Is the South-Mediterranean Canopy-Forming *Ericaria giacconei* (= *Cystoseira hyblaea*) a Loser From Ocean Warming?

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Canopy-forming brown algae support highly productive ecosystems whose decline has been attributed to the interplay of several anthropogenic disturbances. Climate change could have disruptive effects on the biology of these species, but the role of temperature in the development of early life stages is poorly understood. The aim of this study was to assess the response of *Ericaria giacconei*, a winter-reproducing Southern–Mediterranean endemic species, to thermal stress by testing five temperatures (12, 15, 18, 24, and 28°C) on adults and early stages. Chlorophyll *a* fluorescence of adult plants was measured at 0, 24, 72, and 120 h on nine fronds in each of the three aquaria per treatment. To assess egg release, zygote settlement, and embryo growth rate, approximately 1,200 receptacles were cultured on six Petri dishes per temperature treatment, and 10 random subsections of 2×2 mm were examined in three Petri dishes at 0, 20, 44, and 92 h after fertilization. Adult plants showed a plastic physiological response, and thermal stress had no significant effect on PSII efficiency. Embryos fully developed only at 12 and 15°C. Mortality increased at 18 and 24°C, and no zygotes survived at 28°C. In a scenario of further increasing temperatures, the effects of warming could affect the recruitment of *E. giacconei* and increase its vulnerability to further stresses. These effects on the survival of early stages, which are the bottleneck for the long-term survival of the species, should be taken into account in conservation and restoration measures to maintain canopy-forming macroalgal populations and associated biodiversity and ecosystem services.

Keywords: thermal stress, early life stages, photosynthetic efficiency, marine forest, climate change, conservation

INTRODUCTION

Canopy-forming algae of the order Fucales and Laminariales (Phaeophyceae) are among the most ecologically and socio-economically valuable marine species in temperate waters (Steneck et al., 2002; Smale et al., 2013; Bennett et al., 2015). They provide a structural and trophic framework that supports rich biodiversity by providing food, shelter, and habitat for other associated species

(Bustamante et al., 2017; Teagle et al., 2017), and are responsible for nutrient cycling and CO_2 storage (Krause-Jensen and Duarte, 2016; Filbee-Dexter and Wernberg, 2020).

Macroalgal forests are undergoing major regressions worldwide due to a combination of multiple natural and anthropogenic sources of disturbance (Steneck et al., 2002; Strain et al., 2014; Mineur et al., 2015; Krumhansl et al., 2016). In recent years, an increasing number of studies have reported changes in the distribution and abundance of these macroalgal populations as a result of ocean warming and thermal anomalies (especially marine heat waves, MHWs) (e.g., Smale, 2020 and the references therein; Bevilacqua et al., 2019; Savonitto et al., 2021; Verdura et al., 2021). Populations at the edge of their range appear to be particularly affected by this trend (e.g., Viejo et al., 2011; Nicastro et al., 2013; Araújo et al., 2014; Álvarez-Losada et al., 2020; Gurgel et al., 2020). Thermal anomalies may affect the phenology and physiology of these species, impairing their performance, increasing their vulnerability to other stressors, and eventually leading to population declines and local extinction events (Wernberg et al., 2010, 2016; Gouvêa et al., 2017; de Bettignies et al., 2018). These events could also lead to changes in associated species and their interactions (Vergés et al., 2016; Wernberg et al., 2016; Provost et al., 2017), which may ultimately result in detrimental cascading effects on ecosystem functions and the resulting provision of goods and services (Smale et al., 2013; Vergés et al., 2014; Straub et al., 2019). To date, most works addressing the effects of heat stress on the physiology and biology of brown algae have been manipulative and laboratory-based, focusing on kelps, with the 'sporophyte phase' being the most studied (e.g., Wilson et al., 2015; Burdett et al., 2019; Nepper-Davidsen et al., 2019; Fernández et al., 2020; Hereward et al., 2020; Diehl et al., 2021; Umanzor et al., 2021). As for the Fucales, the genus *Fucus* is the most extensively studied, and works on adults predominate over those on early life stages (e.g., Strömgren, 1977; Pearson et al., 2009; Jueterbock et al., 2014; Nielsen et al., 2014; Graiff et al., 2015; Mota et al., 2015; Smolina et al., 2016; Rothäusler et al., 2018; Figueroa et al., 2019). Overall, the trend that emerges is a high sensitivity in the early life stages and a relative ability of adults to grow and survive over broader temperature ranges and to physiologically compensate for thermal stress.

In the Mediterranean Sea, macroalgal forests are dominated by *Cystoseira sensu lato* (*s.l.*) species (Fucales, Phaeophyceae). In recent decades, they have declined or become locally extinct due to anthropogenic pressure (e.g., Thibaut et al., 2005, 2015; Falace et al., 2010; Perkol-Finkel and Airoldi, 2010; Blanfuné et al., 2016). To date, there is little evidence of natural recovery of damaged *Cystoseira* populations (e.g., Munda, 2000; Iveša et al., 2016; Orlando-Bonaca and Rotter, 2018; Medrano et al., 2020), because once losses have occurred, recovery from nearby populations tends to be difficult due to the short dispersal of eggs/zygotes and low connectivity of populations (e.g., Soltan et al., 2001; Buonomo et al., 2017; Capdevila et al., 2018). There is evidence that thermal anomalies and warming can alter the reproductive phenology, germling growth, and viability of *Cystoseira s.l.* species (Celis-Plá et al., 2017;

Capdevila et al., 2018; Savva et al., 2018; Bevilacqua et al., 2019; Cáliz et al., 2019; Mancuso et al., 2019; Savonitto et al., 2021; Verdura et al., 2021). As the Mediterranean Sea is warming faster than the oceans and thermal anomalies occur with increasing intensity, frequency, and duration (Diffenbaugh et al., 2007; Vargas-Yáñez et al., 2008; IPCC, 2019; Pastor et al., 2020; Pisano et al., 2020), examining the response of *Cystoseira s.l.* species to temperature may provide useful insights into their potential future fate under global warming.

The present study focuses on *Ericaria giacconei* Serio et G. Furnari (= *Cystoseira hyblaea* Giaccone), a species endemic to the Sicily Channel (Central Mediterranean Sea) that lives in the intertidal and upper sublittoral at depths of 0.2–1.5 m on semi-exposed and exposed rocky shores. Maximum vegetative and reproductive development occurs in winter, from January to March, when mean seawater temperature at 1 m depth ranges from 14.7 to 16.1°C. This species was described at Punta D'Aliga (southern coast of Sicily, Italy) (Giaccone, 1985), where it is locally extinct (Cormaci et al., 2012). Its current range is fragmented and restricted to two localities: Cap Bon (Kelibia) along the Northern Tunisian coast (Bouafif et al., 2016) and Portopalo di Capo Passero (Isola delle Correnti) in Southern Italy (present study). Its disappearance from the type locality, its limited range, and the fact that it lives in shallow waters raise concerns about the possible fate of *E. giacconei* in the current warming regime. This species, like other *Cystoseira s.l.* species, is listed in some international agreements (e.g., Barcelona Convention, Directive 92/43/EEC), but these are not legally binding.

The objective of this study is to determine the thermal tolerance of both early developmental stages and adults of *E. giacconei*. Adult photosynthetic efficiency and egg release, zygote settlement, and embryo development were examined at five temperature treatments from 12 to 28°C. Evidence for the likely response of this species to projected climate change is provided, together with a thorough discussion on its conservation status. Another outcome of this work is the embryology of *E. giacconei*, which has never been described before.

MATERIALS AND METHODS

Sampling Site

Samples were collected from a semi-exposed rocky shoreline on the southern coast of Sicily (Sicily Channel, Mediterranean Sea: 36°38′49″ N; 15°04′45″ E). On the seabed, sandy substrates covered by *Posidonia oceanica* (L.) Delile alternate with shallow rocky reefs dominated by dense and well-structured stands of *E. giacconei* in the upper subtidal. This species also occurs in the intertidal, replacing the typical fringe of *Ericaria amentacea* (C. Agardh) Molinari et Guiry as previously described by Giaccone (1985) at the type locality.

The mean seawater temperature on the Ionian coast of Sicily at 1 m depth is 15.6°C in winter, with values ranging from 15.1 to 16.7°C, and 25.5°C in summer, with values ranging from 22.0 to 27.5°C (Clementi et al., 2019).

Experimental Set-up

Approximately 6000 receptacles and 135 primary branches (approximately 10 cm long) of *E. giacconei* were collected in March 2020. Samples were wrapped in aluminum foil, stored at 4°C in the dark and transported to the Phycological Laboratory, University of Trieste, within 24 h after collection. At the laboratory, the receptacles were stored at 4°C for 36 h, while the adult fronds were acclimatized at 18°C for 48 h.

Five temperature treatments were replicated in environmentally controlled rooms: 12°C, i.e., the lowest temperature the species can be exposed to in winter; 15°C, i.e., the average daily seawater temperature during the reproductive period; 18°C, i.e., the average daily temperature in early winter (December); 24 and 28°C, i.e., temperatures the species is normally exposed to in summer. Light intensity was set to 125 µmol photons m^{-2} s^{-1} supplied by LED lamps (AM366 Sicce USA Inc., Knoxville, TN, United States) and measured with a LI-COR LI-190/R Photometer (LICOR-Biosciences, Lincoln, NE, United States); photoperiod was set to 12:12 h light:dark.

For each heat treatment, three aquaria were filled with 10 l of filtered seawater (0.22 µm filter membrane), and each aquarium contained nine adult primary branches. Pumps (Sicce Syncra Nano, Sicce S.r.l., Pozzoleone, IT) were placed at the bottom of each aquarium, to ensure that the medium was properly oxygenated. The experiment on adult fronds lasted 120 h (**Figure 1**).

Early life stages, up to the end of the embryonic stage (i.e., the fall of apical hair; Nienburg, 1931; Galun and Torrey, 1969; Savonitto et al., 2019), were studied for 92 h (**Figure 1**). Six replicate Petri dishes per treatment were filled with 10 ml of filtered seawater (0.22 µm filter membrane) and incubated at the five temperatures listed above. Each Petri dish was seeded with approximately 200 receptacles.

To counteract evaporation, additional aquaria filled with filtered seawater were kept at the same temperatures to refill the experimental aquaria and Petri dishes.

Response Variables
Adult Plants

Chlorophyll *a* fluorescence (Chl$_a$F) of each adult specimen was measured at the end of acclimation (t_0) and after 24 h (t_1), 72 h (t_2) and 120 h (t_3) using a Photosynthetic Efficiency Analyzer Fluorimeter Handy-PEA (Hansatech, King's Lynn, United Kingdom). Measurements were taken after a 30 min dark adaptation using the standard Handy-PEA clip. A saturating red-light pulse of 3500 µmol photons m^{-2} s^{-1} for 0.8 s was emitted to obtain the 0JIP fluorescence transient, i.e., the time resolved Kautsky induction, and hence F_m (transient maximum Chl$_a$F level). F_0 (minimum Chl$_a$F level), needed to calculate F_v (variable Chl$_a$F level, i.e., F_m−F_0) and thus F_v/F_m (maximum quantum yield of PSII photochemistry), was measured 50 µs after the onset of illumination. The performance index (PI$_{abs}$) was also calculated from the analysis of the Chl$_a$F transient from F_0 to F_m, the so-called JIP test (Strasser et al., 2000; Bussotti et al., 2010). PI$_{abs}$ is calculated from three independent expressions related to (a) the density of reaction centers, (b) the maximum quantum yield of primary photochemistry, and (c) the efficiency of the electron transport chain between PSII and PSI (Strasser et al., 2000). PI$_{abs}$ is commonly used to test the effects of environmental factors such as temperature, salinity and high intensities of visible and UV-light on the viability and efficiency of the photosynthetic apparatus (Misra et al., 2001).

Early Developmental Stages

Receptacles were removed from Petri dishes after fertilization (AF; 30 h after seeding). To avoid experimental bias and to ensure that the receptacles of all thermal treatments had the same reproductive potential (RP), it was estimated as follows:

$$RP\ (conceptacles\ mg^{-1}) = \frac{mean\ no.\ of\ conceptacles\ receptacle^{-1}}{mean\ receptacle\ dry\ weight}$$
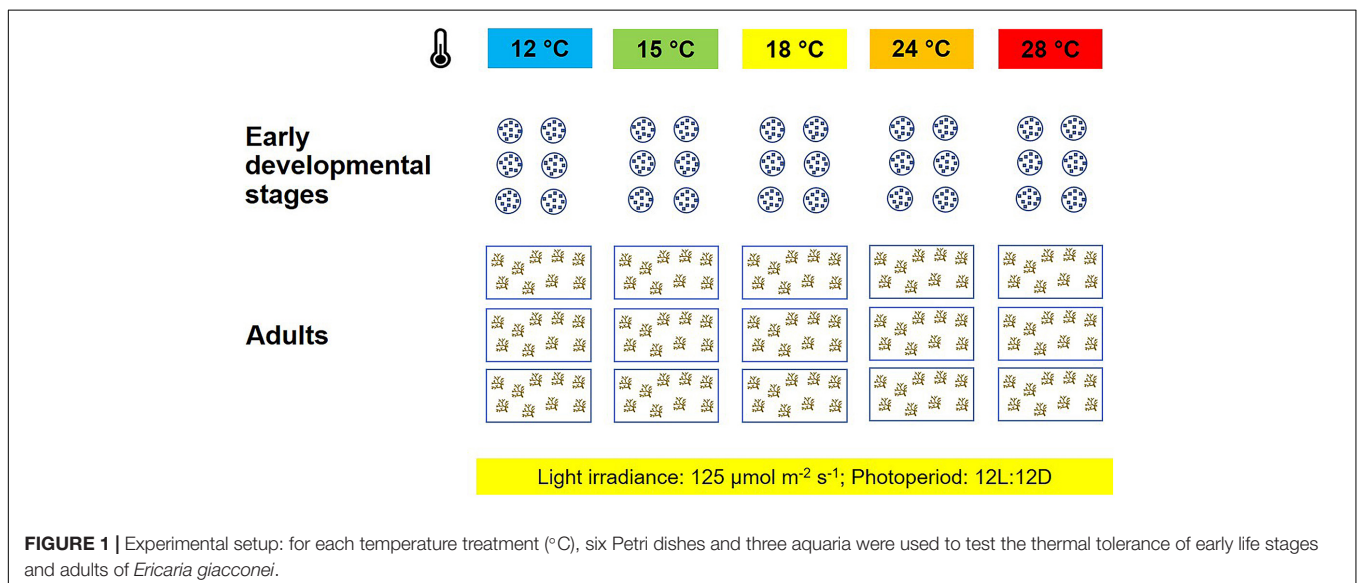
FIGURE 1 | Experimental setup: for each temperature treatment (°C), six Petri dishes and three aquaria were used to test the thermal tolerance of early life stages and adults of *Ericaria giacconei*.

The number of conceptacles per receptacle was counted under a stereomicroscope (Leica MZ 6, Leica Microsystems, Wetzlar, Germany). Receptacles were then dried at 70°C for 48 h.

To quantify egg release and zygote settlement at different temperatures, 10 subareas of 0.2×0.2 cm^2 in three Petri dishes were randomly selected per treatment and photographed under a stereomicroscope with a Nikon Coolpix 4500 camera (Nikon Corporation, Tokyo, Japan) at each sampling time. To reduce stress on the algae, photographs were taken within a few minutes. Three Petri dishes were randomly selected to assess egg release and the remaining three were used to assess zygote settlement. Photographic sampling was carried out at the time of fertilization (i.e., 30 h after seeding) and 20 h AF. The digital images were analyzed to count the number of specimens in each subarea. The counts were then extrapolated to the entire culture area (i.e., 23.76 cm^2). Release (RE) and settlement (SE) efficiencies were calculated as follows:

$$RE \ (eggs \ mg^{-1}) = \frac{no. \ of \ eggs \ cm^{-2}}{mean \ receptacle \ dry \ weight \ cm^{-2}}$$

$$SE \ (zygotes \ mg^{-1}) = \frac{no. \ of \ zygotes \ cm^{-2}}{mean \ receptacle \ dry \ weight \ cm^{-2}}$$

Embryo growth was assessed by taking digital images of 10 randomly selected subareas (0.2×0.2 cm^2) under an inverted microscope (Leica DM IL LED, Leica Microsystems, Wetzlar, Germany) using a Canon Powershot G9 camera (Canon Inc., Tokyo, Japan) at 20, 44, and 92 h AF. In each subarea, the percentage of unfertilized eggs (= stage 0), zygotes (= stage 1), two-celled embryos (= stage 2), multicellular embryos (= stage several), multicellular embryos with rhizoids (= stage rhizoids), dead embryos (= stage dead), deformed dead embryos (= stage deformed dead), and deformed living embryos (= stage deformed living) were counted (**Supplementary Figure 1**).

To describe the embryo development, additional dedicated slides were seeded with receptacles at 15°C (i.e., the average seawater temperature during the reproductive period) and observed over time under an inverted microscope.

Statistical Analysis

Repeated-measures ANOVA was used to test the effects of temperature (five levels: 12, 15, 18, 24, and 28°C) and time (three levels: t_1, t_2, and t_3) on F_0, F_m, F_v/F_m, and PI_{abs} ($n = 27$). The assumption of normality of response variables was tested with the Shapiro–Wilk test. In all cases, the assumption of normal distribution was fulfilled. Tuckey's HSD *post hoc* test was used to examine pairwise significant differences between treatment combinations.

One-way ANOVA was performed to test for differences between temperature treatments on RP, RE, and SE. The assumption of normality of response variables was tested with the Shapiro–Wilk test. In all cases, the assumption of normal distribution was fulfilled. Significant terms were examined by performing a *post hoc* pairwise *t*-test to compare the different treatments. Cochran's *C*-test (Underwood, 1997) was used to test the assumption of homogeneity of variances prior to analysis. For RP, data were square root-transformed to remove heterogeneous variances. To explain the observed bell-shaped patterns, a quadratic regression model was fitted to RE and SE against temperature.

Distance-based permutational multivariable analysis of variance (PERMANOVA, Anderson, 2001) was used to test for differences in temporal patterns of embryonic development between treatments. Data from treatments at 28°C were not included in the analysis since the number of settled zygotes at 20 h AF was extremely low (mean 0.7 zygotes/subarea ± 0.1 SE), and zygote mortality at later sampling times was 100%. The analysis was based on Bray–Curtis dissimilarities (Bray and Curtis, 1957) on untransformed data, and each term in the analysis was tested by 5,000 random permutations. The design for the analysis included two crossed factors: Treatment (Tr, four levels, and fixed) and Time (Ti, three levels, and fixed), with $n = 3$. Non-metric multidimensional scaling ordination (nMDS) of the Tr × Ti centroids was used to represent the multivariate patterns.
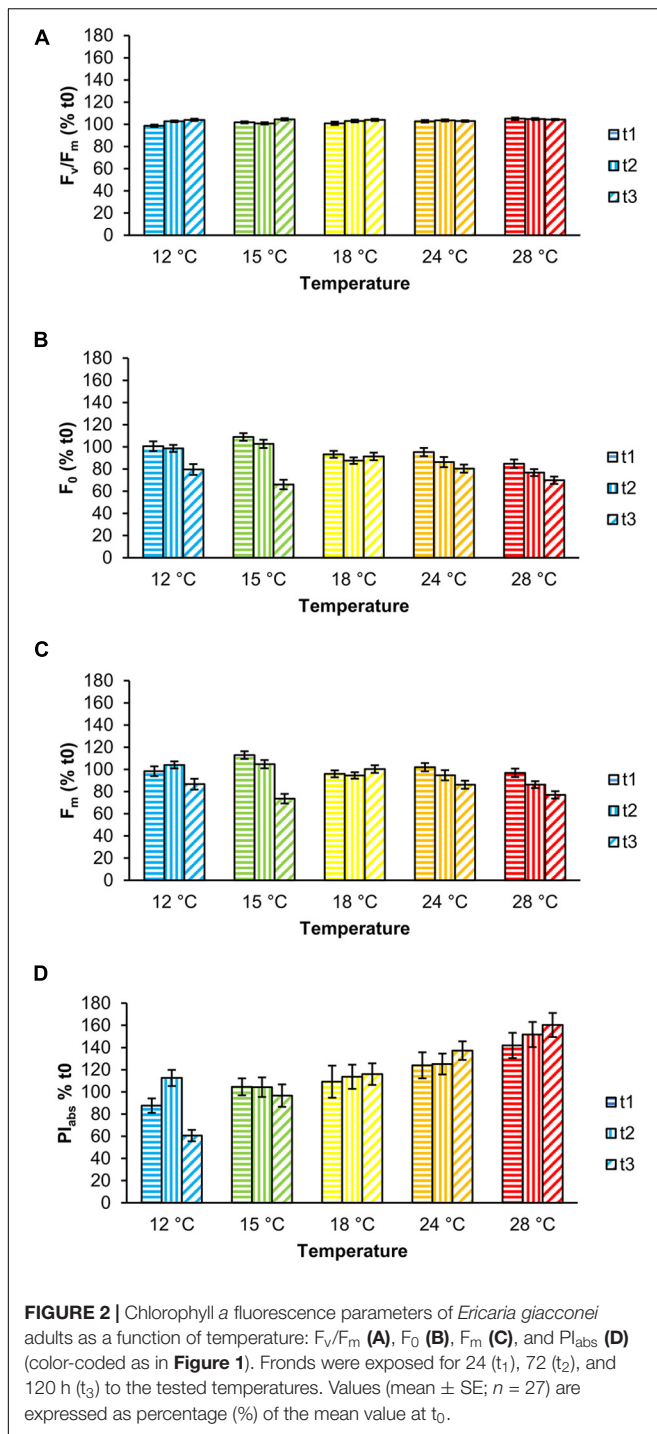
RESULTS

After acclimation, the adult primary branches of *E. giacconei* had F_v/F_m values ranging from 0.606 to 0.768, attesting the viability and good physiological status of the photosynthetic apparatus of the samples.

F_v/F_m values were stable throughout the experiment, although slight but significant changes were observed as a function of temperature and time (**Figure 2A**, **Table 1**, and **Supplementary Table 1**). Specifically, at 12°C F_v/F_m statistically increased over time by 5.3% (**Supplementary Table 1**). The interaction between temperature and time had a significant effect on F_0 and F_m (**Table 1** and **Supplementary Table 1**); from t_1 to t_3, both parameters were stable at 18 and 24°C, whereas F_0 significantly decreased in samples at 12 and 15°C and F_m at 15 and 28°C (**Figures 2B,C** and **Supplementary Table 1**). PI_{abs} was only affected by temperature (**Table 1** and **Supplementary Table 1**): it was highest at 28°C and gradually decreased from 24 to 15°C, with the lowest values at 12°C at t_3 (**Table 1**, **Figure 2D**, and **Supplementary Table 1**).

Ericaria giacconei has branched pigmented antheridia and ovoid oospheres retained in the conceptacle (**Supplementary Figure 2**). The following embryological traits were observed: the first and second division of the zygote are parallel to each other and the third division is perpendicular to the previous ones. The embryo development takes place directly on the substratum: four primary rhizoids are formed fixing it (**Supplementary Figure 3**).

The RP did not differ significantly among thermal treatments, making them comparable at the beginning of the experiment (**Table 2** and **Figure 3A**).

No significant effects of temperature were detected on RE (**Figure 3B** and **Table 2**). In contrast, temperature significantly affected SE (**Figure 3C** and **Table 2**). Specifically, SE at 28°C was lower than all other treatments. RE (**Figure 3B**) and SE (**Figure 3C**) showed a bell-shaped response to temperature, and

FIGURE 2 | Chlorophyll *a* fluorescence parameters of *Ericaria giacconei* adults as a function of temperature: F_v/F_m **(A)**, F_0 **(B)**, F_m **(C)**, and PI_{abs} **(D)** (color-coded as in **Figure 1**). Fronds were exposed for 24 (t_1), 72 (t_2), and 120 h (t_3) to the tested temperatures. Values (mean ± SE; $n = 27$) are expressed as percentage (%) of the mean value at t_0.

the quadratic model fitted to the data explained 51 and 61% of the variability for RE and SE, respectively (**Table 3**).

PERMANOVA on embryo status revealed a significant Tr × Ti interaction (**Table 4**), indicating that temporal patterns of embryonic development differed significantly between temperature treatments. These differences were evident in the nMDS ordination of Tr × Ti centroids (**Figure 4**). The centroids of 12 and 15°C clustered alongside those of 18 and 24°C, the

latter also showing marked separation between 20 and 44–92 h AF. These differences were mainly due to the fact that at 20 h AF a higher percentage of eggs, zygotes, or two-celled embryos were found in the treatments at 18 and 24°C than in those at 12 and 15°C (**Figure 4A**). In contrast, multicellular embryos or rhizoids were found in the treatments at 12 and 15°C in each time interval (**Figure 4B**), suggesting that the development rate was faster at lower temperatures. In addition, embryo mortality was consistently higher at 18 and 24°C than at 12 and 15°C, with the highest percentage of dead embryos recorded at 24°C (**Figure 4C**).

DISCUSSION

Climate change, coupled with multiple anthropogenic and natural stressors occurring in coastal ecosystems, poses a major threat to the long-term survival of marine forests. From this perspective, studying species vulnerability to temperature stress can provide relevant insights that can be used to make more robust and integrated predictions for marine forest conservation and management.

In our experiment, adults of *E. giacconei* were not negatively affected by temperatures, indicating an expected ability to acclimatize to a wide range of temperatures typical of the Mediterranean Sea and especially the intertidal zone. All temperatures to which thalli were exposed had a statistically significant, but not physiologically relevant effect on F_v/F_m (max increase +5% at 12°C, from 0.659 to 0.694; max decrease −1% at 28°C, from 0.731 to 0.725), which remained generally steady and within the range of values indicative of a healthy PSII, i.e., >0.6 (e.g., Celis-Plá et al., 2014; Smolina et al., 2016; Falace et al., 2018b; Savva et al., 2018; Cáliz et al., 2019; Verdura et al., 2021). In contrast, temperatures above 18°C caused an almost equal decrease in F_0 and F_m (**Figures 2B,C**). Photosystem II is considered the most heat-sensitive component of the photosynthetic apparatus, especially at the level of the oxygen-evolving complex (Oukarroum et al., 2016). Impairment of this component leads to a progressive decrease in electrons entering the electron transport chain from PSII until its complete inactivation (Allakhverdiev et al., 2008). Several parameters of the fast Chl_aF transients, such as the maximal and basal fluorescence (F_m and F_0) and the derived maximum quantum yield (F_v/F_m), are the most appropriate tools for detecting early effects of heat stress, as they have been shown to correlate with heat sensitivity/tolerance (Allakhverdiev et al., 2008). In particular, the increase in F_0 is closely related to the temperature at which PSII is inactivated (Yamane et al., 2000). For the aforementioned reasons, this could be interpreted as a transient adaptation of the photosynthetic apparatus to the temperature change rather than heat stress.

Several works reporting the effects of temperature on photosynthetic efficiency of brown algae have shown that adults are generally tolerant of temperature fluctuations. For instance, Chl_aF of *E. selaginoides* adults was not affected after exposure to temperatures up to 28°C for 15 days (Cáliz et al., 2019). Savva et al. (2018) reported that F_v/F_m of *Cystoseira compressa* exposed

TABLE 1 | Summary of repeated measures ANOVAs on F_v/F_m, F_0, F_m, and PI_{abs}.

			F_v/F_m			F_0			F_m			PI_{abs}		
	Num df	Den df	SS	Error SS	F	SS	Error SS	F	SS	Error SS	F	SS	Error SS	F
Time	2	256	232	6787.1	4.3684*	25644	67433	48.6773***	20596	96958	27.1894***	4452	651958	0.8740[NS]
Treatment	4	128	349	3708.9	3.0138*	13972	43935	10.1769***	6561	49513	4.2402**	197157	362166	17.4203***
Time × Treatment	8	256	368	6787.1	1.7343[NS]	17155	67433	8.1408***	16436	96958	5.4246***	37618	651958	1.8464[NS]

[NS], not significant; *P < 0.05; **P < 0.01; and ***P < 0.001.

TABLE 2 | Summary of ANOVAs on reproductive effort, release efficiency, and settlement efficiency.

	Reproductive effort			Release efficiency			Settlement efficiency		
df	SS	MS	F	SS	MS	F	SS	MS	F
Treatment4	0.3	0.1	2.148[NS]	19893.0	4973.0	2.664[NS]	27108.0	6777.0	3.997*
Residual10	12.5	1.2		18665.0	1867.0		17041.0	1704.0	
Pairwise t-test	–			–			28°C≠12°C = 15°C = 18°C = 24°C		
Shapiro–Wilk test	W = 0.919[NS]			W = 0.907[NS]			0.944[NS]		
Cochran's C-test	C = 0.705*			C = 0.533[NS]			C = 0.528[NS]		
Transformation	Square root			None			None		

The assumption of normality was checked through the Shapiro–Wilk test. Pairwise tests were also reported. For RP, Cochran's C-test was not significant after data transformation. [NS], not significant; *P < 0.05.

from 12 to 34°C maintained values close to the optimum in the range of 19.2–30.9°C. Similarly, Mancuso et al. (2019) observed an increase in F_v/F_m in the field up to 28°C when the algae were submerged, and a marked decrease during tidal emersion only when air temperature exceeded 28°C. Accordingly, populations of *Fucus serratus* from southern areas of North Atlantic showed a decrease in PI_{abs} only when temperatures ranged from 28 to 36°C (Jueterbock et al., 2014), although *F. serratus* is a cold-affine species. In our case, adults of *E. giacconei* showed higher PI_{abs} at the upper extreme of the tested temperature range (**Figure 2D**), suggesting that they have better PSII efficiency in warm seasons. Negative effects on Chl_aF parameters were observed in *Fucus distichus* only when thalli were exposed to temperatures 10–15° above their optimum (Smolina et al., 2016), and in *E. selaginoides* when dissolved CO_2 and nutrients were also altered (Celis-Plá et al., 2017).
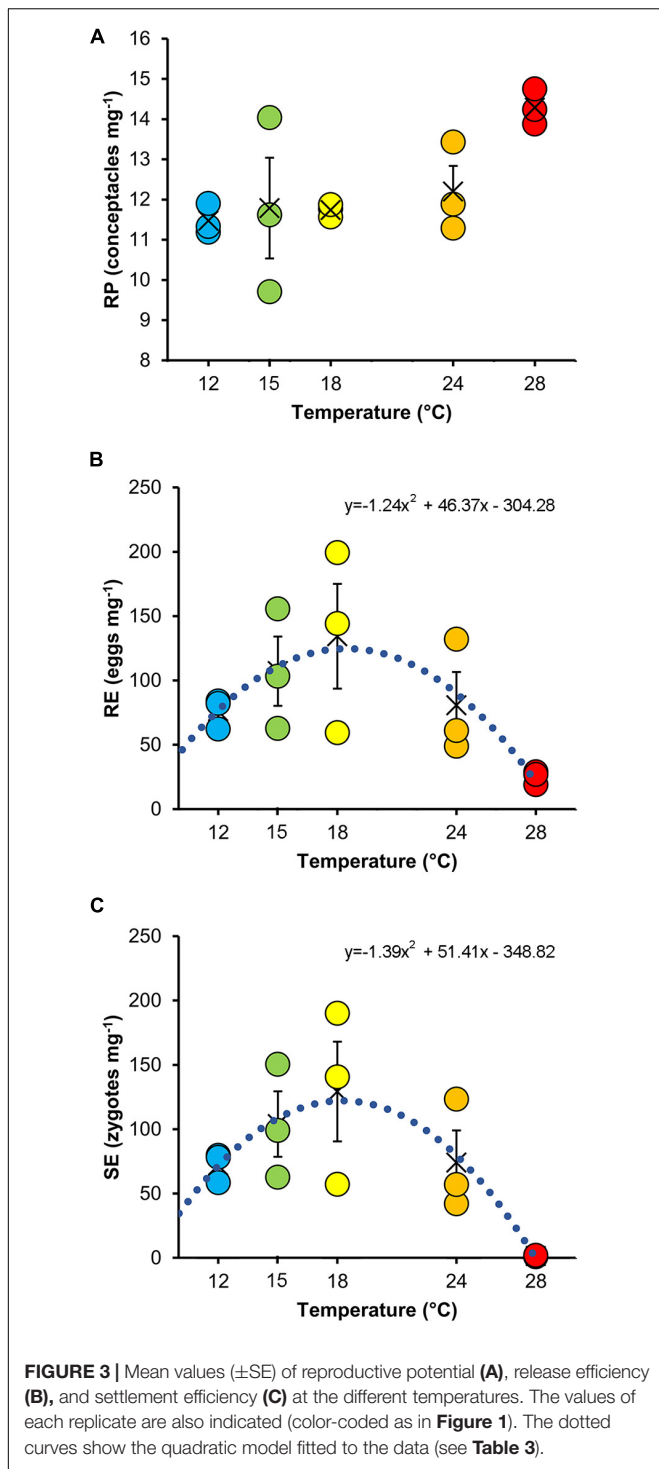
The tolerance of adult thalli of *E. giacconei* and the other intertidal *Cystoseira s.l.* species to temperatures up to 28°C might be related to an adaptation to the highly dynamic habitat they colonize. Indeed, the intertidal is characterized by large temperature fluctuations due to tidal cycles, especially during the warmer months. Notably, during summer tidal cycles, at the site where *E. giacconei* was sampled, these algae can experience temperatures ranging from 28°C (seawater temperature) at 1 m depth at high tide to 41°C (air temperature) at low tide within a few hours (Servizio Informativo Agrometereologico Siciliano, 1995; Clementi et al., 2019). In contrast, species that are not adapted to such extreme environmental changes might be more sensitive to temperature increases. For example, Verdura et al. (2021) reported that adults of the subtidal species *Ericaria crinita* showed a marked decrease in biomass, F_v/F_m, and C:N ratio during a 30-day period at 28°C. Similarly, Sato et al. (2020) observed a decrease in PSII efficiency in the subtidal

kelp *Saccharina sculpera* maintained at temperatures ≥28°C, while the optimal range for the tested population was 22–24°C.

Despite the high tolerance of *Cystoseira s.l.* adults, especially of intertidal species, to temperature fluctuations, little is known about the possible effects on early developmental stages and developmental processes. Apart from the oldest embryological studies (e.g., Guern, 1962; Colombo et al., 1982; Gil-Rodríguez et al., 1988; Motta et al., 1988; Alongi et al., 1999), the embryogenesis of many *Cystoseira s.l.* species is still poorly known (Falace et al., 2018a; Savonitto et al., 2019). Based on reproductive traits and zygote division sequence, *E. giacconei* fits into the first embryological group described by Guern (1962), which includes most *Cystoseira s.l.* species (e.g., *Ericaria mediterranea*, *Gongolaria elegans*, and *E. selaginoides*).

Regarding the effect of seawater temperature on early developmental stages, we found that the eggs' release efficiency did not vary significantly among the tested temperatures. However, greater exudate production was observed at higher temperatures (**Supplementary Figure 4**). Exudates, typically phlorotannins, are released by macroalgae under stress conditions (Sieburth and Jensen, 1969; Kroes, 1970; Abdala-Díaz et al., 2006). The settlement efficiency of the zygotes of *E. giacconei* increased from 12 to 18°C, but no statistically significant difference was found, then it started to decrease (24°C) and dropped significantly at 28°C. Remarkably, the extremely low settlement efficiency at 28°C was due to the fact that eggs and zygotes had undergone cell lysis and clustered together (**Supplementary Figure 5**).

The detrimental effect of heat was even more pronounced during germling development. Embryos were able to fully develop only at 12 and 15°C, while mortality increased sharply at 18°C and all germlings died at 28°C. The highest

FIGURE 3 | Mean values (±SE) of reproductive potential **(A)**, release efficiency **(B),** and settlement efficiency **(C)** at the different temperatures. The values of each replicate are also indicated (color-coded as in **Figure 1**). The dotted curves show the quadratic model fitted to the data (see **Table 3**).

TABLE 3 | Summary of quadratic regression fitted to data of release and settlement efficiency against temperature.

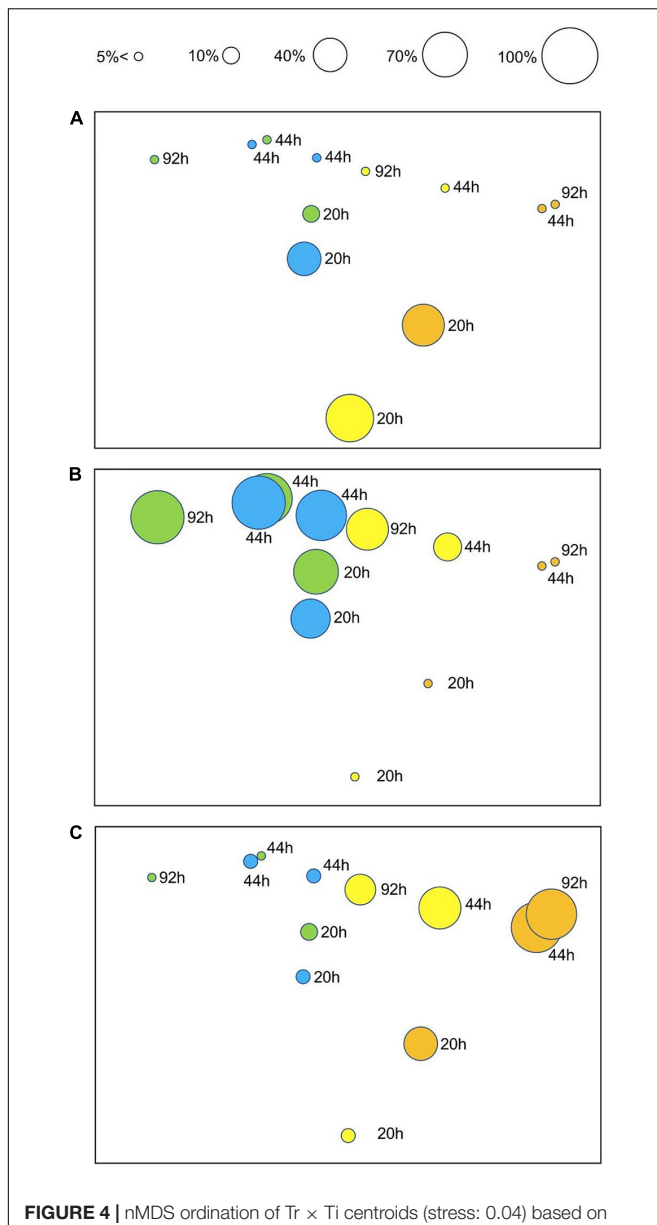|	Multiple R-squared	*F*	*P*
Release efficiency	0.51	6.272	0.014
Settlement efficiency	0.61	9.577	0.003

TABLE 4 | PERMANOVA testing for differences in the proportion of different developmental stages of embryos at varying times and temperature treatments after fertilization.

Source	df	SS	MS	Pseudo-*F*	*P* (perm)
Time	2	19,282.0	9641.0	23.973	0.000
Treatment	3	4104.0	1368.0	3.402	0.000
Time × Treatment	6	18,611.0	3102.0	7.713	0.000
Residual	347	139,550.0	402.2		

Analysis was based on Bray–Curtis dissimilarities and untransformed data, with 5,000 permutations.

To date, very few studies have investigated the potential effects of warming on the early life stages and in adults of *Cystoseira s.l.* species (e.g., Cáliz et al., 2019; Capdevila et al., 2019; Verdura et al., 2021). These studies focused specifically on the effects of high temperatures on the settlement and survival of recruits, showing that higher temperatures lead to embryo death. In particular, a tolerance threshold of 24°C was found in *Ericaria zosteroides* (as. *C. zosteroides*), a deep-sea species (Capdevila et al., 2019), and 28°C in *Ericaria selaginoides* (as *C. tamariscifolia*) (Cáliz et al., 2019) and *Ericaria crinita* (Verdura et al., 2021), two species from shallower waters. These results are only partially consistent with ours, as almost all germlings in this study failed to settle or survive at 28°C. However, in contrast to previous studies, we tested a broader temperature range and found that although *E. giacconei* is an intertidal to upper sublittoral species endemic to the southern Mediterranean, and thus hypothetically adapted to high temperatures, its thermal optimum is at much lower temperatures (12–15°C) than the other *Cystoseira s.l.* species examined.

Our findings suggest that *E. giacconei* is a stenothermic, cold-adapted macroalga that requires an extremely narrow range of low temperatures for embryonic development and survival. These results support the findings of Bouafif and Langar (2019) who, by modeling the potential spatial distribution of *Cystoseira s.l.* species in Tunisia, reported that *E. giacconei* occurs only in the colder waters of northern Tunisia. Sites where *E. giacconei* thrives could represent climatic refugia where the species still survives (e.g., Lourenço et al., 2016; Abelson et al., 2020; Verdura et al., 2021). The Sicilian Channel is characterized by a surface current called "Modified Atlantic Water" (MAW), forming two flows: one along the Sicilian shelf and the other off the Tunisian coast (Robinson et al., 1999; Béranger et al., 2004; Jouini et al., 2016). The complex bathymetry, as well as the water circulation, favor a semi-permanent upwelling regime, which is enhanced by local winds (e.g., Mistral) along the southern coast of Sicily. Therefore, the interplay of surface currents and upwelling provides lower sea surface temperatures along the coast (Raffa et al., 2017), but cannot prevent the occurrence of adverse climatic conditions.

development rate observed at 15°C (highest percentage of embryos with rhizoids already after 20 h AF) suggests that this temperature represents the thermal optimum for reproduction and development of the early life stages. Actually, it corresponds to the mean seawater temperature during the winter months when the species reproduces.

FIGURE 4 | nMDS ordination of Tr × Ti centroids (stress: 0.04) based on Bray–Curtis dissimilarities (untransformed embryo development data). The ordination plot is presented in three versions highlighting three developmental stages, with superimposed bubbles, indicating the corresponding percentage of embryos in earlier (cumulative for stages 0, 1, and 2) **(A)** and later (cumulative for stages "several" and "rhizoid") **(B)** developmental stages, and dead (cumulative for stages "dead" and "deformed dead") embryos **(C)** for each time point (20, 44, and 92 h AF) and treatment (color-coded as in **Figure 1** for 12, 15, 18, and 24°C).

Exceptionally high temperatures for several consecutive days during the reproductive season (e.g., **Supplementary Figure 6**) may actually lead to massive mortality of zygotes/embryos, thus defeating the reproductive efforts of the species. Furthermore, the negative effects of warming on recruitment could be exacerbated by other stressors that have been shown to negatively affect the early developmental stages of *Cystoseira s.l.*, such as herbicides and pollutants (de Caralt et al., 2020).

From this point of view, the recruitment of new individuals seems to be the real bottleneck for the population dynamics of *E. giacconei*, as recruitment failures, if they occur over several years, can lead to lower population densities, ultimately affecting their long-term survival.

The stenothermic nature of the early life stages and the warmer sea areas that evenly surround the few localities with favorable conditions make this species a dotted endemism (Giaccone and Di Martino, 1996). Consequently, *E. giacconei* may become extinct if climate change continues with the current pattern. In the Sicilian Channel, several studies have already reported the disappearance of infralittoral stenoecious species of *Cystoseira s.l.* due to the increase in sea surface temperature and changes in deep circulation (Alongi et al., 2004; Catra et al., 2006; Serio et al., 2006), further evidence of the tropicalization process caused by climate change affecting the Mediterranean Sea (Boero et al., 2008; Furnari and Cormaci, 2009; Marbà et al., 2015).

Together with all Mediterranean species of *Cystoseira s.l.* (except *C. compressa*), *E. giacconei* is included in the "List of Threatened or Endangered Species" of Barcelona Convention (modified Annex II of the "Protocol on Specially Protected Areas and Biological Diversity"; United Nations Environment Agency, 2019; Verlaque et al., 2019), but its conservation status has not yet been defined by the IUCN (like the fucoid *Sargassum*, see Thibaut et al., 2016). In our opinion, *E. giacconei* should be included in the IUCN Red List of Species (International Union for Conservation of Nature, 2021) and classified as Critically Endangered due to its limited distribution and high vulnerability. As a conservation strategy, the climate refugia that ensure the persistence of *E. giacconei* should receive the highest level of protection.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AF, MT, and GA conceived the ideas and designed the methodology. GM and GA collected samples in the field. AF, GS, and MS performed the experiments in aquaria. FC and SB performed the statistical analysis. AF led the writing of the manuscript. AF, GM, GS, MS, FC, SB, and MT contributed significantly to the draft of the manuscript and approved the submitted version. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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