggs were able to hatch at temperatures up to 28 ºC, although the incidence of abnormal larvae was very high at, or over, 27 ºC (Fig. 4a and b). Larvae had no chance of surviving below 16 ºC or above 28 ºC (Fig. 4c).

4. Discussion

In this study, we describe egg size, the thermal range for viable egg hatching, and the time to reach each developmental stage of AB and ABFT. Our findings show differences between both species. Eggs' size was larger in AB than in ABFT. AB eggs hatched at a colder temperature range than ABFT eggs. In both species, both the time to reach different egg developmental stages and the time to hatching decreased with increasing temperatures. At similar temperatures, AB eggs need longer timings to reach different developmental stages and to hatching than ABFT.

In most species, the time to hatching is inversely correlated to temperature and positively correlated to egg diameter; thus, for the same species at the same temperature larger eggs take extra time to hatch (Pauly and Pullin, 1988). We found significant differences in egg size between the two species; eggs were larger in AB than in ABFT (1.29 and 1.07 mm, respectively). These differences may explain the greater DT of AB compared to ABFT at the same temperature, as it was reported by Bonislawska et al. (2000) in different species. However, the larger size of newly-hatched AB larvae (Ortega, 2015) and their faster growth rate later during the larval stage (Reglero et al., 2014a; Blanco et al., 2017) may compensate for longer egg DT.

In our study, times to hatching were longer than those reported in the only other experimental study that estimated hatching times for ABFT between 20 and 26 ºC (Gordoa and Carreras, 2014). The authors of this study reported hatching times of 45 h at 20.5 ºC, 33 h at 23ºC and 23 h at 26ºC, while we observed times to hatching of 52.5 h at 20.5 ºC, 44 h at 23 ºC, and 35 h at 26 ºC. These differences could be due to the larger size of ABFT eggs in our study (1.07 mm versus 1.0 mm in Gordoa and Carreras, 2014), as larger eggs may take longer to hatch than smaller eggs (Pauly and Pullin, 1988). In salmon (Pepin et al., 1997) also showed that larger egg size are usually correlated to longer development time and larger larval size. Similarly to other species (Chambers and Leggett, 1996), larger ABFT adults produce larger eggs than smaller ABFT adults (Ortega, 2015). Our broodfish (150–180 kg) were larger than the 75–125 kg adults of Gordoa and Carerras (2014). On the other hand, the

time to acclimate the eggs to the experimental temperature in our study may have resulted in a slight increase in time to hatching.

In the present study, the size of ABFT and AB eggs and their times to hatching were within the ranges of other tuna species, although we recorded slightly longer times relative to those of Pacific bluefin tuna, southern bluefin tuna, and yellowfin tuna (Fig. 5, Miyashita et al., 2000; Margulies et al., 2007;Wexler et al., 2001;Woolley et al., 2009). Pacific bluefin tuna eggs are typically 1.01–1.02 mm (Miyashita et al., 2000; Partridge, 2013) and southern bluefin tuna eggs are never larger than 1.00 mm.

In this study, the viable hatching rate temperatures of ABFT ranged between 19 ºC and 32 ºC, with an optimal temperature to hatching of 23–26 ºC. These results are very similar to the optimal temperature reported for other bluefin tunas. Miyashita et al., (2000) reported an optimal temperature of 23–27 ºC for Pacific bluefin tuna (*Thunnus orientalis*). Woolley et al., (2009) reported an optimal temperature of 23–25 ºC for southern bluefin tuna (*Thunnus maccoyii*). The optimal temperatures for the topical yellowfin tuna (*Thunnus albacares*) were 27–30 ºC (Wexler et al., 2011) or 23–26 ºC (Kim et al., 2015).

The reduction in thermal tolerance revealed by abnormal larvae at extreme temperatures has also been reported in other species. For example, the gilthead sea bream, *Sparus aurata,* spawns between 14 and 26 ºC, but deformity rates are usual outside the 16–22 ºC range (Polo et al., 1991). Similarly, Atlantic cod (*Gadus morhua*) eggs hatch between −1 ºC and 12 ºC but the incidence of abnormal larvae was low at 4–8 ºC (Galloway et al., 1998). Here we show that viable hatching rate ranges can be significantly lower than total hatching rates, which should be taken into account in experimental studies.

Tuna and billfish larvae have a narrower temperature range than adults (Boyce et al., 2008), but the temperature range for hatching is wider than that for larval development. However, as has been documented in the Mediterranean Sea, tunas spawned in a narrower temperature range which maximize the chances of larval survival. AB larvae have been found between 17–25 ºC (Reglero et al., 2014b, 2018a; Ortega, 2015), and ABFT larvae also at 20–27 ºC (Reglero et al., 2014b; Ortega, 2015).

Data on development, hatching, and survival responses of fertilized ABFT and AB eggs to various temperatures is of broad interest to improve our understanding of egg fitness in these species. Including these estimates into modelling tools has the potential to improve stock recruitment, develop better indices of annual recruitment, and explain the mechanisms behind the possible impact of future changes (Reglero et al., 2018b). The results obtained in this study constitute an important tool for aquaculture. Or result can promote a successful hatchery production of ABFT larvae, as in practice temperature ranges must be narrowed to maximize hatching rate and larvae survival rates.

CRediT authorship contribution statement

Aurelio Ortega: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Writing – original draft. **Fernando de la Gándara:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. **Patricia Reglero:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. **Gabriel Mourente:** Conceptualization, Investigation, Supervision, Writing – review & editing. **Edurne Blanco:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgments

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