Parthenosporophytes of the brown alga *Ectocarpus siliculosus* exhibit sex-dependent differences in thermotolerance as well as fatty acid and sterol composition

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Short title: Sexual differences in thermotolerance in *Ectocarpus siliculosus*

ABSTRACT

In the filamentous brown alga *Ectocarpus siliculosus*, male and female sex is expressed during the haploid parthenosporophyte phase of the life cycle. Here, we found that male parthenosporophytes displayed thermotolerance whereas female specimens displayed severely reduced viability at 25ºC and 28ºC. Profiling of polyunsaturated fatty acids showed that n-3 and n-6 were the predominant species in male and female parthenosporophytes, respectively, and that the n-3/n-6 fatty acid ratio was not affected by a temperature change. Both male and female parthenosporophytes contained the sterols fucosterol, cholesterol, and ergosterol, but these were present at higher levels at 10–25ºC in female specimens than in males. Thus, these fatty acids and sterols would be expected to make the membranes more rigid in the female compared to the male, which is opposite to the paradigm that increased rigidity confers thermotolerance. Our results suggest that the sex-dependent thermotolerance in *E. siliculosus* parthenosporophytes is not explained by the relationship between membrane fluidity and differences in fatty acids and sterol compositions.

Key words: *Ectocarpus siliculosus*, parthenosporophyte, sex, heat stress, thermotolerance, fatty acid, sterol

1. Introduction

Changes in temperature are a common environmental stress for poikilothermal organisms. There is growing evidence for a close relationship between membrane physical state and temperature sensing, response, and tolerance in microbes and multicellular organisms (Carratù et al., 1996; Horváth et al., 1998; Örvar et al., 2000; Sangwan et al., 2002; Königshofer et al., 2008; Leach and Cowen, 2014; for review, Vigh et al., 1998; Murakami et al., 2000; Mikami and Murata, 2003; Upchurch, 2008; Ruelland and Zachowski, 2010; de Mendoza, 2014; Török et al., 2014). The physical state of the membrane can affect the stability of the membrane, as well as transport of molecules through membranes (Lande, 1995; Tillman and Cascio, 2003; Luneva et al., 2007; Weijers, 2012). Cold and heat stress induce changes to the membrane physical state by activation of signal transduction pathways in the organism to adapt to stress conditions and induce tolerance. The major determinants of membrane physical state are lipids, in particular fatty acids and sterols. In general, the number of fatty acid double bonds is increased and decreased under cold and heat stress, respectively, which promotes higher membrane fluidity or rigidity, respectively (Mikami and Murata, 2003). Membrane fluidity is also influenced by sterol compositions (Ford and Barber, 1983; Mora et al., 1999; for review, Hartmann, 1998), whereby an increase or decrease in sterols promotes higher rigidity or fluidity, respectively. Therefore, fluctuation in fatty acid and sterol compositions under stress conditions are important indicators of the responsiveness and adaptability of poikilothermal organisms to temperature changes.

Seaweeds are poikilothermal multicellular algae that live primarily in marine

environments. Past studies have analyzed seasonal changes in fatty acid composition in various kinds of seaweeds (Gerasimenko et al., 2011, 2014; Marinho et al., 2015; Gerasimenko and Logvinov, 2016); however, the relationship between membrane fluidity and heat stress tolerance, or thermotolerance, is poorly understood. To date, there is no direct evidence that temperature changes lead to a modification of lipid composition in seaweeds. Although temperature is a major environmental factor that influences seaweed growth and survival, a number of further environmental factors, including salinity and nutrient availability, are potential stressors. Thus, to understand the contribution of membrane physical state towards seaweed thermotolerance, it is important to control other experimental conditions and investigate how temperature affects fatty acid and sterol composition using temperature-controlled incubators in a laboratory.

Ectocarpus siliculosus is marine photosynthetic seaweed that is widely employed as a model brown algal organism because it is easy to cultivate and has low space requirements when grown in laboratory incubators (Charrier et al., 2008; Charrier et al., 2012; Bogaert et al., 2013). In addition, *E. siliculosus* genomic information is available and a number of organism-specific genetic techniques have been developed (Charrier et al., 2012; Coelho et al, 2012). Gene information (Le Corguillé et al., 2009; Cock et al., 2010) and genetic mutants showing morphological and life-cycle defects are also currently available (Peter et al., 2008; La Bail et al., 2011; Coelho et al., 2011). Moreover, bioinformatics and transcriptome analyses have identified genes involved in metabolic processes including abiotic stress response and carbon storage (Dittami et al., 2009; Michel et al., 2010). Since brown algae are taxonomically classified as

Heterokonts, which are distantly related to the Archaeplastida including red and green algae and terrestrial green plants, the study of cellular, biochemical, and molecular biology processes in these organisms has led to an accumulation of knowledge about unique systems of physiological regulation (Charrier et al., 2012; Coelho et al, 2012; Bogaert et al., 2013; Cock et al., 2014). However, little is known about the differences in stress responses between male and female *E. siliculosus* parthenosporophytes.

In the present study, we explored thermotolerance mechanisms in *E. siliculosus* by analyzing the effect of heat stress on parthenosporophyte viability and fatty acid and sterol compositions of the membrane in both male and female specimens. Our results indicate that thermotolerance differs between male and female parthenosporophytes and that it is not associated with variation in fatty acid and sterol compositions of the membrane.

2. Materials and methods

2.1. Strains and culture conditions

Male and female gametophyte strains (KU-1371 and KU-1372, respectively) of *E. siliculosus* were purchased from the Kobe University Macro-Algal Culture Collection (KU-MACC) in June 2013, and maintained separately in the laboratory as male and female parthenosporophytes. Culture conditions were identical to those previously described for red seaweeds (Mikami et al., 2009). Briefly, these strains were grown in artificially synthetic seawater at 15°C under irradiation with 70 μmol m^{-2} s^{-1} provided

by cool white fluorescent lamps with a photoperiod of 10 h light:14 h dark. The medium was bubbled continuously with filter-sterilized air and changed weekly.

2.2. Experimental treatment of cultures

To analyze the effect of culture temperature variation on viability and fatty acid and steroid compositions in both male and female gametophytes, replicated ca. 0.1 g of parthenosporophytes were incubated at 5, 10, 15, 20, 25, and 28°C for 2, 5, and 7 days in 9 cm petri dish cultures containing 50 ml of artificial sea water. Samples were taken directly from these cultures for viability tests and 7 day-samples were used for analyses of fatty acid and sterol compositions of the membrane following lyophilization as described below.

2.3. Viability test

Experimental samples were stained with artificial sea water containing 0.05% evans blue (Wako Pure Chemical Industries, Japan). After staining for 20 min at room temperature, samples were gently rinsed with artificial sea water to remove the excess of evans blue and mounted on a slide with artificial sea water. To count the number of blue-stained dead cells, a total of three samples were observed per treatment and photographed using an Olympus IX73 light microscope in conjuction with an Olympus microscope digital camera DP22/DP27 via the standalone connection kit DP2-SAL. These observations were repeated three times.

2.4. Fatty acid analysis

Fatty acids were analyzed essentially according to the method described by Tanaka (2016). Dried algae (20 mg each) and 100 μL internal standard solution containing heptadecanoic acid (10 mg/mL) were placed into separate 15 mL glass centrifuge tubes, and 5 mL of chloroform/methanol (2:1 v/v) was added to each tube. Samples were then exposed to a supersonic wave for 10 min, diluted with the addition of 0.75 mL of distilled water, and centrifuged for 5 min at 2,500 × *g*. The subsequent upper layer was removed by an aspirator and the remaining lower layer containing lipids was transferred to another tube via filtration using a cotton plug. The resulting extract solution was evaporated using a centrifuged concentrator (Taitex, Koshigaya, Japan). The dried extracted lipids and 0.1 mL (10 mg/mL) of heptadecanoic acid as an internal standard were hydrolyzed in a screw-capped glass tube with 0.75 mL of 0.5 mol/L potassium hydroxide in methanol at 100°C for 9 min. The reaction mixture was then added to 1 mL of 14% BF_3 in methanol at 100°C for 7 min. After heating, 3.0 mL of hexane and 2.5 mL of saturated sodium chloride solution were added to the mixture, which was then mixed and centrifuged at 2,500 × *g* for 10 min. The upper layer containing fatty acid methyl esters (FAMEs) was transferred to a Sep-Pak silica column (630 mg; Waters), which was pre-washed with hexane, and FAMEs were eluted with 8.0 mL hexane:diethyl ether (96:4 v/v). The eluted solution was evaporated until dry using a centrifuged concentrator, and dried FAMEs were dissolved in 100 μL of acetone for gas–liquid chromatography (GLC) analysis. The GLC system included a gas chromatograph (GC-14A; Shimadzu) equipped with a flame ionization detector and a capillary column (TC-FFAP, 30 m × 0.25 mm i.d.; GL Science, Tokyo, Japan). The

column temperature was programmed for a linear increase of 2°C/min from 180 to 230°C. The injection and detector port temperatures were both 250°C. The carrier gas was nitrogen, and the flow pressure was 5.0 mL/min. FAMEs were identified on the chromatogram by conventional methods based on the retention time of standards.

2.5. Sterol analysis

Sterol compositions were analyzed according to the method described by Ito (2017). Dried algal samples (20 mg) were placed into 15 mL glass test tubes with 100 μL of 1.0% sodium chloride in water and 2.0 mL of 3.0% pyrogallol in ethanol. This solution was saponified by heating at 70°C for 30 min after the addition of 200 μL of 60% (w/v) potassium hydroxide in water. After heating, 4.5 mL of 1.0% sodium chloride in water and 3.0 mL of n-hexane/ethyl acetate (85:15, v/v) were added to the reaction mixture, subjected to centrifuge at 1,500 × *g* for 10 min. The upper layer was transferred to another tube and the solvents were evaporated using a centrifugal concentrator. After concentration, the unsaponifiable substance was reacted with 1-anthroyl cyanide (ACN). For unsaponifiable substance, a volume of 100 μL containing 50 μg/mL of compounds, 100 μL of benzene, 100 μL of ACN (8.0 mg/mL benzene), and 100 μL of quinuclidine (4.8 μg/mL acetonitrile) as a catalyst for the fluorescence reaction were placed into a 15 mL glass test tube. This solution was then reacted by heating at 50°C for 40 min. The reaction was terminated by adding 500 μL methanol. A 10 μL aliquot of the resulting mixture was injected into the HPLC system. The HPLC system included an L-7100 pump, an L-7300 column oven, an L-7200 auto-sampler, an L-7480 fluorescence detector (Ex 370 nm and Em 470 nm) (Hitach High-Technologies Corporation, Tokyo,

Japan), and a 7725i Rheodyne sample injector connected to a 10 µL sample loop (Rheodyne, Rohnert Park, CA, USA). This system was equipped with a C-30 column (Develosil XG-30M-5, 250 mm × 4.6 mm × 5 µm particle sizes, Nomura Chemical Corporation, Aichi, Japan). The column was set inside the column oven and kept at 40°C. The mobile phase was acetone/acetonitrile/hexane/water (71:20:4:5, v/v) and the flow rate was maintained at 1.0 mL/min. One analysis required 50 min, and new samples were injected at intervals of 10 min. The data were processed by CDS-Lite ver. 5.0 chromatography software (LASOFT Corporation, Chiba, Japan).

2.6. Statistical analysis

All analyses were performed in triplicate for both male and female parthenosporophytes and all results are presented as the mean ± standard deviation (S.D.). All statistical analyses were performed using Statcel for Windows (OMS Ltd., Saitama, Japan). One-way ANOVA was followed by the Tukey–Kramer test for multiple comparisons. Differences were considered significant when the calculated *p* value was less than 0.05.

3. Results

3.1 Male and female parthenosporophytes exhibit differences in thermotolerance

Cultures of male and female *E. siliculosus* parthenosporophytes grown at 15°C were subsequently incubated for a further 2, 5, or 7 days at variable temperatures, namely 5°C, 10°C, 15°C (control), 20°C, 25°C, or 28°C. Evans blue staining, which reveals cell

death with blue color, indicated that the heat stress at 25°C and 28°C caused increased cell death, respect to control (15°C), in particular in female specimens (Fig. 1A), whereas the culture growth at 5°C, 10°C, 15°C, and 20°C did not affect either male or female parthenosporophyte viability following 7 days culture (for 15°C and 20°C, see Figs. 1A and 1B, respectively; for 5°C and 10°C, data not shown). Moreover, as shown in Fig. 1B, although both male and female parthenosporophyte viability was gradually reduced by an increase in temperature, female specimens displayed lower thermotolerance compared to that in male specimens. For example, extensive cell death was observed in female parthenosporophytes incubated for 5 and 7 days at 28°C, whereas male parthenosporophytes had an approximate 50% survival rate under the same temperatures (Fig. 1B). Thus, there is a sex-dependent difference in the level of thermotolerance in *E. siliculosus* parthenosporophytes.

3.2. Differences in fatty acid composition between male and female parthenosporophytes

In this study, the effect of temperature on fatty acid composition in male and female *E. siliculosus* parthenosporophytes was analyzed. In normal conditions, subsequent comparisons revealed that both sexes had an identical fatty acid profile, in that palmitic acid (16:0) was the most abundant saturated fatty acid and oleic acid (18:1n-9), linoleic acid (18:2n-6), α-linolenic acid (18:3n-3), eicosenoic acid (20:1n-9), arachidonic acid (20:4n-6), and eicosapentaenoic acid (20:5n-3) were the most abundant polyunsaturated fatty acids (PUFAs) (see Tables 1 for male and Table 2 for female). Thus, male and female *E. siliculosus* parthenosporophytes are constitutively rich in n-3 and n-6 PUFAs.

In addition, the relative amounts of major fatty acids did not vary in cultures grown at 5–20°C; however, saturated fatty acid (16:0) and unsaturated fatty acids (18:1n-9, 18:2n-6, 18:3n-3, 20:1n-9 and 20:4n-6) were slightly increased and decreased, respectively, following culture growth at 28°C, although the amount of 20:5n-3 was not changed by heat stress (Fig. 2, Tables 1 and 2). Moreover, minor saturated fatty acids like 12:0, 13:0, 15:0, and 18:0 were also increased at 28°C, which contributed the increase in the saturation level at that temperature (Fig. 2). Notably, there were differences in fatty acid composition observed between male and female parthenosporophytes, which were respectively a higher abundance of 18:3n-3 and 18:2n-6, and a higher n-3/n-6 PUFA ratio in male parthenosporophytes (Fig. 2, Tables 1 and 2). These unique fatty acid compositions were unaffected by temperature changes (Fig. 2, Tables 1 and 2).

3.3. Differences in sterol composition between male and female parthenosporophytes

Sterols, such as cholesterol, and phytosterols, such as stigmasterol, β-sitosterol, campesterol, ergosterol, and fucosterol, were simultaneously analyzed via a recently developed method based on high-performance liquid chromatography (HPLC) with fluorescence (FL) detection (Ito et al., 2017). A subsequent analysis of chromatograms revealed the composition of major sterols in both male and female parthenosporophytes, which showed that the fucosterol content was greater than that of both ergosterol and cholesterol (Fig. 3, Table 3). Furthermore, campesterol, β-sitosterol, and stigmasterol were detected only in trace amounts (Table 3). As shown in Fig. 3, high ergosterol and

cholesterol contents were measured in female specimens compared to male specimens, in particular in cultures grown at 25°C; however, culture growth at 28°C caused an increase in ergosterol and cholesterol in both male and female parthenosporophytes (Fig. 3, Table 3). By contrast, fucosterol content was reduced by culture growth at 28°C (Fig. 3, Table 3). Despite the presence of these differences for each sterol, in terms of the total amount of sterols, it is difficult to find relevance between a higher content of sterols and sensitivity to 25°C in female specimens, whereas a relationship between a low abundance of sterols and sensitivity to 28°C in both sexes seems to be reasonable.

4. Discussion

Marine temperature varies within daily and monthly periods and affects the growth and survival of poikilothermal organisms because it influences physiological homeostasis. Thus, temperature change is recognized by organisms as an environmental stress that has the potential to cause physiological disorders. However, there are specific responses and adaptations to unfavorable temperatures to cope with the adverse effects of heat stress, such as the modulation of fatty acid composition and the activation of transcription factors that promote heat stress–inducible gene expression (Upchurch, 2008). In this study, we examined the effects of temperature variation on survival of parthenosporophytes of the brown alga *E. siliculosus* and their related fatty acid and sterol compositions. Our results showed sex-dependent differences in thermotolerance at 25°C and 28°C (Fig. 1A and B); however, it is difficult to attribute low thermotolerance in females to temperature-dependent alterations in fatty acid and sterol compositions, as described below.

Our results show that n-3 and n-6 fatty acids are the most abundant in male and female parthenosporophytes, respectively, whereas the relative abundance of saturated and unsaturated fatty acids was not influenced by temperature changes in both sexes (Fig. 2, Tables 1 and 2). Overall, the fatty acid profile in male parthenosporophytes is composed of fatty acids with a higher number of double bonds than those in female parthenosporophytes (Fig. 2, Tables 1 and 2), suggesting membrane fluidity in males and rigidity in females, respectively. It seems that there is indeed a correlation between thermotolerance and a higher abundance of n-3 PUFA in males, whereas low thermotolerance in females is associated with a higher abundance n-6 PUFA (Figs 2, 3, Tables 1, 2, 3). However, thermotolerance in male parthenosporophytes that possess membranes with greater fluidity does not agree with previous reports which stated that membrane rigidity caused by high saturated fatty acid content is important for thermotolerance (Carratù et al., 1996; Horváth et al., 1998; Vigh et al., 1998; Murakami et al., 2000; Königshofer et al., 2008). Indeed, it has been reported that artificial decreases in the number of unsaturation bonds in membrane fatty acids can decrease the expression of a fluidization-inducible gene (Vigh et al., 1993), indicating a direct correlation between fatty acid saturation level and membrane fluidity. Thus, it is possible that the modulation of membrane fluidity under varying temperature is not involved in *E. siliculosus* parthenosporophyte thermotolerance, although changes in membrane fluidity should be confirmed directly.

Our data also indicated that the unsaturated fatty acid content in both sexes did not change in cultures grown under low temperatures (Fig. 2, Tables 1 and 2). Again, this

result does not agree with previous reports which stated that membrane fluidity caused by high unsaturated fatty acid content plays a key role in cold tolerance (Örvar et al., 2000; Sangwan et al., 2002; Mikami and Murata, 2003). However, in *E. siliculosus,* fatty acid composition is also not affected by hyposaline and oxidative stress conditions, although hypersaline and copper stresses do have an effect (Dittami et al., 2011; Ritter et al., 2014), suggesting the presence of regulatory mechanisms for abiotic stress responses without changes in the fatty acid composition in *E. siliculosus,* which could explain why low temperatures have a minimal effect on fatty acid composition in parthenosporophytes. In addition, seasonal variation in fatty acid profiles were reported in the sporophytes of other brown seaweeds, such as *Saccharina* and *Sargassum*, with higher contents of saturated fatty acids observed in warm seasons (Gerasimenko et al., 2014; Marinho et al., 2015; Gerasimenko and Logvinov, 2016). Therefore, it is possible that there are differences in responsiveness of *E. siliculosus* parthenosporophytes and sporophytes to temperature stress in terms of fatty acid profile changes.

In addition to fatty acids, we simultaneously analyzed sterols in parthenosporophytes using our novel HPLC-FL method, which has previously been successfully used to quantify six sterols in green, red, and brown algae (Ito et al, 2017). FL detection is known to be highly sensitive and selective, and is thus suitable for the analysis of low-concentration compounds in a small sample. Furthermore, by using an FL detector, the HPLC-FL method is able to detect sterols at equivalent or greater sensitivity compared to that possible using GC-FID, HPLC-UV, and LC-MS methods. To the best of our knowledge, this study is the first description of sterol composition in *E. siliculosus*. We demonstrated that fucosterol was the predominant sterol and ergosterol

338 and cholesterol were detected as minor sterols in *E. siliculosus* parthenosporophytes
339 (Fig. 3, Table 3). This sterol profile is comparable to those previously reported for other
340 brown seaweeds (Aknin et al., 1992; Fleury et al., 1994; Sánchez-Machado et al., 2004;
341 Kumari et al., 2013; El Shoubaky and Salem, 2014). Sterol type was not altered by heat
342 stress, although a reduction in fucosterol content and an increase in ergosterol and
343 cholesterol contents were observed in both male and female cultures grown at high
344 temperatures (Fig. 3, Table 3). In particular, female parthenosporophyte membranes
345 contained high amounts of these sterols when grown at 25°C, suggesting they had
346 higher membrane rigidity compared to that in male parthenosporophytes at the same
347 temperature. Thus, there is a correlation between thermosensitivity and membrane
348 rigidity caused by relatively high amounts of sterols in female parthenosporophytes at
349 25°C. However, this result is inconsistent with previous reports indicating that the
350 increase in sterol contents results in the increase in membrane rigidity (Ford and Barber,
351 1983; Mora et al., 1999) and enhanced thermotolerance under high temperature
352 conditions is acquired with increased membrane rigidity by increasing saturated fatty
353 acid/unsaturated fatty acid ratio (Carratù et al., 1996; Horváth et al., 1998; Vigh et al.,
354 1998; Murakami et al., 2000; Königshofer et al., 2008). Despite the decrease in sterol
355 contents in both male and female parthenosporophytes at 28°C, there was
356 sex-dependent differences in theremotolerance at that temperature (compare Figs. 1 and
357 3), for which we propose a contribution of constantly high amounts of n-3 fatty acids in
358 male (Fig. 2).
359
360 Taken together, although female parthenosporophytes display lower thermotolerance
361 compared to that in males and compositions of fatty acids and sterols were modulated

under high temperature, the relationships between low thermotolerance and membrane rigidification in *E. siliculosus* contrast to previous findings in many poikilothermal organisms. Thus, we propose that the sex-dependent difference in *E. siliculosus* thermotolerance may not be attributed to membrane fluidity based on our observed compositions of fatty acids and sterols; however, it remains unknown why females display low thermotolerance at 25°C and 28°C. Therefore, it is necessary to confirm if parthenosporophytes respond to temperature stress and, if so, the regulatory mechanisms behind such responses should be resolved by analyzing temperature-dependent changes in metabolic activity and gene expression.

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548

549 **Figure legends**

550

551 Fig. 1. Differences in thermotolerance between male and female parthenosporophytes of
552 *E. siliculosus*. (A) Visual comparison of thermotolerance at 25°C and 28°C by staining
553 with evans blue. Photos at 15°C as controls are also indicated for both male and female
554 parthenosporophytes. Dead cells are stained in blue color. Bar: 100 μm. (B) Relative
555 viability of parthenosporophytes after incubation under various temperature conditions
556 for various durations. Values are means ± SD (n=3). Alphabetical different letters
557 denote a statistical significant difference ($p < 0.05$, one-way ANOVA). Black and white
558 bars represent male and female parthenosporophytes, respectively.

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560 Fig. 2. Comparison of fatty acid compositions between male and female
561 parthenosporophytes of *E. siliculosus*. Relative values of saturated, monounsaturated,
562 and polyunsaturated fatty acids are compared among seaweeds separately incubated
563 under various temperature conditions for 7 days. Relative contents of n-3 and n-6
564 polyunsaturated fatty acids and n-3/n-6 ratios are also indicated. Black and white bars
565 represent male and female parthenosporophytes, respectively. Values are means ± SD
566 (n=3). Alphabetical different letters denote a statistical significant difference ($p < 0.05$,
567 one-way ANOVA).

568

569 Fig. 3 Comparison of sterol compositions between male and female
570 parthenosporophytes of *E. siliculosus*. Contents of fucosterol, ergosterol, and
571 choelesterol are indicated for seaweeds separately incubated under various temperature
572 conditions for 7 days. Black and white bars represent male and female

573 parthenosporophytes, respectively. Values are means ± SD (n=3). Alphabetical different
574 letters denote a statistical significant difference ($p < 0.05$, one-way ANOVA).
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Table 1. Fatty acid compositions of male parthenosporophytes of *Ectocarpus siliculosus* under various temperature conditions for 7 days. Values are the percentage of total fatty acids, and represent means ± SD from triplicate replicated experiments.

	5°C	10°C	15°C	20°C	25°C	28°C
12:0	1.1±0.2	1.2±0.1	1.1±0.1	1.2±0.3	1.1±0.2	2.6±0.5
13:0	1.1±0.1	1.1±0.1	1±0.1	1±0.1	1.2±0.1	2.7±0.4
14:0	1.5±0.2	1.5±0.1	1.4±0.2	1.4±0	1.7±0.2	1.6±0.2
14:1	0.1±0	0.3±0.1	0.2±0	0.2±0.2	0.2±0.2	0.6±0.2
15:0	2.2±0.1	1.8±0.2	2±0.2	1.8±0.4	2.3±0.3	4.6±0.3
15:1	4.1±0.2	3.2±0.4	3.5±0.5	3.2±0.9	4.3±1	8.4±1.3
16:0	12.5±0.3	12±0.3	12.3±0.9	12.7±0.4	13.6±0.5	14.1±1
16:1	0.4±0.2	0.3±0.1	0.3±0.3	0.3±0.1	0.2±0	0.5±0.1
18:0	0.7±0	0.7±0.1	0.8±0.2	0.7±0.1	1±0.2	1.4±0.1
18:1n-9	4.5±0.2	4.7±0.2	5.3±0.5	5.3±0	4.5±0.3	3.8±0.2
18:1n-7	1.1±0.1	0.9±0.1	0.8±0.3	0.6±0.1	1.4±0.5	4.1±0.9
18:2n-6	8.5±1.1	9.6±0.7	9.7±0.5	10.2±0.3	10±0.4	7.8±0.1
18:3n-6	1.1±0	1±0	1.1±0.1	1.1±0	0.9±0	1±0.1
18:3n-3	22.8±0.3	24.1±0.7	23.8±1.5	24.1±1.4	23.6±1.2	16±0.1
20:1n-9	15.3±1.2	14.5±0.2	14.9±1	15±0.3	13.3±1.3	10.5±0.5
21:0	0.1±0	0.1±0	0.1±0	0.1±0.1	0±0.1	0.1±0
20:2n-6	0.1±0.1	0.1±0	–	0±0.1	0.1±0.1	0.1±0
20:3n-6	0.2±0	0.6±0.7	0.1±0	0.1±0	0.2±0	0.1±0
20:4n-6	10.8±0.2	10.5±0.4	9.9±0.6	9.3±0.1	8.4±0.4	8.5±0.5
22:1n-9	0.1±0	0.1±0.1	0.1±0	–	0.1±0.1	0.2±0
20:5n-3	11.3±0.2	11.5±0.3	11.3±0.8	11.3±0.2	11.5±0.3	11.1±0.6
24:0	0.1±0	–	–	–	–	–
24:1n-9	–	–	0.1±0.1	0.1±0	–	–
22:5n-3	0.1±0.1	–	0.1±0.1	0.1±0.1	0.2±0.2	0.1±0
22:6n-3	0.3±0.2	0.2±0.3	0.1±0	–	0.1±0.1	0.1±0.1
Saturates	19.2±0.3	18.4±0.4	18.7±1.6	19±0.5	20.9±0.7	27.2±1.6
Monoenes	25.7±0.9	23.9±0.7	25.3±0.7	24.7±0.5	24.1±0.7	28.1±2.3
Polyenes	55.1±0.8	57.7±1.1	56.1±2.2	56.3±0.8	55±1	44.8±0.9
n-3	34.4±0.6	35.9±1.2	35.3±1.9	35.5±1.2	35.3±0.9	27.3±0.5
n-6	20.6±1.2	21.8±0.5	20.8±1	20.8±0.4	19.7±0.8	17.5±0.4
n-3/n-6	1.7±0.1	1.6±0.1	1.7±0.1	1.7±0.1	1.8±0.1	1.6±0

602 **Table 2.** Fatty acid compositions of female parthenosporophytes of *Ectocarpus*
603 *siliculosus* under various temperature conditions for 7 days. Values are the percentage of
604 total fatty acids, and represent means ± SD from triplicate replicated experiments.

	5℃	10℃	15℃	20℃	25℃	28℃
12:0	1.2±0.1	0.8±0.5	1.1±0.2	1±0.4	1.8±1.3	1.8±0.6
13:0	1.1±0	1.1±0.1	1.1±0.1	1±0.2	0.8±0.5	2.1±0.1
14:0	1.9±0.1	1.7±0.2	1.8±0.1	1.6±0.1	1.5±1.2	2.2±0.2
14:1	0.2±0.1	0.2±0.2	0.2±0.1	0.1±0.1	0.1±0.1	0.3±0
15:0	1±0.7	1.7±0.5	1.9±0.4	1.4±1.1	1.5±1.3	3.7±0.4
15:1	3.1±0.9	2.7±1.2	3.4±0.8	4.1±0.8	2.9±2.6	7±1.5
16:0	11.3±0.4	11.6±0.4	12±0.7	12.4±1.3	15±1.1	15.4±0.5
16:1	0.6±0.1	0.4±0.1	0.4±0.1	0.3±0	0.8±0.5	0.7±0.2
18:0	0.9±0.1	0.8±0.1	0.9±0.1	0.9±0.1	1.6±0.3	2±0.4
18:1n-9	5.7±0.6	5.8±0.2	6.1±0.3	6±0	6.5±0.2	6.8±0.6
18:1n-7	1±0.4	0.7±0.2	0.6±0.1	0.6±0.1	1.2±0.1	4.1±0.6
18:2n-6	15±1	15.5±0.5	15.2±0.2	15.1±0.8	15.6±0.8	12.7±1.3
18:3n-6	1.7±0	1.7±0	1.6±0.1	1.5±0.1	1.4±0.1	1.4±0.1
18:3n-3	20.9±0.4	20±0.4	19.5±0.4	20.3±0.2	18.7±0.9	10.6±0.2
20:1n-9	11.4±0.6	11.6±0.3	11.6±0.3	11.3±0.6	8.8±1.1	7.3±0.7
21:0	0.1±0.1	0.1±0	0.1±0	0.1±0.1	0.1±0.1	0.1±0
20:2n-6	0.1±0	0±0.1	0±0.1	–	0±0.1	–
20:3n-6	0.2±0.1	0.2±0.1	0.2±0	0.2±0	0.2±0	0.2±0
20:4n-6	12.1±0.6	12.3±0.1	11.6±0.4	11.4±0.4	10.5±0.4	10.7±0.7
22:1n-9	0.1±0.1	0.1±0.1	0.1±0	0.1±0.1	0.1±0	0.1±0
20:5n-3	9.9±0.5	10.5±0.3	10.1±0.7	10.1±0.3	9.8±0.6	9.2±0.2
24:0	–	–	0.1±0.1	0.2±0.3	0.1±0.1	0.1±0.1
24:1n-9	–	0.1±0	0.1±0.2	–	0.1±0.1	0.2±0.1
22:5n-3	0.2±0.1	0.1±0	0.1±0.1	0.1±0.1	0.5±0.3	0.3±0.2
22:6n-3	0.3±0.3	0.1±0	0.1±0.1	0.2±0.2	0.4±0.2	0.9±0.8
Saturates	17.6±0.2	17.9±0.3	19±1	18.6±0.8	22.3±0.3	27.4±1.3
Monoenes	22.1±1.1	21.6±0.7	22.5±0.6	22.5±0.7	20.6±2	26.6±1.8
Polyenes	60.3±1.1	60.4±0.7	58.6±1.5	58.9±1.2	57.2±1.9	46±1.5
n-3	31.3±0.8	30.7±0.6	29.9±1	30.7±0.7	29.4±1.2	21.1±0.9
n-6	29±1.5	29.8±0.7	28.7±0.6	28.2±0.9	27.8±1	24.9±0.6
n-3/n-6	1.1±0.1	1±0	1±0	1.1±0	1.1±0	0.8±0

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607 **Table 3.** Sterol compositions of male and female parthenosporophytes of *Ectocarpus*
608 *siliculosus* under various temperature conditions for 7 days. Values are quantified
609 content (μg/mg tissue), and represent means ± SD from triplicate replicated
610 experiments.

	5℃	10℃	15℃	20℃	25℃	28℃
Male						
Ergosterol	0.25±0.04	0.12±0.02	0.27±0.21	0.19±0.06	0.17±0.02	0.51±0.07
Fucosterol	4.86±0.47	3.19±0.18	3.62±0.91	4.05±0.95	2.75±0.3	1.53±0.29
Cholesterol	0.08±0.02	0.04±0.01	0.07±0.05	0.07±0.02	0.05±0	0.15±0.02
Stigmasterol	–	–	–	–	–	–
Campesterol	0.01±0	0.01±0	0.01±0	–	0.01±0.02	0.02±0.01
β-sitosterol	0.01±0	–	–	–	–	–
Female						
Ergosterol	0.19±0.04	0.21±0.02	0.23±0	0.26±0.05	0.33±0.06	0.48±0.04
Fucosterol	3.98±0.79	4.06±0.52	3.76±0.11	4±0.6	3.48±0.4	1.21±0.03
Cholesterol	0.07±0.01	0.08±0.01	0.08±0	0.1±0.02	0.12±0.02	0.16±0.02
Stigmasterol	–	–	–	–	–	–
Campesterol	–	–	–	–	–	–
β-sitosterol	0±0.01	–	–	0.01±0	0.01±0	0.03±0.01

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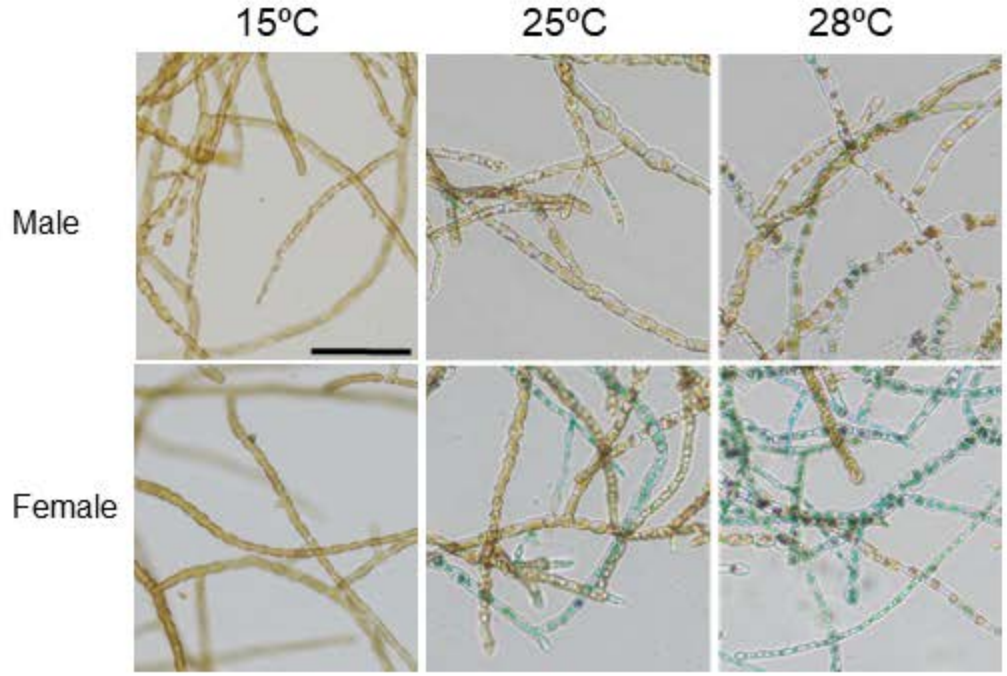
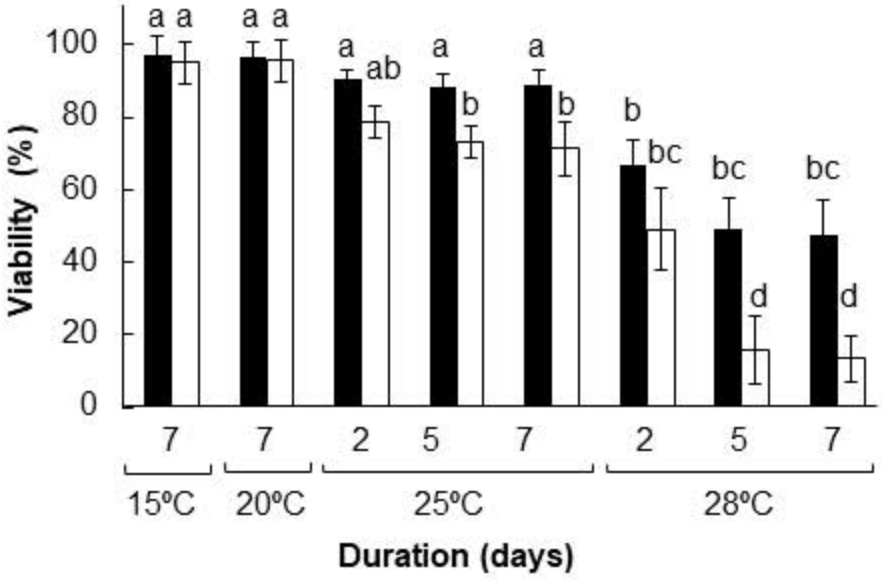
A


15ºC
25ºC
28ºC
Male
Female

B


Viability (%)
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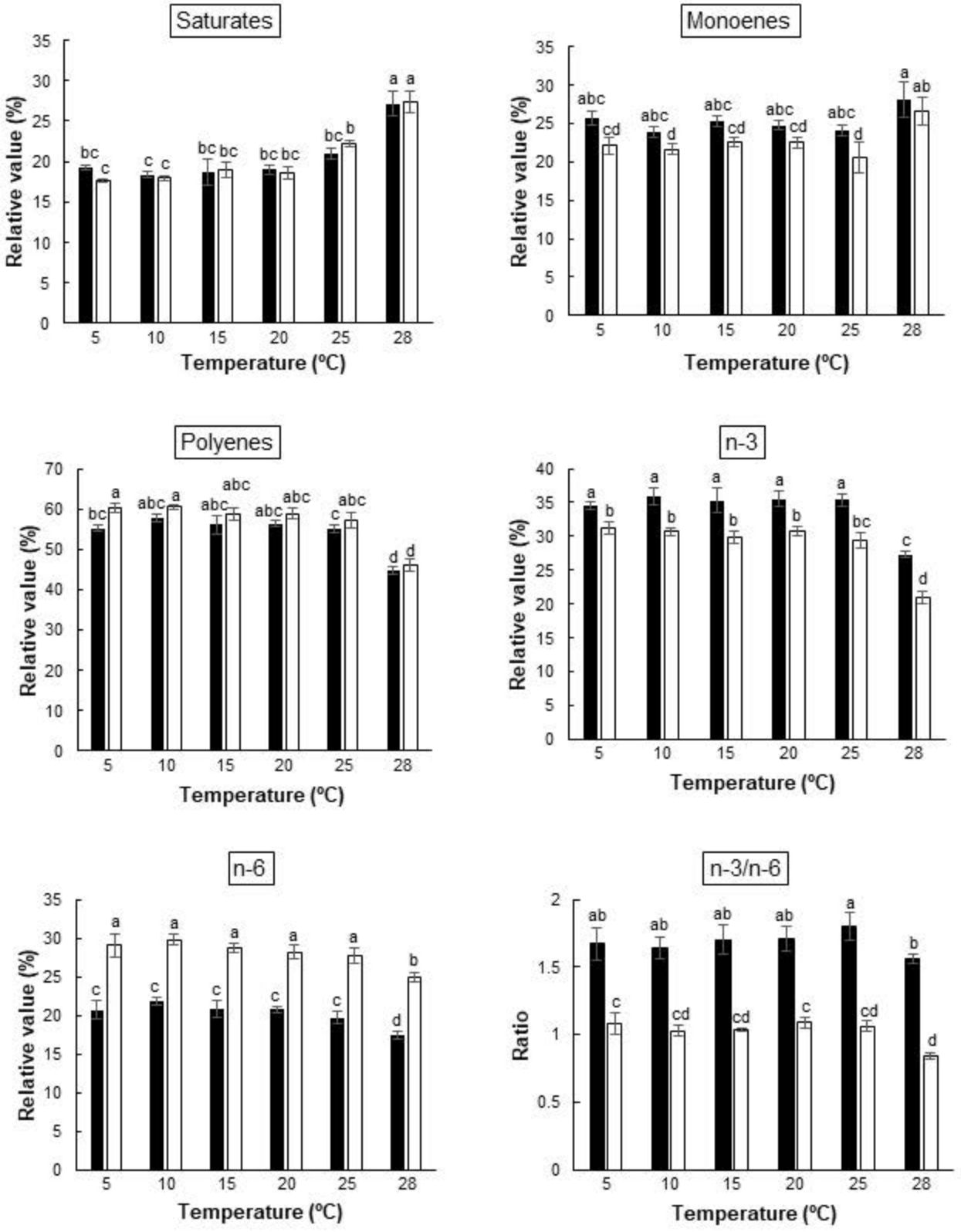
28ºC
Duration (days)

Fig. 1


Saturates
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Monoenes
Relative value (%)
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Polyenes
Relative value (%)
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n-3
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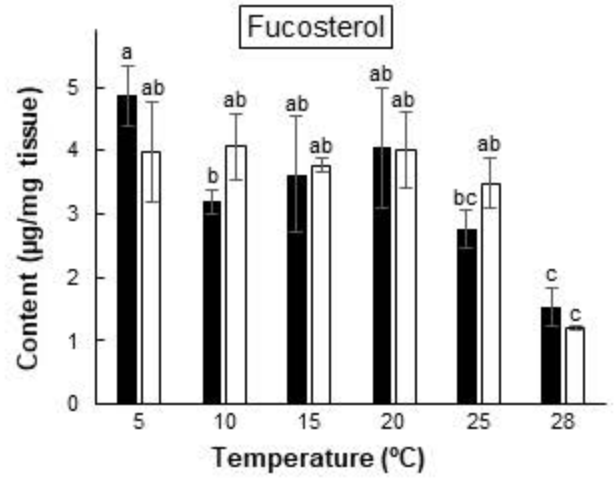
n-6
Relative value (%)
Temperature (ºC)
n-3/n-6
Ratio
Temperature (ºC)

Fig. 2


Fucosterol
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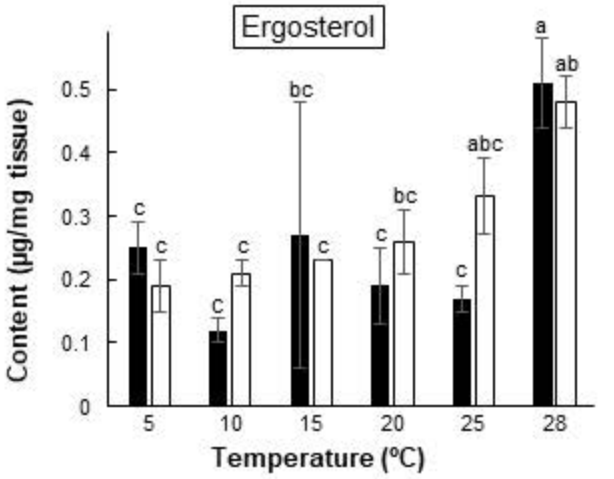
Temperature (ºC)


Ergosterol
Content (µg/mg tissue)
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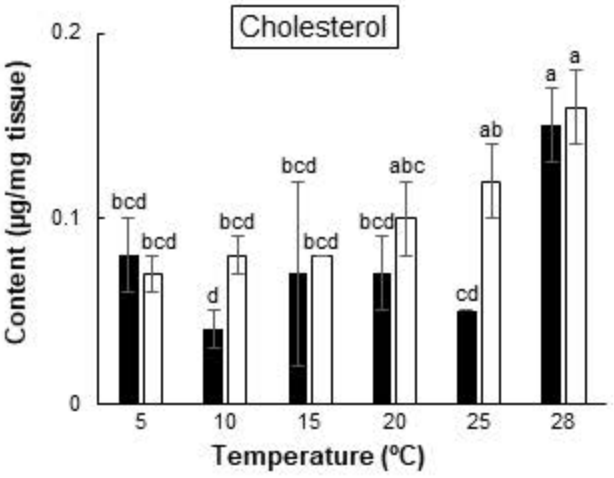
Temperature (ºC)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Content (µg/mg tissue)
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bcd
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bcd
bcd
abc
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ab
a
a
5
10
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20
25
28
Temperature (ºC)

Fig. 3

Highlights

- Female *Ectocarpus siliculosus* parthenosporophytes have low thermotolerance.
- Membranes of female specimens contain a higher proportion of saturated fatty acids.
- Female specimens contain higher amounts of sterols compared to that in males.
- Membranes of female specimens are thought to be more rigid than those of males.
- The level of thermotolerance cannot be explained by membrane fluidity in *E siliculosus*.