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Too Hot to Handle: Effects of Water Temperature on the Early Life Stages of *Gongolaria barbata* (Fucales)

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Abstract: *Gongolaria barbata* plays a crucial role as a habitat-forming Fucales species in the Mediterranean Sea, thriving in shallow, sheltered coastal regions, where it exhibits optimal growth in a temperature range of 10 to 25 °C. In the northern Adriatic Sea, a semi-enclosed part of the Mediterranean, there has been a remarkable increase in seawater temperatures in recent decades, often exceeding 28 °C in summer. These high temperatures pose a significant threat to the vulnerable early life stages of *G. barbata*. This study delves into the effects of four temperatures (15, 18, 24, and 28 °C) on the growth of *G. barbata* over its first 16 days, closely monitoring mortality, deformities, and overall survival. Our experiments reveal that higher temperatures can result in deformities and increased mortality of germlings. Notably, a temperature of 28 °C resulted in the death of all germlings within the first week, whereas those exposed to 24 °C survived until the second week, albeit with significant deformities prior to death. In contrast, germlings cultivated at 15 and 18 °C exhibited normal development with minimal deformities. These results highlight the susceptibility of the early life stages of *G. barbata* to temperature-induced stress and provide valuable insights into the potential consequences of rising seawater temperatures in the Mediterranean.

Keywords: *Gongolaria barbata*; increased temperature; early stages; deformities; mortality

1. Introduction

The Mediterranean Sea, with its rich tapestry of habitats, hosts a diverse array of species. Notably, the brown algae of the order Fucales are foundational organisms that shape shallow rocky reef ecosystems. These seaweeds form complex three-dimensional canopies, akin to marine forests, with the *Cystoseira s.l.* species (comprising genera *Ericaria*, *Gongolaria* and *Cystoseira*) playing a pivotal role [1,2]. These marine forests make an important contribution to coastal ecosystem services [3], supporting primary production, promoting nutrient cycling, attenuating waves, sequestering carbon, and fostering biodiversity [4–7].

Cystoseira s.l. species exhibit a wide distribution throughout the Mediterranean Sea and Eastern Atlantic, with several species endemic to the Mediterranean region. Among the 16 accepted species of the genus *Gongolaria* [8], *Gongolaria barbata* (Stackhouse) Kuntze inhabits the Levantine Sea in the east [9], as well as areas along the African coasts at the south (Algeria [10], Libya [11], Morocco [12], Tunisia [13]), and extends to the northern coastlines encompassing Croatia [14–16], France [17], Greece [18], Italy [19,20], Slovenia [21], and Spain [22]. Along the Romanian Black Sea coast, *G. barbata* is the only remaining representative of *Cystoseira s.l.* [23]. Additionally, it is found around the islands of Malta [24], Corsica [25], Sardinia, and Sicily, as well as the Balearic Islands [26]. In Menorca, the restocked population of *G. barbata* has expanded significantly over the past 10 years, with recruits and juveniles dominating the population and some individuals reaching large size classes [26]. Its distribution also reaches into the Atlantic, including the Canary and

Salvage Islands. In the Mediterranean region, it is classified as threatened by the Barcelona Convention (Annex II; UNEP/MAP, 2013; [27]), together with other species of *Cystoseira s.l.* (except *Cystoseira compressa)* [28].

The extensive distribution of *G. barbata* underscores its adaptability, phenotypic plasticity, and ecological resilience across diverse marine environments. However, this species, that was previously common in the northern Adriatic, is now found only at few sites with scattered populations [21,29]. In the Gulf of Trieste, which represents the species' northernmost point of distribution, three distinct forms are nowadays observed, whether attached or detached [30]. The new proposed nomenclature for these forms comprises *Gongolaria barbata* f. *aurantia* (Kützing) Falace, Alongi, et Kaleb for the detached, free-living form in a lagoon environment, and *G. barbata* f. *hoppei* (C. Agardh) Falace, Alongi, et Kaleb and *G. barbata* f. *turneri* (J. Agardh) Falace, Alongi, et Kaleb for two morphologically different attached forms living in coastal waters [30].

Regrettably, there has been a decline in and local extinction of almost all *Cystoseira s.l.* species in recent decades due to a combination of natural and human impacts [21,31–36] such as climate change, overfishing, coastal development, etc.

In the Mediterranean, changing sea temperatures, alongside other abiotic and biotic factors, are altering the distribution and abundance of algal species [16,37,38]. These shifts have the potential to reshape local biodiversity and community structures, affecting ecosystem resilience. Changes in seawater temperatures directly affect the reproduction of macroalgae by affecting gametogenesis, fertilization, and growth during early life stages [39–42]. These changes can have far-reaching effects on population dynamics and long-term viability. The maintenance of populations of foundation species depends on the persistence of adults and the successful recruitment of early life stages. While adults generally exhibit a wider range of thermal resistance, early life stages are more sensitive [40,43–45] and often represent a 'bottleneck' in the development and maintenance of algal populations [46–48]. Unusual temperature patterns, including thermal anomalies and ocean warming, also pose significant threats to *Cystoseira s.l.*, impacting reproduction, growth, survival, and ecological dynamics [29,44,49–54]. Consequently, temperature-induced changes may trigger cascading effects on organisms that rely on *Cystoseira s.l.* for habitat and food [55].

The effects of increased temperatures surpass the direct impacts on brown seaweeds [56]. Observations of *G. barbata* in the northern Adriatic Sea in the last 5 years show that the main threat to the remaining populations is the high grazing pressure by the herbivorous fish *Sarpa salpa* (pers. obs., [57]). Ocean warming, a prominent facet of global climate change, disrupts ecosystem dynamics by influencing metabolic processes, nutrient availability, and the behavior of key herbivorous grazers, including sea urchins and herbivorous fish [21,57–59]. As these grazers become more active in response to higher temperatures, they may consume more biomass, potentially leading to overgrazing and the degradation of macroalgal forests [60]. In addition to higher activity of grazers, chemical defenses are reduced under climate stressors such as elevated pCO_2 and reduced salinity. *Gongolaria rayssiae* (Ramon) Molinari & Guiry, an endemic species along the Levantine coast, has shown a decline in carbon sequestration rates under high temperatures [61], while *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, growing along the Italian coastline, showed a decrease in photosynthesis when exposed to air temperatures above 28 °C [53].

In the Mediterranean Sea, where accelerated warming and more frequent thermal anomalies are occurring [62–64], understanding the response of *Cystoseira s.l.* species to temperature changes is crucial. Although the adult algal stages are more extensively studied, it is important to study temperature effects also on early life stages [56]. Studies on *Ericaria giacconei* D. Serio & G. Furnari showed sensitivity to elevated temperatures that lead to massive mortality of zygotes and embryos [56]. This is particularly important, as recruitment plays a crucial role in maintaining healthy brown algal forests.

This study aimed to assess the effect of elevated temperature on germination and early thallus ontogeny of *G. barbata* in the northern Adriatic Sea, where the species still forms

some algal forests in the shallow infralittoral zone [15,16,21,29,65–67]. In this area, the species reaches its maximum vegetative and reproductive development from late winter to late spring/beginning of summer (February–May [29]), when sea temperatures range between 8 and 23 °C. The study deals with the development of zygotes and germlings at four different temperature treatments ranging from 15 to 28 °C, corresponding to the currently possible temperatures in the northern Adriatic Sea. Growth and survival of germlings were monitored for 16 days in mesocosms to assess the critical temperature threshold for the early stages of this species. The study provides evidence for the likely response of this species to projected climate change and highlights the detrimental effects of increased seawater temperatures on the early life stages of *G. barbata*.

2. Methods and Materials

2.1. Study Area

The study area is located in Slovenia, in the northernmost part of the Adriatic Sea. The Slovenian coastal sea encompasses the southern part of the Gulf of Trieste, with a coastline of about 46 km. This region is characterized by a shallow coastal sea, reaching a maximum depth of 37 m and an average depth of 18.7 m, making it one of the shallower areas in the northern Adriatic Sea. The seabed along the coastline is predominantly rocky and consists of alternating layers of Eocene flysch, sandstone, and soft marl, which contribute to the ecological complexity of the region [68,69]. Due to its shallowness, the Gulf of Trieste undergoes significant fluctuations in water temperature and salinity throughout the year. These fluctuations are mainly influenced by the inflow of freshwater, primarily from the Isonzo River and by the bora wind blowing offshore. The average surface water temperature is around 25 °C in summer, occasionally reaching up to 28 °C, while, in winter, it can drop below 8 °C [70]. The average salinity in this area is 37–38 °C [71,72]. Temperature stratification occurs in the water column between April and September, with a seasonal thermocline in spring [73]. The northern Adriatic is additionally influenced by intense surface and lateral fluxes, and has highest amplitudes between high and low tide (average = 88 cm [69]) in the Adriatic Sea. In the surface layer, the water circulation is clockwise, while, in the lower layer, circulation is anticlockwise [74].

2.2. Reproductive Material Collection

The selection of the donor site was based on criteria established in previous studies [65,66] and SCUBA diving surveys in spring (February–May) 2023. Only healthy and dense underwater forests of *G. barbata* were selected as suitable donor populations. For this experiment, apices with mature receptacles of *G. barbata* were harvested from the donor population in Izola (coordinates: 45°32.653, 13°40.554; Figure 1), which is known for dense populations of *G. barbata* and *Cystoseira compressa* (Esper) Gerloff & Nizamuddin.

To verify fertility, a few receptacles were collected on a preliminary dive. A dissection of a few receptacles was performed to examine them under a stereomicroscope. Once the fertility of receptacles was confirmed, a dive was planned to collect the reproductive material needed for the experiment. On 15 May 2023, the receptacles were collected at an average depth of 2 m by SCUBA divers. The fertile apical parts with the receptacles were carefully cut off with scissors and placed in mesh bags, which were stored on the boat in plastic buckets filled with seawater. Within 30 min, they were taken to the laboratory of the Marine Biology Station (NIB).

In order to avoid negative effects on the donor population, up to 10% of the fertile apices per algal thalli were collected. Notably, the collection of receptacles posed a challenge due to the reduced availability caused by the observed overgrazing by herbivorous fish [66].
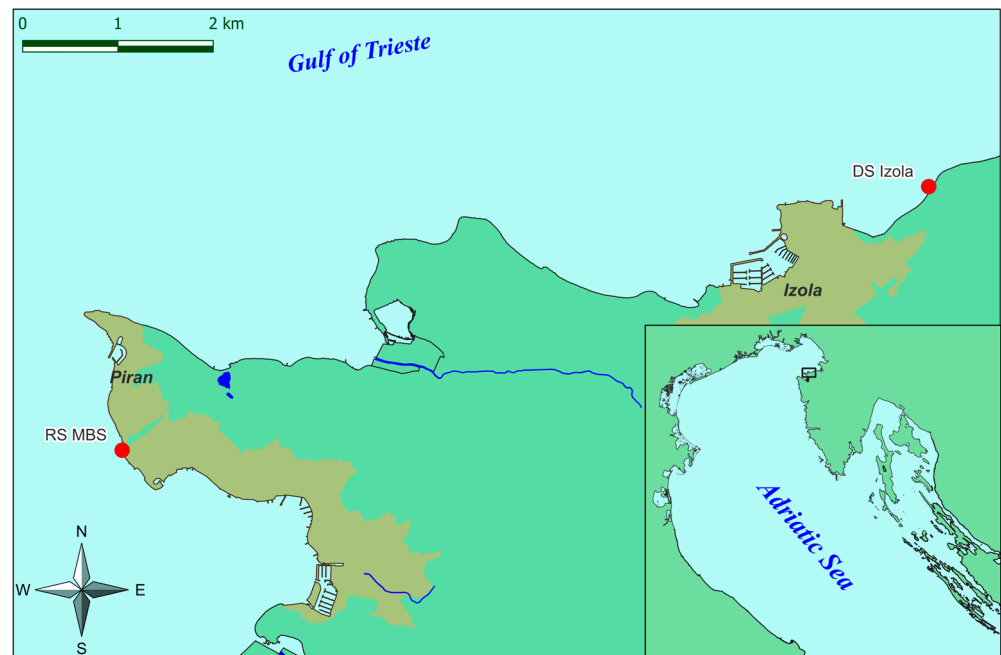
Figure 1. Study area, with the location of the donor site (DS Izola) for *Gongolaria barbata* and the laboratory of the Marine Biology Station Piran (RS MBS). Light blue represents sea, dark blue represents river/lake and brown represents urban areas.

2.3. Laboratory Work

In the laboratory, apices with mature receptacles from the donor population were cleaned of epibionts with a soft brush, rinsed with filtered seawater, wrapped in aluminum foil, and stored overnight (24 h at approximately 4 °C). This thermal shock triggered the release of gametes from the conceptacles, allowing fertilization [42]. Twelve experimental aquaria (Figure 2), each measuring 50 cm × 20 cm × 20 cm, were set up in an environmentally controlled room, with three aquaria assigned to each temperature condition (15, 18, 24, 28 °C). Temperatures were selected according to the normal spring temperatures in the Gulf of Trieste (between 15 and 18 °C) and the possible highest temperatures at the end of spring or beginning of summer (24 and 28 °C). All aquaria and equipment were previously cleaned with 5% solution of sodium hypochlorite (bleach). All aquaria were covered with plexiglass lids to prevent water evaporation and changes in salinity. The temperature of the environmentally controlled room was set at 15 °C, resulting in three aquaria without water heater. In the remaining nine aquaria, NEWA Therm Next 50W water heaters were installed and adjusted to specific temperatures (18, 24, or 28 °C). These water heaters were positioned beneath a zinc wire net stand, on which 10 clay tiles (diameter = 6 cm, with a central hole of 0.6 cm) and 3 Petri dishes (of the same diameter) per aquarium were arranged. Five to six apical parts (approx. 3 cm in length) with, altogether, ten receptacles were placed on each clay tile and Petri dish. Petri dishes were used for measurements and monitoring of germlings' development during the first 5 days because the germlings were too small to be visible on the granulated surface of clay tiles. On the first day, aquaria were filled with UVC-sterilized water to a maximum height of 2 mm above the apices. The photoperiod was set to 15:9 light/dark cycle, which mimics day length during gamete release, fertilization, and early life growth stages of *G. barbata* in the northern Adriatic Sea. The light intensity was set to approximately 140 μmol photons m^{-2} s^{-1}. For illumination, 4 Osram Fluora Florescent tubes with power of 36 W, length of 120 cm, and luminous flux of 1400 lumens per container were used.
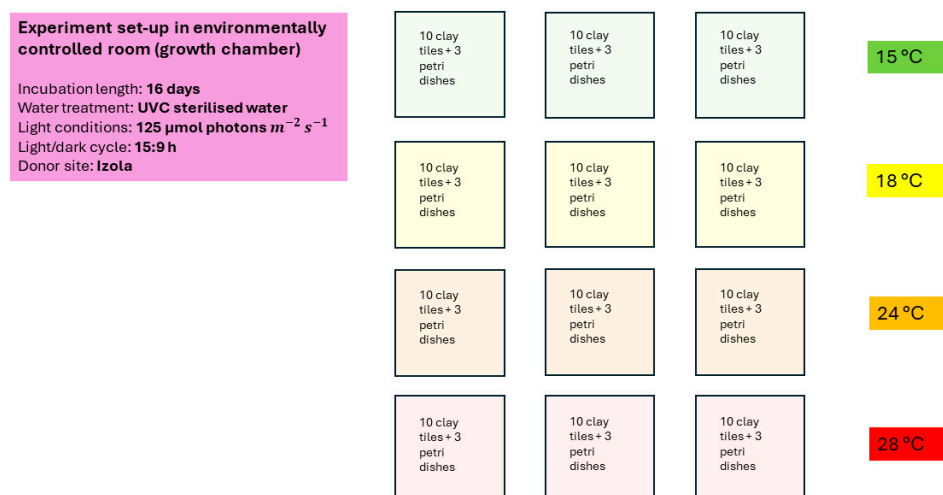
Figure 2. Ex situ cultivation scheme for *G. barbata* at the Marine Biology Station Piran. Each box represents one aquaria with clay tiles and petri dishes which are used as a substrate for *G. barbata* germlings. Three replicate aquaria per each temperature regime were used (green = 15°C, yellow = 18°C, orange = 24°C, red = 28°C).

Receptacles were left on clay tiles for two days to allow gamete release. Following this period, the receptacles were removed, and 1 L of UVC-sterilized seawater per aquarium was added (maintaining a level of approximately 2 cm above the clay tiles). Air pumps and bubblers were then installed in each aquarium and GeO_2 was added to prevent diatom growth. After removal of the receptacles, an area of 0.2 cm^2 was photographed on one randomly selected tile per replicate aquaria of each temperature regime at 24 h intervals. Measurements were taken over a period of 16 days, which was adequate to monitor the mortality of germlings at different temperatures. Water change was performed every 3 days. Length of randomly chosen 25 germlings per each aquarium (75 altogether per each temperature regime) was measured using a stereomicroscope (Olympus SZX16). Average size and percentage of alive, dead, and deformed germlings were calculated. The criterion for determining mortality was the absence of pigmentation, indicating that the germling had entered a state of decomposition. Germlings exhibiting abnormalities such as irregular cell divisions or concave and convex parts were classified as deformed rather than dead (and were not included in either the alive or dead category, as we were unable to detect this). In the calculation of average size, all randomly chosen germlings were included, dead or alive. Percentages of dead, alive, and deformed germlings were calculated on the randomly chosen area of 0.2 cm^2. In the first 5 days, the lengths of the germlings were measured on Petri dishes, while, from the 6th day onward, the measurements were taken on germlings grown on clay tiles.

2.4. Photo-Processing and Statistical Analyses

Photographs from the culture in thermostatic chambers were processed using the ACDSee program. The number of alive, deformed, and dead germlings was counted in the photographed area of 0.2 cm^2, and the percentage of alive, dead, and deformed germlings was calculated for each day and for 4 different temperatures.

Statistical analyses were performed using R 4.2.2 (R Core Team, 2020, Vienna, Austria). A two-way robust ANOVA [75] was performed for the main effect and interaction to check for differences in thalli length of *G. barbata* germlings between two fixed factors: day (with 14 levels) and temperature (with 4 levels: 15, 18, 24, 28 °C). The test was chosen because it is an extension of the one-way ANOVA that examines the influence of two different categorical independent variables on one continuous dependent variable. The two-way ANOVA aims to assess not only the main effect of each independent variable, but also whether there is an interaction between them. Tukey's multiple comparisons of means

were used to compare temperatures and days independently. One-way robust ANOVA and subsequent post hoc tests [76] were performed to check for differences in thalli length of *G. barbata* germlings between different treatment at specific time intervals. Analyses were performed using the WRS2 and AICcmodavg packages [77]. Data were then graphically represented with boxplots using the ggplot2 package [78]

## 3. Results

Survival and Growth of Germlings in Mesocosm

A significant difference in germlings' growth was observed in the four different temperature regimes (ANOVA $F_{(3,1742)} = 2274.1$, $p < 0.0001$; Table 1), with significant interaction between the factors 'day' and 'temperature' (ANOVA $F_{(36,1742)} = 164.95$, $p < 0.0001$; Table 1). Notably, on the second day less than half germlings growing at 24 °C and 28 °C survived (Figure 3), and the longest germlings were measured at 18 °C and 24 °C (Figure 4, Tukey's Test, $p < 0.05$, Table 1). By the third day, the germlings growing at 15 °C had outgrown the germlings cultured at 24 °C and 18 °C (Figure 4). The maximum length reached by the germlings was 523 μm, measured on the 16th day at 15 °C. In comparison, the germlings at 18 °C reached a maximum length of 340 μm on the 16th day. Germlings at 24 °C survived only until the 13th day and reached a maximum length of 187 μm. The germlings at 28 °C reached their maximum length of 222 μm on the fourth day, and no living germlings were found after the sixth day (Table 2).

Table 1. Results of two-way robust ANOVA, and Tukey's pairwise comparison of different treatments on selected days. Df = degrees of freedom, Sum Sq = sum of squares, Mean Sq = mean of the sum of squares. Significance codes: '***': <0.0001; '**': <0.001; '*': <0.01; '.': <0.05; NS: not significant.

2-Way ANOVA						
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F Value**	***p*-Value**	**Significance**
Temperature	3	6,041,970	2,013,990	2274.1	<0.0001	***
Day	12	490,429	40,869	46.15	<0.0001	***
Temperature × Day	36	5,258,925	146,081	164.95	<0.0001	***
Residuals	1742	1,542,751	886			
Pairwise comparison						
Day 2				Day 5		
	15 °C	18 °C	24 °C		15 °C	18 °C	24 °C
18 °C	.			18 °C	***		
24 °C	.	NS		24 °C	***	***	
28 °C	NS	***	**	28 °C	***	***	.
Day 7				Day 12			
	15 °C	18 °C	24 °C		15 °C	18 °C	24 °C
18 °C	.			18 °C	**		
24 °C	***	***		24 °C	***	***	
28 °C	***	***	***	28 °C	***	***	.
Day 14				Day 16			
	15 °C	18 °C	24 °C		15 °C	18 °C	24 °C
18 °C	*			18 °C	***		
24 °C	***	***		24 °C	***	***	
28 °C	***	***	NS	28 °C	***	***	NS
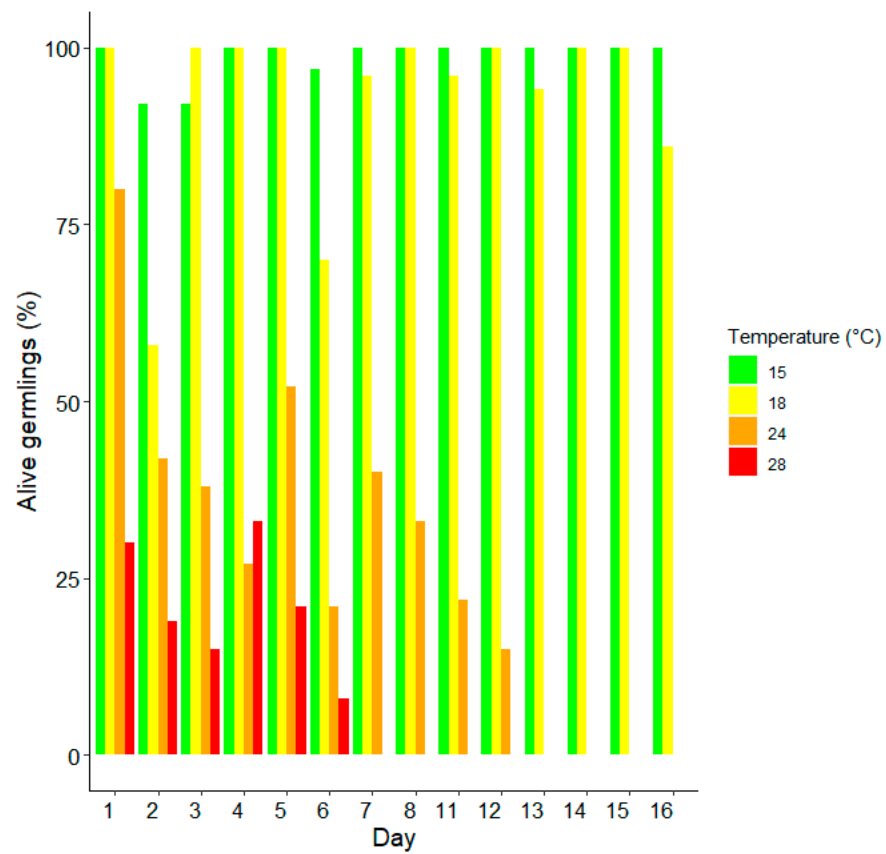
Figure 3. Percentage of alive germlings of *G. barbata* at 15, 18, 24, and 28 °C. The number of living, deformed, and dead germlings was counted daily in a randomly photographed area of 0.2 cm^2.
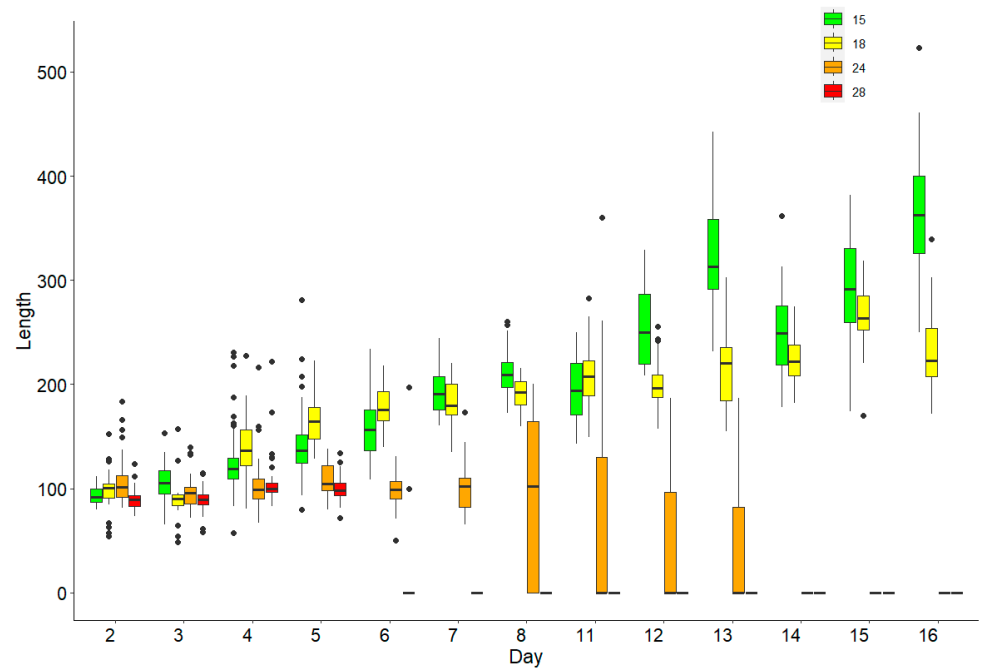
Figure 4. Growth of *G. barbata* germlings (length in μm) in the mesocosms at 15, 18, 24, and 28 °C. The length of living germlings was measured daily in a randomly photographed area of 0.2 cm^2. Boxplots display median, first and third quartiles (boxes), the largest value no further than 1.5× inter-quartile range from the third quartile (upper whisker), the smallest value at most 1.5× inter-quartile range below the first quartile (lower whisker), outliers (dots).

Table 2. Gongolaria barbata germlings' growth at 15, 18, 24, and 28 °C. Average size ± SD and percentage of alive, deformed, and dead germlings on a specific day, calculated from measurements of 25 randomly chosen germlings at each temperature.

Day	Temperature	Average Size [μm] ± SD	%Alive	%Deformed	%Dead
2	15	93.4 ± 8.6	92	8	8
	18	99.1 ± 15.4	58	0	42
	24	106.6 ± 22. 8	42	20	58
	28	90.5 ± 10.0	19	45	81
5	15	130.0 ± 33.8	100	0	0
	18	120.2 ± 35.8	100	0	0
	24	107.7 ± 20.2	52	38	48
	28	96.2 ± 11.0	21	10	79
7	15	196.7 ± 24.9	100	0	0
	18	182.6 ± 19.4	96	0	4
	24	102.4 ± 24.2	40	13	60
	28	98.2 ± 45.4	0	0	100
12	15	252.5 ± 36.7	100	0	0
	18	212.9 ± 35.3	100	0	0
	24	198.6 ± 58.8	15	18	75
	28	0	0	0	100
14	15	367.9 ± 62.5	100	0	0
	18	235.6 ± 42.7	100	0	0
	24	0	0	0	100
	28	0	0	0	100
16	15	367.9 ± 62.5	100	0	0
	18	235.6 ± 42.7	86	9	14
	24	0	0	0	100
	28	0	0	0	100

On the fifth day, germlings growing at temperatures below 20 °C were developing faster and healthier than those at higher temperatures (Table 1, Tukey's Test, $p < 0.05$, Table 2). All germlings growing at 15 and 18 °C were alive and no deformed germlings were found (Figure 3). The percentage of alive germlings was lower at higher temperatures (Figure 3, Table 2); a mere 15% of the counted germlings were alive at 28 °C, with others either dead or severely deformed (Figure 5).

Also, on the seventh day, all germlings observed at 15 and 18 °C were still alive and healthy. In contrast, all germlings growing at 28 °C were already dead, while some of germlings were still alive at 24 °C, with half of them severely deformed (Table 2, Figure 5).

On the 12th day, the average size of germlings reached over 200 μm at 15 °C; those growing at 18 °C were slightly smaller, while those growing at 24 °C were, on average, half of that size (Tukey's Test, $p < 0.05$, Table 1).

On the 14th day, all seedlings growing at temperatures below 20 °C were still alive and healthy, while the seedlings growing at temperatures above 20 °C (at 28 °C and 24 °C) were dead.

On the 16th day, all the germlings at 15 °C were alive. At 18 °C, the vast majority of the germlings were alive, and nearly one-tenth were deformed. Overall, the rate of deformities

was higher in germlings grown at 24 and 28 °C, and the same was true for the mortality rate (Figures 4 and 5).
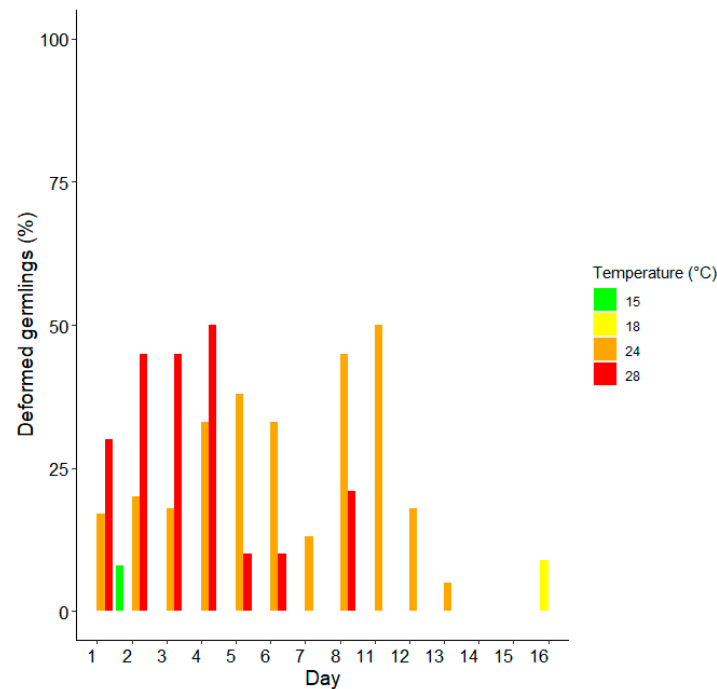
Figure 5. Percentage of deformed *G. barbata* germlings at 15, 18, 24, and 28 °C. The number of deformed germlings was counted daily in a randomly photographed area of 0.2 cm^2.

The percentage of deformed germlings was highest at 24 and 28 °C, 1–2 days before the critical point at which all germlings died (Figures 3 and 5). Germling mortality was highest at 28 °C, followed by germlings grown at 24 °C. Deformations and deaths also occurred occasionally at 15 and 18 °C (Figures 3 and 5).

4. Discussion

The algal populations most negatively affected by water warming and most vulnerable to future marine heatwaves are likely to be populations of temperate canopy-forming kelps and fucoids [14,79–81]. Conversely, as temperatures rise, turf-forming seaweeds, especially invasive species, may increase in abundance and act as competitors, which can lead to replacement of long-lived, complex habitat-forming seaweeds with smaller ephemeral species [81]. In general, high mortality of shallow water fucalean species (such as *Ericaria crinita* (Duby) Molinari & Guiry) has been observed in the northwestern Mediterranean Sea due to rising summer temperatures [14,54]. Several studies have shown that temperatures above 28 °C are detrimental to Fucales [37,82,83]. Regional extinctions of kelps and fucoids following marine heatwave events are likely to have long-term consequences for the ecological structure and functioning, reducing habitat structure and effective services that seaweed-dominated reefs provide [81]. Marine heatwaves are linked to many other potential stressors such as increased herbivory [84–86], altered current patterns [87,88], changes in water clarity, solar radiation [89], and desiccation stress [90] in the intertidal zone, which makes it difficult to study temperature effects in situ.

Gongolaria barbata is a geographically widespread species that belongs to the warm-temperate Mediterranean-Atlantic group [59,63] that requires temperatures of at least 10 °C for reproduction and development of germlings. It is a monoecious species, with oogonia and antheridia that are fertile in the conceptacles from the end of winter to the end of spring [91]. After the fertilization, heavy and large zygotes exhibit highly restricted dispersal; therefore, they fall on the substrate a few centimeters to meters away from the parental thallus. A day or two after fertilization, a typical Sargassous germling with four

primary rhizoids attaches to the rocky surface where it starts growing [42]. Rhizoids are later transformed into discoid holdfast. An earlier study from the Gulf of Trieste reported that the vegetative development of *G. barbata* is maximal at 14 °C [77], which is aligned with the findings on *G. barbata* in the Aegan Sea [59]. Optimal temperatures for adult germlings of *G. barbata* are known to vary between 15 and 25 °C [59,73], while its upper temperature limit was reported to be at 30 °C [37].

The reproductive season of *G. barbata* is becoming increasingly unpredictable, as it can be altered by the general increase in water temperatures and/or marine heatwaves, as recently observed in the northern Adriatic [44,92]. Targeted laboratory experiments, such as those conducted in our study, are useful to provide new evidence of the negative effects of increased temperatures without possible interactions with other factors. The temperatures tested in our study (15, 18, 24, and 28 °C) were chosen according to the possible temperatures in the months of *G. barbata* reproduction in the northern Adriatic (March–May [29,66,93]), considering the 10-year data series of the oceanographic buoy Vida (in Piran, Slovenia data from the oceanographic buoy Vida; https://www.nib.si/mbp/en/; accessed on 8 December 2023) and the predicted temperatures in the Mediterranean in the context of climate change.

While global air and sea surface temperatures have already risen by 0.4–0.8 °C in the past century and are predicted to rise by a further 2–3 °C in the coming century [76–78], the temperature of the Mediterranean Sea is predicted to rise even more than the average, i.e., between 1.3 and 6.3 °C from 1900 to 2100 [94]. In recent decades, higher seawater temperatures have been observed in the northern Adriatic Sea (see Figures S1 and S2). In the Gulf of Trieste, the shallowest part of the northern Adriatic, summer temperatures have already increased by 2.6 °C from 1991 to 2003 [95]. The average winter temperature (December–February) in the last decade (2013–2023) recorded by the oceanographic buoy Vida was 11.5 °C. This stands in stark contrast to the period before 1970 when average winter temperatures in the Gulf of Trieste rarely exceeded 10 °C. Notably, in the last decade, the average annual winter temperatures have consistently surpassed the 10 °C mark (see Figure S1; [96]). Additionally, the Gulf of Trieste experienced several marine heatwaves in the summer and autumn of 2023. In October 2023, the lowest temperature in this area was 25 °C, exceeding the normal temperatures for this time of year, which range between 16 and 22 °C (data from the oceanographic buoy Vida; https://www.nib.si/mbp/en/; accessed on 8 December 2023).

While increased temperatures can initially benefit seaweeds [97] by accelerating their metabolic processes [98–100], affecting nutrient uptake and biochemical pathways and increasing photosynthetic rates [99,101], their beneficial effects are present only until temperature optimum is reached and then rapidly decline at temperatures above optimum [99]. Prolonged exposure to temperatures above optimum can lead to decreased photosynthetic efficiency and photoinhibition [98], posing a challenge for sustainable primary production. Our results are consistent with this statement, as, at the beginning of germling growth (second day), germlings growing at 24 °C reached the largest length and were the most developed, followed by germlings growing at 18 °C (Figure 4). But, from the third day onward, the germlings cultured at 15 °C grew faster than those at 18, 24, or 28 °C. The reason for this could be related to metabolic stress, which disrupts essential physiological functions and results in reduced growth, deformations, and mortality [102].

A recent study on the cultivation of *G. barbata* even reported that, for thalli collected in the Gulf of Trieste, the development of fertile receptacles followed by the zygote release occurred exclusively at 14 °C [91]. Our experiments have shown that a temperature of 18 °C is still suitable for normal germlings' development, but germlings at 15 °C developed faster and reached larger lengths than others (see Figures 3 and 4). Prolonged exposure to temperatures above 18 °C during the reproductive period may also lead to widespread mortality of zygotes/embryos and reduce the reproductive success of the species. Results of our study confirm that temperatures above 24 °C inhibit the development of *G. barbata* germlings. All germlings growing at temperatures of 24 °C and above were dead after two

weeks. This finding aligns with similar observations on other *Cystoseira s.l.* species. For example, *Ericaria zosteroides* (C. Agardh) Molinari & Guiry, identified as a deep-sea species, showed a tolerance threshold of 24 °C [41], while *Ericaria selaginoides* (Linnaeus) Molinari & Guiry (previously *Cystoseira tamariscifolia*) [52] and *E. crinite* [54], species from shallower waters, exhibited a threshold of 28 °C. The settlement efficiency of *E. zosteroides* zygotes decreased at 20 °C and mortality significantly increased at 24 °C [41], while settlement efficiency of *Ericaria giacconei* zygotes increased from 12 to 18 °C, started to decrease at 24 °C, and dropped significantly at 28 °C, where most of the eggs and zygotes had undergone cell lysis [56]. The embryos of *E. giacconei* fully developed only at 12 and 15 °C, while mortality increased sharply at 28 °C [56]. Survival rate and growth of *Ericaria amentacea* (C. Agardh) Molinari & Guiry (previously *Cystoseira amentacea*) embryos was significantly higher when growing at 20 °C than at 24 °C [42]. However, the mortality was quite high, averaging 50% under all conditions (20 and 24 °C, low and high light), which can be attributed to the high stochastic gamete and zygote mortality observed in the natural environment of *Cystoseira*, but also to the fact that temperatures above 20 °C are at the upper temperature limit for germlings [42].

During our experiment, there was also a significantly higher degree of deformations observed among the germlings growing at 24 and 28 °C compared to those at 15 and 18 °C (see Figure 5). On the fourth day (Figure 6), the germlings developed normally at 15 and 18 °C, whereas many at higher temperatures were either deceased or deformed. The first sign that the seedling is not developing normally is usually bleaching, an indication that the alga is losing its photosynthetic pigments from the tissue [53,103]. If the bleached tissue cannot recover, this represents a disruptive stress that can lead to death [49]. In our experiment, loss of pigments was observed in germlings growing at 24 and 28 °C (Figures 6 and 7), as also seen in *E. giacconei* [56]. Consistently with other studies, our results show that higher temperatures can significantly impact the settlement and survival of recruits. Reduced recruitment of new individuals due to temperature anomalies or other pressures is therefore a critical bottleneck for the population dynamics of *G. barbata* and other *Cystoseira s.l.* species. Prolonged recruitment failures over several years can lead to reduced population densities that ultimately threaten long-term survival.
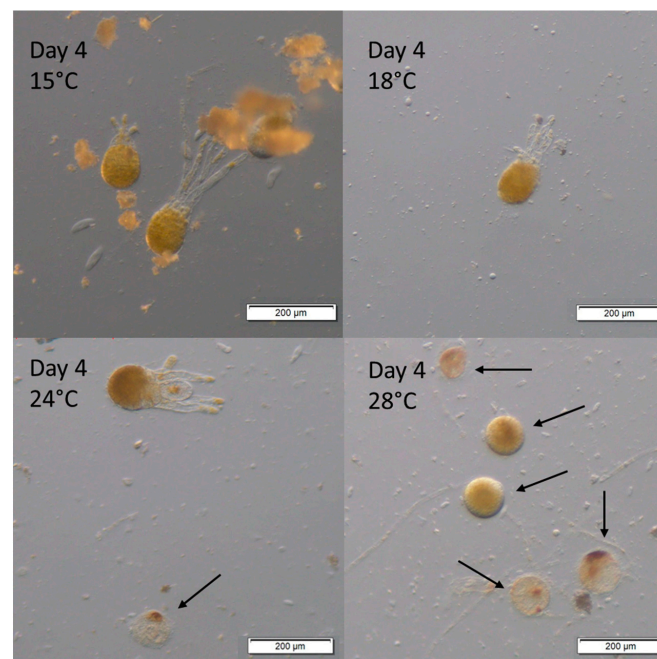
Figure 6. Germlings grown on Petri dishes at 4 different temperatures (15, 18, 24, and 28 °C) on day 4 of the experiment. Arrows point at dead germlings.
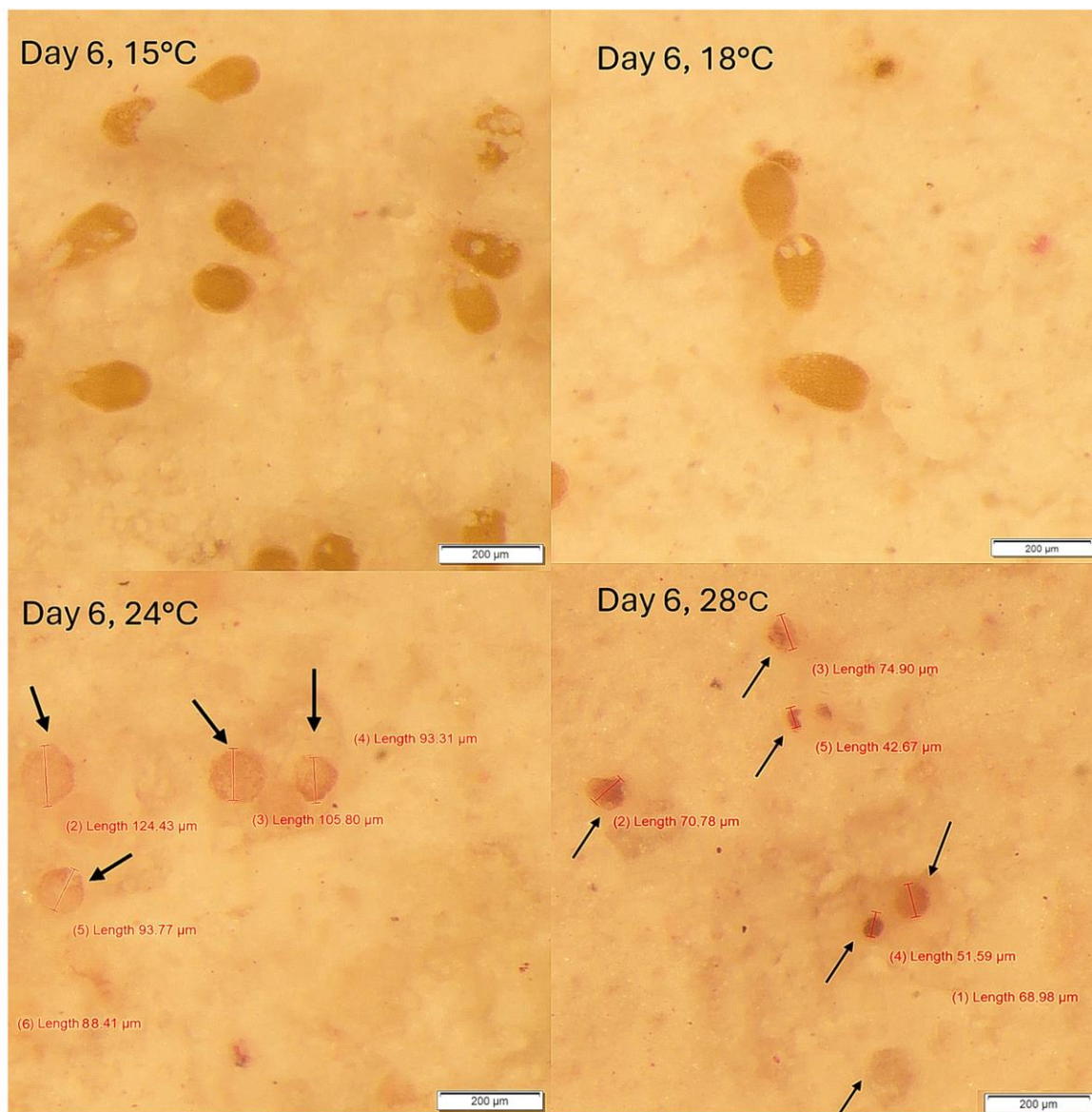
Figure 7. Germlings grown on clay tiles at different temperatures (15, 18, 24, and 28 °C) on day 6 of the experiment. Arrows point at dead germlings.

While each species has its specific temperature niche, temperature tolerance is also influenced by the species' thermal history, namely, the temperatures to which the population has been acclimated [51]. For instance, certain *G. barbata* populations flourish in the extremely shallow lagoons of the Adriatic Sea, where summer temperatures can easily exceed 28 °C and may even heat up to 34 °C for several days [14]. *Gongolaria barbata* growing in the lagoon exhibits a broader temperature tolerance compared to other populations, capable of withstanding temperatures even below 0 °C [14]. However, temperatures below 10 °C and above 28 °C have been shown to be detrimental, especially over longer periods [14,37,82,83]. Freezing temperatures may lead to the loss of lateral branches and tissue damage that is indicated by darkening of the cauloid [37], while high temperatures may result in cauloids overgrown by epiphytes and also loss of lateral branches [14]. When conditions return to the optimal temperature range of 10–25 °C, the damage on *G. barbata* thalli recovers. Even under normal thermal conditions, *G. barbata* loses phylloids and branches every autumn and epiphytes grow on its cauloid [93]. New growth begins in early spring and the frond reaches its largest size in spring or summer [93], which shows that the species is phenotypically acclimated to temperature variations. Seaweeds exhibit three

types of temperature responses: (1) genetic adaptation, which means that algae undergo long-term evolutionary changes to adapt to local conditions over thousands to millions of years; (2) phenotypic acclimation, which means that the phenotype adjusts in response to environmental temperature variations over hours to days; and (3) physiological regulation, which involves immediate responses of algae to temperature changes that occur within seconds to minutes [101]. The ability of adult plants to regenerate after prolonged exposure to very low or high temperatures suggests genetic adaptation to the conditions of the northern Adriatic Sea [14].

Our findings indicate that early life stages of *G. barbata* are particularly vulnerable and cannot survive at temperatures exceeding 24 °C. This vulnerability may be due to such high temperatures being abnormal during the period of zygote settlement and germling development of *G. barbata* in the Mediterranean (see Figures S1 and S2, based on [96]).

Our experiment highlights that *G. barbata* requires temperatures around 15 °C for successful reproduction and the healthy development of germlings. However, temperatures surpassing 24 °C during germling development drastically decrease survival rates, with the majority perishing within the first 1–2 weeks. At temperatures of 24 °C and above, germlings experience bleaching, deformation, and, ultimately, death. Further research is needed to understand the complexity of these responses. This knowledge is crucial for predicting and managing the impacts of climate change on marine ecosystems, where canopy-forming brown algae play an indispensable role in maintaining ecological balance and resilience. In the context of continued warming in the northern Adriatic Sea, *G. barbata* may face long-term survival challenges. While adult algal populations are more adaptable and can withstand shorter periods of higher temperatures [14,37], we demonstrated that the early life stages of this species are very sensitive to thermal anomalies.

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