



Can thermal anomalies impair the restoration of *Cystoseira s.l.* forests?

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ABSTRACT

Marine macroalgal forests are facing unprecedented challenges worldwide due to the accelerating impacts of climate change. These ecosystems play a crucial role in supporting biodiversity, coastal ecosystem functions and services, and are indeed object of several conservation and restoration measures. The Mediterranean Sea is warming faster than the oceans and thermal anomalies are occurring with increasing intensity, frequency and duration. Along the Mediterranean coasts, *Cystoseira sensu lato* species are the main representatives of macroalgal forests and their decline has been widely documented. Some relevant achievements in the implementation of ecological restoration have been obtained, but rising temperatures and the occurrence of thermal anomalies increasingly threaten the success of these restoration attempts. In the summer of 2022, *ex-situ* restoration actions of *Ericaria amentacea* were carried out by collecting fertile material from three donor sites of the Italian coasts along a latitudinal gradient, during the period of sexual maturity (June/July). Noteworthy during the summer of 2022, anomalous thermal conditions were recorded at the donor sites, with sea surface temperatures exceeding the climatological mean up to 4.3 °C and heatwaves lasting up to 78 days. Our results suggest that these thermal anomalies may have affected the culture of the embryos in both the pre- and post-zygotic phases, resulting in significantly low culture efficiency at the three donor sites. The reproductive structures showed some abnormalities, fertilization of eggs was lower and embryo growth was slower, resulting in lower percent cover of seedlings on the tiles and lower survival rate. The observations underscore the vulnerability of Mediterranean algal forests to global change and highlight additional challenges for their restoration due to the increasing frequency and severity of thermal anomalies, emphasizing the need for adaptive strategies and a comprehensive understanding of the species in a changing climate. Marine forest restoration requires long lasting projects, to allow for long-term monitoring and better understanding the biology of the species and for mitigating stochastic events that can cause the temporary failure of efforts.

1. Introduction

There is growing evidence that the Mediterranean is warming faster than the oceans and that thermal anomalies are occurring with increasing intensity, frequency and duration as a result of climate change (Rosselló et al., 2023; Pastor and Khodayar, 2023; Dayan et al., 2023; Hamdeno and Alvera-Azcarate, 2023). Extreme events such as marine heatwaves (MHWs) pose significant threats to marine biodiversity, ecosystem structure and functioning (Hobday et al., 2016; Wernberg et al., 2016a, b; Smale et al., 2019; Garrabou et al., 2022; Holbrook

et al., 2020). There are different definitions of MHWs: Meehl and Tebaldi (2004) defined it as a period of at least three to five days in which the mean or maximum temperature anomalies are at least 3–5 °C above normal (considering the previous 30 years). Hobday et al. (2016) proposed a definition for MHWs that can be used for comparative studies with regional and biological applications. They based their definition on that of Perkins and Alexander (2013), who defined an atmospheric heatwave as at least three consecutive days exceeding a calendar day threshold defined as the 90th percentile value for temperature. Due to the inherently longer time scales of oceanic variability, Hobday et al.

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(2016) qualitatively defined MHWs as discrete, long-lasting, anomalous warm-water events that occur at a specific location, where the sea surface temperature (SST) rapidly exceeds a threshold value for more than five consecutive days and can be caused by a combination of atmospheric and oceanographic processes (Hobday et al., 2016). On a global scale, a long-term increase in the frequency of occurrence of MHWs has been observed (Hobday et al., 2018; Oliver et al., 2018), triggering the need to explore the biological impacts of these thermal anomalies, particularly on foundation species (Smith et al., 2023a; Wernberg et al., 2024).

Along the Mediterranean rocky coasts, the main foundation species belong to the *Cystoseira sensu lato* complex (i.e., *Cystoseira*, *Ericaria* and *Gongolaria*) (Molinari-Novoa and Guiry, 2020). These algal forests provide relevant ecosystem services by contributing to coastal primary production and nutrient cycling and supplying food, shelter, nursery and habitat for many vertebrate and invertebrate species (De La Fuente et al., 2019a; Smith et al., 2023b). These ecosystems are affected by declines and losses on a large scale (Thibaut et al., 2005; Blanfuné et al., 2016; Mariani et al., 2019). Direct habitat destruction, sedimentation, pollution and overgrazing by herbivorous fish, sea urchins and other invertebrates are acknowledged as the main drivers (Sala et al., 1998; Hereu et al., 2004; Arévalo et al., 2007; Mangialajo et al., 2008; Perkol-Finkel and Airolidi, 2010; Sales et al., 2011; Vergés et al., 2014), with climate change including MHWs, posing an additional threat (e.g., Celis-Plá et al., 2017; Bevilacqua et al., 2019; Falace et al., 2021; Verdura et al., 2021; Monserrat et al., 2022; de Caralt et al., 2023; Boudouresque et al., 2024).

Over the last decade, efforts have been implemented to restore degraded marine algal forests in the Mediterranean Sea, using both *in-situ* (Verdura et al., 2018) and *ex-situ* (Falace et al., 2018) techniques. Some relevant achievements concern the establishment of culture protocols for some of the species (Falace et al., 2018) and the layout of the deployment at sea, also preventing grazing pressure (De La Fuente et al., 2019b; Clausing et al., 2023; Monserrat et al., 2023). Yet, there is still a very large stochasticity that jeopardizes restoration efforts. For example, large variability in the reproductive potential of donor populations has been detected on a very small spatial scale (Orlando-Bonaca et al., 2022) and thermal anomalies have been observed, leading to fertility period shifts and post-zygotic abnormalities with death or slow growth of embryos (Bevilacqua et al., 2019; Savonitto et al., 2021; Falace et al., 2021).

From a restoration perspective, extreme climatic events, such as the occurrence of storms, temperature increases and MHWs, can, for example, hamper the survival of outplanted seedlings (De La Fuente et al., 2019b) or affect the growth of embryos or alter the reproductive phenology of species (Falace et al., 2021; Bevilacqua et al., 2019; Savonitto et al., 2021). These effects on the survival of the early stages, which hinder the long-term development of species, also represent a bottleneck for restoration efficiency.

In the summer of 2022, different *ex-situ* restoration activities were implemented for *Ericaria amentacea*, an intertidal species whose restoration is largely established (Falace et al., 2018; De La Fuente et al., 2019b; Clausing et al., 2023). Fertile apices were collected at three sites along a latitudinal gradient (Ligurian Sea: Bogliasco in Liguria; Tyrrhenian Sea: Capri in Campania; Ionian Sea: Brucoli in Sicily) in June/July, when the species is known to achieve sexual maturity. Noteworthy, a very anomalous thermal condition was observed in 2022, when the Mediterranean experienced a “never-ending summer”, with sea surface temperatures up to 5 °C above average and recurrent thermal anomalies (Rosselló et al., 2023; Pastor and Khodayar, 2023; Boudouresque et al., 2024). Also at local scale, summer SST at the collecting sites largely exceeded the average of the last 30 years, which can negatively affect the reproductive phenology and reproductive success of *E. amentacea*.

In the present study, we compare the performance of *E. amentacea* cultures from the three different donor sites and with those from

previous years and relate them to MHW dynamics.

2. Material & methods

2.1. Donor sites

The collection of the apices with reproductive structures of *Ericaria amentacea* for the establishment of cultures (Falace et al., 2018) was carried out in summer 2022 (Supplementary Table 1), when the thalli are usually fertile (Supplementary Table 2).

For the *ex-situ* cultures, apical fertile branches were collected from healthy populations (Fig. 1) at:

- Bogliasco (GE, Italy - 44°22'31.7"N 9°04'32.5"E) on the 3rd of July; apices were also collected in 2018, 2019, 2020, 2021 (e.g., De La Fuente et al., 2019b). Bogliasco (Pontetto) is a rocky shore close to Genoa (around 15 km from city centre). It is a moderately urbanised area, with inhabitant density of around 1000 inhab/km². It is not subject to any protection measures, and it is usually a quite attended place during summer months by swimmers and sun-bathers. It is characterised by a low tidal range (30 cm), for this reason the barometric tide and wind-driven currents are the major hydrodynamic forces in this area.
- Capri (Tyrrhenian Sea, Italy - 40°32'55.5"N 14°11'53.0"E) on the 28th June; Capri Island is located in the southern part of the Gulf of Naples. It is about 10.4 km² wide and 6 km long. The island is exposed to several anthropogenic pressures, especially in summer due to mass tourism. The coastal area is also heavily affected by over exploitation of the date mussel *Lithophaga lithophaga*. The surface circulation in this area is dominated by strong currents that allow the exchange of water masses between the coast and the open sea (Violante et al., 2020).
- Brucoli (Ionian Sea, Italy - 37.284167 °N, 15.187778° E) on the 9th June; the bay of Brucoli is located between Catania and Syracuse on the east coast of Sicily. The south-western part of the basin is interrupted by the estuary of the Porcaria Channel. The Bay of Brucoli is a Site of Community Importance (SIC-ITA090026 “Fondali di Brucoli – Agnone”), which is part of the Natura 2000 network.

The thalli of *E. amentacea* were considered reproductive when at

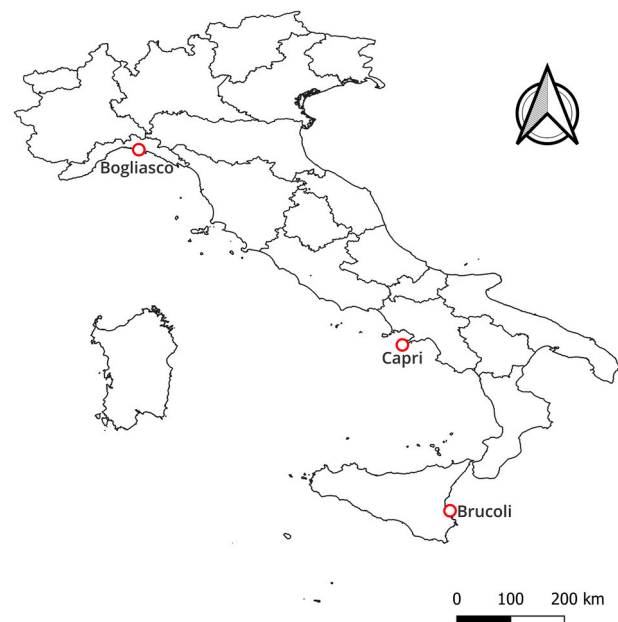


Fig. 1. Map of donor sites distributed along the Italian coast.

least 70% of the primary branches bore developed conceptacles (Fig. 2 A). Maturity was assessed in the laboratory on 500 conceptacles for each site. Thin (50 μm) longitudinal cuts of the apices with conceptacles were made using a cryomicrotome (Leica CM3050 S) (Fig. 2B and C). Apices were mounted on stubs with OCT mounting medium (VWR Chemicals) and serial sections were made from the tip to the base of the apices. The sections were observed and photographed with an inverted microscope (Leica, DM IL LED). The maturity was assessed in the conceptacles where the median section runs through the ostiole to the base of the conceptacle (i.e., on the opposite side of the ostiole; see Fig. 2D, Supplementary Fig. 1A for details). For the *ex-situ* culture, 1000 apices were collected in Bogliasco and 300 in Capri and Brucoli.

2.2. Temperature data

Sea surface temperature (SST) was used to investigate the intensity and duration of the anomalously warm water event detected in the 3 donor sites in summer 2022 and, for comparison across different years, in Bogliasco from 2018 to 2022. Satellite-derived temperature data were obtained for each donor site and for each investigated year. Additionally, satellite-derived SST data were obtained over the period 1982–2015 for the assessment of climatological means (that should be measured over at least 30 years). For detecting MHW events the definition provided by Hobday et al. (2016) was used: MHWs are periods in which daily mean temperatures exceeded the 90th percentile for at least 5 consecutive days relative to the 30-year climatological baseline, calculated from satellite-derived SST data. We decided to use the standardised metrics proposed by Hobday et al. (2016) because they greatly facilitate inter-comparison between events, locations and times. The datasets used to detect MHW and plot the graphs are provided by “Simons, R.A. 2020. ERDDAP. [https://coastwatch.pfeg.noaa.gov](https://coastwatch.pfeg.noaa.gov/erddap)

Monterey, CA: NOAA/NMFS/SWFSC/ERD”. The “ncdcOisst21 NrtAgg” product by NOAA provides “1/4-degree Daily Optimum Interpolation Sea Surface Temperature (SST)”. It is “created by interpolating and extrapolating SST observations from multiple sources, resulting in a smoothed complete field. The data sources are satellite and *in situ* platforms (i.e., ships and buoys), and the specific datasets employed may change over time.” (NOAA website).

2.3. Laboratory cultures

After collection, the apices were transported in the dark and under cold conditions (4 $^{\circ}\text{C}$) to the laboratory facilities at Genoa University (for the Bogliasco donor site) or to Trieste University (for the Capri and Brucoli donor sites). Both facilities are equipped with a temperature/light-controlled room: temperature was kept at 20 $^{\circ}\text{C}$; light was provided directly to the aquaria by LED lamps (Maxspect RSX 150) and no external light sources were present.

Transportation from the donor sites to the facilities took place within 10 h of collection (by car or plane). To induce the release of gametes, the apices were stored at 4 $^{\circ}\text{C}$ for 24 h. The apices were then placed on clay tiles (3–4 per clay tile) and left 24 h for zygote fertilisation. In the facilities of Genoa, 276 tiles were seeded with apices collected in Bogliasco, and in Trieste 60 tiles were seeded with apices from Capri and 60 with apices from Brucoli. Apices that were damaged or overgrown by epiphytes were not used for the seeding phase.

Zygotes were cultured according to the *ex-situ* protocol for *E. amentacea* (Fig. 2 H, I) provided in Falace et al. (2018). In brief, the filtered and Von Stosch-enriched seawater was renewed every 3 days to minimize nutrient limitation. Each time the culture medium was renewed, the tanks were randomly repositioned. Temperature was kept constant at 20 $^{\circ}\text{C}$ (± 0.2) and light intensity was equal to 125 μmol

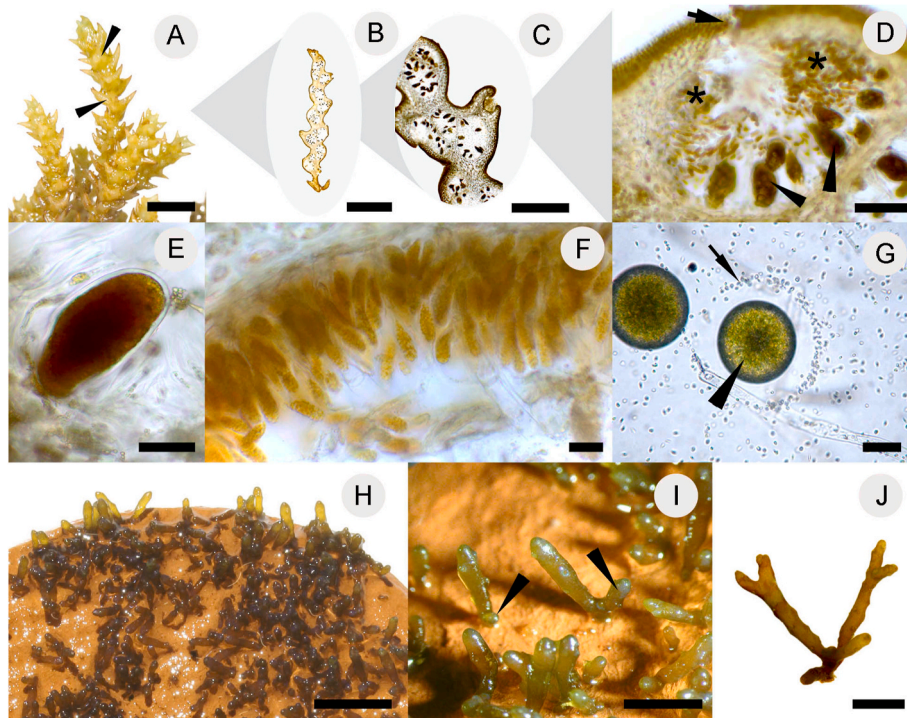


Fig. 2. *Ericaria amentacea* reproductive features and seedlings cultured for *ex situ* restoration. A. Apex with conceptacles at the base of spinose appendages (arrowhead). Scale bar = 3 mm. B. Longitudinal section of a fertile apex with conceptacle chambers. Scale bar = 2 mm. C. Detail of the conceptacles. Scale bar = 500 μm . D. Median section of a conceptacle through the ostiole (arrow) with antheridia (asterisks) covering the roof and oogonia (arrowheads) at the base. Scale bar = 100 μm . E. Mature oogonium. Scale bar = 50 μm . F. Pigmented antheridia covering the roof of the conceptacle. Scale bar = 20 μm . G. Fertilized egg (arrowhead) surrounded by an outer membrane blocking other male gametes (arrow) to prevent polyspermy. Scale bar = 50 μm . H. Seedlings cultured in the laboratory on clay tiles. Scale bar = 1.5 mm. I. Seedlings with lateral branches at the end of the laboratory cultures. Scale bar = 400 μm . J. Seedling after one month in the field. Scale bar = 1 mm.

photons $\text{m}^{-2}\text{s}^{-1}$ (light/dark cycle of 15:9 h).

The culture lasted 3 weeks. At the end of the culture period, photos of the tiles were taken to assess the percent cover of the seedlings.

In addition, the development of the seedlings in the Capri culture was analysed after two weeks. The percentage of each stage of development was calculated according to Falace et al. (2018), for 5 randomly selected areas ($5 \times 5 \text{ mm}^2$) on 3 tiles in triplicate. The output of the Bogliasco culture (in terms of percentage of seedling coverage) was compared with the results of cultures carried out in previous years (2018, 2019, 2020, 2021) with apices collected at the same donor site following the same protocol and using the same laboratory facilities.

2.4. Data analyses

Differences in percent cover of seedlings on tiles seeded with apices from Bogliasco (number of replicates, $n = 70$) and Capri ($n = 37$) were tested through Kruskal-Wallis non-parametric test, given the violation of normality and homogeneity of variance assumptions (tested through Kolmogorov-Smirnov and Bartlett test respectively). Brucoli was not considered for statistical analyses as no seedlings survived up to the third week of culture. Similarly, the differences in Bogliasco culture outputs among years (2018 $n = 157$; 2019 $n = 112$; 2020 $n = 79$; 2021 $n = 202$; 2022 $n = 70$) were tested by way of Kruskal-Wallis non-parametric test. Data are displayed through boxplots with medians highlighted in bold and bars representing the 25% and 75% quartiles, while whiskers representing the lowest and highest data points.

In the Capri culture, seedlings belonging to the following three developmental stages (i.e., I-round-shaped, II-elongated, and III-elongated with branching, Falace et al., 2018) were identified at week 2 and the percent cover of each stage on tiles was quantified. Data are displayed through boxplots, compared with 2018 data obtained using the same lab facilities and culture conditions (2018 $n = 30$; 2022 $n = 45$).

Statistical analyses and plots were performed with R software (R Core Team, 2021). The “stats” and “ggplot2” packages were used to perform analyses and produce boxplots. The “heatwaveR” package was used to calculate and display marine heatwaves over the considered period. Among the several metrics calculated through “heatwaveR” package, for each MHW event, the duration of the event (days), its mean and maximum intensity ($^{\circ}\text{C}$), the onset and decline rate ($^{\circ}\text{C}/\text{days}$) were considered.

3. Results

3.1. Thermal anomalies

In 2022, 6 MHW events were recorded in Bogliasco, with one exceptionally long and intense event, lasting 78 days and 4.3°C above the climatological mean (max intensity, MI), occurring from May 26 to August 11 (Fig. 3A, Supplementary Table 3). Noteworthy are three other very long-lasting events: one from January 18 to February 22 (36 days, 1.2°C MI), one from August 20 to September 26 (38 days, 2.9°C MI) and one from October 20 to December 17 (59 days, 2.7°C MI).

Seven MHW events occurred in 2022 in Capri (Fig. 3B, Supplementary Table 4). The two most intense events occurred from June 1 to 10 (10 days) and from June 25 to July 10 (16 days), with max intensity

values of 3.3°C and 3.6°C , respectively. Two other less intense but longer events occurred from July 20 to August 12 (24 days, 2.6°C MI) and from August 18 to September 19 (33 days, 2.5°C MI).

In Brucoli, 5 MHW events were recorded in 2022 (Fig. 3C, Supplementary Table 5). The most intense and long-lasting event, which exceeded the climatological mean of 2.8°C (MI), occurred from July 13 to September 22 (72 days). Another particularly intense event occurred in June, which exceeded the climatological mean (MI) by 2.4°C and lasted from June 23 to July 08 (16 days).

In the period 2018–2022, the most intense event in Bogliasco occurred in 2019 and exceeded the climatological mean of 4.5°C (MI; Fig. 4B, Supplementary Table 3). The event lasted 20 days, from June 25 to July 14. As far as MHW duration, the longest event recorded in the period under consideration was in 2018 and lasted 89 days, from July 7 to October 3, (Fig. 4A, Supplementary Table 3). The second exceptionally long-lasting event was recorded in 2022, with a duration of 78 days, from May 26 to August 11, (Fig. 4E, Supplementary Table 3). This was also the second most intense event exceeding the climatological mean of 4.3°C (MI). The highest number of events within a year was recorded in 2019 (7 events), followed by 2022 (6 events). Nevertheless, the number of days exceeding the climatological mean were definitely higher in 2022 (223 days) than in 2019 (97 days).

3.2. Reproductive and early life-stage anomalies

In well-developed and mature conceptacles of *Ericaria amentacea* ($420\text{--}698 \mu\text{m}$ high; $297\text{--}504 \mu\text{m}$ wide), the antheridia cover the roof of the conceptacle near the ostiole on 30–50% of the conceptacle wall (Fig. 2D). At maturity, each antheridium ($32\text{--}35 \mu\text{m}$ long and $11\text{--}13 \mu\text{m}$ wide) contains 64 antherozoids (male gametes), which turn orange at maturity due to the pigmented stigma (Fig. 2F). Before fertilization, the membrane at the tip of the antheridia ruptures and releases the motile male gametes. On the side opposite to the antheridia at the base of the conceptacle, an average of 7 oogonia (female gametes) are formed. Mature oogonia are $125\text{--}149 \mu\text{m}$ long and $54\text{--}70 \mu\text{m}$ wide (Fig. 2E).

Deviations from the described reproductive traits (see Fig. 2 and Supplementary Table 3) were considered as anomalies. Examination of the maturity of the conceptacles showed that 65% of the conceptacles from Bogliasco were mature conceptacles, 7% were still developing, while 28% had a reduced number of oogonia (maximum three), but mature orange-coloured antheridia with intact tips (Fig. 5A and B). The low number of oogonia resulted in a lower incidence of seedlings on the culture tiles (Fig. 5C).

Despite the higher proportion of mature conceptacles (92%) in the apices of Capri (Fig. 5D), impairments in fertilization and seedling development were observed. The male gametes showed reduced motility, which probably led to poor fertilization of the eggs (Fig. 5E) and consequently to a low number of zygotes. In addition, a delay in seedling development was observed compared to Falace et al. (2018) and another culture from Bogliasco fertile apices performed in 2018 (author’s unpublished data), using the same lab facilities and culture conditions (Fig. 6). After 2 weeks of culture, 76% of the seedlings were still round (stage I) (Fig. 5F), 23.5% were elongated (stage II) and only a smaller number (0.6%) showed lateral branches (stage III). At the end of the culture, the seedlings growing on the tiles (Fig. 5G) were $376 \pm 0.12 \mu\text{m}$ long.

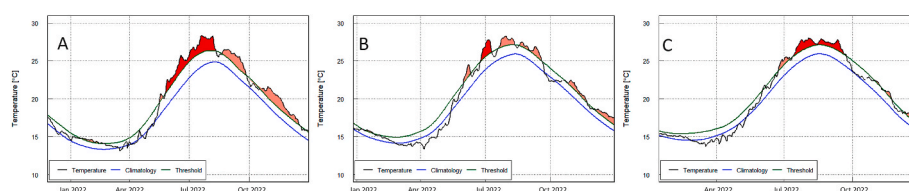


Fig. 3. MHW in the 3 donor sites: A: Bogliasco, B: Capri, C: Brucoli.

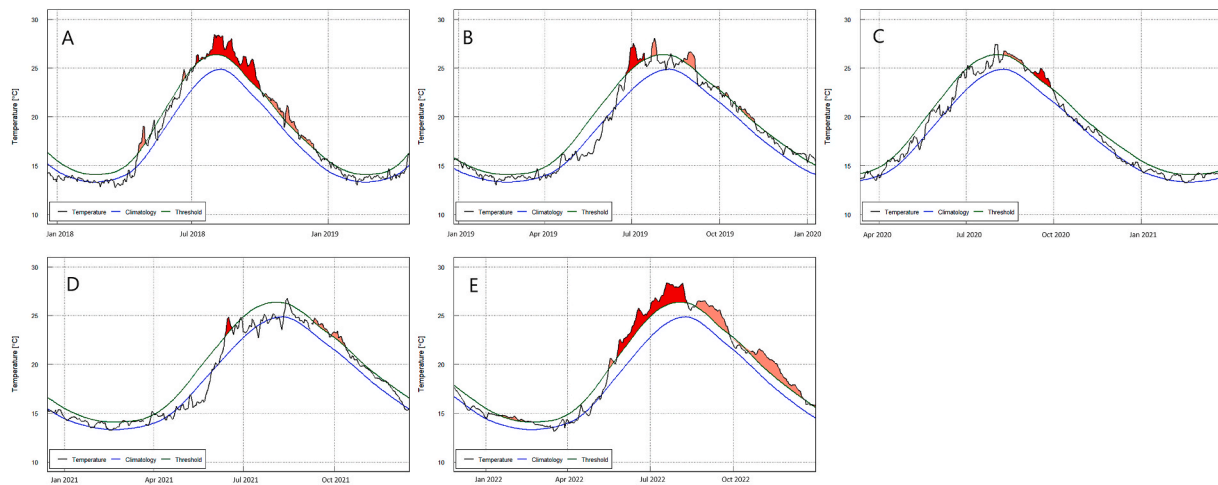


Fig. 4. MHW in Bogliasco in 2018 (A), 2019 (B), 2020 (C), 2021 (D) and 2022 (E).

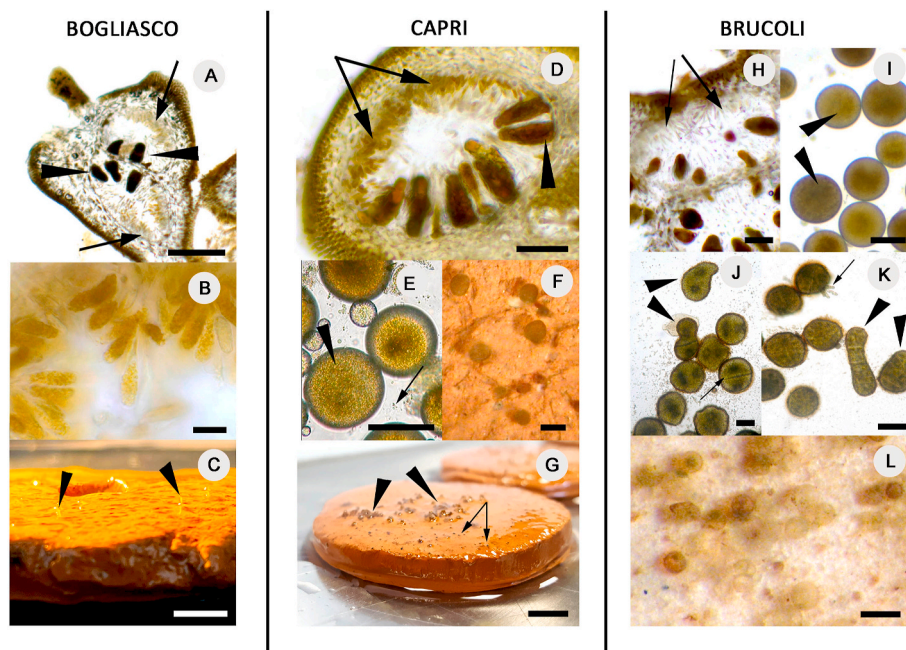


Fig. 5. Morphological anomalies of conceptacles and early-life stages in *Ericaria amentacea* from Bogliasco, Capri and Brucoli. A. Section through conceptacles with two or three oogonia (arrowheads) and mature antheridia on the roof covering over 40% of the chamber (arrows). Scale bar = 500 μ m. B. Detail of antheridia with intact membrane at the tip and pigmented antherozoids. Scale bar = 20 μ m. C. Embryos (arrowheads) on a tile at the end of the culture. Scale bar = 5 mm. D. Section through a mature conceptacle with antheridia (arrows) on the roof and numerous oogonia at the base (arrowheads). Scale bar = 100 μ m. E. Unfertilized oogonium (arrowhead) and antherozoids (arrow). Scale bar = 100 μ m. F. Round seedlings after two weeks of culture (stage I). Scale bar = 200 μ m. G. Tile with live seedlings (arrowheads) and unfertilized eggs or dead embryos (arrows) at the end of culture. Scale bar = 5 mm. H. Median section of a conceptacle with oogonia at the base and paraphyses on the roof (arrows) with only a few scattered antheridia. Scale bar = 50 μ m. I. Unfertilized oogonia. Scale bar = 100 μ m. J. Embryos with first division (arrow), some deformed (arrowheads). Scale bar = 100 μ m. K. One week old embryos with four primary rhizoids (arrow), some of which are deformed. Scale bar = 100 μ m. L. Tile after ten days of culture with dead seedlings. Scale bar = 200 μ m.

The Brucoli apices had mainly mature conceptacles (74%), some were still developing (9%) and conceptacles with anomalies were also observed. In 17% of the conceptacles with mature oogonia (Fig. 5H), the antheridia covered less than 5% of the conceptacle wall and were scattered between the paraphyses (Fig. 5H). Most of the released eggs remained unfertilized (Fig. 5I) and when fertilization occurred, the zygotes did not undergo the second and third divisions or were deformed (Fig. 5J). In the one-week-old embryos, the secondary rhizoids did not develop (Fig. 5K) and an almost total mortality occurred within 10 days of culture (Fig. 5L).

3.3. Culture outputs

In the Bogliasco culture, the seedlings of *Ericaria amentacea* were present on 70 out of the 276 tiles at the end of the cultivation period (25% of survival). In the Capri culture, seedlings survived on 37 out of 60 tiles (61.7%), while no seedlings survived in the Brucoli culture (0%).

The percentage cover of seedlings on the tiles was extremely low (Fig. 7), reaching a maximum of 4.38% in the Capri culture. The average percent cover values were 1.52% (± 0.95 standard deviation) for the Capri culture and 0.16% (± 0.27 standard deviation) for Bogliasco. The Kruskal-Wallis test showed significant difference between the two sites

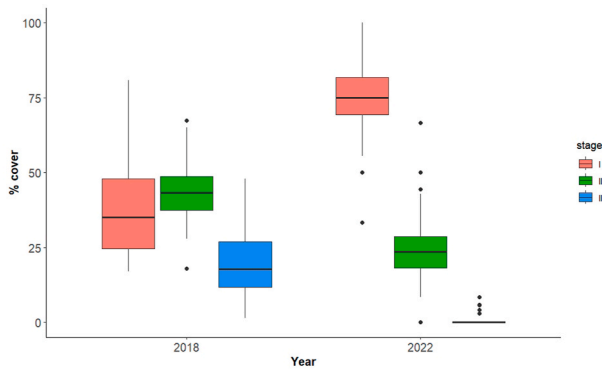


Fig. 6. *Ericaria amentacea* seedling stages after two weeks of culture: I-round-shaped, II-elongated, and III- elongated with branching. 2022 data is from Capri derived cultures; 2018 from Bogliasco derived cultures, authors' unpublished data.

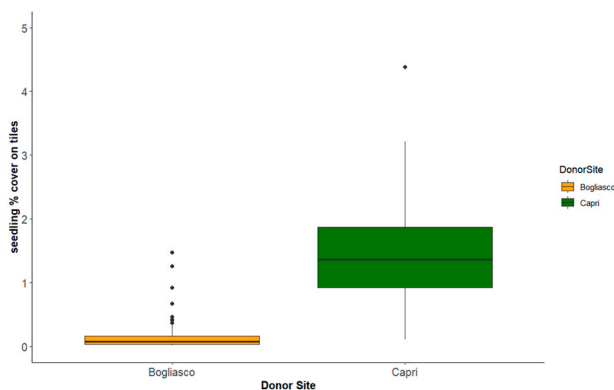


Fig. 7. Boxplot of seedling percent cover on the tiles of the Bogliasco and Capri donor sites.

(p-value <0.001).

For the Bogliasco donor site, a large variability in crop yield was observed with significant differences between years (p-value <0.001). In particular, the percent cover of seedlings in the 2022 culture was significantly lower compared to all previous years (Fig. 8).

4. Discussion

At the three considered donor sites along the Italian coasts, a peculiar and worrying dynamic of sea surface temperature thermal anomalies

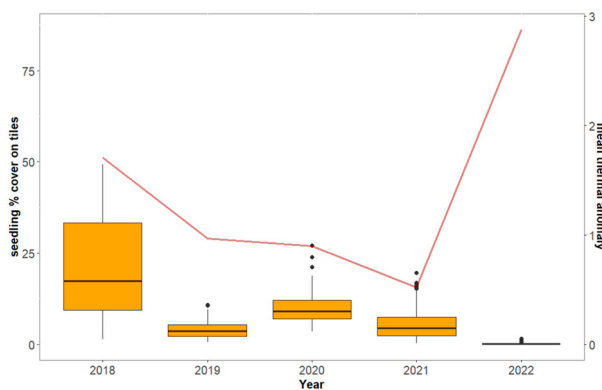


Fig. 8. Boxplot of seedling percent cover on tiles in Bogliasco cultures over years (2018–2022) and relative mean thermal anomaly (red line) over the reproductive period (01 May - 31 July 2022).

was observed in 2022. Exceptionally long-lasting and intense MHW events were recorded, extending over the whole year, especially in the north-western basin, *i.e.*, in the Ligurian Sea (223 days with sea surface temperatures above the climatological mean in Bogliasco). The higher MI recorded in the northern site, Bogliasco, is referred to lower baseline values, based on the climatological mean of the area (average value over the considered period in Bogliasco: $18.3\text{ }^{\circ}\text{C} \pm 4.2$) compared to the southern sites (average value over the considered period in Capri and Brucoli: $19.4\text{ }^{\circ}\text{C} \pm 4.3$ and $19.4\text{ }^{\circ}\text{C} \pm 4.2$ respectively). In fact, a general positive warming trend was observed across all the Mediterranean basins, but with the Ligurian sea showing the highest trend achieved overall (García-Monteiro et al., 2022).

Temperature is one of the main factors controlling reproductive and vegetative phenology in algae (Eggert, 2012). It has been shown that high temperatures can affect both the reproductive potential and the development of early life stages in many seaweeds (Bartsch et al., 2013; Andrews et al., 2014). In temperate seaweeds abrupt warming of seawater can both advance and delay the timing of reproduction (de Bettignies et al., 2018) and suppress or enhance reproduction and recruitment success (de Bettignies et al., 2018; Muth et al., 2019). While recent studies have shed some light on the reproduction of *Cystoseira s.l.* under the influence of high temperatures or MHWs (Bevilacqua et al., 2019; Savonitto et al., 2021; Falace et al., 2021) this aspect remains largely unexplored. Our results suggest that the thermal anomalies that occurred at the donor sites may have impaired the culture of *E. amentacea* in both the pre- and post-zygotic phases, resulting in significantly lower culture efficiency. In Bogliasco, we observed more than one third of poorly developed conceptacles or with developed antheridia but few eggs. In Capri, the observed poor motility of male gametes resulted in lower fertilization of mature eggs. In Brucoli, although only 26% of the conceptacles were not well developed, *i.e.* there were no or few developed antheridia, fertilization was the lowest of the three cultures and embryo growth was the slowest resulting in a lower percent cover of seedlings on the tiles and a lower survival rate.

Negative effects of high seawater temperatures on gamete development were observed by Falace et al. (2021) in *Ericaria giacconei*: at $28\text{ }^{\circ}\text{C}$, the settlement efficiency of zygotes dropped significantly because eggs and zygotes underwent cell lysis and clustered together. A blocked karyogamy (*i.e.*, fusion of cell nuclei during fertilization) as a result of heat stress was evidenced in several brown algae (Nagasato et al., 1999). Additionally, at $28\text{ }^{\circ}\text{C}$, no survival of germlings was recorded. Similarly, Verdura et al. (2021) exposed organisms (adults and recruits) of *Ericaria crinita* to $28\text{ }^{\circ}\text{C}$ aiming to provide evidence of the impact of a MHW event (occurred in summer 2015). After 15 days of exposure at $28\text{ }^{\circ}\text{C}$ photosynthetic efficiency of adults was significantly reduced, and after 30 days 70% of biomass loss was observed, mainly in branches, where reproductive structures develop. Recruits' survival was instead drastically impaired after only 5 days of exposure at $28\text{ }^{\circ}\text{C}$. In another controlled laboratory experiment, exploring the impacts of warming on early life stages of the deeper subtidal *Ericaria zosteroides*, settlement and survival of germlings were both affected by increased temperatures ($20\text{ }^{\circ}\text{C}$ and $24\text{ }^{\circ}\text{C}$) over a six-weeks period (Capdevila et al., 2019).

A detrimental effect of a MHW in terms of shifting of the reproductive period was reported for *Gongolaria barbata* in the Gulf of Trieste: a winter thermal anomaly of $\pm 2.65^{\circ}\text{C}$ lasting for more than one month led to the development of reproductive structures and mature receptacles also on the adventitious branches on thalli in the winter resting *habitus* (Bevilacqua et al., 2019). An *ex-situ* restoration effort was carried out taking advantage of this early fertility and the performance was compared to that performed later during the regular reproductive season. Lower efficiency of the latter was found, both in terms of zygote release and growth (Savonitto et al., 2021), stressing the impairment of the reproductive effort because of the performance of the anomalous, early, one.

In the present study, the abnormalities found in the reproductive structures resulted in poor performance of the cultures. Despite the

culture from apices collected in Capri showed significantly higher percent cover compared to Bogliasco ones, both cultures achieved a cover of less than 2% (with an outlier of 4.38 % recorded in Capri). No germling development was recorded on the tiles from the Brucoli site.

The Bogliasco donor site has been used for the production of cultures for *ex-situ* restoration (ROC-POP Life project) since 2018. Across years, we recorded very variable values in terms of percent cover of seedlings on the tiles, with the highest values observed in 2018 ($20.8\% \pm 13.7$) and lowest in 2022 ($0.16\% \pm 0.27$). Both years had peculiar characteristics in terms of thermal anomaly. In 2018, we recorded the longest MHW event, with a duration of 89 days, but with a MI of $0.6\text{ }^{\circ}\text{C}$ less than in 2022 (MI = $3.7\text{ }^{\circ}\text{C}$ in 2018; MI = $4.3\text{ }^{\circ}\text{C}$ in 2022). Additionally, this long-lasting MHW event started on 7 July (Supplementary Table 4), a time that should be after the reproductive peak in *E. amentacea*, when the fertile structures are already fully developed (Supplementary Table 2). In 2022, instead, we observed an almost continuous MHW from mid-May to the end of the year (Supplementary Table 4), therefore, in the first months, overlapping with the reproductive development period of *E. amentacea* (Supplementary Table 2). This could be, at least, one of the reasons for this strong difference between the crop performance between the two years.

In 2019 and 2021, MHW events occurred in Bogliasco from mid-June and we obtained a seedling coverage of $4.1\% \pm 2.4$ and $5.4\% \pm 4$, respectively. In 2020, instead, we recorded higher germling cover values ($9.9\% \pm 4.7$) and a low intensity of MHWs, which also occurred only after 10th August.

Early MHW events seem to be the main driver influencing crop yields, as they may directly affect the maturation of receptacles and gametes. This finding also seems to be supported by the cultures from 2022 for the Capri donor site. Here, an early MHW event was observed in 2022, starting on June 1, during the maturation period (Supplementary Table 5). Reduced motility of the male gametes was observed, which probably impaired fertilization of the released eggs. Further studies are needed to investigate whether heat stress directly affects the viability and/or chemotaxis of male gametes in *Cystoseira* species or whether it affects the synthesis of sex pheromones released from eggs, which are crucial in the early stage of fertilization in brown algae (Gang Fu et al., 2014; Kinoshita et al., 2016; Kinoshita et al., 2017). In addition, the development of seedlings after 2 weeks of culture was defined based on the percentage cover of different stages. In 2022, we observed a delay in the development of the early life stage, with most seedlings remaining roundish (*i.e.*, first stage) and not reaching the elongated stage with branching (*i.e.*, third stage).

Increased sea surface temperature combined with an anomalous increase in air temperature during successive emersion cycles has been found to play an important role in the physiological stress and survival of *Ericaria selaginoides* in the intertidal zone (Román et al., 2020). At the Brucoli sampling site, a prolonged and extremely high thermal excursion between air temperature and SST, not observed in the other two donor sites, occurred in spring/summer (Supplementary Fig. 2), which could explain the observed anomalous conceptacle development and consequent failure of the culture effort.

The findings of the present study suggest that thermal anomalies, especially MHWs, may impair the restoration efforts of *E. amentacea* in the Mediterranean Sea, one of the sea basins that is experiencing faster global driven changes (García-Monteiro et al., 2022). Therefore, knowledge gaps and adaptive strategies need to be implemented to gain valuable insights for management of restoration actions.

Further efforts should aim to investigate the effects of MHWs on reproduction in *Cystoseira s.l.* species, which might respond differently to these extreme climatic events depending on the species. In particular, the role of abrupt thermal stress in triggering fertility in interaction with i) other extrinsic factors (*e.g.*, day length, light quality, nutrients), ii) and intrinsic factors (*e.g.*, genetic control, endogenous signalling molecules; Liu et al., 2017) should be clarified. The timing of reproduction can be altered by MHWs, so it can be unpredictable even in areas where

phenology is well known (Bevilacqua et al., 2019).

To optimize the *ex-situ* harvesting, the collection of fertile apices must be done during the maximum reproductive period. Therefore, monitoring the reproductive phenology (*i.e.*, the percentage of branches with conceptacles) and maturity (*i.e.*, the percentage of conceptacles with mature antheridia and oogonia) is crucial. However, to determine the sexuality (*i.e.*, hermaphroditic, male or female conceptacles), observations should be made on the median longitudinal section that runs through the ostiole to the base of the conceptacle (Supplementary Fig. 1A). Following Savageau (1912), we strongly recommend avoiding the examination of non-median transverse or longitudinal sections of the conceptacle (see Supplementary Figs. 1B and C), as they could lead to confusion of a hermaphrodite conceptacle with a unisexual one. In this study, the non-median sections also appeared to be unisexual (both male and female, in the same conceptacle), as shown in Supplementary Figs. 1B and C. If the cut occurs near the roof, where the antheridia form, the conceptacle could be mislabelled as male (as shown in Supplementary Fig. 1B), whereas if cut is done near the base of the conceptacle, where the oogonia develop, it could be misinterpreted as female (Supplementary Fig. 1C) (Savageau, 1912). Misinterpretation of male unisexual conceptacles also appears to be the case in a section in Rindi et al. (2023) (Fig. 2B, D and 5G), where the cut appears to be near the roof, as shown in our Supplementary Fig. 1B. Moreover, monitoring fertility and maturation, although necessary, is not sufficient as it must be coupled with fertilization tests, since the presence of fully mature conceptacles is not a guarantee of reproductive success, as already shown by Savonitto et al. (2021) and in the present study.

To intercept premature reproduction triggered by an abrupt increase in temperature in a donor population, an early warning protocol should be introduced in the context of *Cystoseira s.l.* restoration, reducing the risk of failure of cultivations. Another way to overcome the limitation of seedling availability caused by thermal anomalies could be the development of laboratory protocols that induce fertility under controlled laboratory conditions. In a recent study, the use of an algal biostimulant induced fertility in *Gongolaria barbata* demonstrating its potential for restoration applications (Kaleb et al., 2023).

While 'future-proofing', which focuses on the restoration of naturally adapted or tolerant populations, has been proposed to overcome the climate-induced loss of kelp forests (Layton et al., 2022), this does not appear to be a viable option for the Mediterranean *E. amentacea*, as reproductive anomalies have been observed at all three donor sites from the northern to the southern distribution of the species along the Italian coast. Multiple donor sites should be considered, possibly differing in terms of vulnerability to MHW occurrence, although the dynamic nature of the marine environment and the unpredictability of thermal anomalies can frustrate expectations, as in the present study.

Policy implications must be considered to integrate climate change vulnerability of restoration efforts considerations into restoration and conservation strategies. Marine forest restoration requires long lasting projects, both to allow for long-term monitoring and better understanding the biology of the species and for mitigating stochastic events that can cause the failure of efforts in a given year. Some degree of flexibility should also be considered, perhaps in the selection of the target species: a range of species could be considered, of course according to their past presence in the area and the relevance of their ecological role. Such flexibility could buffer species specific effects of MHWs or of other climatic anomalies.

CRediT authorship contribution statement

Jacopo Cimini: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Valentina Asnagli:** Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Maria-chiara Chiantore:** Writing – original draft, Validation, Supervision,

Project administration, Funding acquisition, Conceptualization. **Sara Kaleb:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alessandra Onida:** Writing – original draft, Investigation, Formal analysis, Data curation. **Annalisa Falace:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2024.106537>.

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