Aquat. Living Resour. 2025, 38, 18
© P.S.E. zu Ermgassen et al., Published by EDP Sciences 2025
https://doi.org/10.1051/alr/2025016

Aquatic Living Resources
Available online at:
www.alr-journal.org

Research Article Open Access

Identifying common factors resulting in hatchery crashes during the production of *Ostrea edulis* for ecological restoration in Europe

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Received 17 January 2025 / Accepted 10 September 2025

Handling Editor: Ryan B Carnegie

Abstract – The European flat oyster, *Ostrea edulis*, once formed extensive reefs along European coasts. These reef ecosystems are now functionally extinct, but support for their restoration is rapidly growing. Efforts are currently limited by a bottleneck in *O. edulis* supply. *O. edulis* is a challenging species to produce in a hatchery. Currently unknown causes of high mortality and hatchery crashes must be addressed to meet the increased demand for spat from the ecological restoration sector. Here we present the results of a collaborative effort between nine European hatcheries and marine research laboratories to share recent experiences, as well as production and protocol-related data. We found that crashes were widespread and suspected to be caused by diverse culprits, including *Vibrio* species, predatory zooplankton, and poor water quality. A Boruta regression analysis of production data identified eleven factors including water temperature in the larval tank, broodstock origin, and number of broodstock as being potentially important in explaining crashes during the larval growth phase. Eight factors including duration of larval growth stage and larval density at transfer to the settlement tank, were identified as potentially important in explaining

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crashes during the settlement phase. When applied to larval mortality data of batches that did not crash, the Boruta analysis identified 13 factors, including water temperature, broodstock and larval densities and broodstock origin in determining larval mortality during the larval growth stage and two factors, number of larvae in the initial larval release and broodstock origin, during the larval settlement phase. This research highlights the value of knowledge exchange between hatchery facilities in overcoming spat production problems, identifies factors that may contribute to increased larval mortality and the risk of larval crashes, as well as the importance of developing collaborative research programmes to provide for greater commonality in data collection for future coordination and production analysis.

Keywords: Seed production / larval mortality / oyster restoration / European flat oyster / shellfish / aquaculture production / production analysis

1 Introduction

The European flat oyster, *Ostrea edulis* (Linnaeus, 1758), was historically abundant throughout European coasts and seas, forming extensive reef ecosystems that were the basis of profitable fisheries (Thurstan et al., 2024). As a result of overexploitation, the ecosystem previously formed by *O. edulis* has collapsed, with remnant populations forming habitat patches over relatively small areas (zu Ermgassen et al., 2024). Recognition of the degraded state of this habitat (zu Ermgassen et al., 2024; OSPAR Commission 2009), alongside a growing interest from both policymakers and the public to restore European seas (EU Commission 2020, EU Commission 2024), has resulted in increasing efforts to recover populations of native oysters (Pogoda et al., 2019; Bos et al., 2023). The scope to grow restoration efforts is, however, currently limited by a bottleneck in the production of *O. edulis* for restoration purposes (Colsoul et al., 2021; zu Ermgassen et al., 2023a).

Aquaculture research into *O. edulis* production had, until recently, stalled in Europe following the switch of focus in commercial aquaculture to the more prolific and faster-growing Pacific cupped oyster (*Magallana gigas,* formerly *Crassostrea gigas*) in the 1970s (Colsoul et al., 2021, Pouvreau et al., 2023). A recent resurgence in research activity relating to *O. edulis* hatchery production is resulting in progress towards addressing critical knowledge gaps and increasing the reliability and cost-effectiveness (e.g. Helm and Bourne 2004, González-Araya et al., 2012, Maneiro et al., 2017). Nevertheless, high mortality events (termed "crashes") remain a prevalent feature of the production of *O. edulis* in Europe (zu Ermgassen et al., 2023b).

To meet the growing demand for native oyster habitat restoration in Europe, several aquaculture facilities have re-focused on the production of *O. edulis* spat, and several more have been established specifically for this purpose (Kamermans et al., 2020). Hatcheries that are working with the restoration community are researching how to best meet the unique and specific needs of restoration, which differ from the traditional table market in terms of biosecurity requirements, choice of settlement substrate and broodstock selection for genetic diversity and/or local adaptation (Lallias et al., 2010, zu Ermgassen et al., 2023a). These developments are critical as restoration efforts move from pilot to large-scale operation.

Efforts to re-establish consistent and reliable production of *O. edulis* spat in hatcheries have been hampered by recurring high mortality events (zu Ermgassen et al., 2023b). Such mortality events are a feature of many bivalve aquaculture systems, including *Crassostrea virginica* on the Atlantic coast of the USA (Gray et al., 2022). Gray et al. (2022) described a collaborative effort by diverse hatcheries along the east coast USA to identify potential causes and commonalities of hatchery crashes in *C. virginica* production. Their research characterised crashes and identified data needs which they recommend be the subject of future research. Furthermore, the initial collaboration provided a clear baseline for understanding the scale and ubiquity of hatchery crashes along the Atlantic coast of the US. Since then, a formal research collaboration among research and commercial hatcheries along the US Atlantic coast has emerged to explore production variability, provide diagnostic services (i.e. pathology, water chemistry, etc.), and share knowledge on how best to avoid crash events and smooth production rates (USDA Northeast Aquaculture Regional Center Award #123476-Z5220211). To date, this program consists of approximately 30 hatcheries (~40% Private Hatcheries) that are interested or actively participating (i.e. submitting anonymized larval and water samples) in the program titled *The Bivalve Hatchery Health Consortium (BHHC): Managing larval mortalities in Bivalve Hatcheries*.

Adopting a similar approach to Gray et al. (2022) the present study presents production data from nine European hatcheries/laboratories producing *O. edulis* for ecological restoration. It represents the first transdisciplinary attempt to examine hatchery-scale husbandry techniques and culture conditions and to understand commonalities in *O. edulis* production and crash events across Europe. This effort may act as a springboard for further collaborative research and knowledge exchange between hatcheries and researchers across Europe, by highlighting critical aspects of the culture process that can be considered in hatchery and protocol design and development.

2 Methods

2.1 Study sites/network

A European collaborative network of hatcheries producing *O. edulis* for restoration purposes was established through an open call in 2021 (zu Ermgassen et al., 2023b). The purpose of the collaboration was to foster knowledge exchange and therefore to collectively make progress towards a more reliable hatchery production of *O. edulis* for restoration purposes. The collaboration included experts from eleven institutions. Nine hatcheries/research laboratories from within the collaboration had produced flat oysters during the period between 2019-2023 and volunteered to contribute data on the protocols used and the larval production through to settlement (where applicable).

The hatcheries/laboratories were located across Europe, in Denmark, England, Germany Italy, The Netherlands (N=3), Spain and Wales (Fig. 1). All participating hatcheries/laboratories were situated in transitional waters with the exception of the International Marine Centre (IMC), situated at full salinity on the coast of Sardinia, and Instituto Español de Oceanografia (IEO), situated on the hypersaline Mar Menor lagoon. Seven of the participating hatcheries/laboratories were research hatcheries, while the two participating Dutch hatcheries, Stichting Zeeschelp and Roem van Yerseke produce *O. edulis* alongside other shellfish species commercially.

2.2 Data collection

All participating facilities contributed summary descriptions of observed mass mortality events (100% mortality) and high mortality events (>70% mortality) since 2019, as well as the suspected reasons for the mortality events occurring. In addition, a joint database was designed to capture 2021-2023 hatchery production data. The scope of this database was broad, as we sought to identify data that are commonly recorded across hatcheries. Details of feeding protocols, cleaning protocols, hatchery equipment and biosecurity protocols, as well as broodstock and larval measurements, were submitted. A full list of data we sought to collate is included in the supplementary materials.

Larval mortality during the growth phase was calculated as the decrease in larval abundance between larval release and transfer to the settlement tanks. Larval release date refers to the day when hatcheries observed larvae on their sieves collecting outflowing water of the broodstock tanks. Larval release events in *O. edulis* are usually completed within a few hours, with repeated larval ejections at short and long intervals. While premature swarming can be observed in stressful conditions, only viable swarming events were included in the reported data, with potential premature release identified by examination of the larval length (Tab. S1). Larval collection sieves were examined daily.

Hatcheries used moderately differing developmental indicators to determine when to move the larvae into settlement tanks. While several hatcheries considered the percentage of eyed larvae as an indicator or readiness to settle, thresholds varied from 50% of larvae noted to have an eyespot and/or a foot (dependent on the hatchery), to when the first settlement was observed in the larval tank. We were unable to correct for this inconsistency with regards to when larvae were counted in this study, and while we feel the comparison of larval mortality across hatcheries is still valid, future research efforts should seek a more consistent approach to improve the robustness of the data. This inconsistency does not affect data relating to larval crashes that occurred before transfer to the settlement tank.

3 Data handling

All contributed data were reviewed to ensure conformity in units and interpretations across datasets. In general, the participating hatcheries handled each larval release event as an independent batch, or series of independent batches through to larval mortality and/or settlement. As such, it was possible to trace each settlement or larval mortality recording through to the broodstock that contributed. One participating hatchery (IEO) mixed larval batches at the settlement stage. In this case, the maximum of the two contributing values was used in the analysis of settlement data. This was the case for: duration of conditioning, number of broodstock, release number and week of year of larval release, mean broodstock size, and % larval mortality pre-transfer to the settlement tank.

4 Statistical analysis

Crash timing since larval release by temperature and the proportion of active batches that crashed by week of the year were plotted for all submitted data, including years with complete or near-complete production failure. This database consisted of 387 batches across seven hatcheries, of which 71 (from four hatcheries) were deemed to have crashed. One hatchery (HOH) was not included, as the crash date was not specified by batch in the submitted dataset.

The properties of the data were explored using Boruta for variable selection, random forest classifiers (Kursa and Rudnicki, 2010). Data for years where a hatchery suffered complete or near complete production failure were excluded from statistical analysis of percent mortality during the growth phase. This included data from the Helgoland Oyster Hatchery (2021 and 2022), the Solent Oyster Restoration Hatchery (2022) and the International Marine Centre (2021). Data from Roem van Yerseke was not included as it was not possible to accurately determine the percentage of larval mortality from that facility. Only four hatcheries (IEO, IMC, DTU and SORP) were able to supply complete data on larval mortality during the settlement stage of production.

Boruta random forest was used to determine which variables might be important predictors of larval crashes occurring and of larval mortality during both the larval growth and settlement stages. Starting with the Boruta-identified variables, linear models were fitted to further examine the potential relationships between the explanatory variables and larval mortality. Data were non-normally distributed, therefore logit linear models were fitted to predict the logit mortality rate for batches that did not crash. The least significant terms were sequentially removed to identify the minimum adequate model in each case. There was insufficient data available to apply a binomial general linear model to the “crash” versus “no crash” dataset. Analyses were performed in R version 4.2.3.

5 Results

5.1 Characterising larval crashes

All participating hatcheries reported having suffered crash events in recent years, with the presence of *Vibrio* spp., contamination by zooplankton larval predators and poor water quality most commonly cited as the suspected causes (Tab. 1). Events with both rapid and gradual mortality were reported. There appeared to be no clear relationship between the proportion of larval batches that suffered mass mortality or high mortality and biosecurity measures in place. Rather, there were variable crash-frequencies from year to year within individual hatcheries. Protocols involving more frequent

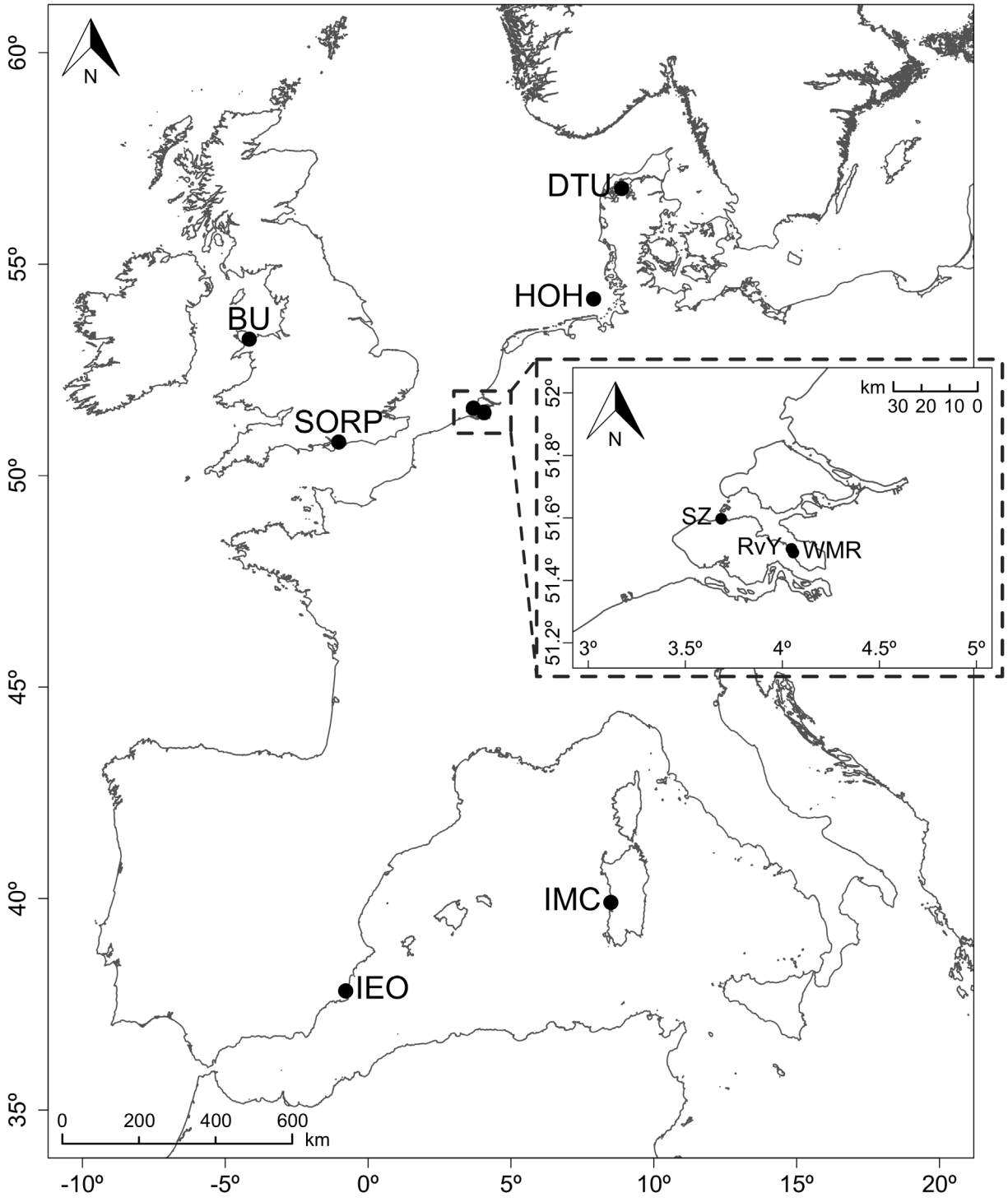

Fig. 1. Map with locations of hatcheries within the collaboration. BU = Bangor University (Wales), DTU = Danish Shellfish Center hatchery at Technical University of Denmark (Denmark), HOH= Helgoland Oyster Hatchery at Alfred Wegener Institute (Germany), IEO = Instituto Español de Oceanografıa (Spain), IMC = International Marine Centre (Italy), Roem van Yerseke (The Netherlands), SORP = Oyster hatchery of Solent Oyster Restoration Project (England), SZ= Stichting Zeeschelp (The Netherlands), WMR= Wageningen Marine Research (The Netherlands).

Table 1. Summary of larval crashes experienced by European hatcheries 2019–2023. Mass (100% mortality) and high mortality events resulting in a discontinuation of the batch are grouped chronologically by hatchery. Timing of the crash is given in days since larval release. Where both rapid and gradual decline is listed, these refer to different batches within the same year of production, unless explicitly stated. Facilities: BU= Bangor University, DTU= Technical University of Denmark, HOH= Helgoland-Oyster-Hatchery- Alfred-Wegener-Institute, IEO= Instituto Español de Oceanografía, IMC= International Marine Centre, RvY= Roem van Yerseke, SORP= Solent Oyster Restoration Project Hatchery, SZ= Stichting Zeeschelp, WMR= Wageningen Marine Research.

Facility	Location	Year	Crash symptoms	Timing (days)	Culprit
BU	Menai Bridge, UK	2022	High mortality, gradual decline.	6–13	Vibrio presence detected
BU	Menai Bridge, UK	2022	Gradual decline in survival.	10–15	Unknown
BU	Menai Bridge, UK	2022	Rapid decline in survival (70%−100% mortality).	>14	Zooplankton contamination. UV failure when stocked
BU	Menai Bridge, UK	2022	Gradual decline in survival.	10–31	Unknown
DTU	The Limfjord, DK	2023	High mortality, gradual then rapid decline.	5–8	Unknown, suspected presence of predators
HOH	Helgoland, GER	2021 & 2022	Mass mortality, rapid decline.	4–10	Unknown, suspected poor water quality
IEO	Mar Menor, ESP	2022	Mass mortality, rapid decline.	10–12	Suspect contamination
IMC	Sardinia, IT	2021	Mass mortality, rapid decline.	6–12	Unknown, suspect predation by *Polydora sp.* larvae
IMC	Sardinia, IT	2022	Mass mortality, rapid decline.	12–27	Predation by *Polydora sp.* larvae
RvY	Yerseke, NL	2019	Growth stopped, overreacting velum. Mass mortality. Rapid and gradual decline.	10–18	Vibrio presence detected
RvY	Yerseke, NL	2020	Growth stopped, overreacting velum, high mortality (80–100%). Rapid decline.	7	Vibrio presence detected
RvY	Yerseke, NL	2021	Releases later in season discontinued when >50% mortality reached. Rapid decline.	10–22	Vibrio presence detected
RvY	Yerseke, NL	2022	Growth stopped, overreacting velum followed by high mortality (80–100%). Rapid decline.	7	Vibrio presence detected
SORP	Portsmouth, UK	2021	Mass mortality. Rapid decline.	4–6	Presence of zooplanktonic predators/competitors, unsuitable diet
SORP	Portsmouth, UK	2022	Low growth, high mortality (RAS: 100%, Rapid decline; static: 100%, gradual decline; FTS: 50%, gradual decline).	RAS: 4–6; Static: 5–8; FTS: 10–14	Unknown − suspected water quality
SZ	Kamperland, NL	2023	Ongoing mortality in few cultures, up to 70–95%, gradual decline.	5–14	Contamination in algae cultures
SZ	Kamperland, NL	2022	Increasing mortality in few cultures, >90% till metamorphosis size, gradual decline.	5–14	Weary broodstock
SZ	Kamperland, NL	2021	75% of the cultures showed ‘fading’ larvae, gradual then rapid decline.	5–14	Suspect low feed quality
WMR	Yerseke, NL	2021	Mass mortality, rapid decline.	7	Vibrio presence detected

Table 2. Summary of biosecurity protocols applied across larval batches within participating hatcheries, and the percent of batches which crashed or were discontinued due to high mortality in each case. In batch culture, a complete water change was undertaken at each tank cleaning. BS= broodstock, Fw= freshwater, Sw = seawater, wk = weekly, Hp = hydrogen peroxide, Cl= chlorine. Hatchery abbreviations: BU= Bangor University, DTU= Technical University of Denmark, HOH = Alfred-Wegener-Institute Helgoland-Oyster-Hatchery, IEO=Instituto Español de Oceanografía, IMC=International Marine Centre, SORP= Solent Oyster Restoration Hatchery, RvY= Roem van Yerseke, SZ= Stichting Zeeschlep.

		New broodstock		Biosecurity		Cleaning protocols				Batches crashed/ dis-continued (Total no. batches)
Hatchery	Prod'n yr	On arrival	Days quara-ntine	Water filtration (µm) /UV?	On entering room	Before handling tank	BS tank cleaning (freq.)	BS cleaning (freq.)	Equipment sterilisation	Larval tank cleaning (freq.)	
BU	2022 &2023	Scrub & Fw bath	56 to 190	1 / yes	None	Hand wash soap & Fw, gloves	Wipe clean, siphon (wk)	Scrub (fortnightly)	Hot Fw / Cl & hot Fw rinse	Wipe with Cl, hot Fw, Sw (3/wk)	39% (18) 2022, 0% (10) 2023
DTU	2023	Scrub, $MgCl_2$ & Cl	20 to 400	1 / yes	Virkon S footbath, hand wash ethanol	Gloves w. Ethanol 70%	scrub, Cl, Fw (wk)	scrub, Fw (wk)	Cl or autoclave	Cl (at start)	0% (5)
HOH	2021 & 2022	Scrub & Cl	7 to 15	1/ yes	Virkon S footbath	Hands & gloves w. ethanol	Wash & ethanol wipe (wk)	Scrub & Sw rinse (wk)	Hot Fw, ethanol, Virkon	Empty, rinse Fw, Cl, rinse Sw (2-3/wk)	100% (155)
IEO	2022	Cl	0	0.5 / yes	None	None	scrub,Cl, Fw (3/wk)	scrub,Cl, Fw (monthly)	Cl	scrub, Cl, Fw (2-3 days)	7% (15)
IMC	2021	Scrub	0	1 / yes	Cl footbath, hand wash ethanol	Hand wash soap & Fw, hand wash ethanol	wash soap, Fw (wk)	scrub, Fw (wk)	autoclave/ handwash soap & Fw	scrub, Fw (every 2 days)	100% (7)
IMC	2022 & 2023	Scrub & Hp	0	1 / yes	Cl footbath, hand wash ethanol	Hand wash soap & Fw, hand wash ethanol	wash soap, Fw (wk)	Hp, scrub, Fw (wk)	autoclave / handwash soap & Fw	scrub, Fw (every 2 days)	29% (7) 2022, 37% (27) 2023
RvY	2023	Scrub & Cl	~32	0.2 / yes	Halamid-D footbath	Hand wash Fw	scrub,Cl, Fw (wk)	Cl, scrub, Fw(wk)	steaming or autoclave	scrub, Cl, Fw (2/wk)	0% (12)
SORP	2022	Scrub & Cl	7	1 / yes	Cl footbath, protective footwear	Gloves w. ethanol 70%	Scrub, soap, Fw, Cl, Sw rinse (wk)	Scrub, soap, Fw, Cl, Sw rinse (wk)	Autoclave (glass), Cl (metal/plastic)	Scrub, soap, Fw, Cl, Sw rinse (3/wk)	92% (51)
SZ	2022 &2023	Scrub & Cl	0	0.02/ no	Cl footbath, hand wash	Hand wash Fw	Wash, Cl, dry (wk)	Wash Fw, dry 1h (wk)	steam (glass), Cl (plastic)	Wash Cl, dry 3/wk	25% (8) 2022, 30% (15) 2023

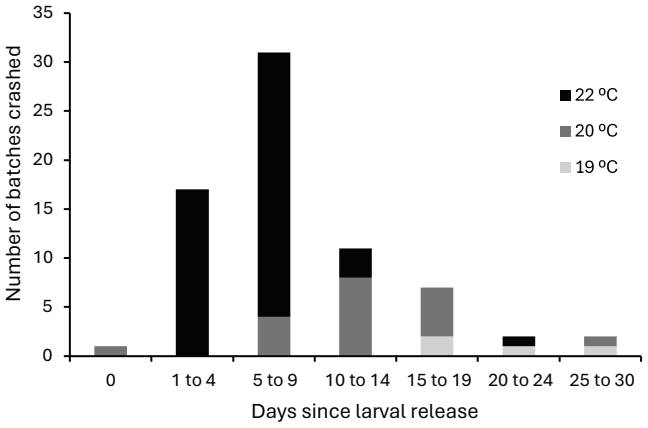

Fig. 2. The timing of crashes following (days since larval release) by temperature at which the larvae are held during the growth phase. Batches were also grown at 25 °C, but suffered no crashes and are therefore not represented in this figure. N = 71 across four hatcheries.

cleaning did not consistently translate into a lower incidence of crash events (Tab. 2).

Crashes most commonly took place in the first two weeks following larval release and temperature appeared to play a role in crash timing, with larvae kept at higher temperatures often crashing earlier (Fig. 2).

5.2 Important variables and predictors of larval crashes and mortality

Participating hatcheries represented a wide range of equipment and protocols, meaning that each hatchery has its own unique set of conditions for larval culture (Tab. 3). There were also few similarities between hatcheries in terms of algal species fed to the oysters (Tab. 4). While operational differences presented a challenge for harmonising the submitted data, 27 variables were reported consistently enough that they could be incorporated into the database for larval mortality during the growth phase (Tab. 5) and 36 variables for the larval settlement phase (Tabs. 5 and 6). Differences in biosecurity protocols were challenging to quantify (Tab. 3) and were therefore incorporated into statistical analyses only in a limited way (Tab. 5).

The Boruta random forest algorithm requires there is no missing data in the dataset, therefore this analysis was run on 93 larval batches reported from five hatcheries that successfully produced larvae and were able to report across all listed variables. Application of the Boruta algorithm to identify factors important in explaining the occurrence of larval crashes during the larval growth phase retained 11 of 27 potential predictors (Tab. 5). The Boruta algorithm identified 13 of the 27 potential predictors as important in explaining the percentage mortality occurring between larval release and transfer to the settlement tanks, for batches that did not crash (Tab. 5). Of these factors, four were retained in the minimum adequate logit linear model: Number of broodstock, temperature at the endpoint of conditioning, whether algal paste was used in broodstock feeding, and salinity at the larval growth stage (Fig. 3, Tab. S2). Given the lack of overlap in hatchery

Table 3. Overview of key hatchery protocols during the broodstock conditioning, larval growth and larval settlement phases of production. L= Local, R = Regional, I = International, C = continuous, P = punctuated, W=weekly, D = daily, FT = flow through, B= batch, RAS= Recirculating Aquaculture System. For hatchery abbreviations see Figure 1.

	Broodstock					Larval growth				Settlement		
Hatchery	Salinity (ppt)	Broodstock	No. algae in feed	Algal paste used	Frequency of feeding	Set up	No. algae in feed	Frequency of feeding	Larval transfer method	Set up	Grading undertaken	Frequency of feeding
BU	32	R	3	Y & N	P - C	FT	2 or 3	2-3/W - C	water	B/FT	no	D
DTU	25-30	L	4	No	C	FT	3	C	water	FT	no	C
HOH	32	I	2	No	C	FT	2	C	water	FT	both	C
IEO	43	L	4	No	C	B	4	D	mesh	B	no	D
IMC	37	L & R	2	No	C	B	2	D	mesh	B	no	D
RvY	32	L	5	Yes	C	FT	5	C	water	FT	yes	C
SORP	33	L & R	2	Yes	C	All	2	2/D - C	mesh	All	yes	D
SZ	30	L	6	No	C	B/FT	4	C	mesh	FT	yes	C

protocols and the imbalance in the number of hatcheries trialing certain factors (e.g. production in and out of season, the use of algal paste in broodstock feeding), it is challenging to confidently determine the nature of the relationship between several of the factors identified as being important in explaining larval mortality. Nevertheless, plots provide some insight into the potential relationship between identified factors and larval mortality, in particular there is some indication in the plots that batches with a shorter broodstock conditioning time, produced in season and produced earlier in the year (week of year) may suffer lower larval mortality rates (Fig. S1). Furthermore, batches kept at a higher temperature during broodstock conditioning and larval growth appeared to have lower mortality rates (Fig. 3a, Fig. S1).

5.3 Important variables and predictors of larval settlement

Application of the Boruta algorithm to the occurrence of larval crashes after larvae were moved to the settlement tanks retained 8 of 36 potential predictors as potentially important in explaining larval crashes during the settlement phase (Tab. 6). In batches that did not crash, the Boruta algorithm identified two factors as important in explaining the percentage mortality of larvae stocked in the settlement tank (Tab. 6; number of larvae in larval release, and broodstock origin). Of these, only the number of larvae in the larval release was retained in the minimum adequate logit linear model (Tab. S3, Fig. 4).

While it was not possible to undertake additional pairwise comparisons on the limited dataset, examining plots of the variables identified as important by the Boruta analysis indicates that higher temperatures during the larval growth stage and shorter time in the larval growth tank both appear to contribute to lower larval mortality in the larval settlement stage. Furthermore, lower larval densities during both the growth and settlement stage were associated with lower larval mortalities, as well as the use of air pumps in the settlement tanks (Fig. S2).

6 Discussion

The focus of this collaboration was to identify the suspected causes of larval crashes and high larval mortality in *O. edulis* hatchery production, and to explore which factors may be important in explaining crashes or high larval mortality across several independent hatchery facilities. Larval crashes and high mortality events were reported from all nine hatcheries/laboratories that produced larvae in recent years (Tab. 1). Yet it is also striking that over the period for which data were collated (2021-2023), several hatcheries recorded extremely low levels of larval mortality across several of their larval batches, with 20% (N=19) of batches overall exceeding the highest survival rates (56%) to eyed larval stage reported for *C. virginica* in Gray et al. (2022). While these survival rates are not directly equivalent due to the differing life histories of *C. virginica* and *O. edulis*, which mean that *C. virginica* survival rates are determined as the percentage of eggs (as opposed to released larvae) that became eyed larvae, such high survival rates are nevertheless a promising indicator that *O. edulis* production has the potential to achieve meaningful yields once crashes and therefore inconsistency in production are addressed.

Unfortunately, high larval mortality events are still a relatively common feature of hatchery production in Europe (Tab. 1). Collating shared experiences across many hatcheries identified a number of common culprits that may have caused the crashes, including *Vibrio* species, predatory zooplankton and poor water quality. It is well established that pathogenic bacteria are associated with decreased growth rates and high larval mortalities of *O. edulis* and have been related to the *Vibrio* spp. bacteria (Jacobs et al., 2020; Lodeiros et al., 1987; Prado et al., 2005). Larvae that are infected with *Vibrio* spp. show a variety of symptoms, e.g. low and erratic swimming activity (Jacobs et al., 2020; Lodeiros et al., 1987; Prado et al., 2005). Moreover, a slimy yellow consistency was observed around the larvae which has been associated with biofilm production of certain *Vibrio* spp. (Karunasagar et al., 1996; Prado et al., 2005). Similarly poor water quality, while a

Table 4. Microalgal species and strains used by participating hatcheries to feed broodstock (bold) or both broodstock and larvae in the years for which data was included in this study. For hatchery abbreviations see Figure 1.

Microalgae species	Cell size range [μm]	Median cell volume [μm^3]	Mean dry weight [$mg/10^6$ cells]	Hatchery							
				IEO	HOH	DTU	IMC	SORP	BU	SZ	RvY
Tisochrysis lutea	4.5	50	0.043	*ECC038*		*CCAP 927/14*			*CCAP 927/14*	*CCAP 927/14*	
Tetraselmis suecica	8	310	0.225	*ECC036*					*CCAP 66/4*	**Seasalter**	**Seasalter**
Diacronema lutheri	3.8	50	0.021	*ECC015*						Seasalter	*CCAP 931/1*
Phaeodactylum tricornutum	4		0.015	*ECC028*				*CCAP 1052/1B*			
Chaetoceros sp.	NA	70	0.03		*CCAP1010/3*	*CCAP 110/38*			*CCAP 1010/3*		
Chaetoceros mulleri	5	70	0.03								*CCAP 1010/3*
Rhodomonas salina	NA	160	0.13		*CCMP1319*	**CCAP 928/27**				**CCAP**	*Seasalter*
Pavlova gyrans	6.0					*CCAP 940/1C*					
Isochrysis galbana	5–6	50	0.012				*NA*	*CCAP 927/1*		*Seasalter*	*CCAP 927–1*
Nannochloropsis oculata	2–5							*CCAP 211/78*			
Nannochloropsis oceanica	2–5									*CCAP 849/8*	
Chaetoceros calcitrans	5.6	70–80								*Seasalter*	*CCAP 1010/11*
Chaetoceros neogracile	5.3	70–80	0.07				*NA*			*CCMP 1425*	
Thalassiosira pseudonana	4.5–5.5	55–70	0.035							*CCAP 1085/12*	
Thalassiosira weisfloggii	4–32	900	0.250							**CCAP**	
Skeletonema marinoi	5–21	85–372	0.05							**CCAP 1077/5**	
Skeletonema costatum	20	85	0.029							**Seasalter**	**RVY**

Table 5. Factors considered during the boruta regression tree analysis of larval crashes and mortality. Significant predictors as identified by the boruta algorithm are listed in order of importance from top to bottom. Terms retained as significant in the general linear model. *** indicates statistical significance at $p<0.001$ and ** at $p<0.01$ in the associated logit linear model. Full results are provided in Table S2.

Possible predictors of hatchery crashes		Significant predictors of:	
Broodstock related factors	Larvae related factors	Hatchery crashes	Mortality in surviving batches
Hatchery	Frequency larval cleaning (days)	Temperature larval tank	Broodstock density***
Year of production	Temperature larval tank (°C)	Broodstock origin	Temperature conditioning [endpoint]***
Broodstock origin	Salinity (ppt) larvae	Broodstock density	Hatchery
Broodstock release number	Larval density at stocking (ind/ml)	Week number larval release	Frequency broodstock cleaning
Quarantine applied	Larval feed delivery frequency	Broodstock release number	Salinity (ppt) larvae***
Frequency broodstock cleaning	Proportion larval feed diatom	Duration of conditioning	Conditioning salinity (ppt)
Broodstock density at larval release	Larval setup	Larval feed delivery frequency	Broodstock origin
In/Out of season	Grading undertaken	Proportion broodstock feed diatom	Algal paste used in broodstock feeding**
Duration of conditioning (days)	Week number larval release	Algal paste used in broodstock feeding	Week number larval release
Conditioning Photoperiod (min light per day)	Number larvae in release (x1000)	Temperature conditioning [endpoint]	Frequency larval cleaning
Temperature conditioning (°C) [endpoint]		In/Out of season	Temperature larval tank (°C)
Conditioning Salinity (ppt)			Duration collection to larval release (days)
Use air pumps broodstock			Quarantine applied
Algal paste used in broodstock feeding			
Feed delivery frequency broodstock			
Proportion broodstock feed diatom			
Duration collection to larval release (days)			

Table 6. Additional settlement related factors considered during the boruta regression tree analysis of larval crashes and mortality. Factors related to broodstock and larval growth stage listed in Table 5 were also considered. Significant predictors are listed in order of importance from top to bottom. *** indicates the term retained as significant in the general linear model at the <0.001 significance level. The full model is given in Table S3.

Possible predictors of hatchery crashes, in addition to those already listed in Table 5		Significant predictors of:	
Settlement related factors		Settlement failure	Percent settled in successful batches
Duration larval tank	Batch, flow-through, or RAS?	% mortality during larval growth stage	Number of larvae in release***
Number larvae in settlement tank (x 1000)	Use of air pumps during settlement	Duration larval tank	Broodstock origin
Feed delivery frequency settlement tank (continuous, or days)	Frequency of settlement tank cleaning	Larval density at transfer to settlement tank	
Larval density at transfer to settlement tank	Cultch soak time (days)	Broodstock density	
	% mortality during larval growth stage	Broodstock origin	
		Use of air pumps during settlement	
		Temperature larval tank	
		Temperature conditioning end point	

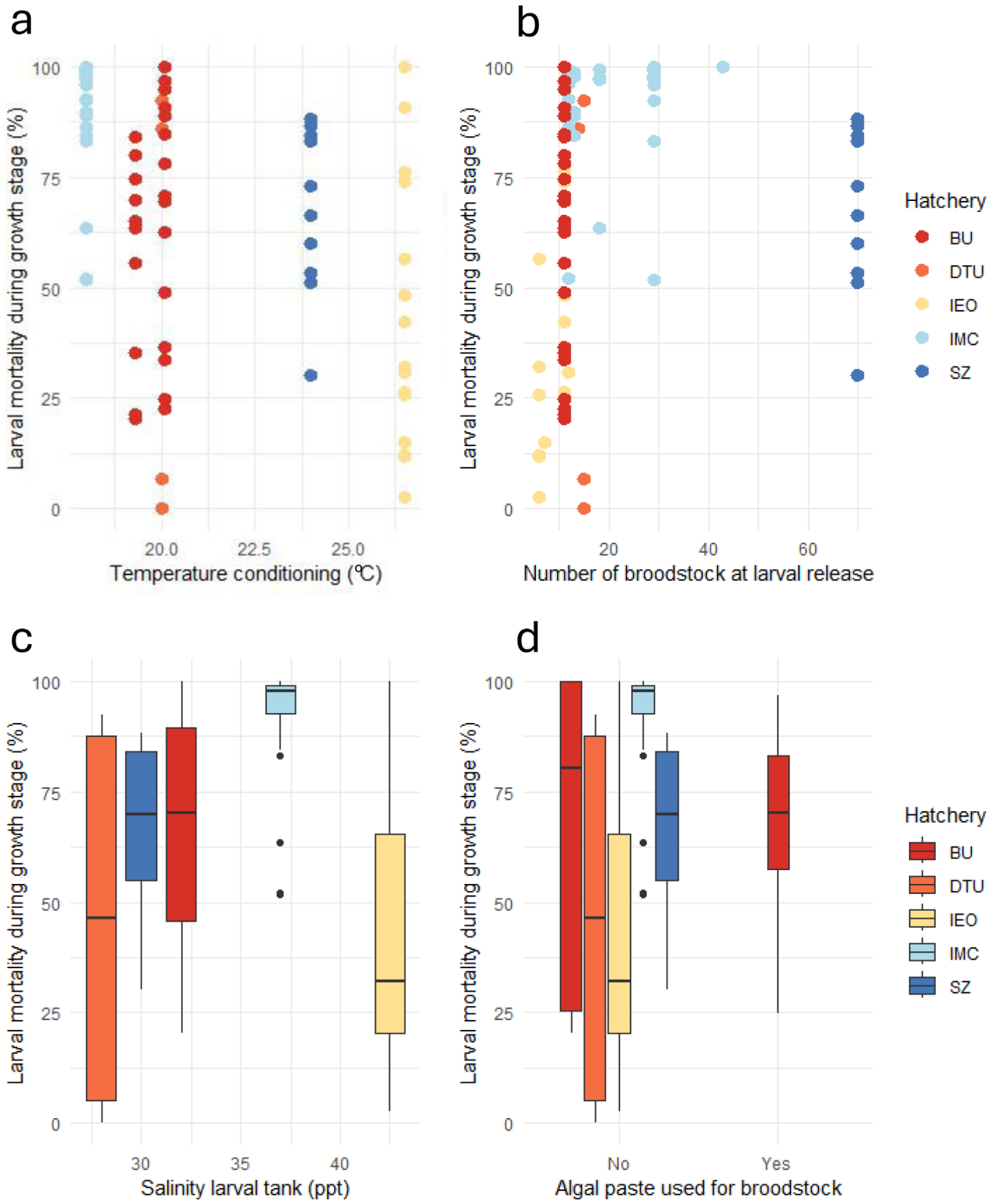

Fig. 3. Larval mortality during the larval growth stage by hatchery against a) water temperature (°C) at the end point of broodstock conditioning b) number of broodstock oysters c) salinity and d) where algal paste was or was not used as part of the broodstock diet. For explanation of hatchery codes see Figure 1. Data are presented as a box-whisker plot when each hatchery is represented by a single value on the x-axis.

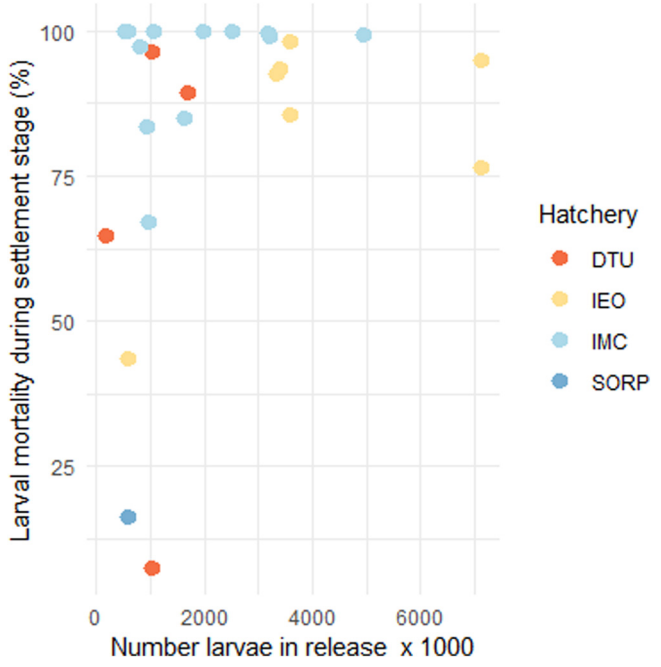

Fig. 4. Scatter plot illustrating the impact of the number of larvae x 1000 in a larval release on larval mortality during the settlement phase of production. For explanation of hatchery codes see Figure 1.

nebulous term, is frequently identified as a likely culprit. Perhaps more surprising, listed among the suspected culprits were predatory zooplankton. *Polydora* spp. construct a U-shaped burrow internally to the oyster shell, and the oyster responds to this parasite by confining it with a conchiolin layer and covering it with nacreous shell (Zottoli & Carriker, 1974). The endolithic lifestyle and burrowing behaviour of *Polydora* in the inner part of the valve, makes direct observation impossible without sacrificing the animal, and at the same time, it protects the *Polydora* against the biosecurity procedures put in place in the hatcheries. Despite the hatcheries' biosecurity protocols before the introduction of broodstock to the hatchery, the presence of this polychaete was reported by a participating hatchery as a potential culprit of hatchery crashes. It was suspected that, once introduced to hatchery, *Polydora* spp adults were able to reproduce, and *Polydora* spp. larvae were observed to predate on the flat oyster larvae.

This collaborative effort also provided a unique opportunity to characterise the diversity of hatchery protocols in use in *O. edulis* hatchery production (Tabs. 2–4). It is evident, for example, that while there is substantial variability between hatcheries in which algal strains are used in feeding broodstock oysters and larvae, most hatcheries work with a relatively small number of algal species (Tab. 4). The large-scale culturing of diverse, high-quality microalgae to meet the nutritional demands of broodstock and larvae is both labour-intensive and costly. Microalgal production has been estimated to account for approximately 30% of total hatchery operating costs (Coutteau and Sorgeloos, 1992). Furthermore, the occurrence of bacterial contamination and algae culture crashes presents a challenge in hatchery operations (Knauer and Southgate, 1999, Vignier et al., 2021). Consequently, the variation in both number and strain of algal species used across many of the participating hatcheries likely reflects both a limited resource availability and operational decisions associated with sustaining stable algal cultures for a variety of uses and in line with the infrastructure of the hatchery.

In characterising the crashes to date, presence of *Vibrio* spp. and predatory zooplankton were most frequently cited as the likely culprit (Tab. 1). However, the suspected culprits causing the larval crashes were, with the exception of predation by zooplankton, usually inferred and could not be definitively confirmed. For example, in the case of *Vibrio* spp., microbial assays were undertaken after crash symptoms were evident, meaning it is not possible to establish cause and effect. This presents a challenge in determining the role of *Vibrio* spp. in the mortality event, as *Vibrio* spp. are also known to be opportunistic and may proliferate as a result of high mortality of larvae (Prado et al., 2005, Cram et al., 2024). Furthermore, it is challenging to confidently identify pathogenic strains of *Vibrio* spp. (Le Roux et al., 2002). More broadly, microbial community structure has recently been found to vary between "good" and "bad" batches of oyster larvae at Horn Point Laboratory prior to a crash event (Cram et al., 2024). These data suggest more studies are warranted to understand the ecology of potentially harmful novel bacteria in larval cultures, examine how their presence in the microbiome leads to batch failure, determine where they enter the hatchery (i.e. source water, broodstock or algae culture), and how they evade mechanical filtration and water sterilization (Cram et al., 2024).

The importance of effectively removing pathogens and predators from incoming water, was illustrated by the high mortalities suffered by participating hatcheries when filtration systems (filtration to <0.02 μm or filtration <2 μm plus UV treatment) failed or were yet to be installed (Tab. 1). There was, however, limited evidence that the nature of biosecurity protocols played a significant role in preventing crashes. Some hatcheries with stringent entry biosecurity measures nevertheless suffered complete or near complete production failure, and some with limited protocols yielded high larval survival (Tab. 2). It is important to state that biosecurity beyond that associated with the treatment of water on entry to the hatchery facilities is nevertheless important in the context of producing oysters for ecological restoration purposes, where reduction of risk associated with the transmission of invasive non-native species and diseases, including those that do not impact oysters directly, is critical to responsible and successful restoration outcomes (zu Ermgassen et al., 2020).

Cleaning protocols and frequency of cleaning differ substantially between hatcheries (Tab. 2). Participating hatcheries noted that some cleaning protocols may induce stress and that their production protocols seek to trade off the frequency of disturbance and handling against the risks posed by reducing the frequency of cleaning. These trade-offs are likely to be hatchery specific, as they are influenced by respective production season, temperature, water quality as well as general hatchery set up. As in Gray et al. (2022) it was also apparent that the elusive "quality" of intake water plays a critical and yet currently unpredictable role in larval survival within the hatchery. Indeed, differences in water quality more generally are suspected to play a role in inter-hatchery differences in larval mortality (Tab. 1). What threshold levels

of contaminants in water present a problem to larval survival are, however, still open questions for research (zu Ermgassen et al., 2023b).

This collaboration provided an opportunity to better characterise the timing of *O. edulis* larval crash events. While crashes occurred throughout the larval growth stage and into settlement (Tab. 1), the greatest proportion of crash events in the dataset occurred between 5–9 days after larval release (Fig. 2). Crashes in the 1–9 days since larval release category were dominated by larvae grown at 22 °C. Two hatcheries (DTU and SZ) grew their larvae at higher temperatures (23−25 °C), but did not suffer any crashes in the years for which data was submitted. The temperature dependence of the timing of crashes implies that crashes may be related to larval development, with larvae grown at cooler temperatures suffering crashes successively later after larval release (Fig. 2).

The Boruta analysis identified 11 factors as being important in explaining crashes and 13 in explaining larval mortality in batches that did not crash. The identified factors provide a starting point for further collaborative investigations, and indicate which factors may play a role in reducing larval mortality. A number of factors were common to both, including the number of broodstock used, whether or not algal paste was used in broodstock feeding, the week of the year in which the larvae were released and the temperature at the end of conditioning.

Broodstock origin was consistently identified as an important factor in explaining larval crashes and mortality during both the growth and the settlement stages of production (Tabs. 5 and 6). While hatcheries each sourced their broodstock from unique locations, several hatcheries were trialling different broodstock origins within the hatchery. In contrast to broodstock origin, hatchery was identified only once as being a potentially important variable (Tab. 5), which emphasises the likely importance of broodstock origin in determining the survival of the larvae produced. Broodstock origin may influence the ability of the larvae to overcome certain environmental stressors (Spencer et al., 2020) depending on different genetic characteristics and may also affect the parental oysters' physiological and reproductive status. For instance, differences in number of mature oocytes produced were observed among broodstock sourced from different populations of *M. gigas* (Chávez-Villalba et al., 2002). This, in turn, can have a significant influence on spawning, larval growth and survival, as observed for the European clam *Ruditapes decussatus*, for which broodstock geographic origin greatly affected larval spawning (number of spawners) in hatchery settings (Matias et al., 2009).

Gray et al. (2022) observed "week of year" to be important in explaining the production yield in *C. virginica* production at the Horn Point Hatchery, the largest hatchery on the Atlantic coast of the USA. Pediveliger yield in the Horn Point Hatchery was clearly lowest at the beginning and end of the production season, which was considered to be associated with attempting to spawn well outside their natural reproductive period. A relationship between week of year or broodstock release number was also suspected in the European context, as it had been observed in some hatcheries that earlier batches appeared to show a reduced risk of crashing (Bakker, Pers. Comm.). This may be attributed to broodstock fitness declining over time in captivity (Helm and Bourne 2004). Although week of year was not identified as being an important factor in determining whether or not a batch crashes, it was identified as an important factor in determining the degree of larval mortality in batches that did not crash. Additionally, whether broodstock were conditioned in or out of season was identified as an important factor in explaining the risk of larval crashes in the growth phase, however, only one hatchery attempted production outside of the natural spawning season. This aspect of production therefore warrants further investigation.

The importance of broodstock condition and larval feed in determining the larval quality (larval growth, survival and settlement) has previously been illustrated for *O. edulis* (González-Araya et al., 2012; da Costa et al., 2023). The analysis undertaken here supports that finding, with factors relating to broodstock feed (proportion of diatoms to flagellates and whether or not algal paste was used), found to be important in potentially explaining larval crashes (Tab. 5). While algal paste is largely seen by hatchery managers as being a less desirable feed type relative to live microalgae, and complete or partial substitution is not currently used on a commercial scale for European flat oyster hatcheries, substitution trials using (centrifuged) algae paste, algae concentrate in viscous solution, and algae powder, are being explored for cost efficiency purposes (Colsoul et al., 2021). In our dataset, algal paste was used experimentally by one hatchery alone, therefore the finding that use of algal paste is important in explaining larval crashes should be viewed as preliminary.

Our analysis identified the number of adults used in broodstock as an important variable in determining hatchery crashes (Tabs. 5–6). Low broodstock densities in hatchery settings, as well as skewed sex ratios and a reduced likelihood of including reproductively competent individuals, can also lead to genetic bottlenecks and inbreeding events. When the ultimate goal of *O. edulis* production is ecological restoration, the preservation of genetic variation is crucial to maintain important traits such as the adaptation to environmental conditions and stress factors (diseases, climate change), which are essential to promote growth rate and long-term survival of oyster spat. The introduction of hatchery-reared oysters in the natural environment may also have further impact on the genetic integrity and diversity of wild stocks, due to their low genetic variation and the disproportionate contribution to the genome of the mixed populations (Saavedra, 1997; Lallias et al., 2010). Therefore, in the context of restorative aquaculture, rearing techniques and protocols established in hatcheries for oyster production, should be adapted to preserve the extant genetic diversity and avoid genetic bottlenecks, differing from the methods used in exclusively commercial production.

Compiling the protocols, measured variables, larval growth, larval mortality and larval settlement data from nine European hatcheries/laboratories rearing *O. edulis* larvae was a challenging but rewarding endeavour. Representatives from the participating institutions unanimously agreed that this effort provided unexpected and fruitful opportunities for knowledge exchange. The process raised several challenges which could be learnt from in future collaborative endeavours. For example, as described in the methods, there were differences in the developmental stage at which larval mortality was assessed, with differing proportions of eyed larvae being used as an indicator for transfer to the settlement

tank. The uncertainty arising from this could also be tackled in future collaborations through agreed data collection protocols, or through additional larval counts at a mutually agreed life stage. Also, the process of including broodstock size measurements was made difficult by hatcheries having different definitions of shell height, length and width, whereas one hatchery used almost exclusively wet weight as the metric of size. This should be addressed in future collaborative research efforts, as size and age of adult oysters can strongly affect their fertility (Walne, 1964). Within the restoration community, monitoring guidelines present a standard language which could be adopted by hatcheries to increase the utility of data (zu Ermgassen et al., 2021). There were also differences in whether shell height or length were measured when assessing larval size. These differences arise from differing methods, with some hatcheries assessing size via sieving, and others measuring a sub-sample under the microscope. Furthermore, hatcheries also measured and counted their larvae at different time points. The lack of consistency in these data precluded the larval size or growth rate from being included in the analysis, although this factor was identified as a likely important variable several times during group discussions.

A further source of uncertainty when comparing data from different hatcheries, was a differing decision threshold regarding when to discontinue batches. While commercial hatcheries cannot afford to continue with batches that show tell-tale signs of trouble, such as gradual but high mortality, overreacting velum and suspicious larval behaviour, research hatcheries may opt to see production through, despite potentially low resulting yields. This does not affect the data set used in this study, as the commercial hatchery that contributed mortality data did not suffer any crashes or high mortality events in the year for which data was supplied. Nevertheless, future efforts should be mindful of these differences during project planning and seek to explore further the nuances between mass and high mortality events, as well as between gradual and sudden mortalities. To date, there is no comprehensive, or perhaps sufficiently nuanced, definition of a hatchery crash. Development of such a definition or definitions may assist in future research, as it is possible that the culprits behind hatchery crashes or high mortality events with different development traits (e.g. affecting larvae at differing developmental stages, gradual or rapid mortality) may themselves be different.

While data availability combined with largely unique hatchery protocols across many variables made it challenging to apply standard parametric statistics and to confidently determine the conditions that most impact larval mortality, a series of factors were identified through the analysis which warrant further investigation (Tabs. 5 and 6). Ideally, such investigation would take place across multiple hatcheries with a similar experimental design, therefore providing power to disaggregate the influence of hatchery location and design from the underlying drivers of larval crash events or high larval mortality. Our observations therefore lead us to the conclusion that more comprehensive and coordinated data collection relating to the occurrence of larval crashes in European hatcheries is warranted, including concerted inter-hatchery research efforts to establish key drivers of larval mortalities. Additionally, investigation into the water quality before and after entry to the hatchery, and the efficacy of biosecurity measures, alongside consideration of water quality, would provide valuable insight into practical and cost-effective actions that can be taken within hatcheries.

While an existing body of literature examines several important aspects for improving hatchery production (e.g. González-Araya et al., 2012; Robert et al., 2017, Colsoul et al., 2021), it is unlikely that there is a single "protocol" for producing native oysters across all of Europe, given the diverse abiotic settings and genetic differentiation across the range of *O. edulis* (Vera et al., 2016, Šegvić-Bubić et al., 2020, Monteiro et al., 2024). Yet we have illustrated that there are commonalities and common risk factors across geographically distinct hatcheries, and our analysis has identified a number of factors which may be important in explaining larval mortality and which are worthy of further examination. As such, greater sharing of data and experience can play a critical role in improving the reliability and consistency of European native oyster hatchery production. Improved hatchery outcomes will aid the broader societal aims of the EU nature restoration regulation and biodiversity strategies.

Acknowledgments

This research was sponsored by the Dutch Ministry of Agriculture, Nature and Food Quality (BO-43-116.01-012). The authors wish to thank Thorolf Magnesen of University of Bergen, Knut Magnus Persson of Scalmarin AS and Dennis Gowland of Northbay Innovations Ltd, for participation in a number of conversations and knowledge exchange, and Moira Kelly for producing several figures.

Data availability statement

Data available upon request.

Supplementary material

Table S1 Table of all the factors relating to hatchery protocols, broodstock and larval release, growth and settlement, requested from participating hatcheries and supplied if available. Factors in green allowed for variables in different compartments to be related to one another.

Table S2 Results from the logit linear model on the percentage mortality during the larval growth stage in batches that did not crash.

Table S3 Results from the logit linear model on the percentage mortality during the larval settlement stage in batches that did not crash.

Figure S1. Plots of all factors identified by the Boruta analysis as important in explaining larval mortality during the larval growth stage but not found to be statistically significant by the logit GLM.

Figures S2. Plots of all factors identified by the Boruta analysis as important in explaining larval mortality during the settlement stage but not found to be statistically significant by the logit GLM.

The Supplementary Material is available at https://www.alr-journal.org/10.1051/alr/2025016/olm.

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Cite this article as: zu Ermgassen PSE, Albentosa M, Bakker N, Barja JL, Blanco A, Bonačić K, Brundu G, Carboni S, Colsoul B, Gair JR, Gray M, Costa Fd, Dubbeldam M, Fabra M, Galley T, Hernandis S, Jones N, Laugen AT, Love R, Malham S, Piñeiro NA, Pogoda B, Preston J, Sas H, Saurel C, Sganga DE, Teng S, Kamermans P. 2025. Identifying common factors resulting in hatchery crashes during the production of *Ostrea edulis* for ecological restoration in Europe. *Aquat. Living Resour.* 38: 18. https://doi.org/10.1051/alr/2025016