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Oyster larvae used for ecosystem restoration benefit from increased thermal fluctuation

Katharina Alter [a,*], Pascalle Jacobs [a,1], Annalisa Delre [a], Bianka Rasch [a], Catharina J.M. Philippart [a,b], Myron A. Peck [a,c]

[a] Royal Netherlands Institute for Sea Research, Department of Coastal Systems, 59, 1790 AB Den Burg, the Netherlands
[b] Utrecht University, Department of Physical Geography, 80.115, 3508 TC Utrecht, the Netherlands
[c] Wageningen University, Department of Animal Sciences, Marine Animal Ecology Group, De Elst 1, 6708 WD Wageningen, the Netherlands

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ABSTRACT

A bottleneck in restoring self-sustaining beds of the European oyster (*Ostrea edulis*) is the successful development and settlement of larvae to bottom habitats. These processes are largely governed by temperature but a mechanistic understanding of larval performance across ecologically relevant temperatures is lacking. We reared larvae at low (20–21 °C) and high (20–24 °C) fluctuating temperatures and applied short-term exposures of larvae to temperatures between 16 and 33 °C to assess vital rates and thermal coping ranges. Larval thermal preference was between 25 and 30 °C for both rearing treatments which corresponded with optimum temperatures for oxygen consumption rates and locomotion. Larvae had 5.5-fold higher settling success, however, when reared at the high compared to the low fluctuating temperatures. Higher mean and periods of increased temperature, as projected in a future climate, may therefore enhance recruitment success of *O. edulis* in northern European habitats.

1. Introduction

The loss of habitat-forming species has contributed to biodiversity loss, weakened resilience against climate change and decreased the provision of ecosystem services in coastal marine systems (Barbier et al., 2011). For example, in European coastal waters and the North Sea, the European flat oyster *Ostrea edulis* (Linnaeus 1758) was once an abundant reef-forming bivalve but is now listed as threatened (OSPAR, 2008, 2013). Habitats created by oysters and other reef-forming species enhance biodiversity by providing refugia and feeding grounds for co-existing species. Moreover, oysters filter phytoplankton, impact nutrient cycles of shelf seas and can mitigate poor water quality caused by excess nutrients (Fulford et al., 2010; Kamermans et al., 2020).

Large-scale restoration of *O. edulis* beds is underway in the North Sea by reintroducing adult oysters and the ultimate success of these efforts will depend on the natural production of larvae by a large number of adults and the subsequent pelagic development and benthic settlement of the larvae (Sawusdee et al., 2015; Bennema et al., 2020). In the past, several oyster restoration projects have failed because adults died after translocation and/or the reproductive success of individuals was poor (Korringa, 1946; Bromley et al., 2016).

During spawning, *O. edulis* females release eggs into their mantle cavity and draw in sperm that was released from males into the ocean. After internal fertilization, females brood their offspring until they developed into D-shape veligers which are released. These pelagic larvae pass through two further stages, umbo veligers and pediveligers, before they settle as spat on, preferably, adult individuals (Bayne, 1969). Juveniles first develop into males and subsequently alternate between sexes which may be dependent on local temperature conditions (Eagling et al., 2018). Also, the time required for larval development depends on temperature while the sum of the temperatures leading up to adult spawning appears to be most important for the timing of larval emergence (Robert et al., 2017; Maathuis et al., 2020). Deep-sea individuals from the English Channel, Scotland and Helgoland are assumed to commence spawning at 12–13 °C, while individuals from France, England and the Netherlands spawn when waters warm to 15–16 °C and

* Corresponding author.
E-mail addresses: Katharina.alter@nioz.nl (K. Alter), Pascalle.jacobs@hz.nl (P. Jacobs), Annalisa.delre@nioz.nl (A. Delre), Bianka.rasch@nioz.nl (B. Rasch), Katja.philippart@nioz.nl (C.J.M. Philippart), Myron.peck@nioz.nl (M.A. Peck).
[1] Present address: HZ University of Applied Sciences, Research Group Aquaculture in Delta Areas, P.O. Box 364, 4380 AJ Vlissingen, The Netherlands.

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individuals at the Norwegian coast spawn at 25 °C (Bromley et al., 2016; Korringa, 1957; Bennema et al., 2020). Larval development is successful at temperatures higher than 17.5 °C, which may explain low recruitment in deep-sea populations and higher recruitment in shallow oyster beds (Bennema et al., 2020). When reared in the laboratory, bivalve larvae often grow best at temperatures well above those which they would experience in nature (Helm et al., 2004). At temperatures between 15 and 30 °C, *O. edulis* larvae of French origin had the highest survival at 25 °C and had the highest settlement success at 30 °C (Robert et al., 2017). Optimum temperatures for adult oysters range between 18 and 24 °C but, similar to juvenile *O. edulis*, adults can withstand temperatures up to 30 °C (Eymann et al., 2020; Kamermans and Saurel, 2022).

Although temperature variation is the norm in nature, the vast majority of the knowledge on the effect of temperature on organisms is based on data collected after exposing individuals to stable temperatures. In most ectotherms, a relationship exists between temperature and performance (from cellular to organismal level) which can, in some animals, be maximized through behavioral thermoregulation (Chapperon and Seuront, 2011a, 2011b). The performance typically increases exponentially from the critical minimum temperature until the thermal optimum and declines rapidly beyond the thermal optimum until the critical maximum is reached (Martin and Huey, 2008).

The optimum temperature of a species exposed to a stable mean temperature can be quite different than that of a species experiencing fluctuations around that mean (Bozinovic et al., 2011; Verheyen and Stoks, 2019). This can be explained by the asymmetry of the performance curve. Increases in temperature above the performance optimum reduce the performance to a larger extent than decreases in temperature below the performance optimum (Martin and Huey, 2008). Hence, in a thermally variable environment, the performance of a species depends on the magnitude of the fluctuation but also on the mean temperature around which the fluctuation occurs.

The majority of studies investigating thermal variability has focused on terrestrial insects. In several fly species, for example, the same magnitude of temperature variation lowered the performance when it was centered at a temperature close to the thermal optimum, yet it increased performance when it was centered at a lower temperature (Bozinovic et al., 2011, Verheyen and Stocks 2019). Similarly, extreme high temperatures were associated with increased recruitment success at the high latitudinal edge of the geographic distribution of marine benthic invertebrates, but other benthic invertebrate species at the more central location within their geographic distribution were negatively affected by the heat wave (Caputi et al., 2016).

An increase in the magnitude and frequency of thermal fluctuation is predicted in a future climate in which global mean temperatures may rise by 4 to 5 °C (RCP 8.5, IPCC, 2021). Thus, understanding the effects of thermal variability and extremes is crucial for predicting how animals will respond to climate change. In general, temperature fluctuations prevail on seasonal to daily scales in temperate regions. In the North Sea, sea surface temperature has historically varied by up to 19 °C across seasons, by 2 to 4 °C across summer weeks and by 1 to 2 °C across summer days with maximum water temperatures reaching 16 to 22 °C (CEFAS buoy data from stations Dogger Bank oyster ground and Noordwijk, supplementary Table A.1, www.cefas.co.uk). Projections for the end of the century (2100) in the southern North Sea suggest that these fluctuations will occur around a 5.4 °C higher mean temperature (Weinert et al., 2016, 2021). In the neighboring Wadden Sea, mean seawater temperatures in June and July, the time of year relevant for *O. edulis* reproduction, reached 20 °C in 2017–2019 (van Leeuwen et al., 2021a, 2021b, 2021c). In 2023, June was the warmest seawater month in the western Wadden Sea in 160 years and seawater temperatures reached 23.5 °C (van Leeuwen, unpublished data). Daily temperature differences ≥4 °C have been measured in 2018, 2020, 2021 and 2023 (van Leeuwen et al., 2021b, 2021d, 2022).

The aim of this study was to quantify vital rates and performance curves of larval *O. edulis* to better understand the impacts of temperature on the potential recruitment success in restoration sites within the North Sea. For this, we monitored the larval release of a local shallow-water *O. edulis* population over a period of two months and used the largest numerical larval batch to test whether larval fitness is constrained by the thermal conditions which they experience in restoration sites. We reared larvae at two fluctuating temperature regimes (low, i.e. 20–21 °C, versus high, i.e. 20–24 °C) and determined growth, development, survival and settlement success, as they are the ultimate measures of successful recruitment and a prerequisite to establish self-sustaining oyster reefs. In addition, performance curves of the aerobic metabolism, i.e. oxygen consumption rates, and behavioral aspects, i.e. swimming speed and swimming directionality of all three larval life-stages, i.e. D-shape, umbo and pediveligers, were measured and their acute thermal preference range was determined to gain a broader understanding of the thermal coping ranges of mechanisms underlying those vital rates of the early life-stages when developing in fluctuating environments.

Based on the large difference between summer temperatures of the North Sea (16 to 22 °C) and the optimal temperature for survival and settlement determined at stable temperatures within a previous laboratory study (25 and 30 °C, Robert et al., 2017), we hypothesized that *O. edulis* larvae will benefit from increased thermal fluctuation due to the periodic exposure to optimal high temperatures.

2. Material and methods

2.1. Vital rates

2.1.1. Broodstock conditioning and larval release

Broodstock ($n = 38$) were from the Royal Netherlands Institute for Sea Research (NIOZ) in-house *O. edulis* population, which was collected on the east coast of the island of Texel, the Netherlands, in 2017 (Jacobs et al., 2020). In February 2021, this broodstock was transferred to a temperature and light-controlled room and maintained at 12 °C (TK 2000, TECO, Italy) in constant darkness. Oysters were submerged in a shallow container placed at the surface of a 1500-L flow-through seawater tank. Seawater (sand filtered) was circulated over the oysters via an airlift. Oysters were fed frozen and thawed algae, provided at a daily ration of 3 mg dry weight (DW) of algae per 100 mg dry flesh weight of oyster (3 % DW*DW^{-1}). The algae were obtained from a local company (Foundation Seashell, Kamperland, the Netherlands) and contained a mixture of *Isochrysis* T-strain, *Pavlova lutheri, Nannochloropsis oceanica, Rhodomonas salina, Chaetoceros calcitrans, Thalassiosira pseudonana, Thalassiosira weisfloggi, Skeletonema costatum* and *Skeletonema marinoi*. In June 2021, the food concentration was increased to 6 % DW*DW^{-1}*day^{-1}, the water temperature was increased to 20 °C within 4 weeks and the light regime was switched to 16 light: 8 dark to induce gonad ripening and spawning. At this time, a sieve with a 100 μm mesh size was fitted below the outflow of the broodstock container. Larvae released by the broodstock were transported with the waterflow and gently collected in the sieve. The sieve was checked each morning and, if larvae were present, a subsample of larvae was counted under a stereo microscope (Stereo Discovery V.8, Zeiss, Germany) and the anterior-posterior "length" and dorsal-ventral "height" (Waller, 1981) of 25 individuals was measured under a microscope (Axio Imager 2, Zeiss, Germany). Larvae released during one day were treated as one batch.

2.1.2. Rearing of the experimental larval batch

Larvae of *O. edulis* were reared in the same temperature and light-controlled room as the broodstock with the same light regime and seawater treatment. In August 2021, 0.5*10^6 larvae (initial density 10 larvae*mL^{-1}) were transferred to conical flow-through seawater tanks (50 L, $n = 6$) at a low fluctuating temperature ($n = 3$) between 20 and 21 °C (mean: 20.6 ± 0.1 °C) and a high fluctuating temperature (n = 3) between 20 and 24 °C (mean: 21.4 ± 0.1 °C). For the low fluctuating treatment, temperature was increased via a titanium heater (100 W) connected to a temperature switch TRD controller (Schego, Germany)

with a hysteresis of 1 °C. For the high fluctuating treatment, a titanium heater (100 W) was connected to a computer-controlled feedback system (IKS Aquastar Industrial, Germany). Cooling occurred passively by the ambient room temperature (13 °C). In the low fluctuating treatment, temperature change was not time-controlled while in the high fluctuating treatment, temperatures were 20 and 24 °C during the night and day, respectively (supplementary Fig. A.1). Temperature within each tank was recorded continuously every 5 min (HOBO Pendant MX Temp, Onset Computer Corp., USA). From the bottom of each tank, air bubbles aerated the water and kept larvae in suspension. Larvae were fed the same algae mixture as the broodstock at a cell concentration of 40,000 algal cells*mL^{-1} using a peristaltic pump (Miniplus 3, Gilson, UK). Tanks were cleaned every 5 days before which salinity, oxygen saturation, pH_{NBS} (HQ40d, Hach, USA), total ammonium (NH_4 + NH_3) and nitrite concentrations were measured (COLOMBO Aquatest Kit, the Netherlands). During the experiment, salinity was 28.0 ± 0.1 PSU, oxygen saturation was 8.5 ± 0.0 mg*L^{-1} and pH_{NBS} was 8.3 ± 0.0 (all mean ± SE). Total ammonium and nitrite concentrations remained below 0.1 mg*L^{-1} and 0.25 mg*L^{-1}, respectively. During cleaning, the air supply was disconnected and the water was drained through the bottom of the tank over a 250 and 100 μm mesh to collect the larvae. The tank was cleaned with chlorine, rinsed with fresh water and left to dry. These are standard hatchery practices that do not influence larval survival (Helm et al., 2004; Wallace et al., 2008). A subsample of the larvae from each mesh size was counted and measured to determine survival, development into competent pediveligers for settlement, and growth. Larval length (n = 25) was averaged for each replicate tank and time point. Pediveligers were classified as competent for settlement when both the foot and eyespot were visible. Alive larvae that had not developed into competent pediveligers were transferred to a clean rearing tank at their respective treatment condition and a subsample of the competent pediveligers was used to determine settlement success (see below). The remaining competent pediveligers were pooled and transferred to larger settlement tanks without further inspection for this study.

2.1.3. Settlement success and post-settlement size

Competent pediveligers for settlement were transferred to a 200-μm sieve (2.45 L volume, 25 cm Ø, 5 cm height) which was hung in a separate rearing tank (n = 3) at the respective temperature treatment. The inflowing water and food supply was placed above the sieve and the air supply from the bottom of the tank was minimized to create a downwelling environment which still maintained sufficient aeration. Ground oyster shells (300 μm, microbrisure, Entre Mer et Terre, France) were placed in the sieve as settling material. The tanks were shaded and left undisturbed for 10 days after which larvae were counted and their behavior was assessed. Larvae were categorized either as settled, crawling, swimming or dead which corresponded to individuals that i) had attached firmly to the oyster shells, ii) had not yet attached firmly to the oyster shells, iii) were predominantly swimming, or iv) had an open shell and were not moving, respectively. The length of settled larvae was measured (n = 7–20 per replicate tank) under a microscope and averaged for each replicate tank.

2.2. Thermal coping ranges

2.2.1. Thermal preference, swimming speed and mean directional change rate

A horizontal thermal gradient was used to determine acute thermal preference range and thermal influence on swimming behavior of D-shape, umbo and pediveligers. The horizontal orientation of the gradient enabled the larvae to select a temperature while the influence of gravity as well as the preference of *O. edulis* larvae to gather at the tank bottom was minimized (Rodriguez-Perez et al., 2020). The thermal gradient was custom build in house at the NIOZ according to specification from Wiggins and Frappell (2000). In brief, a glass tube (40.1 mL volume, 51 cm length, 1 cm Ø) was embedded in a water bath within an u-shaped aluminum block with LED lighting along the left and right horizontal planes. The ends of the aluminum block were connected to a Peltier device or a heating unit. Temperatures were set using a custom-built electronic controller and the linear gradient between 16 and 30 °C established within 30 min. Larval position within the gradient could be viewed through the opening on the top of the aluminum block. A camera (Galaxy S8, Samsung) was installed above the gradient that took images of the gradient at a rate of one per hour throughout each experimental run. In addition, after 1 h, 1-min videos were taken at 5 locations along the gradient which corresponded to 17, 20, 24, 27 and 30 °C. Individuals of D-shape, umbo and pediveligers were evenly distributed throughout the glass tube using a syringe connected to aquarium tubing (n = 3 per life-stage). Larvae were left to move throughout the gradient for three hours after which the temperature was measured every 2 cm along the gradient using a digital thermocouple thermometer. Control runs (n = 2 per life-stage) with a uniform temperature (20 °C) throughout the thermal gradient were conducted to test whether the design of the gradient apparatus had an influence on the larval distribution. Subsamples of larvae were taken at the end of each experimental day to confirm developmental stage and measure larval length (n = 25) under a microscope.

Images captured at the start (0 h) and end (3 h) of each experimental run were used to determine the acute thermal preference range of larvae by counting the number of larvae in 2 cm-sections along the gradient. Numbers of larvae in consecutive sections were summed to determine the temperature range where 50 % of the test-population occurred after the 3 h-exposure (Alter et al., 2017). The average of this temperature range was used to test for differences in the temperature range between treatments and life-stages. Videos taken 1 h after the larvae were introduced into the gradient were used to determine swimming speed and swimming directionality. The 1-min videos were analyzed by automatedly tracking the distance traveled by larvae between every frame (0.03 s) using the plugin TrackMate for Image J (FIJI) (Ershov et al., 2021). The median swimming speed (μm*sec^{-1}) was corrected for larval size by dividing it by larval length (body length (BL)*sec^{-1}). The mean directional change rate is the angle between two links of the swimming path in succeeding frames averaged across the entire track length (Meijering et al., 2012). This value was inverted and the lowest inverted directional change rate was added to each data point and termed swimming directionality in order to achieve positive values to which performance curve models could be fit. Median swimming speeds and swimming directionality of larvae (n = 12–39) of each life-stage were averaged for each temperature (n = 5).

2.2.2. Oxygen consumption rate

Oxygen consumption rate ($\dot{M}O_2$) was measured in D-shape, umbo and pediveligers (n = 5–9 replicates per developmental stage). Subsamples of larvae were taken at the commencement of each experiment to confirm developmental stage and to measure larval length (n = 25) under a microscope. Oxygen consumption rate trials were conducted in a temperature-controlled room at 17, 20, 24, 27, 30 and 33 °C. Two temperatures were tested at the same time and four temperatures were tested in one day. Hence, trials with all six temperatures were accomplished over a period of two days. Pre-experiments showed that $\dot{M}O_2$ did not change significantly within two days of development. Ultra-pure seawater was preconditioned to each of the six temperatures and an unknown number of individuals was transferred from the rearing tanks to the respiration chambers, providing an acute exposure of the individuals to the new temperature. Trials commenced immediately after respiration chambers were hermetically sealed under water. Respiration chambers were 2-mL glass vials with screw caps (PreSens, Germany) that were placed on a sensor dish reader (PreSens, Germany). The sensor dish reader measured the oxygen content within each chamber at a rate of four readings per minute. Larvae swim frequently and stirred the water such that a mechanical stirring mechanism was considered

unnecessary. Lights were switched on during trials and all experiments were conducted during the day. Percentage air saturation (O_2sat) was measured until oxygen dropped below 70 % O_2sat which took approx. 3–5 h. Pre-experiments had shown that critical oxygen levels were below 25 % O_2sat for all veliger life-stages. After the experiments, individuals were preserved in 4 % formalin and subsequently counted (density = 308 ± 13 ind*mL^{-1}). In pre-experiments, these densities did not influence individual $\dot{M}O_2$. Sensors were calibrated in air saturated seawater for 100 % O_2sat and in sodium sulfite-saturated seawater for 0 % O_2sat. Three chambers without larvae served as blanks to account for background respiration. Oxygen consumption rates (in pmol*h^{-1}*ind^{-1}) were calculated from the linear decrease in % O_2sat in each respiration chamber according to Eq. 1 and were averaged for each replicate tank ($n = 3$).

$$\dot{M}O_2 = \frac{FO_2}{t} \times (P_B - P_S) \times \beta_{O_2} \times Vol \times 0.2093 \quad (1)$$

where FO_2 is the change in fractional oxygen concentration over time, t (*sec*), P_B is the barometric pressure (kPa), P_S is the saturation vapor pressure of water (kPa), β_{O2} is the capacitance of water for oxygen, *Vol* is the chamber volume minus the larvae volume (L, assuming 1 g wet mass equals 1 mL) and 0.2093 is the fractional concentration of oxygen in water (Alter et al., 2017). Individual weights were estimated from measured larval length according to Labarta et al. (1999).

2.3. Predicting performance and statistical analysis

We fitted exponential curves to data on larval lengths across developmental time of *O. edulis*. For larval lengths from each rearing tank we fitted the model.

$$Lt = t0 \times e^{k \times t} \quad (2)$$

where *Lt* is the larval length (µm) at time *t*, *t* is the number of days since larval release, *t0* is the larval length at $t = 0$, and *k* is the growth constant.

For data on swimming speed, swimming directionality and $\dot{M}O_2$, thermal performance curves (TPC) were fitted to predict the thermal optima for each parameter. First, data from all three replicate tanks were averaged. Then, TPCs were fitted (*rTPC, nls.multstart*) using nonlinear least squares (NLSS) regression according to Padfield et al., 2021. In brief, out of 24 different TPC models, five models were selected ("Gaussian_1987", "Joehnk_2008", "Oneill_1972", "Pawar_2018", "Weibull_1995") and fitted to data. These five models were chosen because we were interested in the optimum temperature and those models included that parameter in their formulation. Subsequently, the five models were weighted based on the smallest Akaike information criterion correcting for small sample size and the temperature at which swimming speed, swimming directionality and $\dot{M}O_2$ was maximal was estimated using the weighted model (Padfield et al., 2021).

Differences in larval sizes between larval release dates were tested with a non-parametric one-way analysis of variances (ANOVA) (*ARTool*) because homogeneity of variances (Levene's test) was significant. A two-way repeated measures ANOVA was used to test for differences in larval length of the three life-stages at time points of experimental runs. Kaplan-Meier analysis was conducted for data on *O. edulis* cumulative survival and development of competent pediveligers. Larvae that survived and larvae that developed into competent pediveligers were treated as right-censored observations. A two-way ANOVA was used to test if proportions of larvae were statistically different in settlement behaviors between the two rearing temperatures and a two-way repeated measures ANOVA was used to test if rearing temperature and time affected post-settlement larval length.

Differences between the distributions of larvae with and without being exposed to a thermal gradient were tested using Chi-square tests. This was followed by examining the differences in average acute thermal preference range between life-stages and treatments using a two-way ANOVA. The influence of life-stage, treatment and exposure temperature on swimming speed, swimming directionality and $\dot{M}O_2$ data (ln-transformed) was tested with three-way ANOVAs.

Curve fitting and statistical analyses were conducted using R (version 4.2.0). Normality and homogeneity of variances were assessed using the Shapiro-Wilkinson test and Levene's test, respectively. A probability of <0.05 was considered as significant. Bonferroni host-hoc tests were used to detect significant differences between the levels of the variables when independent variables were significant. All data in the text are stated as mean ± SE values.

3. Results

3.1. Vital rates

3.1.1. Larval batch releases and sizes

The broodstock first released larvae 44 days after the temperature was increased, at 19.6 °C. Over a total period of 61 days, oysters released larvae on 14 days. The last two larval batches were released when temperatures were decreased to 18 °C. The number of larvae released per day varied between 0.008*10^6 and 4.7*10^6 (Fig. 1A). Mean larval length varied between 194.3 ± 0.6 µm and 211.9 ± 1.0 µm on day of year (DOY) 232 (batch #12) and DOY 208 (batch #4), respectively (Fig. 1B). The batch with the largest number of individuals (batch #9, DOY 221) was chosen for the larval experiments. Larvae from this batch were similar in length compared to those released on all other days except for those released on DOY 208, which were significantly larger ($p < 0.0001$, Fig. 1B, Table 1).

3.1.2. Developmental time and larval length

Developmental time from release to reaching competence for settlement was seven days longer at the low compared to the high fluctuating temperature treatment (Fig. 2). Larval length at D-shape, umbo and pediveliger life-stages was 202 ± 1, 215 ± 1 and 268 ± 4 µm, respectively, and was similar between treatments (Fig. 2, Table 1).

3.1.3. Survival and development of competent pediveligers

Cumulative survival of larvae until settlement was low and was not influenced by rearing treatment (Kaplan-Meier analysis, Chisq = 0, $p > 0.05$, Fig. 3A). The survival was 4.2 ± 2.1 and 8.5 ± 1.5 % at the low and high fluctuating temperature treatments, respectively. The percentage of larvae that developed into competent pediveligers was 4.2-fold lower in the low (5.9 ± 2.8 %) versus the high (24.6 ± 4.8 %) fluctuating treatment (Kaplan-Meier analysis, Chisq = 41.6, $p < 0.001$, Fig. 3B).

3.1.4. Settlement success and post-settlement size

After 10 days in the settlement tank, length of spat was similar between the two temperature treatments ($p > 0.05$, Fig. 4A, Table 1). Yet, 5.5-fold more larvae ($p < 0.001$) had settled and 0.8-fold fewer larvae were dead ($p < 0.001$) in the high compared to the low fluctuating treatment (Fig. 4B, Table 1). At the low fluctuating treatment, similar proportions of larvae were swimming, crawling and had settled (0.1 ± 0.1 %, 0.4 ± 0.3 % and 3.3 ± 0.4 %, respectively) while at the high fluctuating treatment, the lowest proportion of larvae were swimming and crawling (0.6 ± 0.5 % and 2.1 ± 0.1 %, respectively) and significantly more larvae had settled (18.1 ± 2.9 %, $p < 0.0001$, Fig. 4B, Table 1).

3.2. Thermal coping ranges

3.2.1. Acute thermal preference range

The distribution of larvae along the thermal gradient at the start and end of the experiment was significantly different from another ($p < 0.05$) except for one out of three replicates for D-shape veligers at

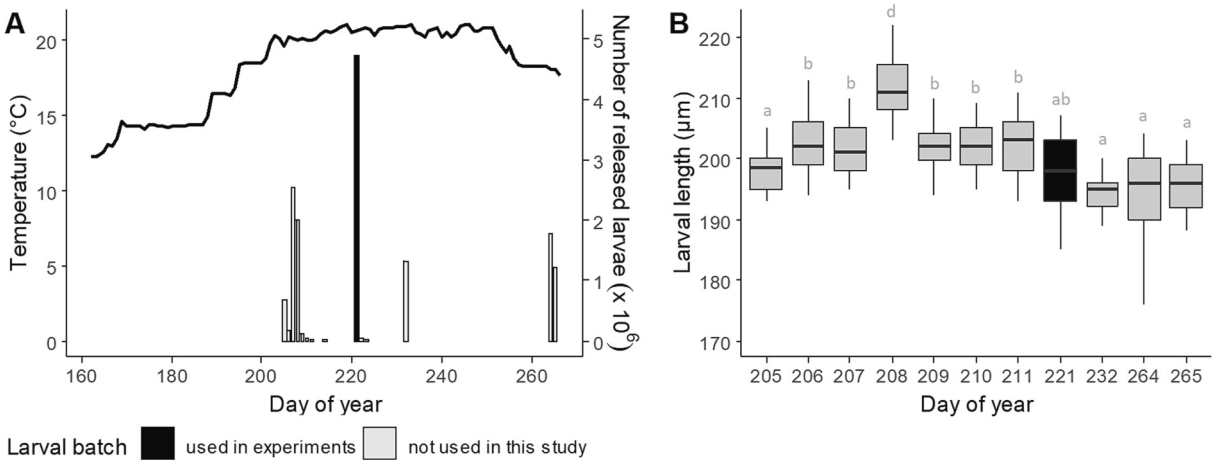

Fig. 1. Temperature (°C) (line) during broodstock conditioning with number of released larvae per day (bar) (A) and larval length (µm) (box) at release (B) over swarming period (day of year (January 1st = day 0)). The box represents the 25th to 75th percentiles. The median is shown as horizontal line. Whiskers represent 10th and 90th percentiles. $n = 25$.

the low fluctuating treatment (Chi-square test, $p > 0.05$). The mean temperature of the acute thermal preference range was higher in pediveligers reared in the high fluctuating treatment compared to D-shape veligers in that treatment ($p < 0.05$) as well as pediveligers at the low fluctuating treatment ($p < 0.05$, Tables 1 & 2).

3.2.2. Swimming speed and directionality

The interaction between treatment and life-stage as well as life-stage and exposure temperature affected larval swimming speed (Fig. 5A-C, Table 1). At each life-stage, swimming speeds were similar between treatments ($p > 0.05$) and at the high fluctuating treatment, swimming speeds were also similar between life-stages ($p > 0.05$). At the low fluctuating treatment, however, swimming speed across exposure temperatures decreased from D-shape to pediveligers ($p < 0.01$). Compared to pediveligers, umbo veligers swam 1.4-fold faster at 17 °C ($p < 0.05$) and D-shape veligers swam 2.2, 2.2 and 1.6-fold faster at 17, 20 and 24 °C ($p < 0.01$), respectively. At 27 °C and 30 °C, swimming speed was similar among life-stages (2.20 ± 0.05 and 2.35 ± 0.06 BL*sec^{-1}, Table 1, Fig. 5A-C). Based on fits of the TPCs, swimming speed was predicted to be highest at ≥30 °C for D-shape and umbo veligers at both treatments and 29.9 °C and 28.4 °C for pediveligers in the low and high fluctuating treatments, respectively (Fig. 5A-C, supplementary Table A.2).

Swimming directionality was influenced by the interaction between treatment and life-stage as well as life-stage and exposure temperature (Fig. 5D-F, Table 1). Directionality of swimming was 1.3-fold higher in pediveligers in the high compared to the low fluctuating treatment ($p < 0.05$). In addition, directionality in pediveligers was 1.4- and 3.0-fold higher than umbo and D-shape veligers at the high fluctuating treatment, respectively ($p < 0.05$). In the low fluctuating treatment, swimming directionality was similar across life stages ($p > 0.05$). At the different exposure temperatures, directionality increased with each subsequent life-stage at the highest temperatures (27 and 30 °C, $p < 0.001$) but not at lower exposure temperatures (Table 1, Fig. 5D-F). Directionality was predicted to be highest at 27.2 and 25.2 °C in D-shape veligers, 26.8 and 29.3 °C in umbo veligers, and 28.4 and 29.5 °C in pediveligers in the low and high fluctuating treatments, respectively (Fig. 5D-F, supplementary Table A.2).

3.2.3. Oxygen consumption rates

Oxygen consumption rates were higher in larvae reared in high compared to the low fluctuating treatment in all life-stages across all exposure temperatures ($p < 0.05$), except for D-shape veligers exposed to 20 and 27 °C ($p > 0.05$, Fig. 6, Table 1). $\dot{M}O_2$ increased with life-stage with 1.4 and 1.5-fold higher $\dot{M}O_2$ in umbo compared with D-shape veligers and with 1.7 and 2.9-fold higher $\dot{M}O_2$ in pediveligers compared to umbo veligers in the low and high fluctuating treatments, respectively (for all $p < 0.001$). TPCs predicted maximal $\dot{M}O_2$ for umbo veligers at or above 33 °C and pediveligers at 29.2 °C for both thermal rearing treatments. For D-shape veligers, $\dot{M}O_2$ was predicted to be maximal at 28.9 and 32.8 °C for larvae reared at low and high fluctuating temperature, respectively (Fig. 6, supplementary Table A.2).

4. Discussion

In the marine environment, solutions to halt biodiversity loss include creating marine protected areas and nature reserves. These activities intend to promote the natural recovery of ecosystem health and, in some areas, natural recovery is supported by actively restoring habitat-forming species (Saunders et al., 2020). These efforts, however, are not always successful due to insufficient knowledge on requirements for the establishment, growth, survival and reproduction of habitat formers (Korringa, 1946; Bromley et al., 2016). A major bottleneck to creating self-sustaining oyster beds, for example, is the successful development and settlement of larvae to bottom habitats, processes largely governed by temperature. In aquaculture, bivalve larvae, including those of *O. edulis*, are reared and grow best at temperatures well above those which they would experience in nature (Helm et al., 2004). Across ecologically relevant temperatures, however, a mechanistic understanding of larval performance is lacking. To better understand how temperature fluctuations in the field could impact the success of oyster restoration, we reared oyster larvae at two thermal regimes that overlapped, yet differed in thermal variability by 3 °C but only by 0.9 °C in their mean temperature. The small difference in temperature resulted in significant and relevant impacts on vital rates, as well as thermal performance curves, especially in the last larval life-stage. The results suggest that *O. edulis* benefitted from the periodically high temperatures by increasing metabolism and swimming performance which translated into more rapid development and higher settlement success.

Settlement and the subsequent metamorphosis are energetically costly processes which involve the reorganization of existing tissues and the formation of new tissue (Labarta et al., 1999; Waller, 1981). During this life cycle transition, $\dot{M}O_2$ increases while energy reserves decrease by up to 65 % and larvae often rely on energy reserves (Lucas et al., 1979, Rodriguez et al., 1990, Videla et al., 1998, Labarta et al., 1999, Garcia-Esquivel et al. 2001). The eastern oyster, *Crassostrea virginica*, for example, still feeds during the searching and crawling stages of settlement but during cementation to the substrate and the early post-

Table 1
Summary of ANOVA results. *Asterisks* indicate significances within a testing treatment. LF = low fluctuating treatment, HF = high fluctuating treatment, D = D-shape veliger, U = umbo veliger, P = pediveliger.

Source	df	MS	F	p-value	Post-hoc
One-way ANOVA					
Larval length at release (non-parametric)					
Batch	10	–	22.094	0.001*	Day 208 > all other batches Day 205, 232, 264 & 265 < day 206, 207, 209–211
Residuals	257				
Two-way ANOVA					
Behavior in settlement tanks					
Treatment	1	0.0	0	0.999	
Behavior	3	10,624.0	1632.57	0.000*	
Treatment × Behavior	3	257.0	39.56	0.000*	LF: swim = crawl = settled < dead HF: swim = crawl < settled < dead settled: LF < HF dead: LF > HF
Residuals	16				
Average acute thermal preference range					
Treatment	1	0.5	1.645	0.226	
Life-stage	2	0.4	1.305	0.301	
Treatment × life-stage	2	2.0	6.131	0.02*	LF: D = U = P HF: U < P P: LF < HF
Residuals	11	0.3			
Two-way repeated measures ANOVA					
Larval length at life-stage					
Treatment	1	5.6	0.206	0.666	
Life-stage	2	1427.7	52.644	0.000*	D < U < P
Treatment × life-stage	2	26.5	0.976	0.430	
Residuals	6				
Post-settlement larval length					
Treatment	1	515.0	1.077	0.358	
Time	1	71,729.0	149.824	0.000*	0 days <10 days
Treatment × Time	1	4.0	0.009	0.929	
Residuals	4				
Three-way ANOVA					
Swimming speed					
Life-stage	2	2.0	38.914	0.000*	
Treatment	1	0.4	7.044	0.010*	
Exposure temperature	4	4.9	96.936	0.000*	
Life-stage × treatment	2	0.4	7.101	0.002*	LF: D > P
Life-stage × exposure temperature	8	0.3	5.499	0.000*	17 °C: D > U > P 20 °C: D > U = P 24 °C: D > P
Treatment × exposure temperature	4	0.0	0.601	0.663	
Life-stage × treatment × exposure temperature	8	0.1	2.03	0.058	
Residuals	60				
Swimming directionality					
Life-stage	2	2180.7	208.412	0.000*	
Treatment	1	26.3	2.513	0.118	
Exposure temperature	4	1180.0	112.774	0.000*	
Life-stage × treatment	2	306.9	29.328	0.000*	LF: D < U = P HF: D < U < P P: LF < HF
Life-stage × exposure temperature	8	130.4	12.461	0.000*	17 °C: D < U 20 & 24 °C: D < U = P 27 & 30 °C: D < U < P
Treatment × exposure temperature	4	14.7	1.400	0.245	
Life-stage × treatment × exposure temperature	8	20.1	1.919	0.074	
Residuals	60				
Oxygen consumption rate					
Life-stage	2	13.0	2937.293	0.000*	
Treatment	1	4.2	954.204	0.000*	
Exposure temperature	5	0.7	242.527	0.000*	
Life-stage × treatment	2	1.0	211.582	0.000*	
Life-stage × exposure temperature	9	0.1	9.554	0.000*	
Treatment × exposure temperature	5	0.0	4.060	0.003*	
Life-stage × treatment × exposure temperature	9	0.0	4.182	0.000*	D: Temp, Treat, Temp * Treat U & P: Temp, Treat
Residuals	68				

settlement development feeding ceases (Baker and Mann, 1994). Results from the present study suggest that the higher $\dot{M}O_2$ in larvae reared at the high fluctuating temperature was sufficient to provide the energy required for metamorphosis resulting in higher settlement and lower spat mortality compared to the low fluctuating temperature.

While feeding rates were not determined in this study, higher horizontal swimming speeds and a more directional swimming of pediveligers at 24 °C could increase rates of prey encounter during the exploratory settlement phase. Any factor, such as warmer temperatures, that increases swimming performance may also directly benefit

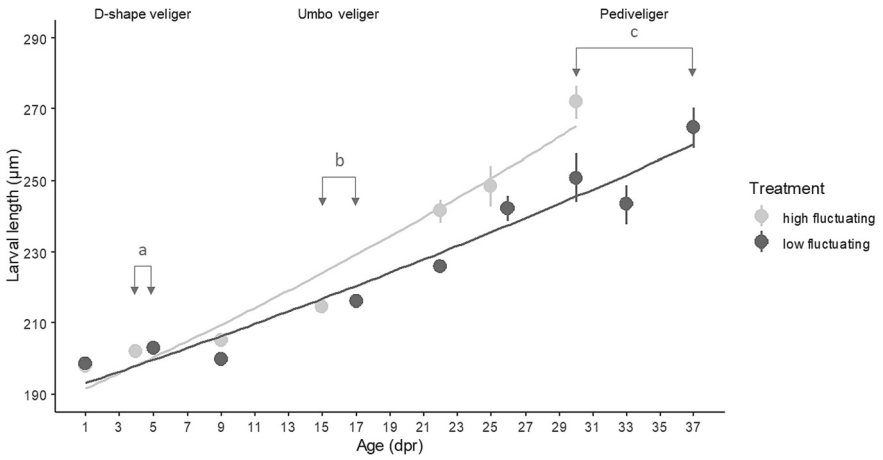

Fig. 2. *Ostrea edulis* larval length (µm) during pelagic development (days post release (dpr)) when reared at low (20–21 °C, dark grey symbols) and high (20–24 °C, light grey symbols) fluctuating treatments. The arrows indicate timepoints at which experiments with the three life-stages were conducted. Note that these are offset on the x-axis for each life-stage between treatments because of longer developmental times in the low fluctuating treatment. Different *lowercase letters* indicate significant differences in larval length between life-stages. Exponential curves were fitted to data and were $y = 191.5\ (\pm 3.7) * \exp^{0.008\ (\pm\ 0.001)*x}$ and $y = 189.4\ (\pm 4.2) * \exp^{0.011\ (\pm\ 0.001)*x}$ for the low and high fluctuating treatment, respectively. Values are mean ± SE, $n = 3$.

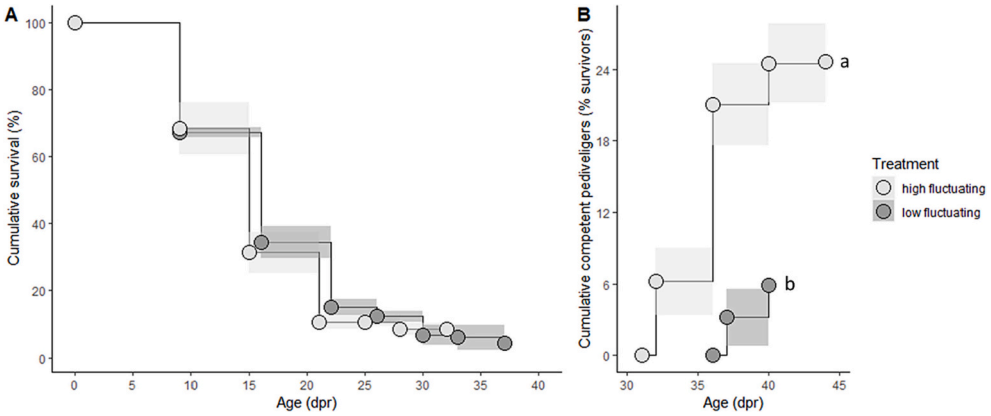

Fig. 3. *Ostrea edulis* cumulative survival (%) during larval development (A) and cumulative development of competent pediveligers (% survivors, B) over time (days post release (dpr)) at low fluctuating (dark grey symbol, 20–21 °C) and high fluctuating (light grey symbol, 20–24 °C) temperatures. Different *lowercase letters* indicate significant differences between rearing treatments. The mean (solid lines) ± 95 % confidence interval (shaded) is shown, n = 3.

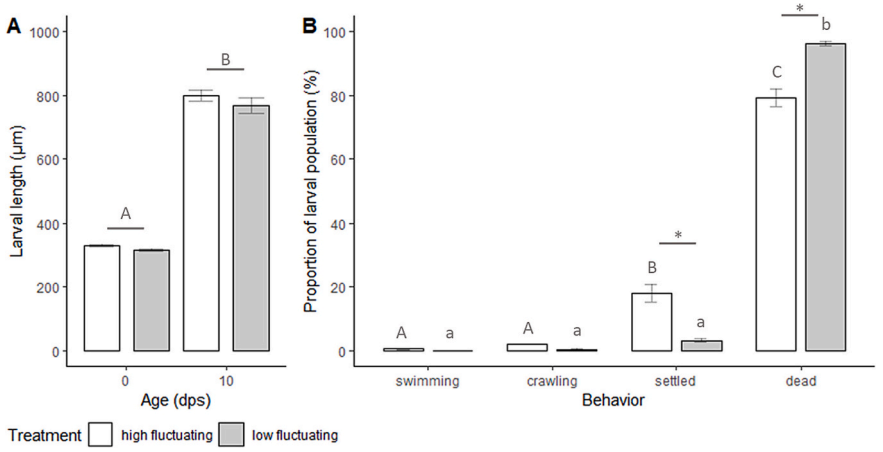

Fig. 4. Shell length (µm) (A) and behavior (B) of proportion (%) of *Ostrea edulis* pediveliger test population (B) after 10 days within settlement tanks (dps). Pediveligers were reared at low (20–21 °C, grey bars) and high (20–24 °C, open bars) fluctuating temperatures. In A, different *capital letters* indicate significant different larval sizes across time. In B, different *capital* and *lowercase letters* indicate significant differences between behaviors within the high and low fluctuating treatments, respectively. *Asterisk* indicates significant differences between treatments for both settled and dead larvae. Values are mean ± SE, n = 3.

Table 2
Average, minimum, maximum and the width (mean ± SE) of the temperature range where 50 % of the total test-population (individuals) of D-shape, umbo and pediveliger of *Ostrea edulis* gathered after three hours when exposed to temperatures between 16 and 30 °C within a thermal gradient. Larvae were reared at a low (20–21 °C) and high (20–24 °C) fluctuating temperature treatment. Different *lowercase letters* indicate significant differences between life-stages within the high fluctuating treatment. *Asterisk* indicates significant differences between treatments for pediveligers.

	Temperature range (°C)				Number of runs (*n*)	Individuals (ind*n^{-1})
	Average	Minimum	Maximum	Width		
Low fluctuating						
D-shape veliger	28.7 ± 0.3	27.2 ± 0.5	30.0 ± 0.0	2.8 ± 0.5	2	703–798
Umbo veliger	28.3 ± 0.7	26.6 ± 1.3	30.0 ± 0.0	3.4 ± 1.3	3	543–760
Pediveliger	27.9 ± 0.1*	25.7 ± 0.2	30.0 ± 0.0	4.3 ± 0.2	3	222–399
High fluctuating						
D-shape veliger	27.8 ± 0.3[a]	25.6 ± 0.6	30.0 ± 0.0	4.4 ± 0.6	3	467–662
Umbo veliger	28.6 ± 0.1[ab]	27.1 ± 0.3	30.0 ± 0.0	2.9 ± 0.3	3	165–918
Pediveliger	29.4 ± 0.0[a]*	28.8 ± 0.0	30.0 ± 0.0	1.2 ± 0.0	3	228–537

settlement by enabling larvae to more rapidly explore and find the suitable settlement location. The increased rate of directionality of swimming observed for larvae in the high fluctuation treatment would be particularly beneficial in nature where larvae will likely encounter a mixture of suitable and unsuitable habitats. *Ostrea edulis* larvae can only successfully settle on hard and clean objects, because organic growth prevents the permanent fixation of the spat (Korringa, 1946; Turner et al., 1994). Often, these objects are the new shell-edges of conspecifics where settlement is particularly high when water retention is low enabling larvae to actively navigate towards their preferred substrates due to the absence of strong currents (Korringa, 1946; Bayne, 1969; Smyth et al., 2020). Although the mechanisms are not fully understood, conspecifics send settlement cues which larvae can follow using rapid vertical swimming. Directional swimming in a horizontal plane, however, may also play an important role during settlement by enabling larvae to navigate over shorter distances to find suitable substrate for attachment (this study, Turner et al., 1994, Rodriguez-Perez et al., 2020). It is also important to note that, at the lowest tested temperatures which are natural temperatures of the North Sea, swimming speeds decreased during development while at temperatures above 24 °C swimming speeds remained high, a pattern also reported for speeds of vertical swimming (Rodriguez-Perez et al., 2020). Together with less directional swimming of pediveligers at colder temperatures, reduced swimming speeds below 24 °C imply that pediveligers have less control over their position in the water column which could hamper settlement success. In agreement, slower swimming speeds in combination with more circular swimming patterns have also been observed in gastropod veligers at unfavorably low temperatures for settlement (Alter et al., 2017).

Growth rates, survival and settlement success will potentially further increase at temperatures higher than 20–24 °C (rearing temperatures in the present study), because swimming performance and $\dot{M}O_2$ of larvae peaked at temperatures higher than 27 °C. In addition, when given a choice of temperatures, larvae preferred temperatures from 26 to 30 °C (note that higher temperatures were not tested). In agreement, when reared at stable temperatures in 5 °C increments between 15 and 30 °C, size at settlement, settlement success and food intake were highest at 30 °C (Robert et al., 2017). Yet, while food intake was linearly correlated with temperature, increments in size at settlement and settlement success were smaller between 25 and 30 °C in comparison to increments between lower temperatures (Robert et al., 2017). This suggests that larvae were approaching their thermal optimum between 25 and 30 °C which agrees with thermal performance curves from the present study.

Projections suggest that maximum (mean) summer bottom temperatures of the southern North Sea which are currently 21.5 °C in non-stratified and 16.5 °C in stratified regions will increase by up to 5.4 °C by 2100 (van Leeuwen et al., 2015; Weinert et al., 2016). Larvae may survive better in those warmer water temperatures by more rapidly passing through the pelagic life-stage in which they are particularly vulnerable to predation. Later in life, however, thermal optima decrease for *O. edulis*. In adults, short term exposure to 25 °C did not cause mortality but increased prey requirements to maintain biomass (Gilson et al., 2021). In accordance, temperatures higher than 24 °C were reported to be suboptimal in adult *O. edulis* acclimated to 12 °C and exposed to a thermal ramping regime. Although heart rate plateaued between 28 and 34 °C, cardiac arrest began at 30 °C, anaerobiosis increased at 26 °C and filtration rate was highest between 18 and 26 °C with a modelled peak at approx. 21 °C (Eymann et al., 2020). Also, Newell et al. (1977) reported highest filtration efficiency for adult *O. edulis* at 20 °C. Hence, there is a trade-off between increased settlement success of larvae and adult performance in habitats with temperatures >25 °C.

In the wild, it has been estimated that approx. 2.5 and 10 % of released *O. edulis* larvae will survive until settlement at 18 and 22 °C, respectively, and of those that survive approx. 1 % successfully settle to benthic habitats (Korringa, 1946). Our results showed that for larvae developing in temperatures representing the lower end of their thermal performance curve (20–21 °C) for locomotion and oxygen consumption, a slight increase in mean temperature (0.9 °C) or an increased magnitude of temperature fluctuation (3 °C) resulted in a >5-fold higher settlement success. Hence, in order to better estimate the recruitment potential within the restoration site in a present and future climate it is important to monitor and thus gain information of the local thermal variability to date to project it for the future as not only magnitude but also duration and periodicity of the thermal variability can influence vital rates (Vasseur et al., 2014). Evidence for differences in vital rates of poikilotherms exposed to fluctuating versus constant temperature is mounting. For example, the growth rate of a subtropical marine coccolithophore held at a fluctuating temperature (either below or above their thermal optimum) was lower than that observed at the same mean but stable temperature (Wang et al., 2019). In that study, a daily fluctuation was less inhibitory than a 2-day fluctuation, indicating less heat damage when warm temperatures were experienced for less time (Wang et al., 2019). Similarly, the growth rate of larvae of a tropical marine gastropod was more reduced in individuals experiencing a 6-day versus a daily fluctuating regime when compared to the mean, constant temperature (Arlauskas et al., 2022). In that study, differences in embryonic developmental time followed the same pattern yet size at hatch was reduced in only the 6-day fluctuating treatment (Arlauskas et al., 2022). Although the majority of research exploring the effects of thermal fluctuation on aquatic animals has been conducted on tropical and subtropical species considered particularly vulnerable since they already live at or near their thermal optima (Deutsch et al., 2008), species at higher latitudes may display the most variable responses to increased thermal fluctuation as well as the largest performance decrements (Kingsolver et al., 2013; Vasseur et al., 2014).

The results of this and other studies highlight the importance of gaining information on the local thermal variability to gauge future

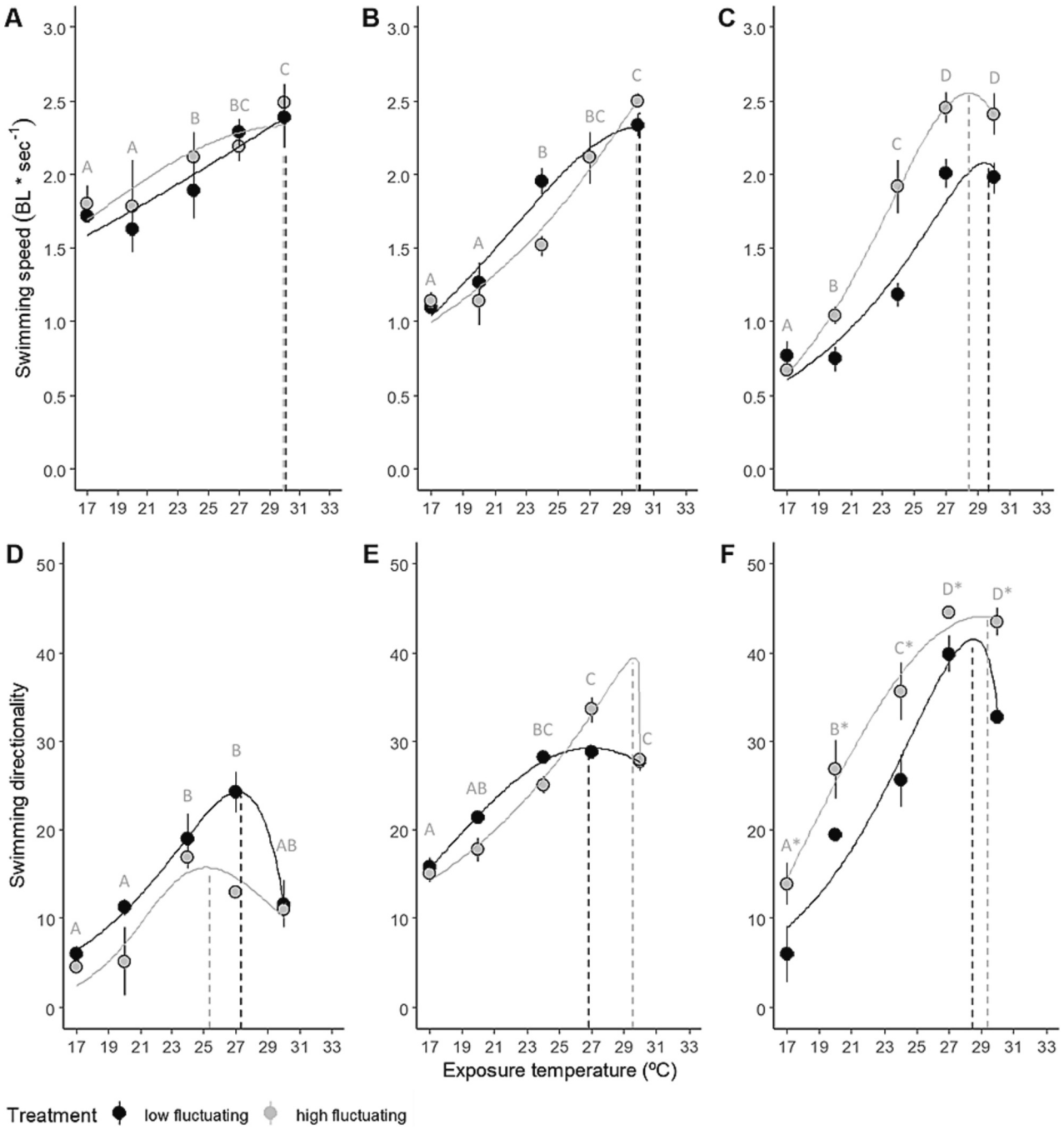

Fig. 5. Median swimming speed (body length (BL) sec^{-1}, A-C) and swimming directionality (D—F) of D-shape veliger (A, D), umbo veliger (B, E) and pediveliger (C, F) of *Ostrea edulis* across different exposure temperatures (°C). Larvae were reared at low fluctuating (20–21 °C, black symbols) and high fluctuating (20–24 °C, grey symbols) temperatures. Different *capital letters* indicate significant differences between exposure temperatures. *Asterisks* indicate significant differences between treatments. Thermal performance curve models were fitted to data (see supplementary Table A.2 for details). Values are mean ± SE, n = 3.

recruitment success at restoration site chosen for *O. edulis* as not only the magnitude but also the duration and periodicity of the temperatures experienced can influence vital rates (Vasseur et al., 2014). With regards to early life stages of *O. edulis*, climate driven intensification of temperature fluctuation would most likely increase growth and survival in this population but the impacts of these changes in thermal environment on later life stages remains to be tested (c.f. Xing et al., 2014).

Larval occurrence as well as larval condition and fitness not only depend on temperature but also on the quality and quantity of prey ingested by them as well as by their parents (Robert et al., 1988; Araya et al., 2012; Maathuis et al., 2020). In the present study, larvae and broodstock were fed a mixture of microalgae that was assumed to be nutritionally optimal. Yet, microalgae were stored frozen before being fed to the oysters which potentially lowered their quality. This, most likely, resulted in the relatively long developmental time and low survival although values for both traits for captively reared *O. edulis* larvae vary widely within the literature (at temperatures between 19 and 25 °C: developmental time: 10–43 days, survival until settlement: 0–100 %; Berntsson et al., 1997, Laing et al., 2005, Acarli and Lok, 2009, Araya et al., 2012, Robert et al., 2017, Jacobs et al., 2020). For some potential restoration sites within the North Sea, it has already been identified that food may be limiting in bottom habitats during gonad ripening as well as

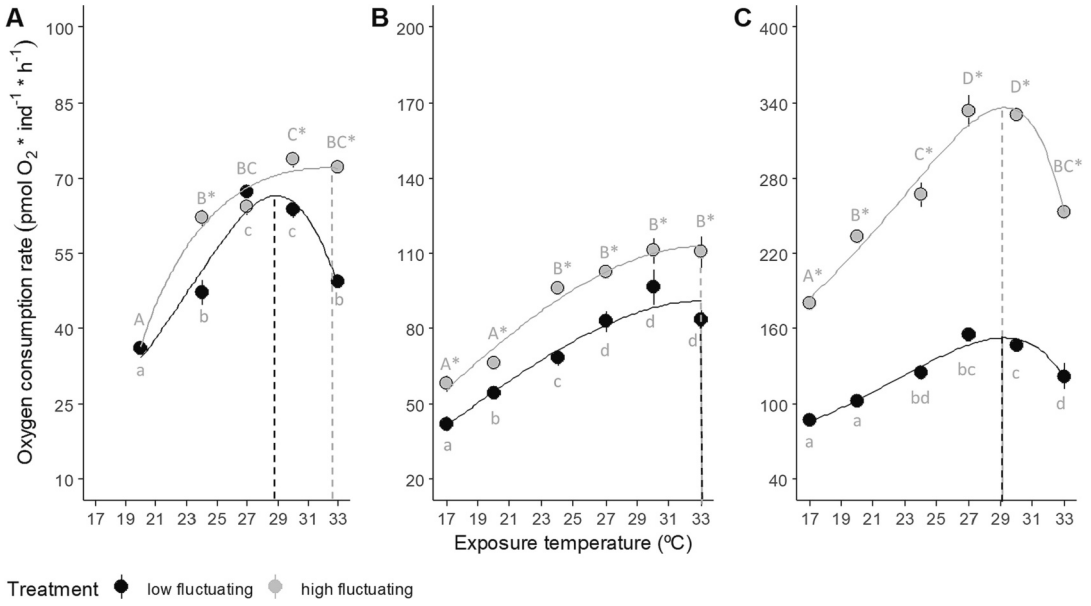

Fig. 6. Oxygen consumption rate (pmol O_2*ind^{-1}*h^{-1}) ($\dot{M}O_2$) of D-shape veliger (A), umbo veliger (B) and pediveliger (C) of *Ostrea edulis* at different exposure temperatures (°C). Larvae were reared at low (20–21 °C, black symbols) and high fluctuating (20–24 °C, grey symbols) temperatures. Within each panel (A-C), different *lowercase* and *capital letters* indicate significant differences in $\dot{M}O_2$ between exposure temperatures within the low and high fluctuating temperature treatment, respectively. *Asterisks* indicate significant differences between rearing treatments at a given exposure temperature. Note the difference in y-axis scale between panel A-C. Thermal performance curve models were fitted to data (see supplementary Table A.2 for details). Values are mean ± SE, n = 3.

during summer months especially when stratification occurs (Kamermans et al., 2018). In this region, a decreasing trend in phytoplankton biomass has been observed and is projected to continue in the future irrespective of the climate change scenario (Beusekom and Diel-Christiansen, 2009; Thewes et al., 2022). In addition, marine heatwaves are projected to increase in frequency and duration in a future climate and within the southern North Sea these events will lead to increased stratification occurrence (Chen et al., 2022). Data on phytoplankton abundance in bottom habitats is scarce in general, in particular with respect to information on small-scale temporal variability (Kamermans et al., 2018). *Ostrea edulis* larvae can survive for several days without food at a wide range of temperatures, yet they do not grow during this time, increasing their risk of predation and mortality (Robert et al., 1988). When selecting restoration sites, it will be important to continue monitoring (and increase the ability to make climate-driven projections of) changes in prey quantity and quality to correctly assess the future suitability of restoration sites.

5. Conclusion

Recent surveys within a Northern Irish Bay have revealed parameters beneficial for self-sustaining *O. edulis* beds. Raised topographical formations create micro-hydrodynamics that trap and concentrate larvae, high shell coverages act as settlement cues and high numbers of fecund in situ adults with <1.5 m distance support successful spawns (Smyth et al., 2020 and references herein). Within the southern North Sea, restoration pilots have started, among others, around wind farms (Bos et al., 2023). The artificial structures can act as particle trap creating areas with retention as low as <1 km (Mayorga-Adame et al., 2022). Indeed, the most suitable locations with regards to larval retention have been identified but recruitment to bottom habitats still fails in restoration pilots (Kamermans et al., 2018; Didderen et al., 2020; Schutter et al., 2021). In accordance, our results showed that current temperatures of the North Sea are not optimal for larval development and settlement. Only small increases in temperature peaks could result in significant and relevant impacts on vital rates especially in the last larval life-stage. While this may be beneficial for oyster recruitment, particularly in warm years, at shallower and hence warmer areas such as the Wadden Sea, or in a future ocean, results of this study can support current restoration efforts best by using the knowledge to optimize larval rearing protocols for hatchery production. For example, in a successful oyster restoration project in Maryland, USA, 164 million spat were produced that settled on oyster shells (spat on shell) and these shells were released into five restoration sites in one year alone to establish self-sustaining oyster beds (Maryland Oyster Restoration Interagency Workgroup of the Chesapeake Bay Program's Sustainable Fisheries Goal Implementation Team, 2021). In Europe, however, only 7 million spat on shell have been produced per year, making the supply of spat on shell a key limiting factor for sufficient active oyster supply to restoration sites (Pogoda et al., 2019; Colsoul et al., 2021). Rearing *O. edulis* early-life stages at their optimum temperatures between 25 and 29 °C can result in fast growth and abundant settlement success, hence improve hatchery yields and ultimately the supply of young oysters to ecological restoration sites to build a large enough population for which sufficient self-recruitment is observed (Alter et al., 2023).

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Credit authorship contribution statement

KA and PJ conceived the study. KA, PJ, BR, AD conducted the laboratory work. KA analyzed the data. KA wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. CJMP, PJ and MAP secured funding.

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.

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References

Acarli, S., Lok, A., 2009. Larvae development stages of the European flat oyster (*Ostrea edulis*). Israeli Journal of Aquaculture–Bamigdeh 61, 114–120.

Alter, K., Andrewartha, S.J., Clark, T.D., Elliott, N.G., 2017. Thermal preference increases during larval development of pure and hybrid abalone. J. Shellfish Res. 36 (1), 141–149. https://doi.org/10.2983/035.036.0114.

Alter, K., Philippart, C.J.M., Teng, S., Bolier, H., Drenth, P., Dubbeldam, M., 2023. Consequences of thermal history for growth, development and survival during metamorphosis and settlement for the European flat oyster. Aquaculture 566, 739174. https://doi.org/10.1016/j.aquaculture.2022.73 9174.

Araya, R.G., Mingant, C., Petton, B., Robert, R., 2012. Influence of diet assemblage on *Ostrea edulis* broodstock conditioning and subsequent larval development. Aquaculture 364, 272–280. https://doi.org/10.1016/j.aquaculture.2012.08.036.

Arlauskas, H., Derobert, L., Collin, R., 2022. Frequency of temperature fluctuations subtly impacts the life histories of a tropical snail. Biol. Bull. 242 (3), 197–206. https://doi.org/10.1086/720129.

Baker, S.M., Mann, R., 1994. Feeding ability during settlement and metamorphosis in the oyster *Crassostrea virginica* (Gmelin, 1791) and the effects of hypoxia on post settlement ingestion rates. J. Exp. Mar. Biol. Ecol. 181, 239–253. https://doi.org/10.1016/0022-0981(94)90131-7.

Barbier, E.B., Hacker, S.D., Kennedy, C., Koch, E.W., Stier, A.C., Silliman, B.R., 2011. The value of estuarine and coastal ecosystem services. Ecol. Monogr. 81 (2), 169–193. https://doi.org/10.1890/10-1510.1.

Bayne, B.L., 1969. The gregarious behaviour of the larvae of *Ostrea edulis* L. at settlement. J. Mar. Biol. Assoc. U. K. 49 (2), 327–356. https://doi.org/10.1017/S0025315400035943.

Bennema, F.P., Engelhard, G.H., Lindeboom, H., 2020. *Ostrea edulis* beds in the Central North Sea: delineation, ecology, and restoration. ICES J. Mar. Sci. 77 (7–8), 2694–2705. https://doi.org/10.1093/icesjms/fsaa134.

Berntsson, K.M., Jonsson, P.R., Wängberg, S.A., Carlsson, A.S., 1997. Effects of broodstock diets on fatty acid composition, survival and growth rates in larvae of the European flat oyster, *Ostrea edulis*. Aquaculture 154 (2), 139–153. https://doi.org/10.1016/S0044-8486(97)00041-0.

Beusekom, J.E.E., Diel-Christiansen, S., 2009. Global change and the biogeochemistry of the North Sea: the possible role of phytoplankton and phytoplankton grazing. Int. J. Earth Sci. 98, 269–280. https://doi.org/10.1007/s00531-007-0233-8.

Bos, O.G., Duarte-Pedrosa, S., Didderen, K., Bergsma, J.H., Heye, S., Kamermans, P., 2023. Performance of European oysters (*Ostrea edulis* L.) in the Dutch North Sea, across five restoration pilots. Front. Mar. Sci. 10, 1233744 https://doi.org/10.3389/fmars.2023.1233 744.

Bozinovic, F., Bastías, D.A., Boher, F., Clavijo-Baquet, S., Estay, S.A., Angilletta Jr., M.J., 2011. The mean and variance of environmental temperature interact to determine physiological tolerance and fitness. Physiol. Biochem. Zool. 84 (6), 543–552. https://doi.org/10.1086/662551.

García-Esquivel, Z., Bricelj, V.M., González-Gómez, M.A., 2001. Physiological basis for energy demands and early postlarval mortality in the Pacific oyster, *Crassostrea gigas*. J. Exp. Mar. Biol. Ecol. 263 (1), 77–103. https://doi.org/10.1016/S0022-0981(01)00300-8.

Bromley, C., McGonigle, C., Ashton, E.C., Roberts, D., 2016. Bad moves: pros and cons of moving oysters – a case study of global translocations of *Ostrea edulis* Linnaeus, 1758 (Mollusca: Bivalvia). Ocean Coast. Manag. 122, 103–115. https://doi.org/10.1016/j.ocecoaman.2015.12.012.

Caputi, N., Kangas, M., Denham, A., Feng, M., Pearce, A., Hetyel, Y., Chandrapavan, A., 2016. Management adaptation of invertebrate fisheries to an extreme marine heat wave event at a global warming hot spot. Ecol. Evol. 6 (11), 3583–3593. https://doi.org/10.1002/ece3.2137.

Chapperon, C., Seuront, L., 2011a. Space–time variability in environmental thermal properties and snail thermoregulatory behaviour. Funct. Ecol. 25 (5), 1040–1050. https://doi.org/10.1111/j.1365-2435.2011.01859.x.

Chapperon, C., Seuront, L., 2011b. Behavioral thermoregulation in a tropical gastropod: links to climate change scenarios. Glob. Chang. Biol. 17 (4), 1740–1749. https://doi.org/10.1111/j.1365-2486.2010.02356.x.

Chen, W., Staneva, J., Grayek, S., Schulz-Stellenfleth, J., Greinert, J., 2022. The role of heat wave events in the occurrence and persistence of thermal stratification in the southern North Sea. Nat. Hazards Earth Syst. Sci. 22, 1683–1698. https://doi.org/10.5194/nhess-22-1683-2022.

Colsoul, B., Boudry, P., Perez-Paralle, M.L., Bratos Cetinic, A., Hugh-Jones, T., Arzul, I., Merou, N., Wegner, K.M., Peter, C., Merk, V., Pogoda, B., 2021. Sustainable largescale production of European flat oyster (*Ostrea edulis*) seed for ecological restoration and aquaculture: a review. Rev. Aquac. 13 (3), 1423–1468. https://doi.org/10.1111/raq.12529.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., Martin, P.R., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proc. Natl. Acad. Sci. U. S. A. 105, 6668–6672. https://doi.org/10.1073/pnas.0709472105.

Didderen, K., Lengkeek, W., Bergsma, J.H., van Dongen, U., Driessen, F.M.F., Kamermans, P., 2020. WWF & ARK Borkum Reef Ground Oyster Pilot: Active Restoration of Native Oysters in the North Sea-Monitoring September 2019. Report 19–227. Bureau Waardenburg, Culemborg.

Eagling, L.E., Ashton, E.C., Jensen, A.C., Sigwart, J.D., Murray, D., Roberts, D., 2018. Spatial and temporal differences in gonad development, sex ratios and reproductive output, influence the sustainability of exploited populations of the European oyster, *Ostrea edulis*. Aquat. Conserv. Mar. Freshwat. Ecosyst. 28 (2), 270–281. https://doi.org/10.1002/aqc.2855.

Ershov, D., Phan, M.S., Pylvänäinen, J.W., Rigaud, S.U., Le blanc, L., Charles-Orszag, A., JRW, Conway, Laine, R.F., Roy, N.H., Donazzi, D., Duménil, G., Jacquemet, G., Tinevez, J.Y., 2021. Bringing Track Mate in the era of machine-learning and deep-learning. bioRxiv. https://doi.org/10.1101/2021.09.03.458852.

Eymann, C., Götze, S., Bock, C., Guderley, H., Knoll, A.H., Lannig, G., Sokolova, I.M., Aberhan, M., Pörtner, H.O., 2020. Thermal performance of the European flat oyster, *Ostrea edulis* (Linnaeus, 1758) – explaining ecological findings under climate change. Mar. Biol. 167, 17. https://doi.org/10.1007/s00227-019-3620-3.

Fulford, R.S., Breitburg, D.L., Luckenbach, M., Newell, R.I., 2010. Evaluating ecosystem response to oyster restoration and nutrient load reduction with a multispecies bioenergetics model. Ecol. Appl. 20 (4), 915–934. https://doi.org/10.1890/08-1796.1.

Gilson, A.R., Coughlan, N.E., Dick, J.T., Kregting, L., 2021. Marine heat waves differentially affect functioning of native (*Ostrea edulis*) and invasive (*Crassostrea [Magallana] gigas*) oysters in tidal pools. Mar. Environ. Res. 172, 105497 https://doi.org/10.1016/j.marenvres.2021. 105497.

Helm, M.M., Bourne, N., Lovatelli, A., 2004. Hatchery culture of bivalves: a practical manual. In: FAO Fisheries Technical Paper, 471. Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 1–203.

IPCC, 2021. In: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., Huang, M., Leitzell, K., Lonnoy, E., JBR, Matthews, Maycock, T.K., Waterfield, T., Yelekçi, O., Yu, R., Zhou, B. (Eds.), Summary for policymakers. In: climate change 2021: the physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change.

Jacobs, P., Greeve, Y., Sikkema, M., Dubbeldam, M., Philippart, C.J.M., 2020. Successful rearing of *Ostrea edulis* from parents originating from the Wadden Sea, the Netherlands. Aquaculture Reports 18, 100537. https://doi.org/10.1016/j.aqrep.2020.100537.

Kamermans, P., Saurel, C., 2022. Interacting climate change effects on mussels (*Mytilus edulis* and *M. Galloprovincialis*) and oysters (*Crassostrea gigas* and *Ostrea edulis*): experiments for bivalve individual growth models. Aquat. Living Resour. 35, 1. https://doi.org/10.1051/alr/2022001.

Kamermans, P., van Duren, L., Kleissen, F., 2018. European flat oysters on offshore wind farms: additional locations: opportunities for the development of European flat oyster (*Ostrea edulis*) populations on planned wind farms and additional locations in the Dutch section of the North Sea. Wageningen Marine Research Report No. C053/18. https://doi.org/10.18174/456358.

Kamermans, P., Blanco, A., van Dalen, P., 2020. Sources of European flat oysters (*Ostrea edulis* L.) for restoration projects in the Dutch North Sea (no. C085/20). Wageningen marine research report no. C085/20. https://doi.org/10.18174/532003.

Kingsolver, J.G., Diamond, S.E., Buckley, L.B., 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. Funct. Ecol. 27, 1415–1423. https://doi.org/10.1111/1365-2435.12145.

Korringa, P., 1946. The decline of natural oyster beds. Basteria 10 (3/4), 36–41.

Korringa, P., 1957. Water temperature and breeding throughout the geographical range of *Ostrea edulis*. Ann. Biol. 33 (1–2), 1–17.

Labarta, U., Fernandez-Reiriz, M.J., Perez-Camacho, A., 1999. Energy, biochemical substrates and growth in the larval development, metamorphosis and postlarvae of *Ostrea edulis* (L.). J. Exp. Mar. Biol. Ecol. 238 (2), 225–242. https://doi.org/10.1016/S0022-0981(98)00171-3.

Laing, I., Walker, P., Areal, F., 2005. A feasibility study of native oyster (*Ostrea edulis*) stock regeneration in the United Kingdom. CARD project FC1016, native oyster stock regeneration: a review of biological, technical and economic feasibility, p. 95.

van Leeuwen, S., Tett, P., Mills, D., van der Molen, J., 2015. Stratified and nonstratified areas in the North Sea: long-term variability and biological and policy implications. J. Geophys. Res. Oceans 120 (7), 4670–4686. https://doi.org/10.1002/2014JC010485.

van Leeuwen, S., Wagemaakers, E., Wernand, M., 2021a. NIOZ jetty hourly data for temperature and salinity for 2017. https://doi.org/10.25850/nioz/7b.b.bc. NIOZ, V1.

van Leeuwen, S., Wagemaakers, E., Wernand, M., 2021b. NIOZ jetty hourly data for temperature and salinity for 2018. https://doi.org/10.25850/nioz/7b.b.cc. NIOZ, V1.

van Leeuwen, S., Wagemaakers, E., Wernand, M., 2021c. NIOZ jetty hourly data for temperature and salinity for 2019. https://doi.org/10.25850/nioz/7b.b.dc. NIOZ, V1.
van Leeuwen, S., Wagemaakers, E., Gerkema, T., 2021d. NIOZ jetty hourly data for temperature and salinity for 2020. https://doi.org/10.25850/nioz/7b.b.1c. NIOZ, V1.
van Leeuwen, S., Wagemaakers, E., Gerkema, T., 2022. NIOZ jetty hourly data for temperature and salinity for 2021. https://doi.org/10.25850/nioz/7b.b.sd. NIOZ, V1.
Lucas, M.I., Walker, G., Holland, D.L., Crisp, D.J., 1979. An energy budget for the free-swimming and metamorphosing larvae of *Balanus balanoides* (Crustacea: Cirripedia). Mar. Biol. 55 (3), 221–229. https://doi.org/10.1007/BF00396822.
Maathuis, M.A., Coolen, J.W., Van Der Have, T., Kamermans, P., 2020. Factors determining the timing of swarming of European flat oyster (*Ostrea edulis* L.) larvae in the Dutch Delta area: implications for flat oyster restoration. J. Sea Res. 156, 101828 https://doi.org/10.1016/j. seares.2019.101828.
Martin, T.L., Huey, R.B., 2008. Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. Am. Nat. 171 (3), E102–E118. https://doi.org/10.1086/527502.
Maryland Oyster Restoration Interagency Workgroup of the Chesapeake Bay Program''s Sustainable Fisheries Goal Implementation Team, 2021. Maryland Oyster Restoration Update: Progress toward the Chesapeake Bay Watershed Agreement's 'Ten Tributaries by 2025' Oyster Outcome.
Mayorga-Adame, C.G., Polton, J.A., Fox, A.D., Henry, L.A., 2022. Spatiotemporal scales of larval dispersal and connectivity among oil and gas structures in the North Sea. Mar. Ecol. Prog. Ser. 685, 49–67. https://doi.org/10.3354/meps13970.
Meijering, E., Dzyubachyk, O., Smal, I., 2012. Chapter nine – methods for cell and particle tracking. Methods Enzymol. 504, 183–200. https://doi.org/10.1016/B978-0-12-391857-4.00009-4.
Newell, R.C., Johson, L.G., Kofoed, L.H., 1977. Adjustment of the components of energy balance in response to temperature change in *Ostrea edulis*. Oecologia 30, 97–110. https://doi.org/10.1007/B F00345414.
OSPAR Commission, 2008. OSPAR List of threatened and/or declining species and habitats. Reference Number: 2008–6. http://www.ospar.org/documents?d=32794.
OSPAR Commission, 2013. OSPAR Recommendation 2013/4 on furthering the protection and conservation of *Ostrea edulis* in Region II of the OSPAR maritime area and *Ostrea edulis* beds in Regions II, III and IV of the OSPAR maritime area. OSPAR (2) 13/4/1, Annex 7. https://www.ospar.org/documents?d=32968.
Padfield, D., O'Sullivan, H., Pawar, S., 2021. *rTPC* and *nls.Multstart*: a new pipeline to fit thermal performance curves in *R*. Methods Ecol. Evol. 12 (6), 1138–1143. https://doi.org/10.1111/2041-210X.13585.
Pogoda, B., Brown, J., Hancock, B., Preston, J., Pouvreau, S., Kamermans, P., Sanderson, W., Von Nordheim, H., 2019. The native oyster restoration Alliance (NORA) and the Berlin oyster recommendation: bringing back a key ecosystem engineer by developing and supporting best practice in Europe. Aquat. Living Resour. 32, 13. https://doi.org/10.1051/alr/2019012.
Robert, R., His, E., Dinet, A., 1988. Combined effects of temperature and salinity on fed and starved larvae of the European flat oyster *Ostrea edulis*. Mar. Biol. 97 (1), 95–100. https://doi.org/10.1007/BF00391249.
Robert, R., Vignier, J., Petton, B., 2017. Influence of feeding regime and temperature on development and settlement of oyster *Ostrea edulis* (Linnaeus, 1758) larvae. Aquac. Res. 48 (9), 4756–4773. https://doi.org/10.1111/are.13297.
Rodriguez, J.L., Sedano, F.J., Garcia-Martin, L.O., Perez-Camacho, A., Sanchez, J.L., 1990. Energy metabolism of newly settled *Ostrea edulis* spat during metamorphosis. Mar. Biol. 106, 109–111. https://doi.org/10.1007/BF02114680.
Rodriguez-Perez, A., Sanderson, W.G., Moller, L.F., Henry, T.B., James, M., 2020. Return to sender: the influence of larval behaviour on the distribution and settlement of the European oyster *Ostrea edulis*. Aquat. Conserv. Mar. Freshwat. Ecosyst. 30, 2116–2132. https://doi.org/10.1002/aqc.3429.
Saunders, M.I., Doropoulos, C., Bayraktarov, E., Babcock, R.C., Gorman, D., Eger, A.M., Vozzo, M.L., Gillies, C.L., Vanderklift, M.A., Steven, A.D.L., Bustamante, R.H., Silliman, B.R., 2020. Bright spots in coastal marine ecosystem restoration. Curr. Biol. 30 (24), R1500–R1510. https://doi.org/10.1016/j.cub.2020.10.056.
Sawusdee, A., Jensen, A.C., Collins, K.J., Hauton, C., 2015. Improvements in the physiological performance of European flat oysters *Ostrea edulis* (Linnaeus, 1758) cultured on elevated reef structures: implications for oyster restoration. Aquaculture 444, 41–48. https://doi.org/10.1016/j.aquaculture.2015.03.022.
Schutter, M., Tonk, L., Kamermans, P., Kardinaal, E., ter Hofstede, R., 2021. EcoScour Project Borssele V: Outplacement Methods of European Flat Oysters. Bureau Waardenburg, Culemborg, pp. 21–328.
Smyth, D.M., Horne, N.S., Ronayne, E., Millar, R.V., Joyce, P.W., Hayden-Hughes, M., Kregting, L., 2020. Wild gregarious settlements of *Ostrea edulis* in a semi-enclosed sea lough: a case study for unassisted restoration. Restor. Ecol. 28 (3), 645–654. https://doi.org/10.1111/rec. 13124.
Thewes, D., Stanev, E.V., Zielinski, O., 2022. Steps toward modelling the past and future North Sea ecosystem with a focus on light climate. Front. Mar. Sci. 9, 818383 https://doi.org/10.3389/fmars.2022.818383.
Turner, E.J., Zimmer-Faust, R.K., Palmer, M.A., Luckenbach, M., Pentchef, N.D., 1994. Settlement of oyster (*Crassostrea virginica*) larvae: effects of water flow and a water-soluble chemical cue. Limnol. Oceanogr. 39 (7), 1579–1593. https://doi.org/10.4319/lo.1994.39.7.1579.
Vasseur, D.A., DeLong, J.P., Gilbert, B., Greig, H.S., Harley, C.D.G., McCann, K.S., Savage, Van, Tunney, T.D., O'Connor, M.I., 2014. Increased temperature variation poses a greater risk to species than climate warming. Proc. R. Soc. B Biol. Sci. 281 (1779), 20132612 https://doi.org/10.1098/rspb.2013.2612.
Verheyen, J., Stoks, R., 2019. Temperature variation makes an ectotherm more sensitive to global warming unless thermal evolution occurs. J. Anim. Ecol. 88 (4), 624–636. https://doi.org/10.1111/1365-2656.12946.
Videla, J.A., Chaparro, O.R., Thompson, R.J., Concha, I.I., 1998. Role of biochemical energy reserves in the metamorphosis and early juvenile development of the oyster *Ostrea chilensis*. Mar. Biol. 132 (4), 635–640. https://doi.org/10.1007/s002270050428.
Wallace, R.K., Waters, P., Rikard, F.S., 2008. Oyster hatchery techniques. SRAC Publication No 4302. Southern Regional Aquaculture Center, Stoneville, MS.
Waller, T.R., 1981. Functional morphology and development of veliger larvae of the European oyster, *Ostrea edulis* Linne. Smithsonian Contributions to Zoology 328, 70. https://doi.org/10.5479/si.00810282.328.
Wang, X., Fu, F., Qu, P., Kling, J.D., Jiang, H., Gao, Y., Hutchins, D.A., 2019. How will the key marine calcifier *Emiliania huxleyi* respond to a warmer and more thermally variable ocean? Biogeo-Sciences 16 (22), 4393–4409. https://doi.org/10.5194/bg-16-4393-2019.
Weinert, M., Mathis, M., Kröncke, I., Neumann, H., Pohlmann, T., Reiss, H., 2016. Modelling climate change effects on benthos: distributional shifts in the North Sea from 2001 to 2099. Estuar. Coast. Shelf Sci. 175, 157–168. https://doi.org/10.1016/j.ecss.2016.03.024.
Weinert, M., Mathis, M., Kröncke, I., Pohlmann, T., Reiss, H., 2021. Climate change effects on marine protected areas: projected decline of benthic species in the North Sea. Mar. Environ. Res. 163, 105230 https://doi.org/10.1016/j.marenvres.2020.105230.
Wiggins, P.R., Frappell, P.B., 2000. The influence of haemoglobin on behavioural thermo- regulation and oxygen consumption in *Daphnia carinata*. Physiol. Biochem. Zool. 73, 153–160. https://doi.org/10.1086/316739.
Xing, K., Hoffmann, A.A., Ma, C.S., 2014. Does thermal variability experienced at the egg stage influence life history traits across life cycle stages in a small invertebrate? PLoS One 9 (6), e99500. https://doi.org/10.1371/journal.pone.0099500.