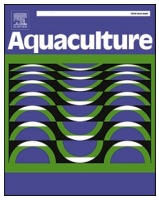
Aquaculture 566 (2023) 739174

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Short communication

Consequences of thermal history for growth, development and survival during metamorphosis and settlement for the European flat oyster

Katharina Alter [a,*], Catharina J.M. Philippart [a,b], Sean Teng [c], Hanno Bolier [c], Pim Drenth [c], Marco Dubbeldam [c]

[a] Royal Netherlands Institute for Sea Research, Department of Coastal Systems, P.O. Box 59, 1790AB Den Burg, the Netherlands
[b] Utrecht University, Department of Physical Geography, P.O. Box 80.115, 3508TC Utrecht, the Netherlands
[c] Stichting Zeeschelp, Jacobahaven 1, 4493ML Kamperland, the Netherlands

ARTICLE INFO

Keywords:
Shellfish
Bivalve
Metamorphosis
Carry-over effect

ABSTRACT

The European Union's Habitats Directive has the obligation to restore natural habitats in order to increase biodiversity within Europe. The European flat oyster *Ostrea edulis* is a favorable habitat builder for ecological restoration for which large amounts of spat are needed. Yet, the supply of spat is a key limiting factor because rearing protocols for *O. edulis* have not been optimized. This study reports on successful hatchery production of *O. edulis* reared at different temperature regimes with the aim to determine the most suitable thermal condition for early-life growth and survival. Larvae were reared at 25 and 29 °C until they were competent for settlement. Then, the larval batch at either temperature was split into two. One half of the batch remained at the original temperature while the other half was exposed to another temperature (25 or 29 °C) until 14 days post settlement. Larval size was similar among temperature treatments until 10 days post release, after which larvae reared at 29 °C were slightly (3%) larger compared to those reared at 25 °C ($p < 0.05$). The percentage of larvae which developed competence for settlement was not different ($p > 0.05$) between 25 °C (37 ± 4%) and 29 °C (33 ± 4%). Two weeks post settlement, size of the spat reared at 29 °C with a larval thermal history of 25 °C was 23% larger ($p < 0.05$) compared to *O. edulis* constantly reared at either 29 or 25 °C. Spat survival, which varied between 29 ± 6% and 56 ± 15% among treatments, was not related to rearing temperatures during the larval and early benthic phase ($p > 0.05$). Spat reached a size at which they are suitable for release into restoration sites within two weeks, resulting in a hatchery period of as little as three weeks. Our results contribute to the optimization of *O. edulis* hatchery protocols to improve yields for a growing demand of spat used in ecological restoration.

1. Introduction

Habitat-forming species increase biodiversity, resilience against climate change and the provision of ecosystem services in marine systems (Barbier et al., 2011). In the North Sea, the European flat oyster, *Ostrea edulis*, is such an ecological keystone species for which the European Union's Directive calls protection, conservation and restoration. Due to the life-history strategy of *O. edulis*, active restoration is a must for their return in locations with absent or low adult individuals and steps for upscaling oyster restoration have to be undertaken in order to establish self-sustaining oyster beds (OSPAR, 2020). One strategy of active restoration involves a large-scale production of pathogen-free early-life benthic oysters (spat) settled on oyster or mussel shells (spat on shell) which can subsequently be released into restoration sites. To date, the supply of spat, however, is insufficient and a key limiting factor for *O. edulis* restoration projects (Pogoda et al., 2019; Colsoul et al., 2021). In Europe, the production is estimated to be approx. 200 million spat/year of which 70 million spat/year is produced in pathogen-free areas and only 7 million spat/year is produced as spat on shell (Kamermans et al., 2020). Yet, in successful oyster restoration projects, for example with the Eastern oyster *Crassostrea virginica* in the USA, 164 million spat on shell were released into five restoration sites in one year (4.8 billion spat on shell since 2015) in Maryland alone with further five restoration sites in Virginia (Maryland Oyster Restoration Interagency Workgroup of the Chesapeake Bay Program's Sustainable Fisheries Goal Implementation Team, 2021). In addition, *O. edulis* populations differ

* Corresponding author.
E-mail address: Katharina.alter@nioz.nl (K. Alter).

https://doi.org/10.1016/j.aquaculture.2022.739174
Received 10 October 2022; Received in revised form 7 December 2022; Accepted 16 December 2022
Available online 19 December 2022

genetically and can be divided into three clusters, i.e. Spanish, Irish/British/French, and Dutch/Danish, which correspond to oceanic fronts characterized by different water parameters such as temperature, salinity and nutrients as well as different stratification (Vera et al., 2016). Translocations between locations is discouraged and the establishment of new hatcheries across Europe is encouraged to produce genetically diverse spat (Pogoda et al., 2019).

Although hatchery protocols for bivalves in general or oysters (such as *C. virginica* in particular) exist, protocols for *O. edulis* are still not reliable (Dupuy et al., 1977; Helm et al., 2004; Kamermans et al., 2020; Colsoul et al., 2021). In a recent review on *O. edulis* spat production, the authors highlight that it remains a challenge to establish high, synchronized and reliable settlement success of larvae which will result in successful growth and survival of spat (Colsoul et al., 2021). Hatchery guides recommend a rearing temperature of 22 °C for *O. edulis* larvae and spat, yet research showed that highest levels of larval survival and settlement success are achieved at temperatures between 25 and 30 °C (Robert et al., 2017). Yet, later in life, performance of adult *O. edulis* peaks at lower temperatures of 20–21 °C, which may indicate that rearing spat at temperatures as high as 30 °C may be unfavorable for growth and survival (Newell et al., 1977; Eymann et al., 2020).

The objective of this study was to assess vital rates of larvae and spat of *O. edulis* under different temperature regimes with the aim to determine the most suitable thermal condition for survival and growth until the market size of 2–4 mm required to increase hatchery production for ecological restoration. In the present study, oyster larvae were reared at 25 and 29 °C until competence for settlement at which time point two temperature treatments were added in which larvae that were reared at 25 °C were transferred to 29 °C and *vice versa* for the spat rearing period of two weeks. This implies that we studied the impacts of two temperatures (25 and 29 °C) during their pelagic phase and four temperature regimes (25/25 °C, 29/29 °C, 25/29 °C, 29/25 °C) during their early benthic phase. Because adult individuals perform better at lower temperatures than larvae (Newell et al., 1977; Robert et al., 2017; Eymann et al., 2020), it was hypothesized that a decrease in temperature when *O. edulis* changes from a pelagic to benthic life stage (so the 29/25 °C treatment) would be the most beneficial for growth and survival of early-life oysters.

2. Material and methods

2.1. Animal rearing

2.1.1. Broodstock

Broodstock conditioning, release and rearing of larvae and spat of *O. edulis* was performed according to standard hatchery practices at the hatchery Stichting Zeeschelp, Kamperland, the Netherlands in August 2022 (see, for example, Jacobs et al. (2020) for a detailed description). In brief, approx. 200 oysters were collected from lake Grevelingen and were tested for *Bonamia ostreae*, the most common parasite for *O. edulis* in the Netherlands (Kamermans et al., 2020). The parasite analysis was performed on individual gill tissue by Wageningen Bioveterinary Research, Lelystad. Only oysters which proved not to be infected were used as broodstock. Boodstock ($n = 70$) were housed in one flow through (2 L/h/oyster) tank containing a sieve at the outflow to collect released larvae that were transported with the water flow. The seawater was sourced from the nearby coastal water (Eastern Scheld), filtered (0.02 μm), heated to 25.3 ± 0.1 °C at a salinity of 30 ± 1 PSU and constantly aerated. The tank was placed in a shaded area subjected to the natural light:dark cycle (14.5: 9.5 h). Broodstock were continuously fed a mixture of life micro-algae (SeaCAPS-system, UK) at a 3% dry weight ration. The algae mixture consisted of approx. 55% diatoms (with equal portions of *Skeletonema costatum, Skeletonema marinoi, Thalassiosira weisfloggi and Chaetoceros neogracile*) and 45% flagellates (with equal portions of *Rhodomonas lens, Isochrysis galbana* and *Pavlova lutheri*). Released larvae were collected from the sieve each day and larvae from one day were used for experiments. It is unknown how many adult individuals contributed to this batch.

2.1.2. Larval phase

Larvae (5 ± 1 ind/ml) were reared in conical flow through tanks (180 L capacity, 20 renewals/day, $n = 6$). Seawater was filtered (0.02 μm), had a salinity of 30 ± 1 PSU and was heated to the treatment temperatures of 25 °C (25.3 ± 0.1 °C, range 24.6–25.7 °C, $n = 3$ tanks) and 29 °C (28.5 ± 0.0 °C, range 28.3–28.6 °C, n = 3 tanks). Throughout the experimental period temperature and pH (8.23 ± 0.05, range 8.02–8.43) were measured every other day using WTW probes (SenTix41 and TetraCon 325, respectively). The live micro-algae diet for rearing the larvae consisted of *C. neogracile* (40%), *I. galbana* (25%), *P. lutheri* (25%), and *R. lens* (10%) and was held constant at an excess density of 40,000 cells/ml. Larvae were transferred to new tanks every other day, whereby they were sieved over a mesh size of 150 μm, 180 μm and 250 μm on day 2, 6 and 9, respectively. Larvae that fell through the mesh size were discarded. A subsample of larvae which remained on the mesh was counted and their anterior-posterior length was measured ($n = 12$ larvae per tank for 6 tanks, subsequently averaged per tank) under a microscope (Olympus CKX41) to determine growth and development into competent larvae for settlement which was considered as larval survival. Individual larvae were considered suitable for settlement ('competent') when eye spots and the foot were present, and they had reached a minimum size of 300 μm.

2.1.3. Spat phase

On day 9 post release (dpr), competent larvae were harvested for determination of spat survival and growth. In conformity with general hatchery practices, only these first maturing larvae were taken because later maturing larvae are generally less successful in metamorphosis and post-metamorphosis survival (Whyte et al., 1992). On that day, a batch of larvae was taken from each tank, divided into two groups and transferred into 12 settlement tanks in total. A group reared at a particular temperature as larvae was further maintained at that temperature ($n = 3$) while the second group (n = 3) was transferred to the other temperature, i.e. 25 °C when larvae were reared at 29 °C and *vice versa*. Different amounts of larvae were taken from the larval tanks because different amounts of competent larvae were available on 9 dpr. This resulted in different stocking densities among replicates within the settlement tanks. A total of 50,000 competent larvae was taken from each of two out of the three larval tanks at both temperatures resulting in a stocking density of 8 ind/ml in the settlement tank. From the third larval tank only 44,500 competent larvae at 25 °C and 29,500 competent larvae at 29 °C were taken which resulted in stocking densities in the settlement tanks of 7 and 5 ind/ml, respectively. Settlement tanks consisted of PVC pipes (20 cm Ø) with a mesh bottom (200 μm) hung into a flow through tank in which the inflowing water and food supply was placed above the sieve to create a down-welling environment. The food ration during the early benthic phase was similar as used for the larvae, but was increased to a density of 60,000 algae/ml. Grind-up oyster shells (200–400 μm, microbrisure, Entre Mer et Terre) were placed in the sieve as settling material. For this study single spat were produced. Sieves were cleaned with seawater every day. After two weeks, subsamples ($n = 10$) of the spat from each tank were counted and their anterior-posterior length and dorsal-ventral height were measured ($n = 20$) under a stereo microscope (Leica MZFLIII) to determine survival and growth. Sizes of spat were averaged for each replicate tank ($n = 3$).

2.2. Statistical analysis

Repeated measures two-way analysis of variances (ANOVA) was used to detect differences in larval size between rearing temperatures during the larval phase. A *t*-test was used to test for differences in larval survival as well as amount of larvae reaching competence on 9 dpr between rearing temperatures. Two-way ANOVAs were used to test for

differences in spat size and spat survival with larvae and spat rearing temperatures as explaining variables. 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. In figures, data is visualized as box and whisker plots where the box represents the 25th to 75th percentiles. The median is shown as horizontal line. Whiskers represent the minimum and maximum values. All sample sizes are $n = 3$.

3. Results

For clarity throughout the results, temperature conditions during the larval and spat phase are stated as a fraction. For example, 25/29 °C refers to 25 °C during the larval phase and 29 °C during settlement, metamorphosis and the spat phase.

3.1. Larval and spat survival

The percentage of larvae that survived the first 14 days after release from the broodstock (the last day of investigation for this trait) was similar between 25 °C (36.6 ± 3.6%) and 29 °C (32.7 ± 3.5%) ($p > 0.05$, Table 1, Fig. 1A). The highest percentage of larvae reaching competence for settlement within one day was 28.4 ± 4.4% at 25 °C and 25.6 ± 2.7% at 29 °C, which occurred at 10 dpr at both temperatures. Competent larvae used for determination of spat growth and survival were collected at 9 dpr which was the second day at which larvae had developed competence for settlement in both temperature treatments with 6.0 ± 0.8% and 4.8 ± 1.1% at 25 and 29 °C ($p > 0.05$, Table 1), respectively. For these individuals, neither larvae nor spat rearing temperature influenced spat survival ($p > 0.05$, Table 1, Fig. 1B). Averaged across all temperature treatments, spat survival of competent larvae was 38.3 ± 4.8%, which was 12.9 ± 1.3% of the initially released and stocked larvae. Although the mean survival of spat was not significantly different between treatments, the 29/25 °C treatment showed a high range in the percentage values of spat that survived the first 14 days after settlement (37–85%) compared to those for the other three temperature treatments (30–40% for 25/25 °C, 22–40% for 25/29 °C, and 25–44% for 29/29 °C; Fig. 1B).

Table 1
Summary of *t*-test and analysis of variance (ANOVA) results. *Asterisks* indicate significances ($p < 0.05$) within a testing treatment. dpr = days post release.

T-test				
Source	df		t	p
Larval survival (%)	4		0.768	0.485
Competence for settlement at 9 dpr	4		0.845	0.446
Two-way ANOVA				
Source	df	MS	F	p
Spat survival (%)				
Larval temperature	1	580.200	2.708	0.138
Spat temperature	1	545.900	2.548	0.149
Larvae × spat temperature	1	222.000	1.036	0.338
Residuals	8	214.300		
Spat length (mm)				
Larval temperature	1	0.078	5.601	0.045*
Spat temperature	1	0.385	27.515	0.001*
Larval × spat temperature	1	0.022	1.548	0.249
Residuals	8	0.014		
Spat height (mm)				
Larval temperature	1	0.087	2.619	0.144
Spat temperature	1	0.644	19.457	0.002*
Larval × spat temperature	1	0.080	2.418	0.159
Residuals	8	0.033		
Repeated measures two-way ANOVA				
Larval length (μm)				
Larval temperature	1	97.200	34.714	0.028*
Age	4	7488.000	565.812	0.000*
Larval temperature × age	4	141.280	4.109	0.042*
Residuals	18	21.000		

3.2. Larval and spat sizes

Larval length was similar ($p > 0.05$) between temperature treatments until 10 dpr, at which time point larvae reared at 29 °C (296 ± 2 μm) were 3% larger compared to those reared at 25 °C (287 ± 2 μm) ($p < 0.01$, Table 1, Fig. 2). The average growth rate of larvae during the pelagic phase at 25 and 29 °C was 10.4 ± 1.4 μm/day and 12.3 ± 1.2 μm/day, respectively. After two weeks in the settlement tanks, spat length was influenced by larval temperature ($p < 0.05$) and spat temperature ($p < 0.001$, Table 1). Spat length was largest in treatment 25/29 °C (2.50 ± 0.10 mm). Compared to that, spat from treatment 29/29 °C (2.26 ± 0.06 mm) and 25/25 °C (2.06 ± 0.04 mm) were significantly smaller ($p < 0.05$ for both, Fig. 3A). Spat height was influenced by spat temperature only ($p < 0.01$, Table 1). For this measure, spat from treatment 25/29 °C (2.77 ± 0.18 mm) was 22% larger than spat from treatment 25/25 °C (2.14 ± 0.02 mm, $p < 0.01$, Fig. 3B).

4. Discussion

The supply of *O. edulis* spat from hatcheries is still a key limiting factor for restoration projects within Europe which aim at increasing biodiversity and its associated benefits for ecosystem health. *Ostrea edulis* oyster beds are not yet self-sustaining and hatchery reared spat is released into restoration sites (Pogoda et al., 2019; Kamermans et al., 2020; Colsoul et al., 2021). Spat used for ecological restoration, has to be pathogen-free and of high genetic diversity and thus should be locally produced (Colsoul et al., 2021). In this study, we demonstrated that rearing *O. edulis* of a pathogen-free Dutch origin at high temperatures of 25 and 29 °C resulted in 30–40% survival of larvae as well as spat. With a switch in temperature from 25 to 29 °C at time of settlement, spat grew to a size at which they are suitable for release into North Sea restoration sites within two weeks, resulting in a hatchery period of as little as three weeks.

When *O. edulis* experienced the lower temperature (25 °C) during the larval phase, spat grew faster at the higher temperature (29 °C) compared to stable temperatures throughout the two life-stages. For species with complex life-stages, larval experiences can influence post-metamorphic fitness, such as growth and survival of juveniles and adults (reviewed in Pechenik, 2006). The effects are usually investigated with regards to nutritional stress during critical periods before metamorphosis, yet other environmental stressors are rarely investigated (Pechenik, 2006). Bivalve larvae often grow best at temperatures well above those which they would experience in nature and those between 25 and 32 °C have been stated as optimal for larvae of the oysters *O. edulis, Crassostrea rhizophorae* and *Magellana gigas* (Helm et al., 2004; Rico-Villa et al., 2008; Robert et al., 2017). These studies, however, did not investigate carry-over effects on the benthic life-stage performance. It may be that larval exposure to 29 °C was energetically suboptimal compared with exposure to 25 °C so that, once transferred to 29 °C at settlement, large larval energy reserves coupled with a more rapid passing through metamorphosis and a higher food intake post-metamorphosis may have boosted rapid early spat growth resulting in largest spat within this treatment group. Metamorphosis is an energetically costly process during which larvae deplete their energy storage of as much as 65% (Rodriguez et al., 1990; Videla et al., 1998; Whyte et al., 1992). After morphological and physiological changes occurred, spat

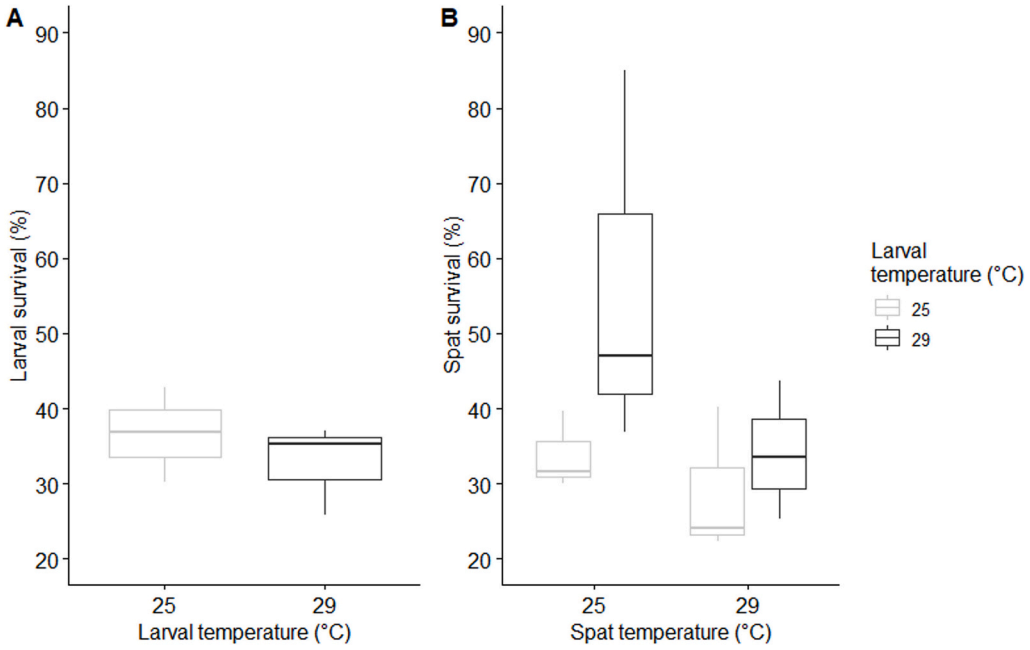


Fig. 1. (A) Percentage (%) of larvae that survived the first 14 days post release from the broodstock and (B) percentage (%) of spat that survived the first 14 days post settlement, when reared at 25°C (grey box) and 29°C (black box). Differences between survival were not significant (see Table 1).

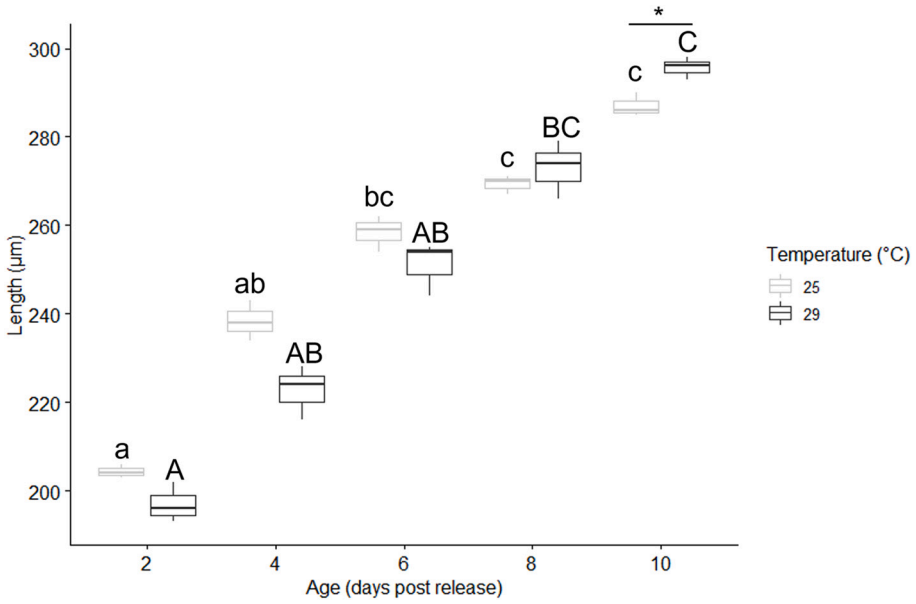


Fig. 2. Larval length (µm) during the pelagic phase when reared at 25°C (grey box) and 29°C (black box). Different *lower-case* (25°C) and *upper-case* (29°C) characters indicate significant differences between the various ages (days post release) for each of the two treatments separately. *Asterisk* (*) indicates a significant difference between the two temperature treatments at ten days post release from the broodstock.

commence feeding again but its rate may not immediately be sufficient for early post-metamorphic growth, resulting in further dependence on stored energy (Whyte et al., 1992). Food intake is positively correlated with temperature in both life stages, yet early spat (determined until 4 days post-metamorphosis) ingested 30% more algae whereas larvae ingested only 10% more algae at 30 °C than at 25 °C (Robert et al., 2017). In addition, experiments with 4 mm spat showed that upon transfer to a 6 °C increase, metabolic costs of spat did not change, and filtration rates increased continuously throughout the three-week experimental period, while a transfer to a 6 °C decrease resulted in reduced metabolic costs but also reduced filtration rates which in the long term were partly compensated (Beiras et al., 1995). Hence, upon transfer from the lower to the higher temperature, competent larvae in the present study might have had a metabolic advantage during the short-term exposure over those that were reared continuously at the higher temperature. Our results show that after rearing *O. edulis* larvae at a temperature commonly used for other oyster species within hatcheries (25 °C) and a transfer of competent larvae to 29 °C results in rapid early spat growth which yields a market sized product (2–4 mm) within the next two weeks (Utting and Spencer, 1991; Helm et al., 2004). It is noteworthy, however, that exposure to temperatures as high as 29 °C become disadvantageous for feeding and thus growth in adult *O. edulis* (Eymann et al., 2020). This time point, when further rearing at high temperatures results in reduced instead of enhanced growth of juvenile

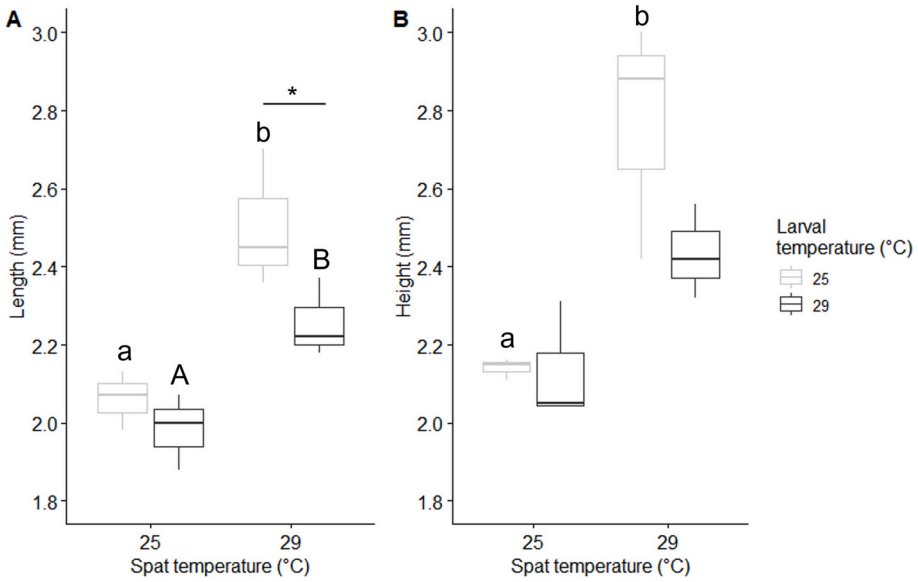


Fig. 3. Larval sizes as (A) length (mm) and (B) height (mm) of spat after being reared at 25°C and 29°C for 14 days, preceded by rearing during the pelagic larval phase at 25°C (grey box) and 29°C (black box). Different *lower case* (25°C) and *upper case* (29°C) characters indicate significant differences in spat size for each larval temperature treatment separately. *Asterisk* (*) indicates a significant difference in spat size within the given spat temperature.

oysters, still needs to be determined. In addition, it further remains to be tested if early spat experiences to high temperatures during the first two weeks post settlement influence later life stages just as it was seen for larval experiences on spat in the present study.

Survival of spat was not different between temperature treatments, yet high stocking densities may have masked a temperature effect. The replicates with the lower initial competent larval stocking density (29/25 °C and 29/29 °C) had the highest spat survival within their respective treatment group. This may suggest that spat survival was density dependent and that this effect was more pronounced at the colder temperature. In theory, growth and ultimately survival decrease when stocking densities become too high so that space and food become limiting factors (Beal and Kraus, 2002). Small oyster spat is considered amenable to high-density stocking and is even layered above another in commercial hatcheries (Helm et al., 2004). At the end of the present study, the rearing tanks held approx. two layers of spat, a stocking density which is rarely used in previous studies in which density dependent effects on growth and/or survival were reported, although they were determined using larger spat (Zorita et al., 2021; Tan et al., 2022). Calculations are necessary to determine which temperature combination, i.e. larval phase at 25 °C with a spat phase of 29 °C which resulted in significantly larger spat, or a larval phase at 29 °C with a spat phase at 25 °C which may lead to increased survival when stocking densities are low, is most cost effective for hatcheries.

Previous research showed that increasing the temperature from the commonly used hatchery temperature for *O. edulis* of 22 to 25 °C yields much higher larval survival and settlement success (Robert et al., 2017). By further adjusting the temperature at settlement, these parameters can be further optimized. In addition, also the growth of spat until market size is improved (this study) which further optimizes hatchery protocols and contributes to a predictable and efficient production of flat oyster spat.

Author statement

Katharina Alter: Conceptualization, data collection, analysis and visualization of data, writing original manuscript, editing of manuscript. **Catharina JM Philippart:** Secured funding, editing of manuscript. **Sean Teng:** Data collection, editing of manuscript. **Hanno Boiler:** Data collection, editing of manuscript. **Pim Drenth:** Data collection, editing of manuscript. **Marco Dubbeldam:** Conceptualization, data collection, secured funding, editing of manuscript.

Funding

This study was supported by the project De Rijke Noordzee, stichting De Noordzee, stichting Ark Nature Development and the regional subsidy grant Zeeland in Stroomversnelling. This research is also a contribution to the European Union's Horizon 2020 research and innovation programme under grant agreement No. 869300 (Climate change and Future Marine Ecosystem Services and Biodiversity – Future MARES). None of these funding sources had involvement in the collection, analysis and interpretation of the data, neither in the writing of the report, nor in the decision to submit the article for publication.

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.

References

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.

Beal, B.F., Kraus, M.G., 2002. Interactive effects of initial size, stocking density, and type of predator deterrent netting on survival and growth of cultured juveniles of the soft-shell clam, *Mya arenaria* L., in eastern Maine. Aquaculture 208 (1–2), 81–111. https://doi.org/10.1016/S0044-8486(01)00900-0.

Beiras, R., Camacho, A.P., Albentosa, M., 1995. Short-term and long-term alterations in the energy budget of young oyster *Ostrea edulis* L. in response to temperature change. J. Exp. Mar. Biol. Ecol. 186 (2), 221–236. https://doi.org/10.1016/0022-0981(94)00159-B.

Colsoul, B., Boudry, P., Pérez-Parallé, M.L., Bratoš Cetinić, A., Hugh-Jones, T., Arzul, I., Merou, N., Wegner, K.M., Peter, C., Merk, V., Pogoda, B., 2021. Sustainable large-scale 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.

Dupuy, J.L., Windsor, N.T., Sutton, C.E., 1977. Manual for design and operation of an oyster seed hatchery for the American oyster *Crassostrea Virginica*. In: Special Report No. 142 in Applied Marine Science and Ocean Engineering. Virginia Institute of Marine Science, Gloucester Point, Virginia, USA.

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.

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

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. Aquacult. Rep. 18, 100537 https://doi.org/10.1016/j.aqrep.2020.100537.

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. doi: 10.18174/532003.

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.

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/BF00345414.

OSPAR, 2020. European flat oyster and *Ostrea edulis* beds. BDC2020, OSPAR.

Pechenik, J.A., 2006. Larval experience and latent effects—metamorphosis is not a new beginning. Integr. Comp. Biol. 46 (3), 323–333. https://doi.org/10.1093/icb/icj028.

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.

Rico-Villa, B., Woerther, P., Mingant, C., Lepiver, D., Pouvreau, S., Hamon, M., Robert, R., 2008. A flow-through rearing system for ecophysiological studies of Pacific oyster *Crassostrea gigas* larvae. Aquaculture 282 (1–4), 54–60 https://doi.org/j.aquaculture.2008.06.016.

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.

Tan, K., Zhai, Y., Zhang, H., Zeng, Z., Ning, Y., Zheng, H.. Effects of culture conditions (stocking density, water depth and aquaculture gear) on the aquaculture performance of a new *Crassostrea angulata* variety "Golden oyster# 1". Aquaculture.739054. https://doi.org/10.1016/j.aquaculture.2022.739054.

Utting, S.D., Spencer, B.E., 1991. The Hatchery Culture of Bivalve Mollusc Larvae and Juveniles, vol. 68. Ministry of Agriculture, Fisheries and Food, Dectorate of Fisheries Research, p. 32.

Vera, M., Carlsson, J., El, Carlsson J., Cross, T., Lynch, S., Kamermans, P., Villalba, A., Culloty, S., Martinez, P., 2016. Current genetic status, temporal stability and structure of the remnant wild European flat oyster populations: conservation and restoring implications. Mar. Biol. 163, 239. https://doi.org/10.1007/s00227-016-3012-x.

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.

Whyte, J.N.C., Bourne, N., Ginther, N.G., Hodgson, C.A., 1992. Compositional changes in the larva to juvenile development of the scallop *Crassadoma gigantea* (gray). J. Exp. Mar. Biol. Ecol. 163 (1), 13–29. https://doi.org/10.1016/0022-0981(92)90144-Y.

Zorita, I., Juez, A., Solaun, O., Muxika, I., Rodríguez, J.G., 2021. Stocking density effect on the growth and mortality of juvenile European flat oyster (*Ostrea edulis* Linnaeus, 1758). Aquacult. Fish Fish. 1 (1), 60–65. https://doi.org/10.1002/aff2.18.