A simulated marine heatwave impacts European sea bass sperm quantity, but not quality

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Abstract

Rapid environmental changes will be the major challenge that most biota will have to deal with in the near future. Extreme events, such as marine heatwaves, are becoming more frequent and could be spatially uniform at a regional scale for a relatively long period of time. To date, most research studies on heatwaves have focused on sessile organisms, but these extreme events can also impact mobile species. Here, a 3-week marine heatwave was simulated to investigate its effects on the male reproductive performance of a Mediterranean Sea emblematic species, the European sea bass *Dicentrarchus labrax*. Males from the control condition (*c.* 13°C) produced significantly more sperm than those exposed to a relatively warm thermal treatment (*c.* 16°C). Nonetheless, neither the percentage of motile spermatozoa nor most of the other sperm motility parameters were significantly affected by the rearing temperature over the whole period. Overall, the results of this study suggest only moderated effects of a potential winter heatwave on the reproductive performance of male European sea bass.

KEYWORDS
global warming, Mediterranean Sea, reproduction, sperm quantity

1 | INTRODUCTION

According to the Intergovernmental Panel on Climate Change technical summary (Arias *et al*., 2021), the global mean of sea surface temperature (SST) has increased by 0.88°C since the beginning of the 20th century. One might consider that this increase would only have minor effects on fish, and would argue that species would have sufficient time to adapt. In reality, this relatively slow increase of the mean hides drastic effects linked to the intensity of marine heatwaves that could last for several days. Indeed, surface ocean marine heatwave days (defined as days exceeding the 99th percentile in SST relative to 1995–2014) are expected to increase by a factor 5–10 by 2100 (Arias *et al*., 2021). Nowadays, the effect of global warming is already preoccupying, and it affects various functions related to fish physiology (McKenzie *et al*., 2021), such as gametogenesis and the capacities of fish to successfully reproduce (Alix *et al*., 2020).

Yet, most of the work has been conducted on females, whereas less is known regarding males (Alix *et al*., 2020). In addition, the few studies investigating the effects of warming on milt quality and quantity have been performed in freshwater fishes with contrasting results (Alix *et al*., 2020). For instance, Ashton *et al*. (2019) detected that milt quantity decreased in the Burbot, *Lota lota* (L. 1758) as the temperature increased (from 2 to 6°C during 21 days), though they did not quantify to what extent. Sperm quantity decreased by a factor of 2 in the common bream, *Abramis brama* (L. 1758), when exposed to +3°C for 10 days (Targońska *et al*., 2014). On the contrary, *Gambusia holbrooki* males (Girard, 1859) exposed to high temperature (30°C) produced about three times more sperm than those kept in colder water (18°C) (Adriaenssens *et al*., 2012). Regarding sperm characteristics, sperm motility of the European grayling, *Thymallus thymallus* (L. 1758) decreased with temperature (Lahnsteiner & Kletzl, 2012). Interestingly, only 1°C of difference was sufficient to trigger the change in

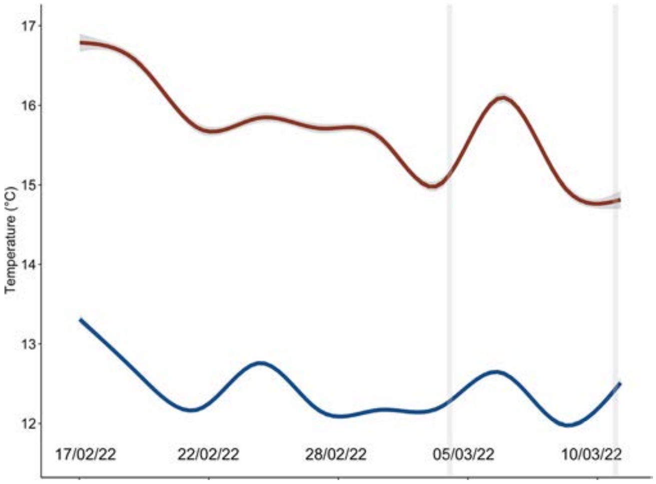

FIGURE 1 Temperatures experienced by adult males *Dicentrarchus labrax* from 17 February 2022 to 11 March 2022. Natural temperature is indicated in blue, and relatively warm temperature is indicated in red. Natural temperature, Warm temperature

motility (Lahnsteiner & Kletzl, 2012), whereas an increase of temperature of 5°C did not change the motility in the sperm of brown trout *Salmo trutta* (L. 1758) (Lahnsteiner & Leitner, 2013).

This study aimed to investigate the effect of high temperature exposure within the natural spawning period (*i.e.*, between January and March, Abascal *et al.*, 2007; Fauvel *et al.*, 1999) of a marine emblematic fish species for the Mediterranean Sea: the European sea bass *Dicentrarchus labrax* (L. 1758). Specifically, the authors tested the effects of an exposure to +3°C over 3 weeks on (a) milt volume production, (b) sperm motility parameters and (c) survival of spermatozoa.

2 | MATERIALS AND METHODS

The study was conducted in February and March 2022 in the Aquaculture Experimental Station Ifremer, Chemin de Maguelone, France. A total of 21 three-year-old males *Dicentrarchus labrax* were randomly divided into two groups in fibreglass tanks (5 m^3) with a continuous supply of sea water (300 l h^{-1}). One group was kept at natural water temperature (around 13°C), and the other one was kept at *c.* +3°C for 3 weeks (Figure 1), thanks to a thermostat (Aquavie ICE 3000, Connaux, France). From 11 February 2022, the temperature was gradually increased at a rate of ±0.5°C day^{-1} during 6 days in the warm condition to reach the desired temperature at the start of the experiment: 17 February 2022 = Day 0. Then, the temperature was manually adjusted each day to follow the natural decrease of temperature observed over time. Temperature was automatically recorded in each tank every 30 min (thanks to a HOBO temperature data logger) during the 23 days of experiment. There were no significant differences in fish weight based on the treatment (natural temperature: 3.5 ± 0.25 kg *vs.* warm: 3.8 ± 0.19 kg; *t*-test *P*-value = 0.3). Fish were fed daily with commercial diet containing 51% protein, 16% fat and 1% fibre. Sperm samples were collected on different fish after 16 and 23 days of rearing at the two different temperatures: natural ($n = 6$ at 16 days and $n = 4$ at 23 days) and warm ($n = 6$ at 16 days and $n = 5$ at 23 days).

Milt samples were collected by manual stripping, taking care of avoiding urine contamination. The ejaculate volume of each male was measured directly during the milt collection using 1 or 5 ml syringes. Each syringe was immediately placed at 4°C until being processed in less than 1 h. To assess sperm concentration, milt samples were diluted at a ratio of 1:2000 (1:20 and 1:100) with formaldehyde-buffered saline. Then, the spermatozoa were counted using a Thoma haemocytometer under an optical microscope at ×400 magnification. The results are expressed as spermatozoa × 10^9 ml^{-1}. Sperm motility was assessed using a CASA (Computer-Assisted Sperm Analysis) system (IVOS II, Hamilton Thorne, Beverly, MA, USA). Milt samples (2 μl) were activated with 1000 μl of seawater solution containing bovine serum albumin 2%, to prevent the sperm sticking to the slide (Pérez *et al.*, 2016). Immediately after sperm activation, 3 μl of the sperm subsample was loaded onto a standard four-chamber slide (Leja Products B.V., Nieuw Vennep, The Netherlands). This was done readily because the duration of sperm motility is particularly low in the European sea bass, tipically between 40 and 50 s (Abascal *et al.*, 2007; Fauvel *et al.*, 1999). The following motility parameters were recorded before 10 s: percentage of total motile sperm, curvilinear velocity (VCL, μm s^{-1}), straight-line velocity (VSL, μm s^{-1}), average path velocity (VAP, μm s^{-1}), linearity (LIN, %), progression (PROG, %), amplitude of lateral head (ALH, μm) displacement, straightness index (STR, %) and wobble (WOB, %). Measurements of sperm kinetic were carried out in triplicate on each of the 21 individuals. In addition, a viability test was carried out using the fluorescence LIVE/DEAD Sperm Viability Kit (Molecular Probes, Invitrogen, Eugene, OR, USA) and flow cytometry on the fish producing sperm after 23 days (11 March). A milt sample (0.5 μl) was incubated with 0.75 μl of SYBR-14 and 0.75 μl of propidium iodide at 10°C in the dark for 15 min. Thereafter, samples were centrifuged at 160 g for 5 min, suspended in phosphate-buffered saline and analysed with a FACS CantoII flow cytometer (BD Bioscience, Franklin Lakes, NJ, USA). Forward and side scatter areas were used to select the cells of interest. Then forward scatter area and forward scatter time-of-flight were crossed to remove doublets allowing an analysis on singulet cells. To consider these repeated measurements on the same individual, all data were analysed with linear mixed-effects models, with individuals as random variables and both treatment (warm or natural) and time (16 or 23 days) as fixed factors. All statistical analyses were performed with R (R Core Team, 2022), and mixed-effects models were performed using the lme4 package (Bates *et al.*, 2015). Plots were done with ggplot2 (Wickham, 2016) and data were given as mean ± S.E.

2.1 | Compliance with ethical standards

All fish were handled in accordance with the Universities Federation for Animal Welfare Use and Care Committee Handbook on the Care and Management of Laboratory Animals (http://www.ufaw.org.uk/pubs.htm#Lab) and following the guidelines for animal

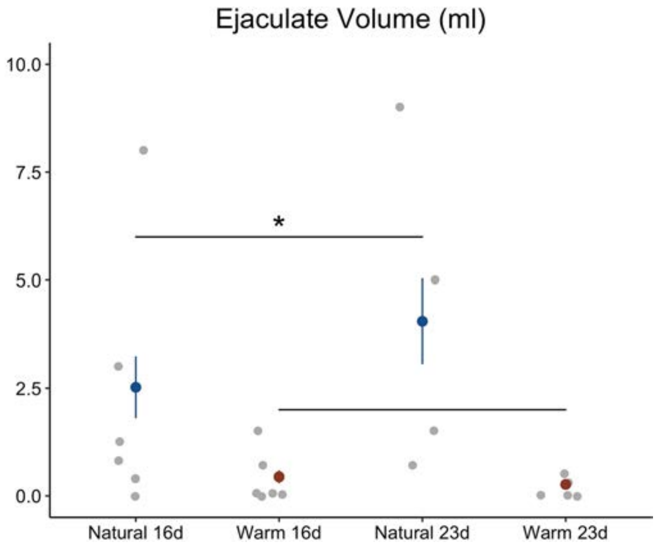

FIGURE 2 Ejaculate volume of adult males European sea bass *Dicentrarchus labrax* collected after 16 and 23 days (16d and 23d) and from the two thermal treatments (natural and warm). Each grey point represents the volume sampled on each individual, whereas the blue and the red points represent the mean ± S.E. of the natural and the warm treatments at each time, respectively. * indicates a significant difference between treatments where $P < 0.05$

experimentation established by Directive 2010-63-EU of the European Union and the corresponding French legislation.

3 | RESULTS

The water temperature of both treatments is shown in Figure 1. Usually, the mean temperature experienced by European sea bass during the winter is considered to be 13°C in the same geographical zone (Abascal *et al*., 2007; Fauvel *et al*., 1999). Here, the monitoring of the fish from 1 January 2022 to 11 March 2022 was in agreement with those previous findings, with a mean natural temperature of 13.1 ± 0.1°C. Overall, fish kept in warm temperatures produced significantly ($P < 0.05$) less volume of sperm when compared to those kept in natural temperatures (Figure 2). Regarding sperm concentration, no significant differences were found among thermal treatments or between experimental days. The percentage of motile spermatozoa was not significantly affected by the rearing temperature or experimental days (Figure 3). For fish kept in warm temperatures, the mean values of spermatozoa velocities (VCL, VSL and VAP) were not significantly different from those kept in controlled condition. The thermal treatment and the experimental time did not affect the indices of sperm motility (LIN, PROG and WOB). Only the ALH was significantly lower in sperm samples of fish kept in warm than those in natural temperatures (Figure 3). The STR variable (VSL/VAP) was significantly higher for warm than control fish (Figure 3). Because only two fish produced sperm after 23 days at warm temperature, the authors only compared sperm viability to two others randomly chosen fish kept at natural temperature. The mean values of sperm viability were close to 65%, with no significant differences between the treatments.

4 | DISCUSSION

The ongoing climate crisis is causing several changes in environmental conditions, including a well-described increase in water temperature. The reproduction of aquatic organism occurs in a limited temperature range (Farhadi & Harlıoglu, 2018; Miranda *et al*., 2013), and for this reason, one might wonder about the potential effects of climate change on gamete quality. For the European sea bass, the usual temperature experienced in the winter is around 13°C in the western Mediterranean zone, and this study shows that an increase of 3°C over a period of 3 weeks reduced significantly the milt volume produced. In river lamprey *Lampetra fluviatilis* (L. 1758) exposed to three different controlled thermal regimes (7, 10 and 14°C) during 6 months, only 30% of males produced milt when temperatures were increased to 10 and 14°C (Cejko *et al*., 2016). Nevertheless, there were no significant differences on the average milt volume, sperm concentration and total sperm production between the three regimes (Cejko *et al*., 2016). It is likely that the observed decrease in milt volume in the present experiment would involve alterations in the endocrine regulation of the hypothalamus-pituitary-gonadal axis. High water temperatures for *Cyprinodon variegatus* (Lacepède, 1803) showed an increase, in males and females, of gene transcripts encoding gonadotropin-inhibiting hormone (*gnih*) and gonadotropin-releasing hormone-3 (*gnrh3*), as well as decreased beta-subunits of follicle-stimulating hormone (*fshβ*) and LH hormone (*lhβ*) only in males (Bock *et al*., 2021). In *D. labrax*, GnIH was shown to decrease the plasma levels of luteinizing hormone LH (Paullada-Salmero *et al*., 2016). LH increases the production of testicular steroids (11-ketotestosterone, KT-11 or 17α, 20β-dihydroxy-4-pregnen-3-one, DHP), which together are clearly involved in the regulation of spermiation in fish. Nonetheless, the mechanisms of action of these hormones on milt hydration and sperm migration to the sperm duct or increase in milt volume are still unclear (Schulz *et al*., 2010).

Regarding sperm concentration, there were no significant differences between temperatures or experimental periods. It should be noted that the sperm concentration recorded in this study was lower than that reported by Fauvel *et al*. (1999) in the same species. Those authors recorded a pic (60×10^9 spermatozoa/mL) in February, which then decreased with the advancement of the reproductive period (Fauvel *et al*., 1999). Because this study was performed in March, it is likely that the lower concentration observed here was only linked to the time-point of sperm collection. Nevertheless, it is important to pinpoint that the sperm concentration of this species shows a great variation between studies (5.5 to 144×10^9 spermatozoa ml^{-1}) (Sorbera *et al*., 1996; Rainis *et al*., 2003). Here, the values obtained are higher than those reported by Asturiano *et al*. (2001) and similar to those obtained by Felip *et al*. (2006).

To date, the effects of temperature changes on sperm motility parameters are scarce in fish species. In this respect, Cejko *et al*.

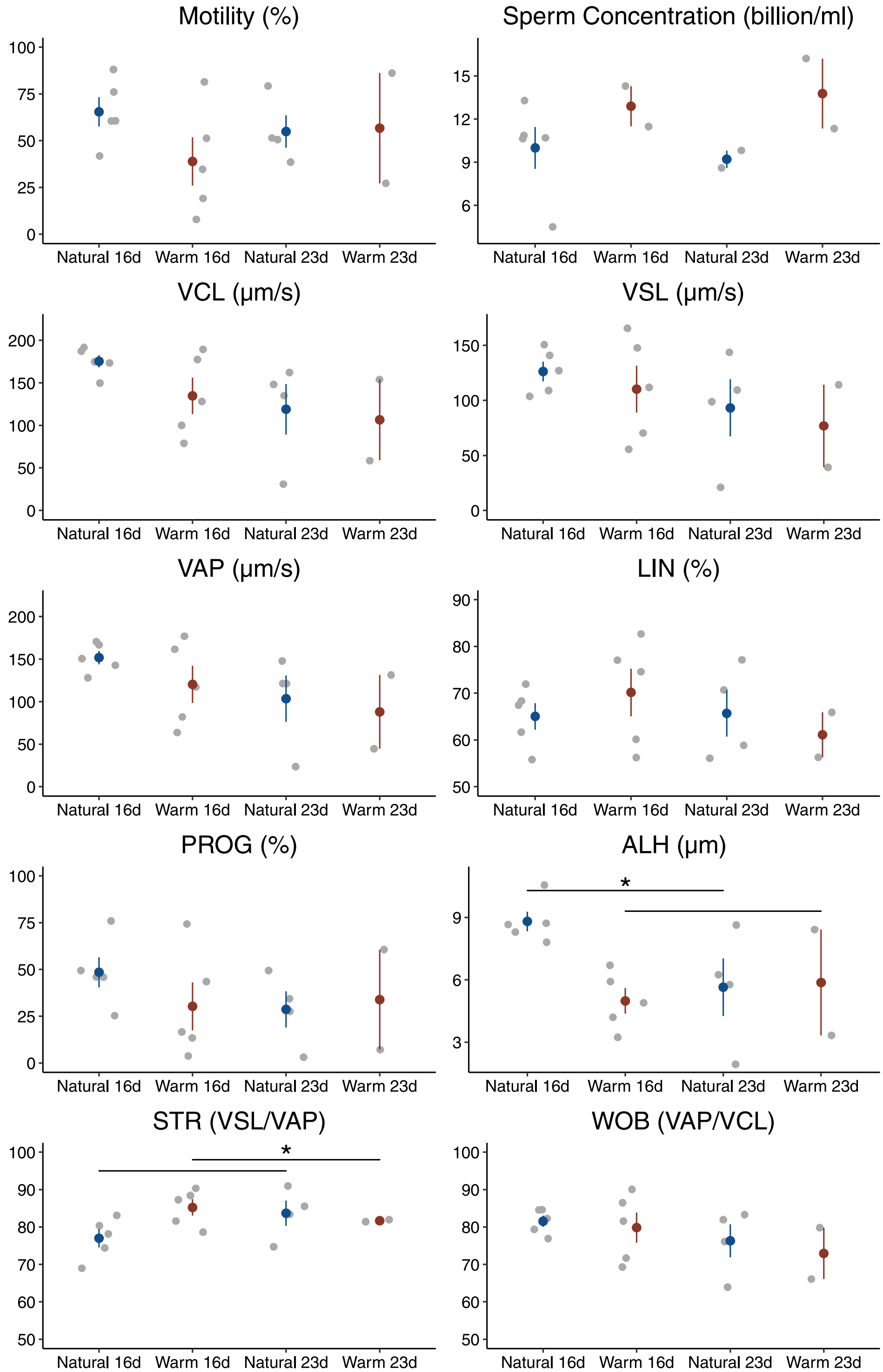

FIGURE 3 Measurements of sperm characteristics using the CASA (Computer-Assisted Sperm Analysis) system (IVOS II, Hamilton Thorne, Beverly, MA, USA). Percentage of total motile sperm, curvilinear velocity (VCL, µm s^{-1}), straight-line velocity (VSL, µm s^{-1}), average path velocity (VAP, µm s^{-1}), linearity (LIN, %), progression (PROG, %), amplitude of lateral head (ALH, µm) displacement, straightness index (STR, %) and wobble (WOB, %), as well as sperm concentration, are represented. Each ejaculate was collected after 16 and 23 days (16d and 23d) and from the two thermal treatments (natural and warm). Each grey point represents the value for one individual, whereas blue and red points represent the mean ± S.E. of the natural and the warm treatments at each time, respectively. * indicates a significant difference between treatments where $P < 0.05$

(2016) detected higher sperm performance [motility, progressive motility (PM) and velocities] in river lamprey maintained at 7°C in comparison to that maintained at 14°C. On the contrary, in *Gambusia holbrooki*, the VSL was significantly higher when the fish were reared at 30°C compared to 18°C. In this study, no significant differences were found for the most important kinetic parameters. Regarding motility, the spermatozoa of the warm treatment presented a mean value that tended to be below that of the natural treatment, and both were lower than those reported by Abascal *et al.* (2007) in the same species, where fresh milt presented 86.67% motility and sperm velocity values similar to those of this study. Mitochondria is important for the activation and sustainability of motility and progressive movement of spermatozoa due to the production of ATP that can be altered using oxidative stress leading to increased reactive oxygen species (ROS) (Figueroa *et al.*, 2017; Sandoval-Vargas *et al.*, 2021). ROS has been reported to affect the spermatozoa, especially due to the presence of a double lipid membrane. The rise in oxidative stress in aquatic species is increased by environmental factors, such as temperature changes, oxygen levels, salinity, pesticides, among others, and because sperm have a low content of cytoplasm, it is the seminal plasma that provides the main defences against ROS (Cabrita *et al*., 2014). Therefore, the low level of seminal plasma in the spermatozoa from the warm treatment probably activated the mitochondrial function before obtaining the samples due to oxidative stress. This explains why the STR index is higher, because the spermatozoa probably managed to go in a straight line with a higher speed at the beginning, but not constantly. This could also explain why they have presented a lower ALH. Confidently, these spermatozoa presented their highest peak of ATP immediately when activated and did not experience a stop of PM, which was rather almost immediate. Several kinetic parameters such as total motility, PM, VCL, VSL VAP, LIN and WOB had been proposed as good indicators of fertility and hatching rates (Gallego *et al*., 2013). Changes in the pattern of STR and ALH parameters have not been significantly related to the sperm function so far.

Regarding sperm viability, there were no significant differences when the fish were reared 3°C above the natural temperature. This might be due to the fact that the maximum temperature evaluated in this study was not above the maximum critical temperature for the species. Sperm membrane damages are usually related to more stressful factors such as cryopreservation or oxidative stress (Sandoval-Vargas *et al*., 2021).

It is evident that warmer-than-optimal temperatures may alter the reproductive capacity in some fish species. In this study, males' *D. labrax* kept at *c*. 16°C showed significantly lesser milt volume and lower values of ALH than those males reared at *c*. 13°C, whereas the STR variable was significantly higher in the warm temperature. It is likely that the difference in the range of temperature evaluated was not enough to lead to drastic impairment of the sperm quality. It is important to stress that the best indicators of sperm quality are not necessarily the motility nor the ability to fertilize an oocyte, but it also relies on the capacity to contribute in the obtention of a high percentage of live and hatched embryos (Pérez-Atehortúa *et al*., 2022). Therefore, future studies should also include this parameter to ensure that sperm quality is not affected by such an increase in temperature. Anticipating potential effects of warming at long term is of vital importance, and more studies are needed to really capture what will happen regarding fish reproduction in this context of global warming.

AUTHOR CONTRIBUTIONS

Benjamin Geffroy: data generation, data analysis, manuscript preparation, funding. Leydy Sandoval-Vargas: data generation, data analysis, manuscript preparation. Myriam Boyer-Clavel: data generation. Maritza Pérez-Atehortúa: manuscript preparation. Stephane Lallement: data generation. Iván Valdebenito Isler: data generation, data analysis, manuscript preparation, funding.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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