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Cite this article: Reglero P *et al*. 2018 Atlantic bluefin tuna spawn at suboptimal temperatures for their offspring. *Proc. R. Soc. B* **285**: 20171405.
http://dx.doi.org/10.1098/rspb.2017.1405

Received: 23 June 2017
Accepted: 4 December 2017

Subject Category:
Ecology

Subject Areas:
ecology, environmental science

Keywords:
phenology, temperature, large migratory fish, apex predator, reproduction, bluefin tuna

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Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.3956182.

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Atlantic bluefin tuna spawn at suboptimal temperatures for their offspring

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Life-history traits such as spawning migrations and timing of reproduction are adaptations to specific environmental constraints and seasonal cycles in many organisms' annual routines. In this study we analyse how offspring fitness constrains spawning phenology in a large migratory apex predator, the Atlantic bluefin tuna. The reproductive schedule of Atlantic bluefin tuna varies between spawning sites, suggesting plasticity to local environmental conditions. Generally, temperature is considered to be the main constraint on tuna spawning phenology. We combine evidence from long-term field data, temperature-controlled rearing experiments on eggs and larvae, and a model of egg fitness, and show that Atlantic bluefin tuna do not spawn to optimize egg and larval temperature exposure. The timing of spawning leads to temperature exposure considerably lower than optimal at all spawning grounds across the Atlantic Ocean. The early spawning is constrained by thermal inhibition of egg hatching and larval growth rates, but some other factors must prevent later spawning. Matching offspring with ocean productivity and the prey peak might be an important driver for bluefin tuna spawning phenology. This finding is important for predictions of reproductive timing in future climate warming scenarios for bluefin tuna.

1. Introduction

Annual cycles in productivity and temperature are important for timing of reproduction [1], and the importance of spatial and temporal match between egg hatching and environmental conditions for offspring survival and recruitment of strong year classes is well known [2,3]. However, the effects of seasonality and environmental variability on the phenology and the annual routines of migratory marine apex predator species are poorly understood. Atlantic bluefin tuna, *Thunnus thynnus*, is categorized as a near threatened species according to the IUCN Red List criteria [4]. Like other bluefin tuna species, the Atlantic bluefin tuna is a long-distance migrant with a narrow environmental window for spawning [5,6], suggesting that spawning conditions are suitable for offspring only during a short period of time and in areas with specific environmental characteristics.

The evolutionary drivers of differences in reproductive phenology across the western (Gulf of Mexico, Slope Sea) and eastern (Mediterranean) Atlantic stocks managed by the International Commission for the Conservation of Atlantic Tunas (ICCAT) are still unknown. Atlantic bluefin tuna of the eastern stock migrate more than 10 000 km in May–June to reach warm confined areas of the Mediterranean Sea to spawn, and then return to their foraging grounds in the North Atlantic during July–August [7,8]. Reproductive schedules are not synchronized between spawning areas within the Mediterranean Sea, and bluefin tuna begin reproduction 2–4 weeks earlier in the Eastern Mediterranean than the Central and Western spawning areas [9]. Western Atlantic bluefin tuna travel from foraging areas to spawning grounds in the Gulf of Mexico in April–June, returning back to feeding grounds during the summer [10]. Recently, another spawning area for the western stock has been reported in the Slope Sea where spawning occurs two months later than in the Gulf of Mexico [11].

This variability in seasonal timing of reproduction may be due to physiological thermal tolerance limits of early life stages, since bluefin tuna larvae generally inhabit a narrower and warmer range of temperatures than adults [5,7,12,13], and consequently all tuna species, temperate and tropical, spawn in warm waters. A correlation between the occurrence of larvae and temperature from scientific survey data [5,14,15] suggests that the confined spawning season could be linked to a physiological tolerance range for larval development. However, the ecological and physiological drivers of this relationship are unknown, and it is difficult to infer exactly how important these thermal constraints are for timing of spawning. Many fish species in temperate areas (0–25°C) tend to spawn at temperatures close to, but slightly colder than those that maximize probability of egg hatching success [16]. Our aim here is to analyse the role of seasonality in temperature for the phenology of bluefin tuna reproduction, and its relative importance in the different spawning areas across the Atlantic Ocean for both stocks. Our hypothesis is that breeding phenology of Atlantic bluefin tuna is optimized to the water temperature such that offspring survival is maximized. Egg hatching success is only one element of egg fitness. In addition, we include temperature-driven egg and larval stage duration and mortality rates to model the consequences of spawning at different times of the year in bluefin tuna.

An integrative approach combining laboratory experiments and field surveys of different life stages of Atlantic bluefin tuna is now possible. Regular spring and summer monitoring cruises both in the spawning areas of the eastern and western bluefin stocks provide time series of larval occurrences. Commercial fisheries allow sampling of mature gonads, and farming cages provide an observatory for recording spontaneous spawning events in adult tuna. Moreover, successful rearing techniques now make laboratory and mesocosm experiments possible [17]. In this study we take advantage of this unique opportunity of combining experiments and field sampling to evaluate the hypothesis that breeding phenology of Atlantic bluefin tuna is optimized to the seasonal cycle of water temperature. This question must be answered to assess future effects of ocean warming on migrant marine species such as tunas. We also consider alternative potential drivers of the spawning phenology such as the synchronization to seasonality in ocean productivity and parental constraints.

2. Material and methods

(a) Field sampling of tuna eggs

Spontaneous spawning was recorded in Atlantic bluefin tuna adults kept in captivity during four years during 2010–2012 and 2015 (electronic supplementary material, table S1). Adult tuna were captured off Balearic Islands waters (western Mediterranean) and moved to Caladeros del Mediterráneo SL fattening facilities. One circular cage (25 m in diameter and 20 m in average depth) with 37 adult bluefin tuna was monitored during 2010–2012. Another circular cage (50 m in diameter and 16 m in average depth) was monitored in 2015. The broodstocks in the cages were fed to satiety once a day on a diet of raw fish. A PVC curtain was installed around the perimeter of the two cages to capture any visible floating eggs that would then be collected manually using plankton nets from the surface of the water [18]. The cages were monitored from the beginning of the spawning season to the end. Spawning events occurred naturally around 02.00–04.00, therefore egg collection was conducted during night time. After collection the eggs were counted in the laboratory. The egg abundance was a rough estimation of the total of eggs released since it could not be ensured all the eggs were caught. These data were only available for the Western Mediterranean Sea.

(b) Field sampling of tuna larvae

Field sampling of larvae was carried out on cruises during spring and summer from 2001 to 2013 in the Western Mediterranean Sea (electronic supplementary material, table S1). The sampling covered synoptically a wide geographical scale (180 × 220 miles) with a 10-nautical-mile separation between stations. From 2001 to 2005, tuna larvae were collected using standard double-oblique hauls down to 70 m depth, with Bongo nets with a mouth diameter of 60 cm equipped with 333 µm meshes [19]. From 2006 to 2013, the larvae were collected using Bongo nets with a mouth diameter of 90 cm equipped with 500 µm meshes hauled throughout the mixed layer, down to approximately 30 m depth, coinciding with the thermocline as determined from CTD profiles on board. One replicate was preserved in 4% buffered formalin in seawater and the second replicate was preserved in ethanol. Once at the laboratory, fish larvae were sorted from the 4% buffered formalin using a stereoscopic microscope, identified and counted to the lowest taxonomic level using determination keys according to available descriptions for the area [20]. The number of Atlantic bluefin tuna larvae identified in each sample was divided by the volume of water filtered to standardize the catches. These data were not available for the Central and Eastern Mediterranean Sea.

In the Gulf of Mexico, larval bluefin tuna samples have been collected annually (except 1985) through the National Marine Fisheries Service South East Area Monitoring and Assessment Program (SEAMAP) [15] beginning around 20 April and extending through the end of May (electronic supplementary material, table S1). Tuna larvae were collected using standard double-oblique hauls down to 200 m depth, with Bongo nets with a mouth diameter of 60 cm equipped with 333 µm meshes. Larval samples were sorted and identified by the Polish Plankton Sorting and Identification Center in Szczecin, Poland. Sample identifications were validated by the Southeast Fisheries Science Center in Miami, Florida, USA. The number of larvae in the Slope Sea was obtained from the literature after cruises conducted in 2013 from the beginning of June to the middle of August [11].

(c) Field sampling of tuna females

A total of 724 female bluefin tuna were sampled in the three main spawning areas of the Western, Central and Eastern

Mediterranean Sea (electronic supplementary material, table S1). From those, 528 were sampled in the Western Mediterranean over 11 years around the Strait of Gibraltar during the tuna trap fishing season in late spring, and at the breeding grounds southwest of the Balearic Islands in early summer. In the Strait of Gibraltar area, the fish were caught by four traps during their reproductive migration towards the Mediterranean Sea. Later in the season, individuals were captured by the purse seine and longline fleets operating off the Balearic Islands. Female gonads from the Eastern and Central Mediterranean spawning grounds were collected under the framework of the project REPRODOTT [21]. During the purse seine fishing season in the years 2003–2005, a total of 64 female bluefin tuna were sampled in the Central Mediterranean and 132 were sampled in the Eastern Mediterranean Sea.

Shortly after capture (within 1–3 h), the animals were weighed to the nearest kilogram and the ovaries (stripped of perivisceral fat) were weighed to the nearest gram. The gonado-somatic index (GSI) for each female was calculated according to the equation $GSI = (W_G/W) \times 100$, where W_G represents the gonad weight and W the total body weight. GSI values above 3 correspond to spawning females (electronic supplementary material, figure S1).

(d) Annual temperature cycles

NOAA CoastWatch Program and NASA's Goddard Space Flight Center (https://coastwatch.pfeg.noaa.gov/erddap/index.html) provided global SST data from NASA's Aqua Spacecraft at 4 km spatial resolution for the Western, Central and Eastern Mediterranean Sea. The data consisted of a composite of 8 days measured by the Moderate Resolution Imaging Spectroradiometer (data from August 2015 accessed on 10 October 2016). The algorithm for retrieving the SST data is based on the brightness temperature at 11 and 12 µm [22] (http://oceancolor.gsfc.nasa.gov/cms/atbd/sst). The time series covering the period from January 2003 to December 2015 were averaged yearly, obtaining the mean and standard deviations within the specific geographical limits of each Mediterranean study areas.

Temperature data for the Gulf of Mexico and the Slope Sea were generated using data from the 1/25° HYCOM analysis/reanalysis (depending on year) at a modelled water depth of 10 m. Average daily temperatures were calculated for the years 1993–2016. Temperature averages for the Gulf of Mexico were calculated for the area surveyed by SEAMAP, the eastern portion of the SEAMAP survey and the western portion of the SEAMAP survey. The Gulf of Mexico was defined as waters north of the Yucatan channel and west of the Straits of Florida. The area surveyed by SEAMAP was defined as the perimeter of the survey points from the spring plankton survey for all years. The area for the Slope Sea was estimated from the larval survey area [11].

(e) Annual chlorophyll values

Chlorophyll values for all of the areas were estimated using NASA's 9 km mapped monthly chlorophyll-a data product [23], which is based on intercalibrated SeaWIFS and MODIS-A satellite data. The area-based extractions were provided by the Coastal and Oceanic Plankton Ecology, Production and Observation Database (COPEPOD; http://www.st.nmfs.noaa.gov/copepod/), which is a global database of plankton survey data hosted by the National Marine Fisheries Service (NMFS) of the National Oceanic and Atmospheric Administration (NOAA).

(f) Annual zooplankton abundance

Monthly changes in zooplankton abundance sampled in the spawning area of the Western Mediterranean Sea were obtained from samplings conducted every 10 days during 1994–2003 using double oblique hauls from 0–70 m depth with a Bongo of 20 cm mouth diameter and mesh size of 250 µm [24]. No time series was available for the bluefin tuna spawning areas in the Central and Eastern Mediterranean Sea.

For the Gulf of Mexico and the Slope Sea, monthly averages of biomass of the entire zooplankton community, measured as bio-volume or average total sample displacement volume ml m^{-3}, was provided by COPEPOD. For the Slope Sea, data summarizes samplings collected by the NEFSC Ecosystem Monitoring Program collecting zooplankton using a bongo net (60 cm diameter, 333 µm mesh) towed obliquely from 200 m (or near the bottom to the surface) since 1977 in the Mid-Atlantic Bay. For the Gulf of Mexico, data from the 333 µm mesh Bongo nets were obtained from plankton surveys throughout the Gulf of Mexico since 1982 within the northwest and northeast off-shelf regions of SEAMAP.

(g) Experiments on egg hatching success and duration

Fertilized bluefin tuna eggs were collected from spontaneous spawning in captive populations placed in cages in 2013 and 2014. Eggs were collected and transported to the experimental facilities, arriving around 1 h later, when the eggs were in the 4–16 cell phase. The eggs were acclimated at the incubation water temperatures by increasing or decreasing the temperature at catch at a rate of 1°C every half hour to the target temperature. The eggs were then distributed among 250 ml flasks with approximately 50 eggs each, at controlled incubation temperatures between 18–33°C, with 3 replicates for each 1°C interval, per temperature. The experiments were conducted in a temperature-controlled room, set at 18°C. Each tank was equipped with a heater to warm the water, a thermostat to maintain the desired temperature and aeration to homogenize them within the tank. Temperatures remained constant throughout the experiment and were monitored continuously every 5 min. When the eggs began hatching, the flasks were controlled hourly. When all the eggs were hatched, the larvae were counted, identifying normal and abnormal larvae to calculate the hatching rate (rate of normal larvae with regard to total inoculated eggs).

(h) Experiments on larval growth rates

Fertilized bluefin tuna eggs were collected from spontaneous spawning in the broodstock cages in 2012 and 2015. The eggs were transferred to sixteen tanks of 1500 l volume with initial larval stocking densities of 7 larvae l^{-1} on average. Water salinity in the tanks was natural in the area and dissolved oxygen concentration was close to saturation. The photoperiod regime was 14 h of light and 10 h of darkness, as observed in nature, with a light intensity in the middle of the water column of approximately 250 lux. Four replicates were conducted for each temperature treatment with average temperatures (±s.d.) throughout the experimental period of 22.9 ± 0.9, 24.9 ± 0.7, 27.3 ± 0.6 and 27.7 ± 0.4°C.

The larvae were fed live prey supplied in excess two times per day. The feeding schedule consisted of enriched rotifers (*Brachionus plicatilis*) with densities within the tanks maintained at 10 rotifers ml^{-1}. Twenty-seven recently hatched larvae were sampled randomly, measured in length and dried at 60°C to obtain dry weight. The larvae in all the tanks were randomly sampled throughout the duration of the experiment. The larvae were sampled just before the lights were switched on to guarantee their stomachs were empty. On the last day of the experiment, all larvae were counted and 50 larvae were randomly subsampled. One of the replicates for the 27.3°C treatment was not considered further in the analyses due to technical problems.

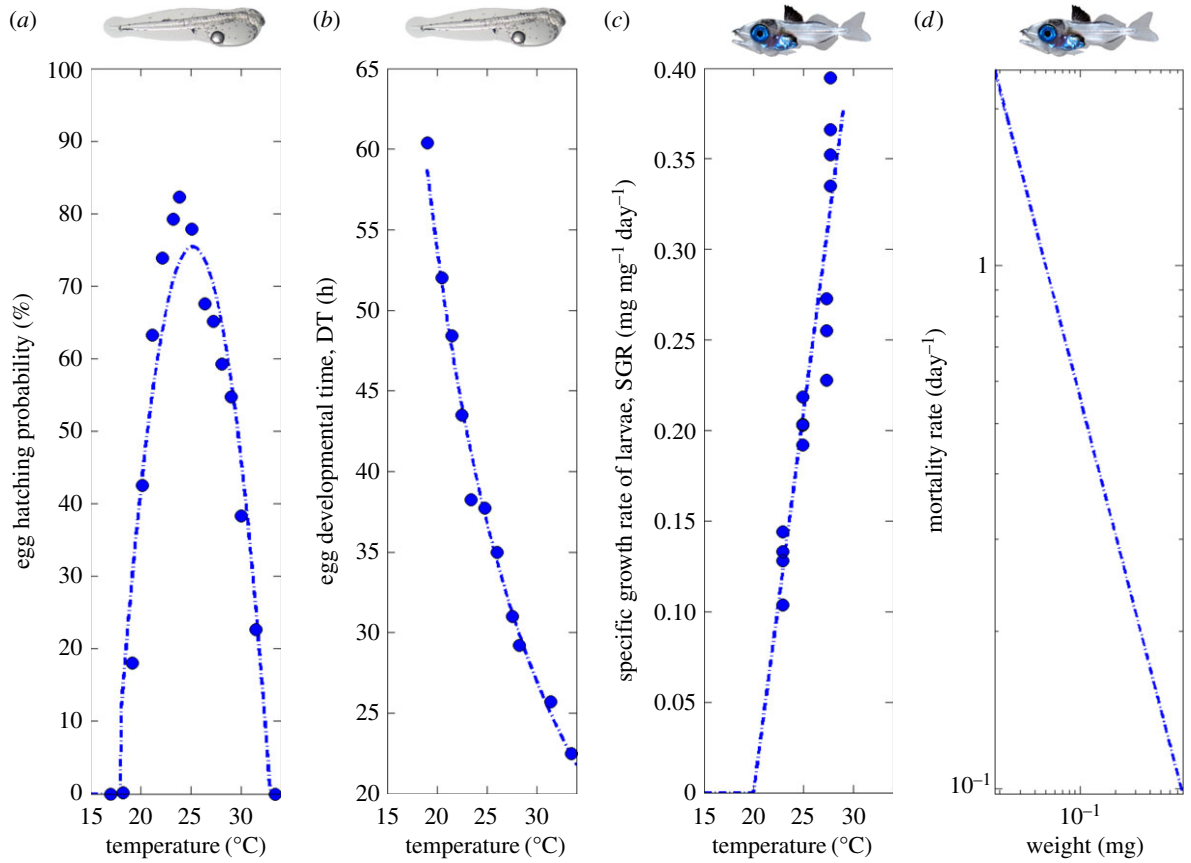

Figure 1. Empirical models from temperature-controlled rearing experiments performed on Atlantic bluefin tuna eggs and larvae. (*a*) The daily probability of egg hatching success (H, %) is temperature (T) dependent ($H = -1.27T^2 + 63.78T - 727.98, r^2 = 0.92, p < 0.001$), and below 19°C and above 32°C all eggs die. (*b*) After 22–60 h (DT, hours) depending on temperature ($\mathrm{DT} = 8787.5T^{-1.701}, r^2 = 0.99, p < 0.001$), eggs hatch into 0.018 mg (± 0.007, $n = 27$) dry weight larvae. (*c*) The larvae grow up to the postflexion stage (0.77 $\pm$ 0.25 mg dry weight larvae, $n = 275$) with a temperature-limited specific growth rate (SGR, mg mg^{-1} d^{-1}) assuming food satiation of ($\mathrm{SGR} = 0.0418T - 0.8355$, $r^2 = 0.84$, $p < 0.001$). (*d*) The larvae are subjected to size-dependent natural mortality [26] $M = 0.00022W^{-0.85}$. Experimental data are shown as blue dots. Lines indicate the fit. (Online version in colour.)

To stun and kill the larvae, a small dose (40 ppm) of clove oil (eugenol) was used.

The larvae were photographed live using a camera (Olympus SC20) connected to a dissecting microscope (Olympus SZ61-TR) and then frozen individually in cryotubes at −80°C for later examination. From images we measured individual standard length from the upper jaw tip to the notochord end using the software Image Pro 6.2. The frozen larvae were rinsed in distilled water, dried at 60°C over 24 h and weighed to estimate dry weight [25]. From the total of 1640 bluefin tuna larvae weighed and measured, 27 larvae corresponding to recently hatched larvae were randomly measured and weighed to estimate the initial larval dry weight (0.018 $\pm$ 0.007 mg) and standard length (3.82 $\pm$ 0.25 mm); 275 larvae corresponding to larvae in the flexion stage were used to estimate the dry weight at flexion (0.77 $\pm$ 0.25 mg) and the standard length at flexion (7.54 $\pm$ 0.46 mm). We fitted an exponential curve between age (experimental day) and dry weight for each tank within treatments and then used the estimated value for the slope in the fit against temperature treatment to estimate the temperature-dependent specific growth rate (electronic supplementary material, table S2).

(i) Model of egg fitness

We used the probability of survival for eggs spawned at different days of the year from hatching until larvae metamorphose and become piscivorous (the postflexion stage) as a proxy to determine the optimal spawning window. We call this egg fitness, and it integrates effects of temperature on hatching success and development time for both eggs and larvae, including mortality costs of longer development times. We developed empirical models of hatching probability (figure 1*a*), egg development time (figure 1*b*) and larval growth rate (figure 1*c*; electronic supplementary material, table S2) based on data from the temperature-controlled rearing experiments conducted with Atlantic bluefin tuna eggs and larvae described above. These temperature-dependent functions for Atlantic bluefin tuna covered the complete thermal range they can be exposed to, unlike earlier laboratory studies in Atlantic or other bluefin tuna species (electronic supplementary material, S1, figures S2–S4). In addition to egg-hatching success, we included the size-dependent mortality rates during the larval stage from a review using data from many species [26], also used in other studies for Atlantic bluefin tuna [27,28]. We assume the daily mortality rate (M) of larvae (dry mass less than 0.77 mg) is size-dependent [26] (figure 1*d*), ranging from 2.3 day^{-1} for eggs to 0.1 day^{-1} for flexion larvae. Field estimates of mortality rates in tuna species are inconsistent across studies and species, ranging from 0.06 to 2.71 day^{-1} [27,28]. In Atlantic bluefin tuna larvae (Western Mediterranean) mortalities are around 0.86 day^{-1} [29], but it is difficult to estimate accurately.

Most of the mortality is likely to take place during the egg and larval stages, and because temperature reduces stage duration, egg and larval survival increase rapidly in warmer water (figure 2*a*). If hatching success is the main (only) driver of timing for spawning, the best temperature is about 25 degrees (figure 2*b*). When we add size-dependent mortality, the integrated survival through egg stage, hatching and larval stage is much higher in warmer water (figure 2*b*). Lower mortality rates [30] reduce the difference in

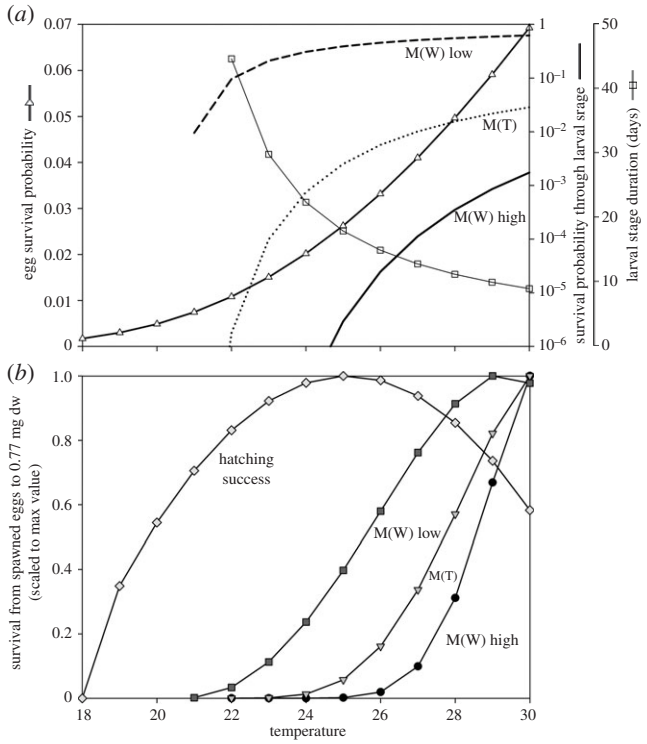

Figure 2. Sensitivity to temperature in bluefin tuna egg and larval survival probability under various assumptions about mortality rates. Survival is sensitive to temperature dependence in stage duration. (*a*) Survival through egg stage, assuming mortality rate is 2.3 day^{-1} [26] and egg development from figure 1*b*. Larval stage duration from hatching to the postflexion stage decrease with temperature (figure 1). Larval survival probability is size-dependent mortality rates for eggs- and larvae (*M* high) [26]; the (lower) general size dependence in fish mortality $M = 0.00526W^{-0.25}$ (*M* low) [30]; and temperature-dependent mortality rate $M = 0.0256 + 0.0123T$ ($M(T)$) [31]. (*b*) Survival of newly spawned eggs through to postflexion stage if hatching success is the only source of mortality ($M = 0$), and for the various assumptions about mortality rates. Values are scaled to the maximum value for comparison.

survival chance over temperatures. We also tried an empirical mortality function where mortality increases with temperature [31], but the integrated survival remains higher in warmer water due to reduced stage duration.

(j) Alternative drivers of reproductive timing

We tested the hypothesis that temperature alone can explain the observed spawning phenology in the eastern and western stocks. By combining the estimated temperature dependence of egg development, hatching success and larval growth with assumptions about size-dependent survival rates we can model the overall survival probability (egg fitness) as a function of spawning date from the annual temperature cycle in each spawning area (electronic supplementary material, figure S5). Then we used a model to assess how final body size, growth and size dependence in mortality rates influenced the optimal spawning time (electronic supplementary material, S2, temperature-controlled rearing experiments, S3, sensitivity simulations). We then compared actual observed spawning dates, seasonal patterns of chlorophyll and zooplankton abundance to see how well the ocean productivity cycle match observed spawning phenology in both eastern and western Atlantic bluefin tuna stocks (electronic supplementary material, table S1).

3. Results and discussion

The optimal spawning window predicted from modelling survival to the postflexion stage (egg fitness) as a function of *in situ* temperatures occurs much later than the observed spawning window (figures 3 and 4). If thermal effects on early life stages were the only driver of egg fitness, then the optimal spawning time in the Western and Central Mediterranean would be mid-August instead of the observed June–July (figure 3*a*,*b*), and July–September instead of the observed May–June in the Eastern Mediterranean and Gulf of Mexico (figures 3*c* and 4*b*,*c*). In the Slope Sea, observed larval occurrences fitted relatively well within the predicted optimal thermal window (figure 4*a*). The field data show that in most areas spawning occurs approximately two months before the predicted temperature-driven egg fitness peak and just after temperatures exceed 20°C, which allows eggs to hatch (figures 3 and 4) with some success. In the Gulf of Mexico, however, temperatures are always above the minimum hatching temperature (figure 4*b*,*c*).

The 20°C limit can explain the delayed onset of spawning from east to west in the Mediterranean Sea. It seems that bluefin tuna spawning respond to the earlier increase in temperature in the Eastern Mediterranean 20°C first on 4, 25 and 29 May in the Eastern, Central and Western Mediterranean respectively (figure 3). The delayed onset of spawning in the Slope Sea relative to the Gulf of Mexico is also explained by the occurrence of the 20°C temperature limit (figure 4). The predicted optimal spawning time is robust to the annual variance of the average year-round temperature (electronic supplementary material, table S3). Spawning occurs before the temperature optimum for the offspring is reached, independently of how mortality is parameterized (electronic supplementary material, figure S5).

The large inconsistency in predicted temperature-dependent egg fitness and observed spawning phenology suggests that tuna spawn as early as possible but after temperatures are above 20°C (figures 2 and 3). If the seasonal temperature follows a similar pattern every year, then a simple heuristic for spawning tuna, considering only temperature, is to spawn as temperatures pass approximately 20°C. Another cue may be the continuous increase in temperature, as seen in Gulf of Mexico (figure 4*b*,*c*). In Mediterranean spawning grounds, rising surface temperatures identify patterns in larval distribution better than the absolute temperature values [32]. Our results show Atlantic bluefin tuna spawn as early as possible, even if this leads to suboptimal temperature exposure. An important component to climate change studies is adult acclimation to warmer temperatures that can result in increased egg survival at the highest temperatures [33]. At the moment it is not feasible to control the temperatures experienced by the adult bluefin tunas in captivity, but egg hatching success could increase at higher temperatures. Our results assume the egg and larval relationships with temperature are the same for the different spawning areas. However, spawning adults may have adapted to local temperature regimes in each area.

The match and mismatch of early life stages to favourable environmental conditions have implications for fish productivity, but it is not trivial to know exactly which factors are important. Clearly, the evolution of spawning dates in

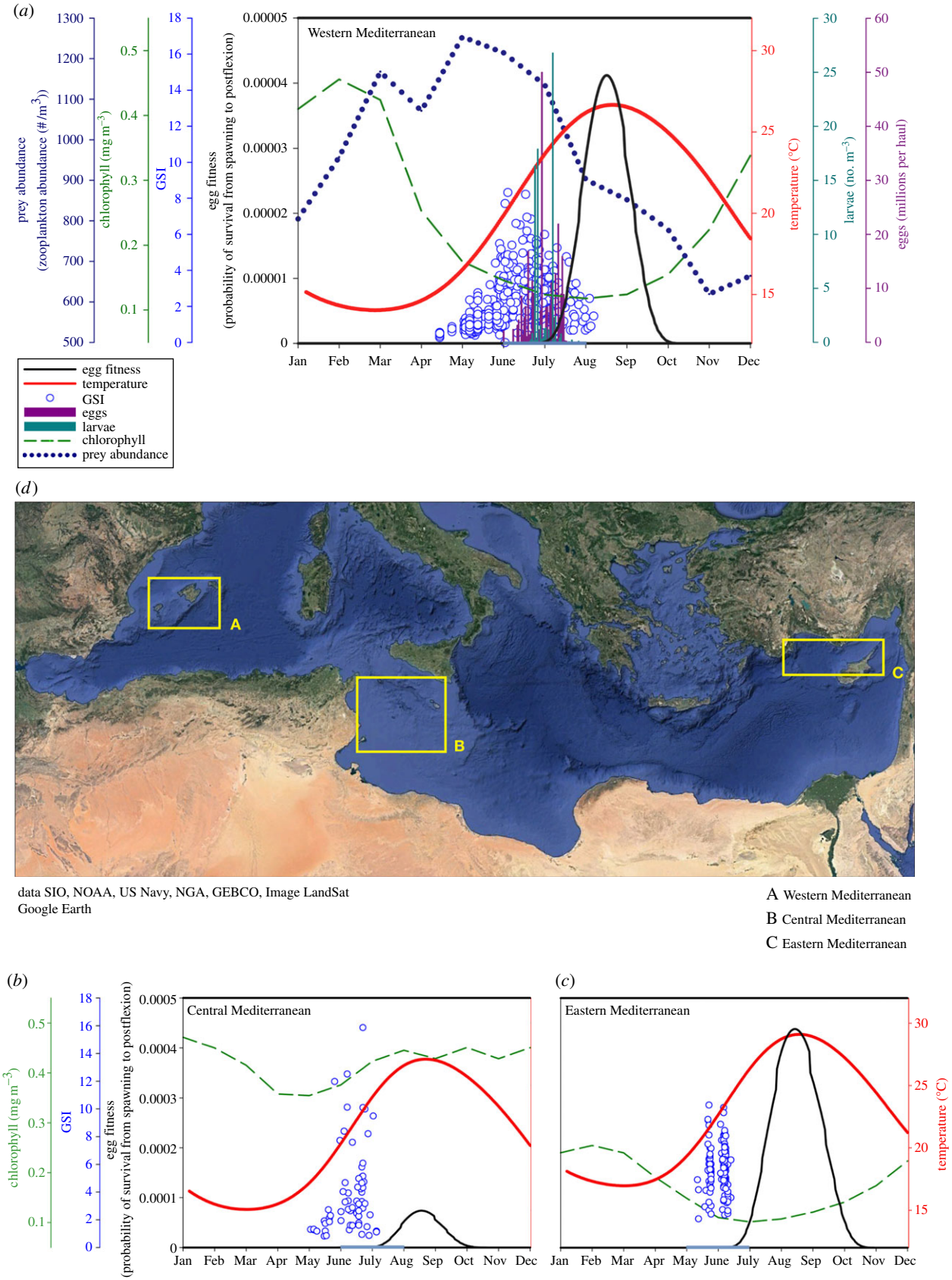

Figure 3. Predicted egg fitness and observed spawning phenology in the bluefin tuna eastern stock. Results are compared across the three major spawning grounds for bluefin tuna located in the (*a*) Western Mediterranean, (*b*) Central Mediterranean and (*c*) Eastern Mediterranean, outlined in yellow on the map (*d*). Legends are common for panels (*a–c*) and indicate for each area the variation in the predicted egg fitness or probability of survival of eggs from hatching to the postflexion stage as a continuous black line, the average temperature (°C) records as a continuous red line, the observed spawning phenology indicated by the gonad data (GSI) shown as open blue dots (528 female bluefin tuna for the Western Med, 64 for the Central Med, and 132 female bluefin tuna for the Eastern Med). Eggs (millions/haul) from spontaneous spawning in cages shown as purple bars and larvae abundances (no. m^{-3}) from research cruises shown as blue bars were only available for the Western Mediterranean study area. Chlorophyll (mg m^{-3}) is shown as a dashed green line for the three spawning areas whereas prey abundance (measured as zooplankton abundance, no. m^{-3}) is shown as a dark blue dotted line was only available for the Western Mediterranean study area. Grey bar on *x*-axis indicates the generally accepted duration of the spawning season.

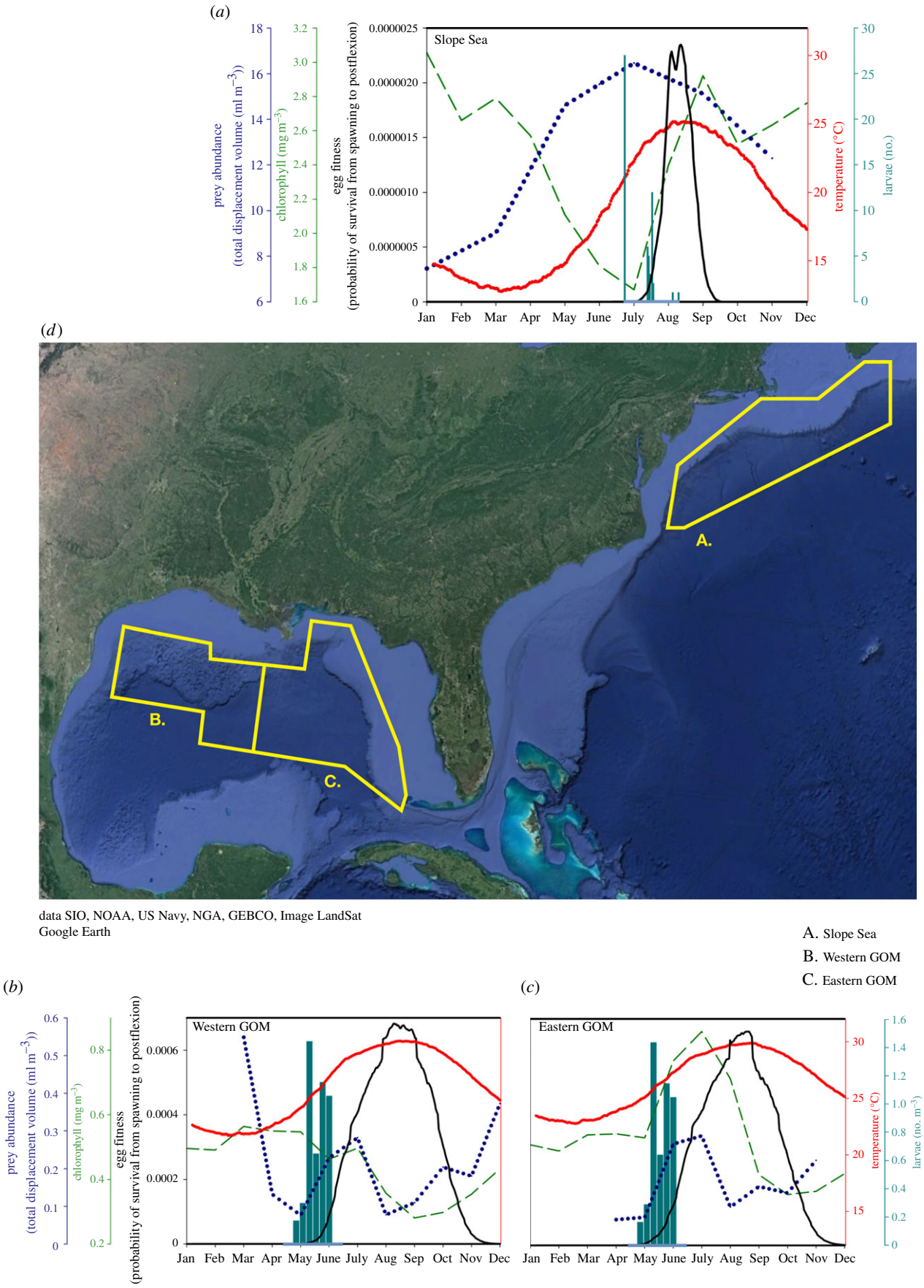

Figure 4. Predicted egg fitness and observed spawning phenology in the bluefin tuna western stock. Results are compared across the three major spawning grounds for bluefin tuna located in the (*a*) Slope Sea, (*b*) Western Gulf of Mexico and (*c*) Eastern Gulf of Mexico, outlined in yellow on the map (*d*). Legends are common for panels (*a*–*c*) and indicate the variation in the predicted egg fitness or probability of survival of eggs from hatching to the postflexion stage as a continuous black line, the average temperature (°C) records for each area as a continuous red line, larvae abundances (no. in (*a*) and no. m^{-3} in (*b*–*c*)) from research cruises shown as blue bars, chlorophyll (mg m^{-3}) shown as a dashed green line and prey abundance (measured as total displacement volume in ml m^{-3}) shown as a dark blue dotted line. Grey bar on *x*-axis indicates the generally accepted duration of the spawning season. Note for the Slope Sea the extent of the spawning ground and the overall duration of the spawning period is still unknown.

Atlantic bluefin tuna is not driven only by thermal tolerance or temperature exposure during early life stages. Instead, there is selection for spawning as early as possible within the viable period of the year. Other alternative drivers of the early spawning could be (i) maximization of larval zooplankton prey availability, (ii) size-dependent cannibalism among larvae, (iii) survival benefits at the juvenile stage of early hatching, (iv) energy constraints on parents or (v) reducing the exposure to larval predators. First, higher temperatures will increase growth and survival only if there is sufficient food. If a trade-off between foraging and survival exists, then higher food abundance always increases larval survival, even beyond satiating prey densities [34]. Atlantic bluefin tuna reproductive schedule may emerge from a trade-off between releasing eggs in the optimal temperature window and matching the larvae with high prey abundances (figures 3 and 4). The spring bloom occurs much earlier than bluefin tuna spawning in all areas, except in the Eastern Gulf of Mexico, indicating chlorophyll may not be a direct cue for spawning (figures 3 and 4) [6]. Second, tuna larvae are voracious piscivores that consume other fish larvae—in many cases other conspecific tuna larvae—and hence earlier spawning would increase the likelihood that offspring are predators rather than prey in trophic interactions [35]. Since an early switch from planktivory to piscivory in the larval stage yields growth and survival advantages [36], early breeding may have evolved from the benefit of consuming other fish larvae in an environment where zooplankton is scarce. Third, juvenile tuna grow at their fastest rates during summer and early autumn, with much slower growth rates in winter [37]. Therefore, a significant survival benefit from being large when winter begins could select for earlier spawning [38]. However, we obtained a similar seasonal egg fitness peak when the target size for fitness assessment was extended to include the juveniles compared to that obtained when the target size for fitness only included the larval stage (electronic supplementary material, table S4), suggesting survival during the juvenile phase is not enough to explain the early spawning phenology. Increasing mortality rates or changing target size for fitness assessment (survival probability) shift the modelled optimal spawning date up to three weeks later and do not eliminate the mismatch with the data (electronic supplementary material, table S4). Fourth, the parents' reproductive energy investment, an average loss of 15–26% of body mass after spawning [39], can limit the duration of reproductive activity since the condition may influence the adults in their migration back to Atlantic feeding grounds just after reproduction [8]. Given the oligotrophy of the spawning areas, the scarcity of food for the parents during spawning could explain the short duration of the reproductive window, but not the timing. The thermal stress on adults, often hypothesized to explain spawning times in the Gulf of Mexico for the western bluefin stock [7], is not likely to set time constraints for reproduction for the eastern stock, since maximum temperatures in the Mediterranean are never above 30°C (electronic supplementary material, figure S6), a temperature beyond which cardiac activity impairment occurs in big tunas [40]. Besides, water at depth may be cooler than at the surface, providing a thermal refuge to spawning adults. Elevated temperatures can have an inhibitory effect on fish [41,42], but the upper limit of temperature for heat-induced gonad degeneration in bluefin tuna has not been accurately established [43]. Finally, the oligotrophic spawning grounds may also be relatively deprived of potential predators on eggs and larvae, but we have few data on the seasonal cycles of their abundance.

Our understanding of how endangered large migratory marine species and top predators in the ocean adapt to environmental change is limited, but it is necessary to assess the synergistic consequences of climate variability and harvesting [44]. Early life stages in Atlantic bluefin tuna may tolerate a scenario of higher temperatures during egg and larval development, but the spawning phenology also suggests that larval fitness depends on seasonal ocean productivity and a match with zooplankton prey. Consequently, both changes in the seasonal production cycle and temperature are needed to forecast how global warming may affect bluefin tuna recruitment success, spawning distribution and migration.

Ethics. All experiments were carried out in accordance with the relevant guidelines on animal experimentation on fish. The methods used in the current study were accepted by the Ministry of Economy, Industry and Competitiveness of Spain and the Steering Committee of the project CTM2011-29525-C04-02.

Data accessibility. Temperature and fitness data supporting this article are available from the Dryad Digital Repository [45] (http://doi.org/10.5061/dryad.mg249).

Authors' contributions. P.R. and Ø.F. developed the concept of the paper. P.R., A.O., E.B. and F.d.l.G. performed the laboratory experiments and analysed the experimental data. F.J.A. and A.M. collected the adult field data and were in charge of the database. F.A., D.A.-B., L.R. and P.R. collected the larval data and were in charge of the database. A.O. and F.d.l.G. collected the egg data and were in charge of the database. R.B. coded the model. D.A.-B. and L.R. collected the environmental data. P.R, F.J.A., M.H. and D.A.-B. performed statistical analyses. All authors discussed the interpretations. P.R. and Ø.F. wrote the paper and all other authors provided intellectual insight and detailed comments.

Competing Interests. We declare we have no competing interests.

Funding. This research has received partial funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 678193 (CERES) and No 675997 (MARmaED) and the Bluefin tuna project. E.B. and M.H. acknowledge the pre-doctoral FPI Fellowship and postdoctoral grant support respectively from the regional government of the Balearic Islands, Conselleria d'Educacció, Cultura i Universitats, selected as part of an operational program co-financed by the European Social Fund. F.J.A. was partially funded by a Marie Curie Intra-European Fellowship (contract PIEF-GA-2012-326455) during the study. The experiments were approved and financed by project ATAME CTM2011-29525-C04-02, funded by the Spanish Ministry of Economy and Competitiveness.

Acknowledgements. We thank the people involved in the extensive field sampling, assistance of the people involved in the experimental work and data processing. Caladeros del Mediterráneo SL provided bluefin tuna eggs for the experiments. MODIS SST data were provided by NOAA CoastWatch Program and NASA's Goddard Space Flight Center. Samples of spawning females in the Eastern and Central Mediterranean were provided by Drs I. Oray, S. Karakulak, R. Vassallo-Agius and A. Corriero under the research project REPRO-DOTT, funded by the European Community Fifth Framework Programme under grant agreement no. Q5RS-2002-0153 and SELFDOTT, funded by the European Community Seventh Framework Programme under grant agreement no. 212797. We acknowledge the MODIS mission scientists and associated NASA personnel for the production of the data used in this research effort. We thank Todd O'Brien for providing data from the Coastal and Oceanic Plankton Ecology, Production and Observation Database (COPEPOD; http://www.st.nmfs.noaa.gov/copepod/). We thank Brian MacKenzie and two anonymous referees for useful comments on the manuscript.

References

1. McNamara J, Houston AI. 2008 Optimal annual routines: behavior in the context of physiology and ecology. *Phil. Trans. R. Soc. B* **363**, 201–319. (doi:10.1098/rstb.2007.2141)
2. Hjort J. 1914 Fluctuations in the great fisheries of Northern Europe viewed in the light of biological research. *Rapports et Procès Verbaux des Réunions du Conseil International pour l'Exploration de la Mer.* **20**, 1–228.
3. Cushing DH. 1990 Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Adv. Mar. Biol.* **26**, 249–293. (doi:10.1016/S0065-2881(08)60202-3)
4. IUCN. 2016 The IUCN Red List of Threatened Species. Version 2016-2. See http://www.iucnredlist.org.
5. Reglero P, Tittensor DP, Álvarez-Berastegui D, Aparicio-González A, Worm B. 2014 Worldwide distributions of tuna larvae: revisiting hypotheses on environmental requirements for spawning habitats. *Mar. Ecol. Prog. Ser.* **501**, 207–224. (doi:10.3354/meps10666)
6. Llopiz JK, Hobday AJ. 2015 A global comparative analysis of the feeding dynamics and environmental conditions of tunas, mackerels, and billfishes. *Deep Sea Res. II* **113**: 113–124. (doi:10.1016/j.dsr2.2014.05.014i)
7. Block BA *et al.* 2005 Electronic tagging and population structure of Atlantic bluefin tuna. *Nature* **434**, 1121–1127. (doi:10.1038/nature03463)
8. Aranda G, Abascal FJ, Varela JL, Medina A. 2013 Spawning behaviour and post-spawning migration patterns of Atlantic bluefin tuna (*Thunnus thynnus*) ascertained from satellite archival tags. *PLoS ONE* **8**, e76445. (doi:10.1371/journal.pone.0076445)
9. Heinish G *et al.* 2008 Spatial-temporal pattern of bluefin tuna (*Thunnus thynnus* L. 1758) gonad maturation across the Mediterranean Sea. *Mar. Biol.* **154**, 623–630. (doi:10.1007/s00227-008-0955-6)
10. Muhling BA *et al.* 2017 Reproduction and larval biology in tunas, and the importance of restricted area spawning grounds. *Rev. Fish Biol. Fisheries* **27**, 697–732. (doi:10.1007/s11160-017-9471-4)
11. Richardson DE *et al.* 2016 Discovery of a spawning ground reveals diverse migration strategies in Atlantic bluefin tuna (*Thunnus thynnus*). *Proc. Natl Acad. Sci. USA* **113**, 3299–3304. (doi:10.1073/pnas.1525636113)
12. Schaefer KM. 2001 Reproductive biology of tunas. In *Tuna physiology, ecology and evolution* (eds BA Block, ED Stevens), pp. 225–272. San Diego, CA: Academic Press.
13. Boyce DG, Tittensor DP, Worm B. 2008 Effects of temperature on global patterns of tuna and billfish richness. *Mar. Ecol. Prog. Ser.* **355**, 267–276. (doi:10.3354/meps07237)
14. Muhling BA, Lee SK, Lamkin JT, Liu Y. 2011 Predicting the effects of climate change on bluefin tuna (*Thunnus thynnus*) spawning habitat in the Gulf of Mexico. *ICES J. Mar. Sci.* **68**, 1051–1062. (doi:10.1093/icesjms/fsr008)
15. Muhling BA *et al.* 2013 Comparison between environmental characteristics of larval bluefin tuna *Thunnus thynnus* habitat in the Gulf of Mexico and western Mediterranean Sea. *Mar. Ecol. Prog. Ser.* **486**, 257–276. (doi:10.3354/meps10397)
16. Tsoukali S, Visser AW, MacKenzie BR. 2016 Functional responses of North Atlantic fish eggs to increasing temperature. *Mar. Ecol. Prog. Ser.* **555**, 151–165. (doi:10.3354/meps11758)
17. De la Gándara F, Ortega A, Buentello A. 2016 Tuna aquaculture in Europe. In *Advances in tuna aquaculture. From hatchery to market* (eds DD Benetti, GJ Partridge, A Buentello), pp. 115–157. New York, NY: Elsevier Academic Press.
18. De la Gándara F, Mylonas CC, Covès D, Bridges CR. 2012 SELFDOTT Report 2010–2011. See http://hdl.handle.net/10508/1118.
19. Alemany F *et al.* 2010 Characterization of the spawning habitat of Atlantic bluefin tuna and related species in the Balearic Sea (western Mediterranean). *Prog. Oceanogr.* **86**, 21–38. (doi:10.1016/j.pocean.2010.04.014)
20. Alemany F. 1997 Ictioplancton del Mar Balear. PhD thesis, University of Illes Balears, Palma de Mallorca.
21. Anon. 2007 REPRO-DOTT Final Report. Reproduction of the Bluefin Tuna in Captivity—feasibility study for the domestication of *Thunnus thynnus*. Contract number: Q5RS-2002-0153. See http://hdl.handle.net/10508/1010.
22. Minnett PJ *et al.* 2004 Sea-surface temperature measurements from the Moderate-Resolution Imaging Spectroradiometer (MODIS) on Aqua and Terra. *IGARSS 04 Proceedings* **7**, 4576–4579.
23. Hu C, Lee Z, Franz B. 2012 Chlorophyll a algorithms for oligotrophic oceans: a novel approach based on three-band reflectance difference. *J. Geophys. Res.* **117**, 1–25. (doi:10.1029/2011JC007395)
24. Puelles MF, Alemany F, Jansá J. 2007 Zooplankton time-series in the Balearic Sea (Western Mediterranean): variability during the decade 1994-2003. *Prog. Oceanogr.* **74**, 329–354. (doi:10.1016/j.pocean.2007.04.009)
25. Seljeset O, Vollset KW, Folkvord A, Geffen AJ. 2010 The role of prey concentration and size range in the growth and survival of larval cod. *Mar. Biol. Res.* **6**, 251–262. (doi:10.1080/17451000903150355)
26. McGurk MD. 1986 Natural mortality of marine pelagic fish eggs and larvae: role of spatial patchiness. *Mar. Ecol. Prog. Ser.* **34**, 227–242. (doi:10.3354/meps034227)
27. Mangel M, Brodziak J, DiNardo G. 2011 Reproductive ecology and scientific inference of steepness: a fundamental metric of population dynamics and strategic fisheries management. *Fish Fish.* **11**, 89–104. (doi:10.1111/j.1467-2979.2009.00345.x)
28. Simon M *et al.* 2012 Effects of stochasticity in early life history on steepness and population growth rate estimates: an illustration on Atlantic Bluefin tuna. *PLoS ONE* **7**, e48583. (doi:10.1371/journal.pone.0048583).
29. Moltó M. 2015 Tracking a high density bluefin tuna larval patch: development of methodologies to calculate daily mortality and growth rates. Master degree in marine ecology, Universitat de les Illes Balears, Spain.
30. Peterson I, Wroblewski JS. 1984 Mortality-rate of fishes in the pelagic ecosystem. *Can. J. Fish. Aquat. Sci.* **41**, 1117–1120. (doi:10.1139/f84-131)
31. Houde ED. 1989 Comparative growth, mortality, and energetics of marine fish larvae—temperature and implied latitudinal effects. *Fish. Bull.* **87**, 471–495.
32. Alvarez-Berastegui D *et al.* 2016 Pelagic seascape ecology for operational fisheries oceanography: modelling and predicting spawning distribution of Atlantic bluefin tuna in Western Mediterranean. *ICES J. Mar. Sci.* **73**, 1851–1862.
33. Donelson JM, Munday PL. 2012 Thermal sensitivity does not determine acclimation capacity for a tropical reef fish. *J. Anim. Ecol.* **81**, 1126–1131. (doi:10.1111/j.1365-2656.2012.01982.x)
34. Fiksen Ø, Jørgensen C. 2011 Model of optimal behavior in fish larvae predicts that food availability determines survival, but not growth. *Mar. Ecol. Prog. Ser.* **432**, 207–219. (doi:10.3354/meps09148)
35. Reglero P, Urtizberea A, Torres AP, Alemany F, Fiksen Ø. 2011 Cannibalism among size classes of larvae may be a substantial mortality component in tuna. *Mar. Ecol. Prog. Ser.* **433**, 205–219. (doi:10.3354/meps09187)
36. Reglero P *et al.* 2014 Size-related differences in growth and survival in piscivorous fish larvae fed different prey types. *Aquaculture* **433**, 94–101. (doi:10.1016/j.aquaculture.2014.05.050)
37. Labelle M, Hoch T, Liorzou B, Bigot J. 1997 Indices of bluefin tuna (*Thunnus thynnus thynnus*) abundance derived from sale records of French purse seine catches in the Mediterranean Sea. *Aquat. Living Resour.* **10**, 329–342. (doi:10.1051/alr:1997036)
38. Hurst TP, Conover DO. 1998 Winter mortality of young-of-the-year Hudson River striped bass (*Morone saxatilis*): size-dependent patterns and effects on recruitment. *Can. J. Fish. Aquat. Sci.* **55**, 1122–1130. (doi:10.1139/f98-017)
39. Rodríguez-Roda J. 1964 Biología del atún, *Thunnus thynnus* (L.), de la costa sudatlántica de España. *Invest. Pesq.* **25**, 33–146.
40. Blank JM *et al.* 2004 In situ cardiac performance of Pacific bluefin tuna hearts in response to acute temperature change. *J. Exp. Biol.* **207**, 881–890. (doi:10.1242/jeb.00820)

41. Wood AW, Van der Kraak GJ. 2001 Apoptosis and ovarian function: novel perspectives from the teleosts. *Biol. Reprod.* **64**, 264–271. (doi:10.1095/biolreprod64.1.264)
42. Pankhurst NW, Munday PL. 2011 Effects of climate change on fish reproduction and early life history stages. *Mar. Freshwater Res.* **62**, 1015–1026. (doi:10.1071/MF10269)
43. Gen K. 2015 Physiology of bluefin tuna reproduction: new insights into reproduction in wild and captive bluefin tuna species. In *Biology and ecology of bluefin tuna* (eds T Kitagawa, S Kimura), pp. 325–354. Boca Raton, FL: CRC Press.
44. Hobday AJ *et al.* 2013 Climate impacts and oceanic top predators: moving from impacts to adaptation in oceanic systems. *Rev. Fish. Biol. Fisheries* **23**, 537–546. (doi:10.1007/s11160-013-9311-0)
45. Reglero P *et al.* 2018 Data from: Atlantic bluefin tuna spawn at suboptimal temperatures for their offspring. Dryad Digital Repository. (https://doi.org/10.5061/dryad.mg249)